BEFORE THE OIL CONSERVATION COMMISSION
COMMENCING NOVEMBER 12, 2024

CASE NO. 23580

WILD EARTH GUARDIANS – PFAS RULEMAKING

PART 3

NEW MEXICO OIL AND GAS ASSOCIATION

NMOGA EXHIBIT E5.7 THROUGH
NMOGA EXHIBIT E11.498

Table 3. Distribution of Σ_{44}PFAS, EOF and UOF among donors who report using select organofluorine pharmaceuticals and those who do not.

	No Reported Pharma Use (n = 10)	Reported Pharma Use (n = 10)
Concentration of Σ_{44} PFAS (ng/mL)		
mean (±SD)	6.54 (±3.55)	9.51 (±7.35)
median	5.87	7.49
range	3.16–14.90	2.88–26.24
Concentration of EOF (ng F/mL) [a]		
mean (±SD)	6.10 (±2.59)	6.93 (±2.76)
median	6.45	6.26
range	2.02–10.04	2.67–11.22
Concentration of UOF (ng F/mL) [b]		
mean (±SD)	3.37 (±2.04)	3.73 (±1.31)
median	2.99	4.02
range	0.94–7.48	1.70–6.05

Notes: [a] EOF was measured using CIC for fluorine.; [b] UOF was determined as the concentration of EOF not explained by fluorine attributable to Σ_{44} PFAS.

The median values of Σ_5 PFAS (SI Table S2) were lower in female donors compared to male donors, which is consistent with data from NHANES (data not shown) [45]. For both males and females in our study, median concentrations of Σ_5 PFAS were lower than national levels reported in NHANES in 2017–2018; however, 100% of donors in our study identified as Black or Hispanic, who have lower median levels of PFAS compared to non-Hispanic white populations in NHANES [45,46].

3.3. Extractable Organofluorine in Serum

Concentrations of EOF observed in our study ranged from <2.02 to 11.2 ng F/mL and were slightly higher amongst the pharmaceutical users (Table 3). Consistent with NHANES, the individual PFAS analytes comprising the majority of identified EOF were linear and branched PFOS, collectively accounting for roughly 50%, followed by PFHxS (23%), and PFOA (14%) (SI Table S2). The concentration of EOF was similar across Black and Hispanic donors (data not shown).

3.4. Unexplained Organofluorine in Serum

The proportion of UOF relative to EOF measured in serum ranged from 15% to 86% (Figure 1), which is comparable with previous studies that show the proportion of UOF ranging from 30% to 70% [26,27]. The distribution of UOF (ng F/mL) in our study was approximately normal and ranged from 0.94 to 7.48 ng F/mL (Table 3). The mean concentration of UOF was slightly lower in serum from Black donors compared to Hispanic donors and slightly greater in females compared to males, but neither difference was statistically significant ($p > 0.05$) (Table S3a,b). On average, study participants above the median age of 48.5 had a 1.4 ng F/mL greater concentration of UOF than those below the median age ($p = 0.056$) (Table S3c). The concentration of UOF and fluorine attributable to Σ_{44} PFAS do not appear to be correlated, with a Spearman correlation coefficient of $\rho = 0.06$ (p-value = 0.82) suggesting that contributors to UOF are not associated with the fluorine attributed to the 44 PFAS measured in serum.

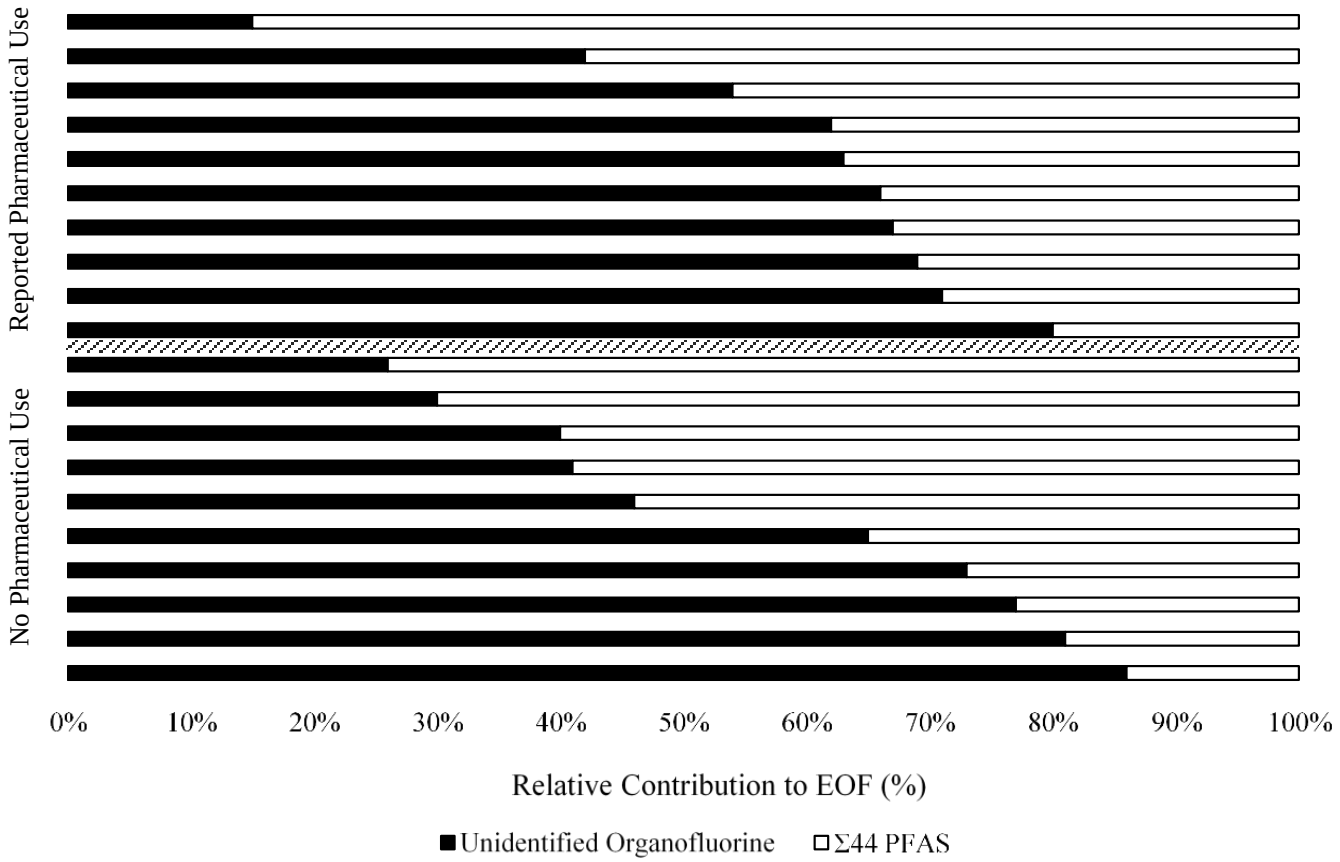

Figure 1. Relative contribution of unidentified organofluorine and Σ_{44} PFAS to extractable organofluorine (EOF) (%) in individual serum samples from donors who report using select organofluorine pharmaceuticals (n = 10) and those who do not (n = 10).

3.5. Linear Regression of UOF on Pharmaceutical Use

Comparing the difference in the concentration of UOF between groups, people who report using organofluorine pharmaceuticals had 0.36 ng F/mL greater UOF, on average, compared to people who reported not using these pharmaceuticals (95% CI: −1.26, 1.96, Figure 2), but the difference was not statistically significant at the $\alpha = 0.05$ level. Adjusting for age had no effect on the relationship between pharmaceutical use and the concentration of UOF (Table 4). Diagnostic tests showed the linear model did not violate regression assumptions. We identified one potential outlier; omitting the observation, the crude mean difference in UOF between pharmaceutical users and non-users increased to 0.81 ng F/mL (95% CI: −0.56 to 2.18).

Table 4. Linear regression estimating the relationship between unexplained organofluorine (ng F/mL) and reported pharmaceutical use, adjusting for age.

Variable	Coefficients (95% CI)	Standard Error
Intercept	2.65 (1.36 to 3.94)	0.61
Organofluorine Pharmaceutical Use	0.36 (−1.14 to 1.85)	0.71
Age [a]	1.43 (−0.06 to 2.93)	0.71

Notes: [a] Model is adjusted for age (above or below median age of 48.5).

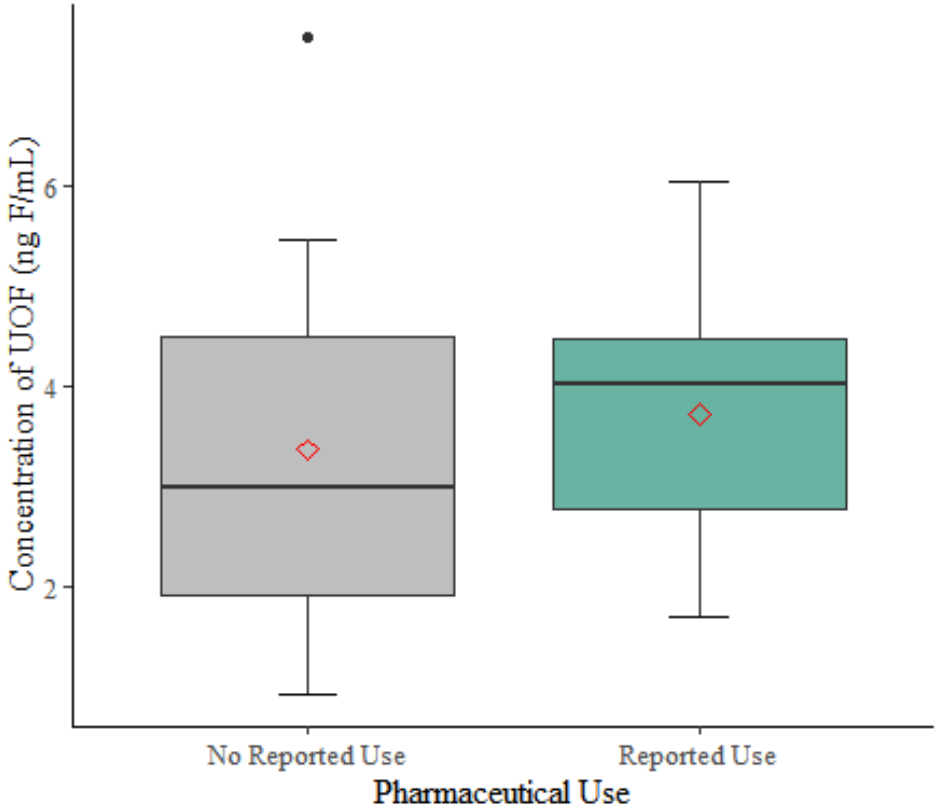

Figure 2. Boxplots of UOF measured in donated serum (n = 20). Whiskers range from 1 SD above and below the mean for each group with the mean and median observation for UOF among reported users of nine organofluorine pharmaceuticals and non-users.

4. Discussion

Previous studies using organofluorine mass balance revealed the occurrence of UOF in environmental and biological matrices [25,47–51], yet the characterization of total and unknown EOF in U.S. serum is not understood. In this study, we show that the concentration of EOF in serum from a sample of U.S. adults is only partially explained by conventional PFAS. The 44 PFAS we targeted account for 14–85% of EOF in serum, comparable with previous findings from China, which showed the concentration of Σ_{10} PFAS accounted for 30–70% of EOF [26], and from Sweden, which showed the concentration of Σ_{61} PFAS accounted for 30–74% of EOF [27]. Substituting zero for left-censored values used to calculate Σ_{44} PFAS in our study may underestimate the fraction of EOF explained by targeted PFAS.

Previous studies in Sweden suggest UOF may differ by sex and age [27]. Bivariate analyses in our study suggested a small difference by sex (UOF slightly increased in females) and a larger difference by age (higher above the median age than below). Age did not appear to confound the relationship between UOF and reported use of organofluorine pharmaceuticals, but the small sample size in this exploratory study limited further examination of possible confounders. Importantly, limited information on the commercial donor population and demographics besides sex, age, and race/ethnicity reduces our ability to generalize results to other populations.

Our results suggest people who reported using organofluorine pharmaceuticals have a slightly greater concentration of UOF (0.36 ng F/mL) compared to those who do not report using these pharmaceuticals. While this difference is consistent with the estimated organofluorine concentrations contributed by some drugs (e.g., Lipitor and Crestor), it is two orders of magnitude lower than some others (Table 1). If taken as prescribed, organofluorine pharmaceuticals should exist in serum at relatively stable levels, and the estimated concentration of organofluorine attributable to some pharmaceutical compounds

exceeded 40 ng F/mL (i.e., Prozac, Januvia). For comparison, the median blood level for PFOS in the general U.S. population in 2017–2018 was 4.30 ng/mL and 1.47 ng/mL for PFOA [45].

There are at least two possible explanations for the discrepancy between the pharmacokinetic estimates and the analysis of EOF in serum: (1) uncertainties in knowledge about pharmaceutical use; and (2) analytical approaches to the quantification of EOF related to pharmaceuticals in serum. We assumed that the pharmaceuticals were in steady state, using the average concentration at steady state to represent the range of levels that would be expected upon continuous administration of a drug, yet we lacked information on the duration, frequency, or compliance of serum donors for the pharmaceuticals they reported using. We also lacked information on socioeconomic status that could influence whether pharmaceuticals are used as prescribed in this population (e.g., adherence) and whether the results can be generalized to other populations. Self-reported pharmaceutical use could introduce non-differential misclassification of exposure if donors did not accurately recall the names of their medications or if they did not truthfully report their medication use (e.g., because of associated social stigma [52]). This misclassification would bias our results towards the null. Furthermore, people may not take the pharmaceuticals as prescribed (e.g., accidentally or intentionally skipping doses), though the slow elimination rates of some organofluorine pharmaceuticals make it likely for the compound to persist in the body for days to weeks even if dosing is skipped or stopped [53].

Discrepancies between the pharmacokinetic estimates and the EOF analysis may also be explained by differences in analytical measurements. We used ion-pair extraction, a method shown to capture some PFAS (neutral, sulfonates, and carboxylates); however, the capability for capturing cationic or zwitterionic compounds varies and depends on chain length [54]. Depending on the functional groups and the dissociation constant, organofluorine pharmaceuticals can be neutral, anionic, cationic, or zwitterionic at physiological pH (Table 1), as can some "PFAS" [1,55]. Since no alkaline buffer was used for the ion-pair extraction, Januvia, Prozac, Citalopram/Escitalopram, and Paxil (Table 1), each of which exist as cations at physiologic pH, may not be captured using conventional extraction methods developed for anionic compounds. It is possible that traditional extraction techniques for anionic compounds do not capture the full suite of organofluorine compounds in a sample, and true EOF is likely much larger, particularly in samples where cationic organofluorine species are present. Furthermore, our analysis was limited to pharmacokinetic estimates for organofluorine from parent compounds, not considering the contributions from fluorinated metabolites that can also accumulate in serum. For example, fluoxetine (Prozac) is extensively metabolized into norfluoxetine, which is measured at concentrations of 72–258 ng F/mL and has a fluorine equivalent of 13–47 ng F/mL [53]. Fluorinated metabolites exist for other organofluorine pharmaceuticals as well, but differences in pharmacokinetics related to age, sex, diet, genetic polymorphisms in metabolizing enzymes, and drug-drug interactions make estimating the organofluorine contribution from active and inactive metabolites more complicated [56]. Therefore, the estimated concentration of organofluorine in serum attributable to pharmaceuticals is likely even greater, with true EOF accounting for contributions from organofluorine pharmaceuticals and metabolites.

Our results suggest that organofluorine pharmaceuticals contribute to EOF, but that a substantial amount of EOF remains unexplained. Large fractions of UOF among people who report not using the nine organofluorine pharmaceuticals suggest other sources of UOF. Other sources of EOF not measured in this study may include pesticides, ultra-short-chain organofluorine compounds such as TFA, as well as PFAS or their precursors, for which analytical standards are not available or have not yet been identified. We did not analyze ultra-short-chain PFAS in our study, though one study in Sweden detected TFA in >60% of blood samples [27]. While short-chain compounds typically have shorter biological half-lives [57], continuous exposure to these compounds in the environment may contribute to EOF.

Recent studies using EOF as a class-based analytical method to screen for PFAS in environmental media may wish to understand the extent to which unknown PFAS contribute to contamination [58]. However, whether organofluorine compounds such as TFA or pharmaceuticals contribute to EOF as "PFAS" depends on the definition of PFAS being used and the user-specific working scope. For example, as written, the definition developed by the U.S. Department of Defense for the purpose of monitoring for PFAS in surface waters includes 94% of organofluorine pharmaceuticals [21]. In this context, measuring the presence of pharmaceuticals could be of great importance, and analyses using EOF to screen for PFAS should consider using multiple extraction methods that can measure anions, cations, and zwitterions because organofluorine pharmaceuticals are present in surface water [21,59]. Non-pharmaceutical organofluorines also exist as cations and zwitterions [1,55]. All of these compounds would contribute to EOF if fully extracted, yet whether they contribute as "PFAS" depends on how PFAS are defined and the context in which they are studied. Future analyses using EOF to screen for PFAS may consider multiple extraction methods to detect these compounds in environmental and biological media.

5. Conclusions

Since the detection of organofluorine in serum in the 1960s, efforts to close the fluorine mass balance gap rely on adequate analytical methods and standards to identify, detect, and quantify compounds of interest. Here, we present an illustrative example highlighting the importance of using appropriate analytical methods for the context of the analysis. The definition of PFAS has important implications for organofluorine mass balance, as the fraction of EOF explained by "PFAS" depends on the definition being used. Depending on the purpose for which a definition is being used (e.g., water quality monitoring, regulatory action to ban PFAS in consumer products) [21], the implications for EOF and the inclusion of cations and zwitterions may vary. Our findings suggest organofluorine pharmaceuticals contribute to EOF in serum, but a large fraction of EOF remains unexplained. Future analyses should consider multiple extraction methods to also include cations and zwitterions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics11050416/s1. Table S1: Analytes for targeted LC-MS/MS and their limits of quantification (LOQ); Table S2: Concentrations of target PFAS per subject (ng/mL), extractable organofluorine (EOF; ng F/mL), and the concentration of fluorine attributable to total PFAS (F44-PFAS; ng F/mL); Tables S3: Unexplained organofluorine (UOF) stratified by sex, race, and age.

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Data Availability Statement: Research data can be found in the Supplemental Information.

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References

1. Glüge, J.; Scheringer, M.; Cousins, I.T.; DeWitt, J.C.; Goldenman, G.; Herzke, D.; Lohmann, R.; Ng, C.A.; Trier, X.; Wang, Z. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environ. Sci. Process. Impacts* **2020**, *22*, 2345–2373. [CrossRef] [PubMed]
2. Sunderland, E.M.; Hu, X.C.; Dassuncao, C.; Tokranov, A.; Wagner, C.C.; Allen, J.G. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. *J. Expo. Sci. Environ. Epidemiol.* **2019**, *29*, 131–147. [CrossRef] [PubMed]
3. De Silva, A.O.; Armitage, J.M.; Bruton, T.A.; Dassuncao, C.; Heiger-Bernays, W.; Hu, X.C.; Kärrman, A.; Kelly, B.; Ng, C.; Robuck, A.; et al. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environ. Toxicol. Chem.* **2021**, *40*, 631–657. [CrossRef] [PubMed]
4. DeLuca, N.M.; Minucci, K.M.; Mullikin, A.; Slover, R.; Cohen Hubal, E.A. Human exposure pathways to poly- and perfluoroalkyl substances (PFAS) from indoor media: A systematic review. *Environ. Int.* **2022**, *162*. [CrossRef]
5. Trudel, D.; Horowitz, L.; Wormuth, M.; Scheringer, M.; Cousins, I.T.; Hungerbühler, K. Estimating Consumer Exposure to PFOS and PFOA. *Risk Anal.* **2008**, *28*, 2. [CrossRef]
6. Strynar, M.; Lindstrom, A.B. Perfluorinated compounds in house dust from Ohio and North Carolina, USA. *Environ. Sci. Technol.* **2008**, *42*, 3751–3756. [CrossRef]
7. Centers for Disease Control and Prevention. Early Release: Per- and Polyfluorinated Substances (PFAS) Tables, NHANES 2011-2018, Dec. 16. 2021. Available online: https://www.cdc.gov/exposurereport/pfas_early_release.html (accessed on 7 February 2022).
8. Grandjean, P.; Budtz-Jørgensen, E. Immunotoxicity of perfluorinated alkylates: Calculation of benchmark doses based on serum concentrations in children. *Environ. Heal.* **2013**, *12*, 35. [CrossRef]
9. NTP. *Toxicity Report Tables & Curves: Perfluorinated Compounds: Carboxylates*; U.S. Department of Health and Human Services: Washington, DC, USA, 2018. [CrossRef]
10. NTP. *Toxicity Report Tables & Curves: Perfluorinated Compounds: Sulfonates*; U.S. Department of Health and Human Services: Washington, DC, USA, 2018. [CrossRef]
11. DeWitt, J.C.; Blossom, S.J.; Schaider, L.A. Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: Epidemiological and toxicological evidence. *J. Expo. Sci. Environ. Epidemiol.* **2019**, *29*, 148–156. [CrossRef]
12. Barry, V.; Winquist, A.; Steenland, K. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. *Environ. Health Perspect.* **2013**, *121*, 1313–1318. [CrossRef]
13. Nelson, J.W.; Hatch, E.E.; Webster, T.F. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. *Environ. Health Perspect.* **2010**, *118*, 197–202. [CrossRef]
14. Centers for Disease Control and Prevention. Biomonitoring Summary | CDC. 2017. Available online: https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html (accessed on 22 October 2022).
15. USEPA. CompTox Chemicals Dashboard, PFASMASTER Chemical List. 2021. Available online: https://comptox.epa.gov/dashboard/chemical_lists/PFASMASTER (accessed on 13 August 2021).
16. OECD. Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance. Available online: https://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/terminology-per-and-polyfluoroalkyl-substances.pdf (accessed on 29 July 2021).
17. Kwiatkowski, C.F.; Andrews, D.Q.; Birnbaum, L.S.; Bruton, T.A.; DeWitt, J.C.; Knappe, D.R.U.; Maffini, M.V.; Miller, M.F.; Pelch, K.E.; Reade, A.; et al. Scientific Basis for Managing PFAS as a Chemical Class. *Environ. Sci. Technol. Lett.* **2020**, *7*, 532–543. [CrossRef]
18. TURI. *TURA Science Advisory Board*. 2021. Available online: https://www.turi.org/Our_Work/Policy/Toxics_Use_Reduction_Act/Councils_and_Committees/TURA_Science_Advisory_Board (accessed on 31 October 2021).
19. Cousins, I.T.; DeWitt, J.C.; Glüge, J.; Goldenman, G.; Herzke, D.; Lohmann, R.; Miller, M.; Ng, C.A.; Scheringer, M.; Vierke, L.; et al. Strategies for grouping per- and polyfluoroalkyl substances (PFAS) to protect human and environmental health. *Environ. Sci. Process. Impacts* **2020**, *22*, 1444–1460. [CrossRef] [PubMed]
20. Library of Congress. S.1790—116th Congress (2019-2020): National Defense Authorization Act for Fiscal Year 2020, Dec. 20. 2019. Available online: https://www.congress.gov/bill/116th-congress/senate-bill/1790 (accessed on 5 March 2022).
21. Hammel, E.; Webster, T.F.; Gurney, R.; Heiger-Bernays, W. Implications of PFAS Definitions Using Fluorinated Pharmaceuticals. *iScience* **2022**, *25*, 104020. [CrossRef] [PubMed]
22. ATSDR. PFAS in the US Population. 2020. Available online: https://www.atsdr.cdc.gov/pfas/health-effects/us-population.html (accessed on 5 January 2022).
23. Taves, D.R. Evidence that there are two forms of fluoride in human serum. *Nature* **1968**, *217*, 1050–1051. [CrossRef] [PubMed]
24. Hansen, K.J.; Clemen, L.A.; Ellefson, M.E.; Johnson, H.O. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ. Sci. Technol.* **2001**, *35*, 766–770. [CrossRef]
25. Miyake, Y.; Yamashita, N.; So, M.K.; Rostkowski, P.; Taniyasu, S.; Lam, P.K.; Kannan, K. Trace analysis of total fluorine in human blood using combustion ion chromatography for fluorine: A mass balance approach for the determination of known and unknown organofluorine compounds. *J. Chromatogr. A* **2007**, *1154*, 214–221. [CrossRef] [PubMed]

26. Yeung, L.; Miyake, Y.; Taniyasu, S.; Wang, Y.; Yu, H.; So, M.K.; Jiang, G.; Wu, Y.; Li, J.; Giesy, J.P.; et al. Perfluorinated compounds and total and extractable organic fluorine in human blood samples from China. *Environ. Sci. Technol.* **2008**, *42*, 8140–8145. [CrossRef]
27. Aro, R.; Eriksson, U.; Kärrman, A.; Yeung, L.W.Y. Organofluorine Mass Balance Analysis of Whole Blood Samples in Relation to Gender and Age. *Environ. Sci. Technol.* **2021**, *55*, 13142–13151. [CrossRef]
28. Miaz, L.T.; Plassmann, M.M.; Gyllenhammar, I.; Bignert, A.; Sandblom, O.; Lignell, S.; Glynn, A.; Benskin, J.P. Temporal trends of suspect-and target-per/polyfluoroalkyl substances (PFAS), extractable organic fluorine (EOF) and total fluorine (TF) in pooled serum from first-time mothers in Uppsala, Sweden, 1996-2017. *Environ. Sci. Process. Impacts* **2020**, *22*, 1071–1083. [CrossRef]
29. Butt, C.M.; Muir, D.; Mabury, S.A. Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: A. review. *Environ. Toxicol. Chem.* **2014**, *33*, 243–267. [CrossRef]
30. Yin, T.; Te, S.H.; Reinhard, M.; Yang, Y.; Chen, H.; He, Y.; Gin, K.Y.-H. Biotransformation of Sulfluramid (N-ethyl perfluorooctane sulfonamide) and dynamics of associated rhizospheric microbial community in microcosms of wetland plants. *Chemosphere* **2018**, *211*, 379–389. [CrossRef] [PubMed]
31. Poothong, S.; Papadopoulou, E.; Padilla-Sánchez, J.A.; Thomsen, C.; Haug, L.S. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. *Environ. Int.* **2020**, *134*, 105244. [CrossRef] [PubMed]
32. Inoue, M.; Sumii, Y.; Shibata, N. Contribution of Organofluorine Compounds to Pharmaceuticals. *ACS Omega* **2020**, *5*, 10633–10640. [CrossRef] [PubMed]
33. Wadhwa, R.R.; Cascella, M. *Steady State Concentration*; StatPearls: Treasure Island, FL, USA, 2022. Available online: https://www.ncbi.nlm.nih.gov/books/NBK553132/ (accessed on 7 November 2022).
34. KEGG. KEGG Drug Database, New Drug Approvals in the U.S. 2021. Available online: https://www.genome.jp/kegg/drug/br08319.html (accessed on 6 August 2021).
35. Agency for Healthcare Research and Quality (AHRQ). Medical Expenditure Panel Survey (MEPS) 2013–2020. 2022. Available online: https://clincalc.com/DrugStats/Top200Drugs.aspx (accessed on 7 November 2022).
36. Wishart, D.S.; Knox, C.; Guo, A.C.; Shrivastava, S.; Hassanali, M.; Stothard, P.; Chang, Z.; Woolsey, J. Drugbank: A comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res.* **2006**, *34*, D668–D672. [CrossRef]
37. Lins, R.L.; E Matthys, K.; A Verpooten, G.; Peeters, P.C.; Dratwa, M.; Stolear, J.-C.; Lameire, N.H. Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hypercholesterolaemic haemodialysis patients. *Nephrol. Dial. Transplant.* **2003**, *18*, 967–976. [CrossRef]
38. Mayo Clinic Laboratories. Test Catalog. *Mayo Foundation for Medical Education and Research*. 2022. Available online: https://www.mayocliniclabs.com/test-catalog (accessed on 8 November 2022).
39. Li, X.-N.; Xu, H.-R.; Chen, W.-L.; Chu, N.-N.; Zhu, J.-R. Pharmacokinetics of rosuvastatin in healthy Chinese volunteers living in China: A randomized, open-label, ascending single- and multiple-dose study. *Clin. Ther.* **2010**, *32*, 575–587. [CrossRef]
40. Martin, P.D.; Mitchell, P.D.; Schneck, D.W. Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA reductase inhibitor, rosuvastatin, after morning or evening administration in healthy volunteers. *Br. J. Clin. Pharmacol.* **2002**, *54*, 472–477. [CrossRef]
41. BioIVT. Biofluids (Blood-Derived), Serum. 2022. Available online: https://bioivt.com/biofluids-blood-derived/serum (accessed on 25 October 2022).
42. Genuis, S.J.; Curtis, L.; Birkholz, D. Gastrointestinal Elimination of Perfluorinated Compounds Using Cholestyramine and Chlorella pyrenoidosa. *ISRN Toxicol.* **2013**, *2013*, 1–8. [CrossRef]
43. Aro, R.; Eriksson, U.; Kärrman, A.; Jakobsson, K.; Yeung, L.W. Extractable organofluorine analysis: A way to screen for elevated per- and polyfluoroalkyl substance contamination in humans? *Environ. Int.* **2022**, *159*, 107035. [CrossRef]
44. Eriksson, U.; Haglund, P.; Kärrman, A. Contribution of precursor compounds to the release of per- and polyfluoroalkyl substances (PFASs) from waste water treatment plants (WWTPs). *J. Environ. Sci.* **2017**, *61*, 80–90. [CrossRef]
45. Centers for Disease Control and Prevention (CDC). National Health and Nutrition Examination Survey Data 2017–2018. National Center for Health Statistics (NCHS). 2020. Available online: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspxBeginYear=2017 (accessed on 11 September 2022).
46. Calafat, A.M.; Wong, L.-Y.; Kuklenyik, Z.; Reidy, J.A.; Needham, L.L. Polyfluoroalkyl chemicals in the U.S. population: Data from the national health and nutrition examination survey (NHANES) 2003-2004 and comparisons with NHANES 1999–2000. *Environ. Health Perspect.* **2007**, *115*, 1596–1602. [CrossRef]
47. Ruyle, B.J.; Pickard, H.M.; LeBlanc, D.R.; Tokranov, A.K.; Tchackray, C.P.; Hu, X.C.; Vecitis, C.D.; Sunderland, E.M. Isolating the AFFF signature in coastal watersheds using oxidizable PFAS precursors and unexplained organofluorine. *Environ. Sci. Technol.* **2021**, *55*, 3686–3695. [CrossRef] [PubMed]
48. Yeung, L.W.Y.; Mabury, S.A.; Mabury, S.A. Are humans exposed to increasing amounts of unidentified organofluorine? *Environ. Chem.* **2015**, *13*, 102–110. [CrossRef]
49. Yeung, L.; Miyake, Y.; Wang, Y.; Taniyasu, S.; Yamashita, N.; Lam, P. Total fluorine, extractable organic fluorine, perfluorooctane sulfonate and other related fluorochemicals in liver of Indo-Pacific humpback dolphins (Sousa chinensis) and finless porpoises (Neophocaena phocaenoides) from South China. *Environ. Pollut.* **2009**, *157*, 17–23. [CrossRef]

50. Schultes, L.; Vestergren, R.; Volkova, K.; Westberg, E.; Jacobson, T.; Benskin, J.P. Per- and polyfluoroalkyl substances and fluorine mass balance in cosmetic products from the Swedish market: Implications for environmental emissions and human exposure. *Environ. Sci. Process. Impacts* **2018**, *20*, 1680–1690. [CrossRef]
51. Schultes, L.; Peaslee, G.F.; Brockman, J.D.; Majumdar, A.; McGuinness, S.R.; Wilkinson, J.T.; Sandblom, O.; Ngwenyama, R.A.; Benskin, J.P. Total Fluorine Measurements in Food Packaging: How Do Current Methods Perform? *Environ. Sci. Technol. Lett.* **2019**, *6*, 73–78. [CrossRef]
52. MacLeod, J.; Hickman, M.; Smith, G.D. Reporting bias and self-reported drug use. *Addiction* **2005**, *100*, 562–563. [CrossRef]
53. U.S. Food and Drug Administration. U.S. FDA Drug Database: Prozac (Fluoxetine Hydrochloride). 2017. Available online: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=018936 (accessed on 28 October 2022).
54. Kaiser, A.-M.; Aro, R.; Kärrman, A.; Weiss, S.; Hartmann, C.; Uhl, M.; Forsthuber, M.; Gundacker, C.; Yeung, L.W.Y. Comparison of extraction methods for per- and polyfluoroalkyl substances (PFAS) in human serum and placenta samples—Insights into extractable organic fluorine (EOF). *Anal. Bioanal. Chem.* **2020**, *413*, 3. [CrossRef]
55. Backe, W.J.; Day, T.C.; Field, J.A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47*, 5226–5234. [CrossRef]
56. Chappell, M.; Payne, S. Pharmacokinetics. *Biosyst. Biorobotics* **2002**, *24*, 61–72. [CrossRef]
57. Pizzurro, D.M.; Seeley, M.; Kerper, L.E.; Beck, B.D. Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria. *Regul. Toxicol. Pharmacol.* **2019**, *106*, 239–250. [CrossRef] [PubMed]
58. Young, A.S.; Pickard, H.M.; Sunderland, E.M.; Allen, J.G. Organic Fluorine as an Indicator of Per- and Polyfluoroalkyl Substances in Dust from Buildings with Healthier versus Conventional Materials. *Environ. Sci. Technol.* **2022**, *56*, 17090–17099. [CrossRef] [PubMed]
59. Furlong, E.T.; Batt, A.L.; Glassmeyer, S.T.; Noriega, M.C.; Kolpin, D.W.; Mash, H.; Schenck, K.M. Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States: Pharmaceuticals. *Sci. Total Environ.* **2017**, *579*, 1629–1642. [CrossRef] [PubMed]

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Critical Review

A Critical Review of the Application of Polymer of Low Concern and Regulatory Criteria to Fluoropolymers

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are a group of fluorinated substances that are in the focus of researchers and regulators due to widespread presence in the environment and biota, including humans, of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Fluoropolymers, high molecular weight polymers, have unique properties that constitute a distinct class within the PFAS group. Fluoropolymers have thermal, chemical, photochemical, hydrolytic, oxidative, and biological stability. They have negligible residual monomer and oligomer content and low to no leachables. Fluoropolymers are practically insoluble in water and not subject to long-range transport. With a molecular weight well over 100 000 Da, fluoropolymers cannot cross the cell membrane. Fluoropolymers are not bioavailable or bioaccumulative, as evidenced by toxicology studies on polytetrafluoroethylene (PTFE): acute and subchronic systemic toxicity, irritation, sensitization, local toxicity on implantation, cytotoxicity, in vitro and in vivo genotoxicity, hemolysis, complement activation, and thrombogenicity. Clinical studies of patients receiving permanently implanted PTFE cardiovascular medical devices demonstrate no chronic toxicity or carcinogenicity and no reproductive, developmental, or endocrine toxicity. This paper brings together fluoropolymer toxicity data, human clinical data, and physical, chemical, thermal, and biological data for review and assessment to show that fluoropolymers satisfy widely accepted assessment criteria to be considered as "polymers of low concern" (PLC). This review concludes that fluoropolymers are distinctly different from other polymeric and nonpolymeric PFAS and should be separated from them for hazard assessment or regulatory purposes. Grouping fluoropolymers with all classes of PFAS for "read across" or structure–activity relationship assessment is not scientifically appropriate. *Integr Environ Assess Manag* 2018;14:316–334. © 2018 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals, Inc. on behalf of Society of Environmental Toxicology & Chemistry (SETAC)

Keywords: Fluoropolymer International regulation Polytetrafluoroethylene Polymer of low concern PFAS

INTRODUCTION

The carbon–fluorine (C–F) bond is the strongest bond between C and another atom, instilling substances that contain a majority of C–F bonds with stability, inertness, and persistence (Banks et al. 1994). Per- and polyfluoroalkyl substances (PFAS) are a large group of highly fluorinated synthetic substances with diverse properties that have been used in a wide variety of industrial and consumer applications since the 1950s (Buck et al. 2011). Within the group are distinct substances with different properties: polymers and nonpolymers; solids, liquids, and gases; persistent and nonpersistent substances; highly reactive and inert substances; mobile and insoluble substances; and toxic and nontoxic chemicals.

The PFAS are a large, diverse group of substances that, in some respects, challenge easy distinction for assessment and management. A clearer understanding of the origin of PFAS found in the environment and assessment of their properties is needed to be able to determine which classes of PFAS require management action. Per- and polyfluoroalkyl substances must be assessed taking into account their differences in chemical, physical, thermal, and biological properties. A single, globally harmonized system for PFAS classification has not yet been defined, resulting in a lack of distinction between PFAS. As regulatory frameworks continue to evolve, such as the Regulation (EC) No 1907/2006 of the European

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Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (RC 2006), more work is needed to distinguish classes of PFAS to ensure that regulations are appropriate in scope and proportionality.

Two long-chain nonpolymer perfluoroalkyl acids (PFAAs), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (both PFAS), found widespread in the environment and living systems, led to regulatory assessment and management efforts in several countries (Buck et al. 2011; OECD 2017; USEPA 2017a). Management actions to curtail manufacture of long-chain PFAAs, including PFOS and PFOA, and substances that may degrade to form them (also known as "precursors") have been taken (EC 2006; ECHA 2015; USEPA 2017a). Both PFOS and PFOA have been determined by regulators to be persistent, bioaccumulative, and toxic (PBT) substances (EC 2006; ECHA 2015). A current concern is the potential for certain side-chain polymer PFAS to degrade in the environment to PFOS and PFOA or lower homologues (Liu and Mejia-Avendaño 2013). In addition, PFOS (a nonpolymeric perfluoroalkyl substance) and related substances have been listed as persistent organic pollutants (POPs) under Annex B of the Stockholm Convention (UNEP 2009), and PFOA and other related substances (UNEP 2011), as well as perfluorohexane sulfonic acid (PFHxS) and related substances are being evaluated for listing (UNEP 2017a). As a result, questions about the health and environmental safety of PFAS as a group have been raised (Scheringer et al. 2014; Blum et al. 2015).

These findings have prompted expanded regulatory interest and concern about PFAS as a group, spurring additional assessment and management actions. The German Environment Agency, Umweltbundesamt (UBA), published a proposal to implement new assessment criteria and procedures for identifying persistent (P), mobile (M), and toxic (T) substances under the European Union REACH chemical registration process (UBA 2017). The UBA has concluded that PM and/or PMT substances constitute "an irreversible threat to sources of drinking water and the quality of drinking water" in Germany. This has prompted the designation of PFAS substances as posing an "equivalent level of concern" under Article 57(f) of REACH and thereby has prompted the need for a new paradigm for chemical assessment and authorization. The Swedish Chemicals Agency, Kemikalieinspektionen (KEMI), announced agreement among 37 government agencies and research institutions in the European Union (EU) to expand cooperation to reduce the risks and increase the knowledge of PFAS, thereby endorsing the UBA view on the hazards posed by all PFAS substances (KEMI Swedish Chemicals Agency 2016). The KEMI announcement indicated that all perfluoralkyl substances should be considered as extremely persistent in the environment, and many are water soluble, mobile in soil, and likely to contaminate waterways and drinking water supplies. A risk assessment report prepared by KEMI is forthcoming (ChemNews 2016).

The PFAS are divided into 2 primary categories: nonpolymers and polymers (Figure 1). Figure 1 shows that these 2 categories are divided into 5 classes of PFAS. The fluoropolymer class of PFAS is the focus of the present paper. The nonpolymer category includes perfluoroalkyl substances and polyfluoroalkyl substances. The polymer category includes fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers. Polymers generally have very different physical, chemical, and biological properties than do nonpolymer chemical substances of low molecular weight. Precise criteria that distinguish polymers from nonpolymers have been established (OECD 1993).

There are distinct differences between the 5 classes of PFAS. For example, PFOA, in the class nonpolymer perfluoroalkyl substances, is small, mobile, and persistent; has been assessed and determined to be a PBT chemical (ECHA 2015); and is in the final stage for recommendation of listing as a POP under the Stockholm Convention (UNEP 2017b). Regulatory and industry management actions on PFOA include precursor substances that may degrade to form PFOA (USEPA 2017a). An example in the class of nonpolymer polyfluorinated substances, 8:2 fluorotelomer alcohol, is known to degrade under environmentally relevant conditions to form PFOA (Liu and Mejia-Avendaño 2013). It is therefore a precursor substance to PFOA and subject to regulatory management (Liu and Mejia-Avendaño 2013). Polymers derived from 8:2 fluorotelomer alcohol are examples of the side-chain fluorinated polymers class. These polymers may degrade to form PFOA and therefore are subject to regulatory management. Lastly, perfluoropolyethers class is a complex class of PFAS, which contains O linkages in the polymer backbone.

In the present paper, we address fluoropolymers, a class of PFAS polymers (Figure 1). Fluoropolymers are high molecular weight solid plastics that have been studied extensively.

The present paper brings together fluoropolymer toxicity data, human clinical data, and physical, chemical, thermal, and biological data for review and assessment to show that fluoropolymers satisfy widely accepted assessment criteria to be considered as "polymers of low concern" (PLC) and to show that fluoropolymers are distinctly different enough from other classes of PFAS to not be grouped with them for hazard assessment or regulatory purposes.

PERFORMANCE CHARACTERISTICS AND USES OF FLUOROPOLYMERS

Since the discovery of polytetrafluoroethylene (PTFE) in 1938 (Plunkett 1987), the use of fluoropolymers has grown considerably to take advantage of their unique physical–chemical, thermal, and biological properties. The 4 fluoropolymers addressed in the present paper, polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), ethylene tetrafluoroethylene (ETFE), and tetrafluoroethylene copolymers with perfluoroalkyl vinyl ethers (e.g., perfluoroalkoxy polymer, PFA), accounted for approximately 70% to 75% of the world fluoropolymer consumption in 2015 (IHS 2016). The representative fluoropolymer discussed in the present paper, PTFE, made up 58% (by weight) of 2015 worldwide fluoropolymer consumption (IHS 2016). Fluoropolymers are high molecular weight plastics with unique properties attributable to the strong C–F bonds, the strongest bond between C and another atom, making

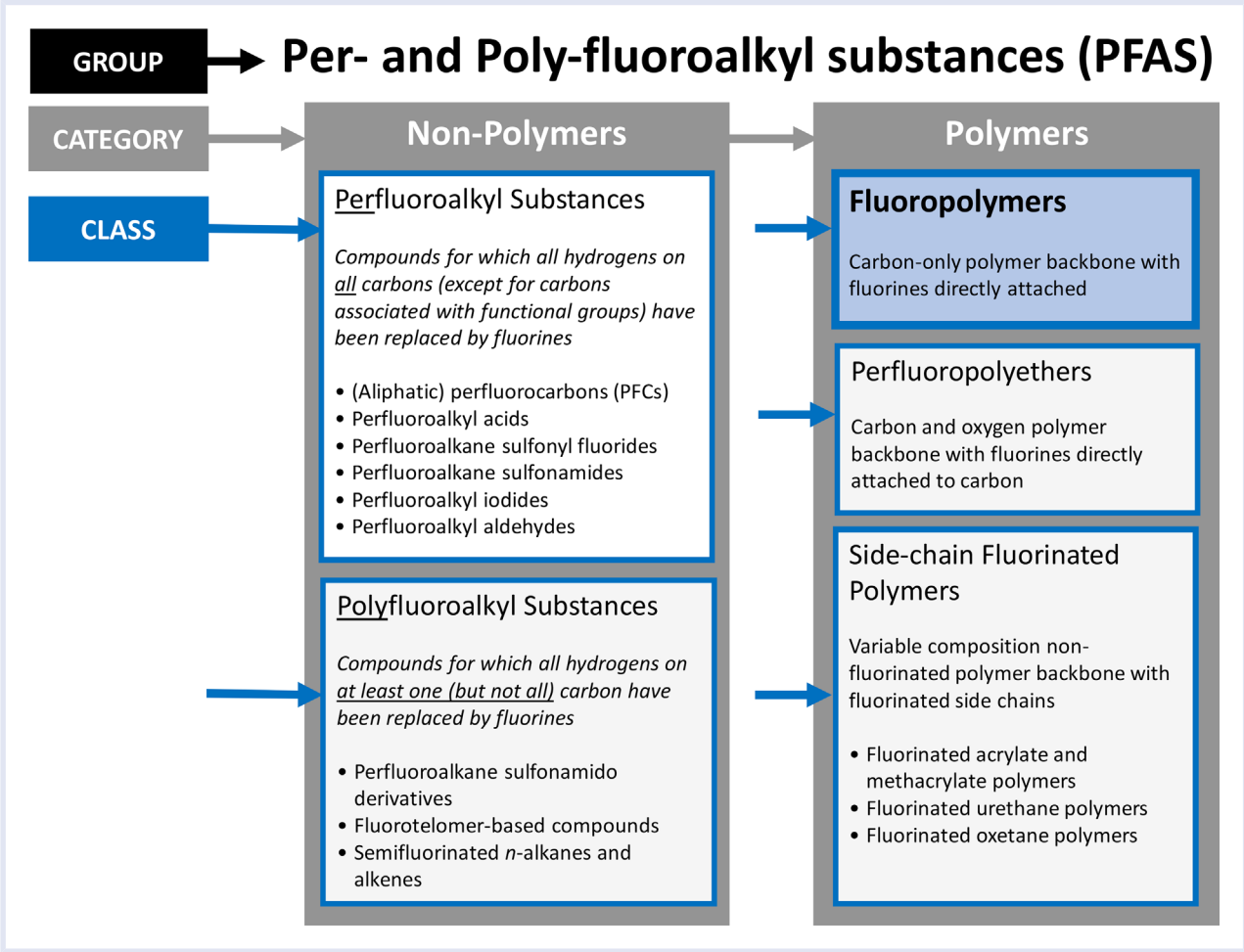

Figure 1. Per- and polyfluoroalkyl substances (PFAS).

them highly stable (Olabisi and Adewale 2015). Carbon atoms alone form the fluoropolymer backbone, each surrounded by an envelope of F atoms. Fluoropolymers are generally very high molecular weight (>100 000 Da); have high thermal, chemical, photochemical, oxidative, hydrolytic, and biological stability; have low flammability, neutral electrical charge, and resistance to degradation; have negligible residual monomers and low molecular weight oligomer content; have limited low molecular weight leachables; and have no reactive functional groups of concern (Gangal and Brothers 2015).

The unique properties of fluoropolymers include durability, mechanical strength, inertness, thermal stability in foreseeable use conditions, and resistance to chemical, biological, and physical degradation (Hougham et al. 1999). Table 1 shows performance characteristics required in various commercial fluoropolymer applications (Gangal and Brothers 2015; Dams and Hintzer 2016). For example, medical devices are successful when they are made from "biocompatible" biomaterials, that is, the material has the ability to perform with an appropriate host response in a specific situation (Williams 1987). The inertness of PTFE allows for its acceptance into the body. Moreover, PTFE flexibility and durability deliver mechanical integrity for the device's lifetime. The microstructure of PTFE can be modified to meet specific physiological needs (e.g., porous and open structure to facilitate tissue ingrowth), enhancing its utility in medical devices. In terms of end-use function, PTFE's inertness, physical properties (Ebnesajjad 2011), and the low level of residual monomer, oligomers, and low molecular weight leachables (Supplemental Data p 32–55) meet the requirements for low levels of contaminants and particulates in manufacturing environments essential for the food and beverage, pharmaceutical, medical, and semiconductor industries (Olabisi and Adewale 2015). Manufacturing applications requiring ultrapure high efficiency particulate air (HEPA) filtration use the finely controlled microporous PTFE membranes. Other components requiring a high degree of contamination control associated with patient care (e.g., dialysis tubing) also find the properties of PTFE essential. Durability in harsh conditions makes PTFE a superior material of choice in aerospace, environmental controls, energy production and storage, and electronics, as well as in technical apparel. The thermal stability of PTFE and FEP fluoropolymers provides improved fire safety risk over other polymers when used in plenums and structural

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Table 1. Fluoropolymer functionality and commercial applications

	Fluoropolymer characteristics									
	Durable		Inert		Functional			Stable		
Commercial application	Mechanical strength	Low particulation	Resistance to chemicals	Nontoxic, biocompatible, biological degradation resistant	Flexibility	Friction resistance	Low dielectric constant	Low leachables	Resistance to photolysis, oxidation, hydrolysis	Stability
Aerospace	X	—	X	—	X	X	X	—	X	X
Automotive industry	X	—	X	—	X	X	X	—	X	x
Medical devices	X	X		X	X	X	—	X	X	X
Pharmaceutical manufacture	X	X	X	X	X	—	—	X	—	X
Consumer outdoor apparel	X	—		X	X	—	—	X	—	—
Technical clothing (military, firefighters, first responders, medical personnel)	X	—	X	X	X	X	—	X	X	X
Consumer electronics	X	—	X	—	X	X	X	—	X	X
Wireless communications	X	—	X	—	X	X	X	—	X	X
Satellite navigation systems	X	—	X	—	X	X	X	—	X	X
Semiconductor industry	—	X	X	—	—	—	X	X	—	—
Building construction	X	—	—	X	X	—	X	—	X	X
Energy production and storage	X	X	—	—	—	—	X	X	X	X
Food and beverage production	X	X	X	X	X	X	—	X	X	—
Food protection and packaging	X	X	X	X	X	—	—	X		—
Drinking water filtration	—	X	X	X	—	—	—	X	X	—
Environmental protection	—	X	X	X	—	—	—	X	X	X

geometries in aviation and standard building construction (Olabisi and Adewale 2015). In addition, chemical resistance to acids, bases, solvents, and chemical attack, combined with its unique conformable strength, makes PTFE an ideal coating for chemical process equipment, lining for process piping, sealants for gaskets and hoses, and fabricated parts for pumps, gears, and other mechanical parts that need this extreme resistance for functionality (Olabisi and Adewale 2015). The low dielectric constant of PTFE ensures the integrity of high speed–low signal loss systems as employed in the aerospace industry for flight controls, communication, and protection from extreme cold, moisture, and altitude changes (Dams and Hintzer 2016). These are lifesaving applications that are used in satellite systems for navigation, wireless communications, in-flight navigation, and shielding from electronic interference. Civil and military aviation depends on reliable performance of these systems for long service hours with minimal maintenance down times. In addition, PTFE provides reduced friction of moving parts (e.g., cable chains), preventing particulation during automated manufacturing in cleanroom environments (Dams and Hintzer 2016). This friction reduction is also uniquely beneficial in light load bearings, gears, cams, and other mechanical machine parts as well as in weaving fibers, yarns, and greases (Dams and Hintzer 2016).

ASSESSMENT OF POLYMERS

History

Prior to the mid-20th century, regulation of new chemical substances, mixtures, and polymers in general was very limited. National chemical inventories were created with notification requirements for new chemical substances, mixtures, and polymers. In the United States, new chemicals submitted to the US Environmental Protection Agency (USEPA) under the Toxic Substances Control Act (TSCA) (USC 1976) for addition to the US chemical inventory are reviewed for potential physical, chemical, and biological effects (environmental and mammalian), as well as for potential exposure to the environment and human populations. Over time, the USEPA regulatory scientists gained enough knowledge through the review of the thousands of data packages to develop tools to assist in the identification of physical–chemical properties, potential hazard, and potential exposure to assist in and expedite the chemical review and assessment process (Auer et al. 1990; Wagner et al. 1995; USEPA 2012; USEPA 2017b).

The predictive power and reliability of these approaches were tested and refined (Wagner et al. 1995). Over time, it was recognized that many of the physical–chemical properties, such as molecular weight, limit the ability of the chemical to cross the cell membrane and therefore limit its bioavailability. Further examination of general physical–chemical properties and their relationship to hazard potential of a given chemical led to the development of general principles or criteria for the identification of chemicals, including polymers, with low hazard potential. These criteria were developed for use by USEPA for its hazard evaluation of new polymers. The USEPA made this methodology available to the public to assist submitters interested in developing low hazard polymers (USEPA 1997a). In 1984, the USEPA published the polymer exemption rule to exempt low hazard polymers from certain notification requirements under the new chemicals program (USFR 1984). The polymer exemption rule incorporated the hazard criteria as part of the criteria to determine eligibility for exemption (USEPA 1997a, 2010).

The hazard criteria that support the PLC concept represent an extension of these principles and practices developed for (nonpolymeric) chemicals and rely heavily on physical–chemical properties that determine a chemical's bioavailability. In 1993, the Organisation for Economic Co-operation and Development (OECD) Expert Group on Polymers found that sufficient data existed to create a consensus document identifying the essential data elements to qualify a polymer as a PLC to human health and the environment (OECD 1993). By 2007, the OECD Expert Group on Polymers agreed that, "Polymers of low concern are those deemed to have insignificant environmental and human health impacts" (OECD 2009). Thus, there was agreement within the OECD that polymeric chemicals meeting these criteria have a low hazard potential. However, the integration of the criteria into a risk management framework may differ from country to country according to their individual regulatory mandate.

In a recent report commissioned by the European Commission (EC) (BIO by Deloitte 2015), the following countries agreed on the polymer properties predictive of adverse human health and environmental hazard: Australia, Canada, China, Japan, South Korea, Philippines, New Zealand, Taiwan, and the United States. Further, the report identified the eligibility criteria to be considered a PLC with respect to potential for adverse impact on health and the environment. The report also compiled existing polymer regulations outside the EU and proposed alternative options for EU polymer registration, including defining a category of a PLC and grouping polymers into families.

The PLC criteria are described in the following section. Note that there are some policy components, such as elemental composition, as well as the physical–chemical attributes, in the PLC criteria.

POLYMER OF LOW CONCERN CRITERIA

Here we describe each of the eligibility criteria for PLC and provide an assessment for the representative fluoropolymer PTFE. We will show that fluoropolymers, including PTFE, satisfy the widely accepted assessment criteria to be considered PLCs (Table 2) and therefore are considered to be of low hazard to human health and the environment.

Polymer composition

The polymer composition criterion requires structure and elemental composition of the polymer be described and identified (e.g., by Chemical Abstracts Service [CAS] number).

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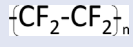
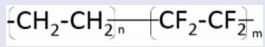
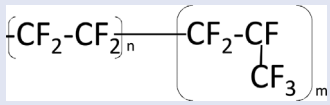
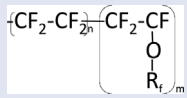
Table 2. Fluoropolymers and PLC criteria

	Fluoropolymers			
	PTFE	ETFE	FEP	PFA
Assessment criteria[a]	CAS 9002-84-0	CAS 25038-71-5, 68258-85-5	CAS 25067-11-2	CAS 26655-00-5, 31784-04-0
Structure				
Polymer composition (must have C, H, Si, S, F, Cl, Br, or I covalently bound to C)	Yes	Yes	Yes	Yes
Molecular weight	389 000–8 900 000[bc]	—	—	—
(M_n > 1000 Da and oligomer content < 1%)	520 000–45 000 000[bd]	530 000–1 200 000[ef]	241 000–575 000[eg]	200 000–450 000[eh]
Molecular weight distribution MW ÷ number average M_n (M_n and heterogeneity of MW distribution indicate if majority are >1000 or <1000 Da, which could penetrate the cell)	2.3[i]	1.4–2.7[f]	1.55–2.09[g]	1.7[j]
Wt % oligomer (see Figure 2) (<5% for <1000 Da oligomers, <2% for <500 Da oligomers)	Negligible	Negligible	Negligible	Negligible
Ionic character (cationic polymers associated with aquatic toxicity; polycationic with adverse human health effect)	Neutral	Neutral	Neutral	Neutral
RFGs[k] (some highly reactive functional groups associated with adverse human health and ecotoxicology effects, e.g., acrylates, isocyanates, anhydrides, aziridines)	<1 (see section *Reactive functional groups and RFG ratio to MW*)	<1 (see section *Reactive functional groups and RFG ratio to MW*)	<1 (see section *Reactive functional groups and RFG ratio to MW*)	<1 (see section *Reactive functional groups and RFG ratio to MW*)
FGEW[k] (typical value) (the lower the FGEW, the more reactive the polymer and the higher the potential for health and environmental impact)	$>10^5$–10^7	$>10^5$–10^6	$>10^5$	$>10^5$
Low molecular weight leachables (MW < 1000 Da able to enter cell)	<1 ppm	No active leachables by USP class VI[l] (121 °C)	No active leachables by USP class VI[l] (121 °C)	No active leachables by USP class VI[l] (121 °C)
Residual monomers (monomers have lower MW than polymers; typically more hazardous than polymers)	<1 ppm	<50 ppb	<50 ppb	<50 ppb
Ratio of residual monomers to molecular weight (typical value) (more low MW monomer content per mole increases bioavailability and hazard potential)	$\sim10^{-13}$ to 10^{-15}	$\sim10^{-13}$ to 10^{-14}	$\sim10^{-13}$	$\sim 10^{-13}$
Structural similarities to RFG of concern (increases potential risk of adverse effects)	None	None	None	None
Reference standard see also ISO 1133 (ISO 2011), ISO 12086 (ISO 2006)	ASTM D 4894 (ASTM 2015a), D 4895 (ASTM 2015b)	ASTM D 2116 (ASTM 2016a)	ASTM D 3159 (ASTM 2015c)	ASTM D 3307 (ASTM 2016b)

(*Continued*)

Table 2. (*Continued*)

	Fluoropolymers			
	PTFE	ETFE	FEP	PFA
Assessment criteria[a]	CAS 9002-84-0	CAS 25038-71-5, 68258-85-5	CAS 25067-11-2	CAS 26655-00-5, 31784-04-0
Physical–chemical properties				
Water solubility (per USP 2011) (water solubility <10 mg/L showed generally low health concerns; 10 mL/L to 10000 mg/L had potential health concern)	Practically insoluble or insoluble (1×10^{-5} mg/L)	Practically insoluble or insoluble	Practically insoluble or insoluble	Practically insoluble or insoluble
Octanol–water partition coefficient, K_{OW} (higher K_{OW} associated with lipophilicity and a high potential to bioaccumulate or bioconcentrate)	NA	NA	NA	NA
Particle size (median mass aerodynamic diameter, MMAD, should be >5 μm)	100–500 μm (powders)	50–250 μm (powders)	50–250 μm (powders)	50–250 μm (powders)
	—	2–4 mm (pellets)	2–4 mm (pellets)	2–4 mm (pellets)
Stability				
Hydrolysis (breaking into M_n < 1000 Da increases hazard potential)	Stable	Stable	Stable	Stable
Light (hν) (breaking into M_n < 1 000 Da increases hazard potential)	Stable	Stable	Stable	Stable
Oxidation (breaking into M_n < 1000 Da increases hazard potential)	Stable	Stable	Stable	Stable
Biodegradation (aerobic and anaerobic) (breaking into M_n < 1000 Da increases hazard potential)	Stable	Stable	Stable	Stable
Thermal stability at normal foreseeable use maximum continuous temp (°C) (breaking into M_n < 1000 Da increases hazard potential)	260	150	200	260
Meets PLC criteria[a] (Y/N)	Yes	Yes	Yes	Yes

ASTM = American Society for Testing and Materials; CAS = Chemical Abstracts Service; Da = dalton; ETFE = ethylene tetrafluoroethylene; FEP = fluorinated ethylene propylene; FGEW = functional group equivalent weight; ISO = International Organization for Standardization; MMAD = median mass aerodynamic diameter; M_n = number average molecular weight; MW = molecular weight; MWD = molecular weight distribution; OECD = Organisation for Economic Co-operation and Development; PFA = perfluoroalkoxy polymer; PFPE = perfluoropolyether; PLC = polymer of low concern; PTFE = polytetrafluoroethylene; PVDF = polyvinylidene fluoride; PVF = polyvinyl fluoride; RFG = reactive functional groups; USEPA = US Environmental Protection Agency; USP = US Pharmacopeia.

[a]See OECD 2009 and BIO by Deloitte 2015 for details on characteristics of a "polymer of low concern."

[b]Molecular weight is number average molecular weight.

[c]Berry and Peterson 1951; Doban et al. 1956.

[d]Suwa et al. 1973.

[e]Molecular weight is weight average molecular weight.

[f]Tuminello et al. 1993.

[g]Tuminello 1989.

[h]Putnam 1986.

[i]Chu et al. 1989.

[j]Frick et al. 2012.

[k]For definition of reactive functional group; lists of low-, moderate-, and high-concern functional groups; and FGEW limits, see USEPA Polymer Exemption Guidance Manual (USEPA 1997b), BIO by Deloitte 2015 (p 191–192), and USEPA 2010. See Supplemental Data.

[l]In the USP<88> testing for "class VI," 2 g of the plastic (e.g., FEP, ETFE, or PFA) were extracted at 121 °C in: 1) 0.9% sodium chloride solution, 2) sesame oil, NF, 3) alcohol saline, and d) polyethylene glycol. The acute systemic toxicity and intracutaneous reactivity tests were conducted with those extracts. The intramuscular implantation was conducted with the plastic. Passing these 3 tests indicates that any leachables were not released in concentrations capable of causing these adverse effects, but does not result in a quantitative concentration of leachables. (See USP 2018.)

Note: The following are not addressed in this paper: PFPEs, side-chain fluorinated polymers, fluoroelastomers, PVF, and PVDF.

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Molecular weight, number average molecular weight, MW distribution, and % oligomer <1000 Da

The number average molecular weight (M_n) and oligomer content are the most commonly used criteria for PLC assessment. The EU assessment report (BIO by Deloitte 2015) states that the "most potential health concern polymers have a number average molecular weight, Mn, < 1000 Da and oligomer content >1%." The higher the oligomeric content, the more likely a polymer is to be a health or ecotoxicological (OECD 2009, p 9). In fact, when comparing the potential health concern of polymers with varying percent oligomer content, "...the distribution of potential health concern polymers showed an increased incidence of higher oligomer content that began at 5% for <1000 Da and 2% for <500 Da oligomeric content" (OECD 2009, p 24).

Molecular weight (MW) is an important predictor of biological effect because very large molecules (>1000–10 000 Da) are too large to penetrate cell membranes (Supplemental Data in Beyer 1993, p 14). Because large molecular weight polymers cannot enter the cell, they cannot react with "target organs," such as the reproductive system, and are not bioavailable. "Therefore, as the M_n of a polymer increases, a reduced incidence of potential health concern effects might be expected" (OECD 2009, p 20).

An additional PLC consideration is the weight percent oligomers <1000 Da. Oligomers may be composed of, for example, dimers, trimmers, and tetramers, meaning they have 2-, 3-, and 4-monomer units, respectively. The EU report (BIO by Deloitte 2015) concluded that most potential health concern polymers have M_n of <1000 Da and oligomer content of >1%: "...the distribution of potential health concern polymers showed an increased incidence of higher oligomer content that began at 5% for <1000 Da and 2% for <500 Da oligomeric content" (OECD 2009, p 24).

Molecular weight distribution (MWD), also known as "polydispersity index," measures the heterogeneity of size of polymer molecules in a polymer. The MWD is an important parameter for predicting potential biological effects of polymers because although M_n may be a large value, low MW oligomers <1000 Da may be present, which could penetrate the cell.

Electrical charge (ionic character)

Electrical charge or ionic character can be anionic, cationic, amphoteric, or nonionic. Specifically, cationic polymers have been associated with aquatic toxicity (Auer et al. 1990; USEPA 1997a). Polycationic polymers that are water soluble or dispersible are of concern due to adverse human health (inhalation) effects (NICNAS 2016).

Reactive functional groups and RFG ratio to MW

A "reactive functional group" (RFG) is defined as an atom or associated group of atoms in a chemical substance that is intended or can be reasonably anticipated to undergo facile chemical reaction (USFR 2012). Some highly reactive functional groups (or a high ratio of RFGs per mole) have been associated with adverse human health and ecotoxicology (e.g., acrylates, methacrylates, isocyanates, anhydrides, aziridines) (USEPA 2010). Methods have been demonstrated to identify the functional end groups on fluoropolymers (Pianca et al. 1999).

The functional group equivalent weight (FGEW) is used to determine if the RFGs in a polymer are substantially diluted by polymeric material to allow the polymer to be a PLC (USEPA 1997b). The FGEW of a polymer is defined as the ratio of the M_n to the number of functional groups in the polymer. It is the weight of a polymer that contains 1 formula weight of the functional group. The FGEW is used as an indication of the degree of reactivity of the polymer; the lower the FGEW, the more reactive the polymer and the higher the potential for health and environmental impact (OECD 2009, p 10).

Low MW leachables

Low MW leachables are chemical molecules, either inorganic or organic, that migrate (i.e., leach) out of the polymer. These could be residual monomers or oligomers resulting from incomplete polymerization processes, surface residues, or other chemicals used in the manufacturing processes (e.g., initiators, catalysts, chain transfer agents, surfactants). Chemical analysis, by techniques such as thermal gravimetric analysis (TGA), gas chromatography mass spectrometry (GC-MS), or liquid chromatography mass spectrometry (LC-MS) are used to identify low MW leachables.

Low MW leachables are critically important to the potential for a polymer to affect health and the environment, given that they may be able to migrate out of the polymer and cross cell membranes to potentially react with biomolecules. In a report to the EU (BIO by Deloitte 2015) the polymer policies for 10 countries around the world, including the EU REACH handling of polymers, were reviewed. The report concluded that "Polymers with <1% MW <1000 Da and low water extractivity are not able to cause systemic effects which are toxicologically or ecotoxicologically relevant."

Monomers, by nature, are reactive. Unreacted monomer left in a polymer may migrate out of the polymer to react with biomolecules to cause potential adverse effects. Regulatory authorities (BIO by Deloitte 2015) and the OECD Expert Group on Polymers (OECD 2009) agree that the residual monomer content of a polymer is critical to determining if it qualifies to be a PLC.

Particle size

Particle size is also a PLC criterion. Particles that are small enough to reach the deep lung upon inhalation are often associated with adverse health effects. Therefore, to qualify as a PLC, median mass aerodynamic diameter (MMAD) of the polymer particle size should be greater than 5 μm.

Structural and elemental composition

In the United States, Chemical Categories of Concern are the result of the review of new chemicals by the USEPA under the TSCA (see https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/chemical-categories-used-review-new). New chemicals submitted to the USEPA

under the TSCA for addition to the US chemical inventory are reviewed for potential chemical, physical, and biological effects (environmental and mammalian). The USEPA groups Premanufacture Notice (PMN) chemicals with shared chemical and toxicological properties into categories, enabling both PMN submitters and USEPA reviewers to benefit from the accumulated data and past decisional precedents, allowing reviews to be facilitated. The categories describe the molecular structure, boundary conditions such as MW, equivalent weight, the log of the octanol–water partition coefficient, log P, or water solubility, and standard hazard (mammalian and ecological) and (environmental) fate tests to address concerns. The categories include chemicals for which sufficient history has been accumulated so that hazard concerns and testing recommendations vary little from chemical to chemical within the category. (See Supplemental Data, p 30, for details on USEPA's chemical categories.)

Elemental composition

The elemental composition is a factor in the assessment of the eligibility of polymers for reduced notification requirements. The exclusion of polymers under this step is not a conclusion of hazard but a determination that the elemental composition does not fall within the parameters of the polymer set under which this rule was formulated, and consequently, these polymers would have to follow the standard notification and review process. These elemental requirements differ across jurisdictions as covered in the report to the EU on global regulatory approaches to polymer assessment (BIO by Deloitte 2015). For example, in the EU under REACH it is proposed that polymers composed from among these elements, covalently bound to C, have reduced hazard: H, N, O, Si, S, F, Cl, Br, or I (BIO by Deloitte 2015). In contrast, the USEPA Polymer Exemption Rule states that a polymer is eligible for reduced agency review when it has at least 2 of the following elements: C, H, O, N, S, or Si (USFR 1995).

Water and lipid solubility and the octanol–water partition coefficient

Water solubility is the extent to which a compound will dissolve in water. According to the OECD 2009 meeting of the Expert Group on Polymers, polymers with "negligible" water solubility, or those described as "hydrophobic" have been represented with a water solubility of 0.000001 mg/L (1×10^{-6} mg/L; assigned arbitrarily) (OECD 2009). That is equivalent to 1 ppt, a very conservative definition.

Based on the data set studied, the OECD Expert Group on Polymers concluded "A higher proportion of polymers with intermediate water solubility values (10 mL/L–10 000 mg/L) displayed potential health concern. Polymers with water solubility <10 mg/L showed generally low health concerns" (OECD 2009, p 10). Although not a solubility metric, a polymer capable of absorbing its weight in water was associated with increased inhalation cancer risk in rats (OECD 2009).

The octanol–water partition coefficient (K_{OW}) is another criterion to assess chemicals and their environmental and health impact. The K_{OW} is a physical–chemical property at equilibrium to represent the lipophilic or hydrophilic nature of a chemical, the distribution of a compound in octanol, representing the lipophilic nature, to its solubility in water, representing the aqueous nature. The higher the K_{OW}, the more lipophilic the compound. Typically, a K_{OW} >5000 or a log K_{OW} >5 means high lipophilicity and, thus, a high potential to bioaccumulate or bioconcentrate. Numerous studies showed that K_{OW} was useful for correlating structural changes of drug chemicals with the change observed in some biological, biochemical, or toxic effect (LaGrega et al. 2010). It has been found to be related to water solubility, soil or sediment adsorption coefficients, and bioconcentration factors for aquatic life. According to the Stockholm Convention, a bioconcentration factor of >5000 and a log K_{OW} >5 is used as a criterion for bioaccumulation.

Stability

Stability is resistance to physical, chemical, or biological transformation. Loss of stability in the polymer breaks it down into smaller pieces, producing low MW species. As was previously described in the Polymer of Low Concern section under the *Molecular weight, number average molecular weight, MW distribution, and % oligomer <1000 Da* heading, molecules with M_n <1000 Da are capable of crossing cell membranes, making unstable polymers potentially hazardous to health and the environment.

Abiotic stability

Polymers are stable; monomers are not. Abiotic degradation may involve sunlight, water, or oxygen. Photochemical transformation is a reaction involving the radiation energy of sunlight (ultraviolet radiation) that may break a bond in a molecule to change it to another chemical entity. Hydrolytic degradation of polymers is another potential way to break the polymer bonds, creating smaller oligomers that may be bioavailable. Chemical oxidation is a reaction involving the loss of electrons from 1 atom to another.

Biotic stability: aerobic, anaerobic, and in vivo

Biotic stability is assessed by whether or not the polymer is degraded by microorganisms under oxygenated (aerobic) or anoxic (anaerobic) conditions; in vitro and in vivo stability studies demonstrate this. In vivo biodegradation involves the breaking of the polymer bonds by the action of bacteria, enzymes, and oxidants within the organism.

Thermal stability

Thermal stability of a polymer can be assessed when used as intended under normal, foreseeable use conditions or in extreme temperatures during disposal, such as by incineration. Thermal stability testing may involve Thermal Gravimetric Analysis (TGA), which determines mass loss over time and temperature of a test substance.

ASSESSMENT OF FLUOROPOLYMERS ACCORDING TO PLC CRITERIA

Characteristics of a PLC have been described in the preceding section. These criteria represent the combined

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experience and knowledge of global regulatory authorities on factors demonstrated to be predictive of health and environmental hazards of polymers (OECD 2009; BIO by Deloitte 2015). Four fluoropolymers were assessed according to the PLC criteria. The results are summarized in Table 2, and an expanded discussion on specific criteria is provided in the remainder of this section.

Polymer composition

Fluoropolymers satisfy the PLC criterion of polymer composition. Polytetrafluoroethylene is a homopolymer of tetrafluoroethylene (TFE). Polytetrafluoroethylene can be a homopolymer (1 monomer) or it can be a modified homopolymer containing TFE widely and not more than 1% of another fluoromonomer (see ASTM 2015). Polytetrafluoroethylene contains only C and F having a $–CF_2–$ backbone terminated on both ends of each polymer chain with $–CF_3$. In unique cases, based on production method and ingredients used, commercial PTFE may have end groups that contain O, H, N, or S, depending on the initiator or chain transfer agent used in polymerization (Pianca et al. 1999). Polytetrafluoroethylene meets the compositional criterion to be a PLC.

Molecular weight, M_n, MWD, and % oligomer <1000

Fluoropolymers satisfy the PLC criterion of MW, M_n, MWD, and % oligomer <1000. Fluoropolymers are practically insoluble in water and all organic solvents. Therefore, standard MW methods are not applicable for fluoropolymers like PTFE and have been replaced by standardized indirect methods that use specific gravity and melt flow index to determine MW of PTFE and fluoropolymers (see Supplemental Data, p 27–28). Standard Specific Gravity (SSG) and Melt Flow Rate (MFR) are more conveniently and frequently used with fluoropolymers rather than rheological and dynamic light scattering methods (Chu et al. 1989; Starkweather and Wu 1989; Tuminello 1989; Tuminello et al. 1993). Polytetrafluoroethylene has an M_n of 500 000 to 9 000 000 Da (Berry and Peterson 1951; Doban et al. 1956; Suwa et al. 1973; Putnam 1986; Chu et al. 1989; Tuminello 1989; Tuminello et al. 1993; Frick et al. 2012). Therefore, PTFE, as a very high molecular weight polymer, cannot cross cell membranes, is not bioavailable, and cannot bioaccumulate or be toxic (see Supplemental Data, p 14). High molecular weight fluoropolymers, such as PTFE, therefore meet the PLC criterion for having MW that prevents them from entering the cells. Polytetrafluoroethylene has negligible (<<1%) oligomeric content (Starkweather and Wu 1989), as does FEP (Figure 2.) In summary, fluoropolymers are high molecular weight polymers with narrow MWD and negligible oligomer content.

Reactive functional groups and RFG ratio to MW

Fluoropolymers satisfy the PLC criterion of RFGs and RFG ratio to MW. Polytetrafluoroethylene most typically has a terminal $–CF_3$ group that is not an RFG. When this is not the case, the most common terminal group is –COOH, which is

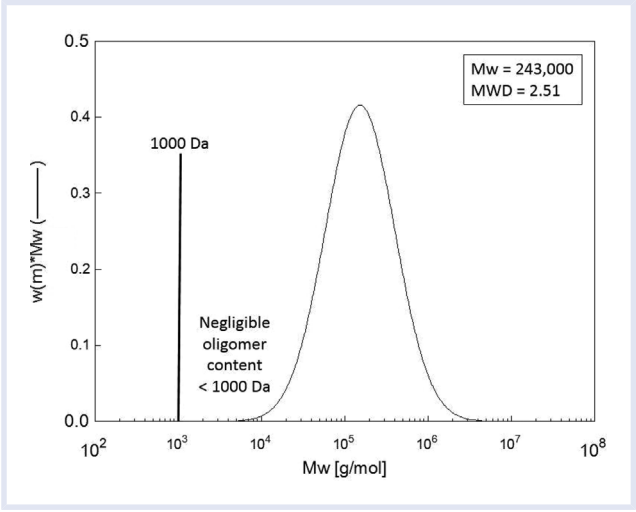

Figure 2. A fluorinated ethylene propylene (FEP) fluoropolymer molecular weight distribution from a rheological study. MW = molecular weight; MWD = molecular weight distribution.

categorized by the USEPA as a low-concern functional group. In unique cases, based on production method and ingredients used, PTFE may have end groups that may contain O, and H, N, or S, depending on the initiator or chain transfer agent used in polymerization. Fluoropolymers have a very high MW, which yields an FGEW on the order of 10^5 or more, well beyond the FGEW threshold of concern.

Low MW leachables

Fluoropolymers satisfy the PLC criterion of low MW leachables. Concentration of leachables from fluoropolymers, particularly PTFE "fine powder" (ASTM [2015] 4895-16 Type I fine powder definition), are typically very low (<1 ppm) (see Supplemental Data). This finding can be explained by the sensitivity of the PTFE polymerization reaction to contamination and is due to the postpolymerization processing steps aggressively exercised to wash out residuals and drive off volatiles. In order to achieve high MW polymerization of TFE, all traces of telogenic H- or Cl-bearing impurities must be removed (Ebnesajjad 2011; Supplemental Data).

In the analysis done on PTFE (see Supplemental Data, p 32), residual TFE monomer was not detected in PTFE resin by headspace GC-MS with a limit of detection of 1 ppm. In addition, publicly available analytical data from independent industry authorities demonstrate that TFE is not detected in finished articles made from fluoropolymers at detection limits down to about 0.01 ppm wt/wt (SPI 2005). Table 3 compares the molecular weight and the 8-h time weighted average (TWA) (American Conference of Governmental Industrial Hygienists [ACGIH], threshold limit value [TLV]), for monomers used to make fluoropolymers (ACGIH 2010). The TWAs are the exposure levels to which a worker could be exposed in an 8-h shift without adverse effects. The monomers have significantly lower MW, have lower TWAs, and are reactive. Note that the fluoropolymers are high MW, have no TWAs, and are inert. Table 3 illustrates that polymers do not have the same health hazards or MWs as their monomers.

Table 3. Fluoropolymer and monomer molecular weight and TLV data

Substance	CAS Nr	Molecular weight	ACGIH TLV 8-h TWA
Monomer: TFE	116-14-3	100	2 ppm
Monomer: Ethylene	74-85-1	28	200 ppm
Monomer: HFP	116-15-4	150	0.1 ppm
Monomer: PPVE	1623-05-8	266	200 ppm (vendor limit)
Polymer: PTFE	9002-84-0	389 000–45 000 000	None
Polymer: ETFE	25038-71-5, 68258-85-5	530 000–1 200 000	None
Polymer: FEP	25067-11-2	241 000–575 000	None
Polymer: PFA	26655-00-5, 31784-04-0	200 000–450 000	None

ACGIH = American Conference of Governmental Industrial Hygienists (ACGIH 2010); CAS = Chemical Abstracts Service; ETFE = ethylene tetrafluoroethylene; FEP = fluorinated ethylene propylene; HFP = hexafluoropropene; PFA = perfluoroalkoxy polymer; PPVE = perfluoropropylvinyl ether; PTFE = polytetrafluoroethylene; TFE = tetrafluoroethylene; TLV= threshold limit value; TWA = time weighted average.

Elemental composition

Fluoropolymers meet the widely accepted elemental composition criterion (BIO by Deloitte 2015). The USEPA, in updating its Polymer Exemption Rule, which applies to new polymers only, changed some review procedures to address certain side-chain fluorinated polymers that may degrade into small, mobile, and persistent substances (USFR 2010). This has contributed to confusion regarding the assessment of fluoropolymers. The exclusion of polymers under this step is not a conclusion of hazard, but a determination that the elemental composition does not fall within the parameters of the polymer set under which this rule was formulated, and consequently, these polymers would have to follow the standard notification and review process.

When USEPA updated the polymer exemption rule in 2010, the agency excluded polymers containing $–CF_3$ or larger chains that are covalently bound to C. The agency's rationale for the change was "...because the Agency has receiving information which suggests that polymers containing PFAS (perfluoroalkyl sulfonates) or PFAC (perfluoroalkyl carboxylates) may degrade and release fluorochemical residual compounds in the environment. Once released, PFAS or PFAC are expected to persist in the environment, may bioaccumulate, and may be highly toxic..." (USFR 2006).

Although USEPA recognized that PFAS and PFAC chemicals with longer C chain lengths (C7 and longer) may be of greater concern, it stated that there is insufficient evidence at this time, however, to definitively establish a lower C chain length limit to meet the "will not present an unreasonable risk" finding, which is the determination necessary to support an exemption under section 5(h)(4) of TSCA. The USEPA believes that it is possible for polymers containing these other types of perfluoroalkyl moieties to also degrade over time in the environment, thereby releasing the perfluoroalkyl moiety (USFR 2006).

The updated USEPA polymer exemption definition in 2010, summarized in the Objective and Rationale section for the Final Rule, may imply that new fluoropolymers with pendant or terminal –CF3 groups, such as FEP, do not meet the polymer exemption eligibility for reduced PMN reporting (USFR 2010). However, the summary definition in USFR (2010) lacks critical context found in the preamble to the Final Rule, which elaborates the conditions that would be necessary to exclude a perfluoro chemical from the polymer exemption:

- The first condition is cited above, "...polymers containing PFAS (perfluoroalkyl sulfonates) or PFAC (perfluoroalkyl carboxylates)..." where the C or S atom is an integral part of the polymer molecule; and
- the second condition notes that, polymers containing fluorotelomers or "...perfluoroalkyl moieties that are covalently bound to either a carbon or sulfur atom where the carbon or sulfur atom is an integral part of the polymer molecule can be attached to the polymers using conventional chemical reactions."

For the PFAS and PFAC as described by USEPA, the agency offers a clarification about the nature of the linkage, stating "How these materials are incorporated into the polymer is immaterial (they may be counter ions, terminal/end capping agents, or part of the polymer backbone)" (USFR 2010). The key characteristic is the presence of a –CF3 group that is attached to, or forms part of, the polymer backbone and "this link (between the polymer backbone and the –CF3 group) is susceptible to degradation and cleavage." (USFR 2010). Thus, in USEPA's review, the presence of –CF3 group is important because it is a structural alert to consider potential degradation products. The USEPA will make a determination whether the potential degradation of the polymer in question presents an unreasonable risk to health and the environment under TSCA. As shown in Table 2, these fluoropolymers are not subject to degradation.

Water and lipid solubility and the octanol–water partition coefficient

Fluoropolymers, such as PTFE, are not soluble in octanol or water. Therefore, it is not possible to measure or calculate a

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K_{OW}. Because solubility in octanol is predictive of lipid solubility, PTFE cannot dissolve in cell membrane lipids to gain access to cellular contents, nor is it small enough to enter the cell due to its very high MW. Because PTFE cannot enter the cells, it is not capable of bioaccumulation or bioconcentration in aquatic life.

Stability

Under normal, foreseeable uses, fluoropolymers are stable. Stability is resistance to physical, chemical, or biological transformation. Loss of stability in the polymer breaks it down into smaller pieces, producing low MW species. Molecules with M_n <1000 Da are capable of crossing cell membranes, making unstable polymers potentially hazardous to health and the environment. Fluoropolymers, in general, have exceptional chemical and thermal stability; that is why they are so unique and useful. This is due to very strong C–F bonds that are stable under even extreme conditions (Gangal and Brothers 2015). Polytetrafluoroethylene is inert and chemically resistant to all solvents except molten alkali metals, chlorine trifluoride, and oxygen difluoride. Polytetrafluoroethylene, as a representative fluoropolymer, has the best chemical resistance of all currently known polymers and is insoluble in all known solvents, including water (Drobny 2006).

Abiotic stability

Polymers are stable; monomers are not. Photochemical transformation is a reaction involving the radiation energy of sunlight (ultraviolet radiation) that may break a bond in a molecule to change it to another chemical entity. Although PTFE will rapidly degrade in ionizing radiation (e.g., gamma radiation or high energy electron-beam radiation), it is resistant to photolysis (Drobny 2006). Photoinduced reactions with fluoropolymers do not occur. In addition, hydrolysis is a reaction involving the breaking of a bond in a molecule using water. The fluorine envelope surrounding the C backbone of PTFE is very hydrophobic. Fluoropolymers, such as PTFE, are hydrolytically stable, water resistant, and are not subject to hydrolysis catalyzed degradation (Arkles 1973). Finally, chemical oxidation is a reaction involving the loss of electrons from one atom to another. Because the C–F bond is one of the strongest known, and F is the most electronegative element, the C–F bond is thermodynamically stable, unfavorable to lose electrons (i.e., to oxidize) (Arkles 1973).

Biotic stability: aerobic, anaerobic and in vivo

Fluoropolymers like PTFE are biologically inert and not degraded by microorganisms under oxygenated (aerobic) or anoxic (anaerobic conditions); in vitro and in vivo studies demonstrate this. In vivo degradation involves the breaking of the polymer bonds due to bacteria and other enzymes and oxidants. For example, PTFE hernia patches explanted from patients and examined by scanning electron microscopy, attenuated total reflectance Fourier transform infrared spectroscopy, modulated differential scanning calorimetry, and optical microscope showed no degradation in vivo (King et al. 2013).

Thermal stability

Fluoropolymers, when used as intended under normal, foreseeable use conditions as specified in Table 2 (or "continuous processing temperature") are thermally stable (Puts et al. 2014). The fluoropolymer industry has provided significant information on appropriate use of fluoropolymers (SPI 2005). Thermal gravimetric analysis determines mass loss over time and temperature of a test substance. Polytetrafluoroethylene is one of the most thermally stable polymers. Polytetrafluoroethylene's continuous processing temperature is 260 °C (SPI 2005). This means that PTFE could remain for decades at 260 °C and not decompose (SPI 2005 see percent mass lost per hour at maximum continuous processing temperature).

Outside of normal, foreseeable use conditions (also known as "misuse"), when fluoropolymers are held at temperatures above their recommended processing temperatures, they degrade. Upon decomposition, fluoropolymers generate volatile degradation products (SPI 2005). At 450 °C, the decomposition of PTFE "only proceeds at a rate on the order of one percent per hour. It is not until considerably above the polymer first-order transition temperature (329 °C) that substantial decomposition is observed" (Arkles and Bonnett 1974). As the temperatures increase above recommended processing temperatures, the rate of generation rises and may sufficiently degrade the polymer to produce hazardous gaseous byproducts and polymer (particulate) fume fever (SPI 2005). Temperature, availability of O, the physical form of the polymer article, and the residence time at elevated temperature factor into the ultimate nature of the decomposition products (SPI 2005), mainly fluoroalkenes, hydrogen fluoride, oxides of C, and lower molecular weight fluoropolymer particulates. For PTFE, TFE is the principle gaseous product observed at temperatures near 330 °C. See Supplemental Data for additional information regarding overheating PTFE.

PRODUCT-SPECIFIC REGULATORY REQUIREMENTS

Certain product-specific regulations, such as those for medical devices and food contact for the United States and the EU, require the development of additional data beyond what is required to conduct a PLC evaluation. The following text will discuss food contact requirements for the United States and the EU, and medical device requirements.

Data requirements for food, pharmaceutical, and medical device applications

There are country-specific data requirements for fluoropolymer use in food, pharmaceutical, and medical device applications because the intended use of these products has the potential to directly or indirectly introduce the product into the human body. An extensive fluoropolymer data set has been developed by W.L. Gore for these uses. The clinical history of the safe implantation of more than 40 million PTFE medical devices over 40 y, extensive toxicity data, preclinical

data, and chemical extractables and migration testing confirmed that fluoropolymers are not bioavailable. Although the data requirements have evolved over time for contacting food, pharmaceuticals, or use in medical devices, the data (some of which are provided in the present article, the Supplemental Data for the present paper, regulatory submissions, and product literature) confirm the conclusion that fluoropolymers are safe for these intended uses and support the conclusion that fluoropolymers should be considered PLCs.

Polymer of low concern data and US and European Union food contact requirements

In general, the data required to support a PLC determination are helpful, but insufficient to qualify a material for food contact use. Submissions to the US Food and Drug Administration (USFDA) to support new food contact substances require extensive data submissions, including, for example, the nature and amount of nonvolatile extractives (USFDA 2017). Fluoropolymers, however, are not new substances in applications where they come in contact with food and have longstanding acceptance by regulators. In the United States, the USFDA is responsible for regulation of materials that come in contact with food and are considered "indirect food additives," specifically polymers (USFR 2016a). Food storage or food packaging materials, such as the fluoropolymers PTFE, FEP, and PFA, are "perfluorocarbon resins" acceptable for use by application and material type, provided they meet the extractable limits specified in the regulation (USFR 2016b).

Similarly, the European Food Safety Agency (EFSA) provides recommendations to the European Commission (EC) within the EU for the regulation of food contact materials, requirements for their evaluation, and authorization of acceptable uses (EC 2004). Polymer clearance is based in part upon the fact that polymers will not migrate into food due to their high molecular weight. The EU focuses on potential low molecular weight moieties, such as residual monomers and leachables, rather than on the polymer itself. The EU food contact regulation requires that monomers, other starting substances, and additives used to produce food contact polymers should be risk assessed and authorized (EU 2011). The regulation lists authorized substances that are permitted to have food contact (EU 2011). This regulation also sets the specific migration limit (SML), which is the maximum permitted amount of substance in food that has been determined not to pose a risk to human health, specifically for individual chemicals (e.g., monomer) (EU 2011). Note that these limits exist whether or not the substance is present in the food contact material (FCM). The monomers, other starting substances, and additives used to produce fluoropolymers for food contact (e.g., PTFE, FEP, and PFA) have been authorized for food contact uses. Representative SMLs for these monomers, additives, and starting substances relevant for fluoropolymers are given in the Supplemental Data (p 14).

Polymer of low concern data and medical device regulatory requirements

Satisfaction of the PLC criteria is insufficient to satisfy medical device requirements. Formal biocompatibility evaluations are required by the USFDA and other global regulatory authorities to support submissions for approval of medical devices and pharmaceuticals (e.g., combination products, such as drug-eluting stents or prefilled single-dose syringes). The International Organization for Standardization (ISO) 10993 Biocompatibility of Medical Devices standards describe a broad array of biocompatibility tests that require consideration for each new device or significant changes to existing devices (ISO 2009). Over the years, medical devices containing PTFE (or expanded PTFE) have been evaluated using ISO 10993 and US Pharmacopeia (USP) Class VI standards (USP 2011) and have been determined to be biocompatible in their intended uses.

The ISO 10993 standards provide guidance for evaluation of the biological response to a medical device. The USFDA, as well as most international regulatory agencies, recognizes and uses ISO 10993 standards to guide safety evaluations of medical devices submitted for their approval. Requirements to demonstrate the biocompatibility of medical devices are set forth in ISO 10993-1, and regulatory authority–specific requirements (e.g., PMDA 2003; USFDA 2016). In addition, country pharmacopeial organizations also specify testing required for biological reactivity of drugs (e.g., US Pharmacopiea, EU Pharmacopiea, Japan Pharmacopiea). The ISO requirements are categorized by the nature of body contact (e.g., mucosal membrane, circulating blood, tissue, bone, dentin) and duration of contact ($<$24 h, $\geq$1 d $\leq$30 d, $>$30 d). Depending on the nature and duration of contact, requirements include cytotoxicity, irritation, sensitization, implantation, acute–subchronic–chronic systemic toxicity, material-mediated pyrogenicity, hemocompatibility (e.g., hemolysis, thrombogenicity, and complement activation), genotoxicity (in vitro and in vivo), carcinogenicity, and developmental toxicity. (See Supplemental Data p 15 for a list of ISO 10993 biocompatibility tests.)

MEETING PLC CRITERIA PRECLUDES A FINDING THAT A CHEMICAL IS OF HIGH CONCERN

Just as regulatory frameworks have mechanisms to identify materials of low concern such as PLCs, they also have mechanisms to identify chemicals of high concern. For example, under REACH, a mechanism exists to identify substances of very high concern (SVHCs). Having demonstrated that fluoropolymers like PTFE should be considered PLCs, we will also demonstrate that these fluoropolymers cannot be SVHCs under REACH, do not meet the PM and PMT criteria proposed by UBA, and do not meet the criteria for listing as a POP under the Stockholm Convention.

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Fluoropolymers and EU REACH SVHC, CMR, PBT, vPvB, and endocrine disrupting chemical (EDC) criteria

According to the European Chemicals Agency (ECHA), SVHCs are defined in Article 57 of Regulation (EC) Nr 1907/2006 ("the REACH Regulation") (EC 2006) and include substances that are

- "Carcinogenic, mutagenic or toxic to reproduction (CMR), meeting the criteria for classification in category 1 or 2 in accordance with Directive 67/548/EEC. This directive was replaced in beginning of 2009 by the new EU regulation (EC) No 1272/2008 on classification, labeling and packaging of chemical substances and mixtures, the so-called CLP Regulation. According to the new CLP Regulation these substances shall be classified as 1a or 1b."
- "Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB) according to the criteria in Annex XIII of the REACH Regulation."
- "Identified, on a case-by-case basis, from scientific evidence as causing probable serious effects to human health or the environment of an equivalent level of concern as those above (e.g., EDCs)."

Under REACH, polymer substances are not registered, but the monomers they are composed of are registered, and the registration must be supported by data submissions that are tiered on the basis of tonnage (see EC 2006, Annex VII). The REACH definition of polymer includes materials with as few as 3 repeating units. But such a small molecule would not meet common industry standard definitions for fluoropolymers (ASTM 2015). It is highly unlikely that fluoropolymers meeting the PLC criteria would exhibit the criteria of an SVHC under REACH. Fluoropolymer data developed for other regulatory needs support the predictive value of the PLC assessment criteria and demonstrate the low hazard potential of this class of PFAS. Due to their physical–chemical properties, PLCs are not bioavailable to cause toxicity or to bioaccumulate. Toxicity study data on PTFE in the Supplemental Data (p 15–27), for example, demonstrate a lack of toxicity, including genotoxicity. Although fluoropolymers are persistent, they are not bioaccumulative or toxic and therefore do not meet the PBT criteria.

Fluoropolymers and German UBA–proposed PMT criteria

As regulatory frameworks continue to evolve, more work is needed in the area of PFAS classification to ensure that regulations are appropriate in scope and proportionality. Although some well-known PFAS would qualify as PM or PMT substances as proposed by the UBA (2017), fluoropolymers do not possess these characteristics. Although fluoropolymers are highly stable (persistent), they do not meet the criteria to be mobile or toxic. To demonstrate this point, PTFE, a high molecular weight fluoropolymer and a member of the PFAS group, is assessed (in the last 4 paragraphs of this section) according to the proposed UBA criteria (UBA 2017).

Briefly, the changes to PM and/or PMT assessment proposed by UBA address applicability, persistence, mobility, and toxicity. The UBA proposes an initial step involving assessment of the chemical composition of a substance to determine if the substance is within the applicability domain of the proposed new assessment criteria. The UBA notes that currently only identifiable organic and organometallic chemicals are considered, and purely inorganic substances or substances of unknown or variable compositions, complex reaction products, or biological material are excluded (UBA 2017).

With respect to persistence, UBA proposes that the criterion for persistence be the same as in Annex XIII of REACH, which considers degradation half-lives in marine water, fresh- or estuarine water, marine sediment, and soil as part of the PBT/very persistent, very bioaccumulative (vPvB) assessment criteria; these degradation half-life criteria range from 40 to 180 d. The UBA proposes that a substance meets the persistent criterion if the degradation half-life in marine water at pH 6 to 8 and 12 °C is higher than 60 d, the half-life in fresh- or estuarine water at pH 6 to 8 and 12 °C is higher than 40 d, the half-life in marine sediment at pH 6 to 8 and 12 °C is higher than 180 d, the half-life in fresh- or estuarine water sediment at pH 6 to 8 and 12 °C is higher than 120 d, or the half-life in soil at pH 6 to 8 and 12 °C is higher than 120 d.

The UBA proposes that the mobility criterion for a persistent chemical should be determined on the basis of 2 considerations. First, the water solubility of a substance at pH 6 to 8 and 12 °C must be greater than or equal to 150 μg/L, and the log K_{OC} at pH 6 to 8 and 12 °C must be less than or equal to 4.5. The UBA notes that the mobility criterion should be applied only to substances that have fulfilled the criterion for persistence.

Lastly, with respect to toxicity, UBA proposes a 5-part test for involving data to understand if the substance is carcinogenic, germ cell mutagenic, or toxic for reproduction; if there is other evidence of chronic toxicity; and if there is evidence for effects on or via lactation. The derived no adverse effect level (DNEL) must be less than or equal to 9 $\mu g \cdot kg^{-1}d^{-1}$. The UBA notes that the first 2 considerations are the same criteria defined in Annex XIII of REACH as part of the PBT/vPvB assessment criteria regarding human health. The next 2 criteria specifically address concerns for drinking water exposure and are based on Regulation EC No 1272/2008 (EC 2008) and Cramer class II (Cramer et al. 1978) for substances exhibiting moderate or low biological activity, respectively. The DNEL criterion is based on Kalberlah et al. (2014).

Regardless of the arguments concerning the scientific foundation and credibility of the changes proposed by UBA to REACH PM and PMT assessment criteria, the central question with respect to PTFE is whether chemical-specific assessment would lead to an outcome different from that assuming PTFE behaved similarly to other PFAS substances. Polymers, including fluoropolymers, are different from non-polymeric chemicals and may be regulated differently. Because of these differences, it is recognized that some data requirements may not be applicable to polymers (EU 2011) For example, as we have shown, the physical–chemical criteria of PLC are predictive of lack of hazard.

With respect to applicability, PTFE is not a substance currently registered under REACH because it meets the REACH definition of a polymer substance: "a molecule that contains a sequence of at least 3 monomer units, which are covalently bound to at least one other monomer unit or other reactant" (EC 2006). However, because PTFE is an identifiable organic substance, the proposed UBA framework for assessment using the proposed PMT criteria would be applicable. Further, PTFE is highly stable and persistent in the environment. It is resistant to thermal degradation, being stable for decades at temperatures up to 260 °C (SPI 2005); is stable in terms of hydrolysis, oxidation, and light (Brydson 1999); and is stable in terms of anaerobic and aerobic degradation (King et al. 2013). Therefore, PTFE would fulfill the UBA's proposed persistence criterion.

In contrast, PTFE is practically insoluble in water and, therefore, is not mobile in the environment. Using the descriptive solubility table for the USP (2011), the water solubility of PTFE would be classified as practically insoluble (1×10^{-5} mg/L or 0.01 μg/L) to very slightly soluble (1×10^{-4} mg/L or 0.1 μg/L) (USP 2011). The mobility of PFTE is 1000 to 10 000× lower than UBA's proposed mobility criterion. Therefore, PTFE does not fulfill UBA's proposed mobility criterion and would not be classified as a PM or PMT substance.

A similar negative finding for PTFE pertains to toxicity. The average molecular weight of PTFE is too large for the polymer to cross a cell membrane, which means it is not bioavailable or toxic. Polytetrafluoroethylene has been tested extensively in the United States and European Union to assess commercial applications for food contact and global medical device regulations (see Supplemental Data for additional details). Results demonstrate the absence of toxicity. Therefore, PTFE does not fulfill UBA's proposed toxicity criterion and would not be classified as a PM or PMT substance (Table 4).

Fluoropolymers and the Stockholm Convention POP criteria

In addition to country and regional regulations, there are global legally binding instruments, such as the United Nations Environment Programme–administered conventions on chemicals and waste (UNEP 2001), such as the Stockholm Convention on Persistent Organic Pollutants. The Convention aims to eliminate POPs by eliminating their production, reducing their use, or limiting their use through a cradle-to-grave approach. For the listing of new chemicals into the Convention, numeric or other criteria have been set for the screening of proposed compounds. Stockholm Convention Criteria (annex D) are compared to those of the USEPA, EU REACH, and the UBA-proposed PMT (Table 4). Fluoropolymers meet the persistence criterion only, not the bioaccumulative, toxic, or mobile criteria.

Fluoropolymers satisfy widely accepted criteria to be considered PLCs. Their physical–chemical properties prevent bioavailability, bioaccumulation, toxicity, and degradation. They have negligible monomer, oligomer, and leachable content and no reactive functional groups with high toxicity. These comparisons of PLC and various regulatory assessment criteria demonstrate that, in the realm of PFAS, high molecular weight fluoropolymers like PTFE have vastly different properties than do other PFAS, and therefore, they are truly a separate class of materials that must be assessed on their own merits as has been done here. They also underscore the value of a global regulatory definition of a polymer.

FUTURE WORK

It is important to acknowledge that the manufacture and end-of-life phases of the fluoropolymer life cycle are not the subject of the present paper. The following reflections are provided on how these may be explored in future work. Fluoropolymer manufacture includes fluoromonomers and a wide array of initiators, catalysts, et cetera, including polymer production aids, some of which are fluorosurfactants (non-polymer PFAS) (see Supplemental Data, p 8, for more information about them). Historically, perfluorocarboxylic acids such as PFOA and perfluorononanoic acid (PFNA) were used as polymer production aids in the manufacture of fluoropolymers. They are no longer used by leading global fluoropolymer manufacturers (USEPA 2017a), who are now using alternative substances such as fluorinated polyether carboxylates (see Supplemental Data Table S2). The toxicological and environmental properties (e.g., persistence, bioavailability, and mobility) of these alternatives are very important. Future work should delve into fluoropolymer manufacture and describe the safety, health, and environmental management practices and controls employed; should describe the applicable regulations; and should assess substances used in fluoropolymer manufacture, their human health and environmental attributes, and their mass balance.

At end-of-life when a fluoropolymer has fulfilled its intended use and will be disposed of, the fate of fluoropolymers should be investigated further. Although there are sufficient data to demonstrate that fluoropolymers such as PTFE do not degrade in the environment or release substances of toxicological or environmental concern (Hintzer and Schwertfeger 2014), the downstream, end-of-life process of incineration merits future work. For instance, at temperatures above 450 °C, PTFE begins to degrade, releasing hazardous substances such as hydrofluoric acid. There are published studies on the incineration of fluoropolymers under normal, foreseeable municipal waste incinerator conditions targeting specific analytes (Taylor 2009). Presently, most legislation addresses the release of hydrogen fluoride (HF) as the only critical parameter; limit values are for stack emissions (e.g., EU 2000). Future work should investigate incineration under a range of relevant foreseeable use conditions to determine more comprehensively the substances formed and their amounts. Such an incineration study is underway with results to be published upon completion (W.L. Gore 2017). In addition, the practice of the open burning of fluoropolymers, or for that matter of any polymer, is unacceptable and unsafe. Responsible incineration of fluoropolymers, adhering to regulatory guidelines, at the end of their life cycle is appropriate.

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Table 4. Comparison of United States, Stockholm Convention, EU REACH, and German Criteria

Criterion	United States[a]	Stockholm Convention[b]	REACH[c,d]	Germany[d,e]
Persistence (half-life)				
P	Water, soil, sediment > 60 d	Water >60 d	Marine water > 60 d	Same as REACH
		Soil, sediment >180 d	Estuarine water > 40 d	
			Fresh or estuarine sediment or soil > 120 d	
vP	Water, soil, sediment > 180 d		Marine, fresh, estuarine H_2O > 60 d	
			Marine, fresh, or estuarine sediment > 180 d	
			Soil > 180 d	
Bioaccumulation				
B	Aquatic BCF > 1000	Aquatic BCF or BAF > 5000	BCF > 2000	
		Log K_{OW} > 5		
vB	BCF > 5000		BCF > 5000	
Toxicity				
	Fish	Toxic or ecotoxic	Long-term aquatic NOEC or EC10 < 0.01	1) Carcinogenic, germ cell mutagenic, or toxic for reproduction[d];
	Low > 10 mg/L	(No numeric criteria)	Classified as carcinogen category 1A or 1B; mutagen 1A or 1B; reproductive toxin 1A, 1B, or 2[d]	2) other evidence of chronic toxicity[d]; and
	Moderate 0.1 mg/L–10 mg/L			3) evidence for effects on or via lactation[d].
	High < 0.1 mg/L			4) DNEL[f] ≤ 9 μg · $kg^{-1}d^{-1}$
			Specific target organ toxicity (STOT RE 1 or 2) upon repeated (chronic) exposure)[d]	
Long-range transport (potential for)		Long-range transport (potential for): Presence through monitoring or modeled data; $t_{1/2}$ (air): 2 d		
Mobility				Mobility: water solubility at pH 6–8, 12 °C, must be ≥150 μg/L, and the log K_{OC} at pH 6–8, 12 °C must be ≤4.5.

BAF = bioaccumulation factor; BCF = bioconcentration factor; DNEL = derived no adverse effect level; EU = European Union; M = mobile; P = persistent; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; STOT RE = specific target organ of toxicity repeat exposure; T = toxic; v = very.
[a]USEPA 1999.
[b]UNEP 2001
[c]ECHA 2014
[d]EC 2008
[e]UBA 2017
[f]Barlow 2005; Kalberlah et al. 2014.

Recycling, reuse, and closed loop systems are alternative options at the end of life. Recent work has shown, on a small scale, the ability to convert fluoropolymers back to their monomers for capture (Schlipf 2014; Invertec 2017). This approach to a closed loop economy for fluoropolymers merits additional work and discussion, as does the recycling and reuse of melt-processable fluoropolymers, such as FEP.

CONCLUSIONS

The present review has brought together fluoropolymer toxicity data, human clinical data, and physical–chemical characteristics, using PTFE as an example to show that fluoropolymers satisfy the widely accepted regulatory assessment criteria to be considered as PLCs. Fluoropolymers are high molecular weight, have narrow molecular weight distribution, and have negligible oligomer content and organic and inorganic leachables. Data show that fluoropolymers have thermal, chemical, photochemical, hydrolytic, and biological stability. Polytetrafluoroethylene has been extensively tested to comply with US and EU food contact and global medical device regulations (e.g., USFDA, CFDA, Korea MFDS, Japan PMDA), including ISO 10993 biocompatibility testing and preclinical animal testing. Toxicology studies on PTFE demonstrate the absence of acute or subchronic systemic toxicity, irritation, sensitization, local toxicity on implantation, in vitro and in vivo genotoxicity, hemolysis, complement activation, or thrombogenicity. The data presented demonstrate that the fluoropolymer class of PFAS is well defined, meets PLC criteria, and should be considered as distinctly different from other classes of PFAS. The grouping of all PFAS together is not supported by the scientific data.

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Data Accessibility—All data and information used in this manuscript have been made available in tabulated form (Tables 1–4) by the authors and are included in the paper and the Supplemental Data.

SUPPLEMENTAL DATA

The Supplemental Data contains descriptive and more detailed information as highlighted in the paper.

Figure S1. Where does polytetrafluoroethylene (PTFE) come from?

Figure S2. Fluoropolymer primer: polytetrafluoroethylene (PTFE) polymerization scheme.

Figure S3. Fluoropolymer primer: polytetrafluoroethylene (PTFE) finishing scheme.

Figure S2. A fluorinated ethylene propylene (FEP) fluoropolymer molecular weight distribution from a rheological study.

Table S1. Polytetrafluoroethylene (PTFE) polymerization and post polymerization aids

Table S2. Alterative fluoropolymer processing aids: Sources of data

Table S3. Solubility table from USP 34 NF 29 General Notices, Section 5.3.0, p 6

Table S4. European Union (EU) specific migration limits (SMLs) for monomers in representative fluoropolymers

Table S5. Biocompatibility tests, conditions, and acceptance criteria results for expanded polytetrafluoroethylene patch

Table S6. US Environmental Protection Agency's (USEPA's) chemical categories of concern, 2010

REFERENCES

[ACGIH] American Conference of Governmental Industrial Hygienists. 2010. TLVs and BEIs: Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH): American Conference of Governmental Industrial Hygienists.

Arkles B. 1973. Recycling polytetrafluoroethylene in polymer science and technology. In: Guillet J, editor. Polymers and ecological problems, Vol. 3. New York (NY): Plenum. p 122.

Arkles B, Bonnett R. 1974. Method for the depolymerization of polytetrafluoroethylene, US 3832411A.

[ASTM] American Society for Testing and Materials. 2015a. ASTM D3159-15, Standard specification for modified ETFE fluoropolymer molding and extrusion materials.

[ASTM] American Society for Testing and Materials. 2015b. ASTM D4894, Standard specification for polytetrafluoroethylene (PTFE) granular molding and ram extrusion materials.

[ASTM] American Society for Testing and Materials. 2016a. ASTM D2116-16, Standard specification for FEP-fluorocarbon molding and extrusion materials.

[ASTM] American Society for Testing and Materials. 2016b. ASTM D3307-16, Standard specification for perfluoroalkoxy (PFA) resin molding and extrusion materials.

[ASTM] American Society for Testing and Materials. 2016c. ASTM D4895–16, Standard specification for polytetrafluoroethylene (PTFE) resin produced from dispersion.

Auer CM, Nabholz JV, Baetcke KP. 1990. Mode of action and the assessment of chemical hazards in the presence of limited data: Use of structure-activity relationships (SAR) under TSCA, Section 5. *Environ Health Perspect* 87:183–197.

Banks RE, Smart BE, Tatlow J. 1994. Organofluorine chemistry: Principles and commercial applications. New York (NY): Springer. 644 p.

Barlow S. 2005. Threshold of toxicological concern (TTC): A tool for assessing substances of unknown toxicity present at low levels in the diet. Brussels (BE): ILSI Europe. ILSI Europe Concise Monograph Series.

Berry KL, Peterson JH. 1951. Tracer studies of oxidation—Reduction polymerization and molecular weight of "Teflon" tetrafluoroethylene resin. *J Am Chem Soc* 73:5195–5197.

Beyer EC. 1993. Gap junctions. *Int Rev Cytol* 137:2.

 DOI: 10.1002/ieam.4035

BIO by Deloitte. 2015. Technical assistance related to the review of REACH with regard to the registration requirements on polymers Final report prepared for the European Commission (DG ENV), in collaboration with PIEP.

Blum A, Balan SA, Scheringer M, Trier X, Goldenman G, Cousins IT, Diamond M, Fletcher T, Higgins C, Lindeman AE, et al. 2015. The Madrid statement on poly- and perfluoroalkyl substances (PFAS). *Environ Health Perspect* 123:A107–A111.

Brydson JA. 1999. Plastics materials. 7th ed. Oxford (UK): Butterworth Heinemann. 920 p.

Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SPJ. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr Environ Assess Manag* 7(4):513–541.

ChemNews. 2016 Jul 7. Sweden's Kemi issues interim report on PFAS. Chemical Watch (Global Risk and Regulation News).

Chu B, Wu C, Buck W. 1989. Light scattering characterization of poly-(tetrafluoroethylene). PTFE in perfluorotetracosane: Molecular weight distribution and solution properties. *Macromolecules* 22:831–837.

Cramer GM, Ford RA, Hall RL. 1978. Estimation of toxic hazard—A decision tree approach. *Food Cosmet Toxicol* 16(3):255–276.

Dams R, Hintzer K. 2016. Industrial aspects of fluorinated oligomers and polymers. In: Ameduri B, Sawada H, editors. Fluorinated polymers, Vol 2: Applications. London (UK): Royal Society of Chemistry. p 1–31.

Doban RC, Knight AC, Peterson JH, Sperati CA. 1956. The molecular weight of polytetrafluoroethylene. Paper presented at 130th Meeting of American Chemical Society; 1956 Sep; Atlantic City, NJ. p 9S.

Drobny JG. 2006. Fluoroplastics. Shropshire (UK): Rapra Technology. p 12.

Ebnesajjad S. 2011. Introduction to fluoropolymers. In: Kutz M, editor. Applied plastics engineering handbook. New York (NY): Elsevier. p 49–60.

[EC] European Commission. 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. [cited 2017 July 12]. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02004R1935-20090807&from=EN

[EC] European Commission. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). [cited 2017 July 12]. https://echa.europa.eu/regulations/reach/legislation

[EC] European Commission. 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance). [cited 2017 July 12]. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32008R1272

[ECHA] European Chemicals Agency. 2014. Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT/vPvB assessment version 2.0 November 2014. [cited 2017 July 12]. https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

[ECHA] European Chemicals Agency. 2015. Opinion of the Committee for Risk Assessment and Opinion of the Committee for Socio-economic Analysis on an Annex XV dossier proposing restrictions of the manufacture, placing on the market or use of a substance within the EU. [cited 2017 July 12]. https://echa.europa.eu/documents/10162/13641/rest_pfoa_compiled_opinions_en.pdf/2f0dfce0-3dcf-4398-8d6b-2e59c86446be

[EU] European Union. 2000. Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste.

[EU] European Union. 2011. European Union Guidance on Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food as regards information in the supply chain available on the food safety part of the website of the European Commission, on the page concerning the legislation on Food Contact Materials. [cited 2017 July 12]. http://ec.europa.eu/food/safety/chemical_safety/food_contact_materials/legislation/index_en.htm

Frick A, Sich D, Heinrich G, Stern C, Schlipf M. 2012. Classification of new melt-processable PTFE: Comparison of emulsion- and suspension-polymerized materials. *Macromol Mater Eng* 297:329–341.

Gangal SV, Brothers PD. 2015. Perfluorinated polymers. In: Kirk-Othmer encyclopedia of chemical technology. New York (NY): Wiley. p 1–68.

Hintzer K, Schwertfeger W. 2014. Fluoropolymers – Environmental aspects. In: Smith Jr DW, Iacano ST, Iyer SS, editors. Handbook of fluoropolymer science and technology. New York (NY): Wiley. p 495–520.

Hougham G, Cassidy PE, Johns K, Davidson T. 1999. Topics in applied chemistry. Fluoropolymers 2: Properties. New York (NY): Springer. 408 p.

IHS Markit. 2016. Fluoropolymers chemical economics handbook. Freedonia Group.

Invertec. 2017. Pilot project: Recycling of fluoropolymers (PTFE). [cited 2017 July 12]. https://www.invertec-ev.de/en/projects/environmental-care/ptfe-recycling/

[ISO] International Organization for Standardization. 2006. ISO 12086-1:2006 Plastics – Fluoropolymer dispersions and moulding and extrusion materials. Part 1: Designation system and basis for specifications.

[ISO] International Organization for Standardization. 2009. ISO 10993-1: Biological evaluation of medical devices. Part 1: Evaluation and testing in the risk management process. Geneva (CH).

[ISO] International Organization for Standardization. 2011. ISO 1133-1:2011 Plastics — Determination of the melt mass-flow rate (MFR) and melt volume-flow rate (MVR) of thermoplastics. Part 1: Standard method.

Kalberlah F, Oltmanns J, Schwartz MA, Baumeister J, and Stiffler A. 2014. Guidance for the precautionary protection of raw water destined for drinking water extraction from contaminants regulated under REACH. Dessau-Roßlau (DE): Umweltbundesamt (UBA). Report FKZ 371265416.

Kemi Swedish Chemicals Agency. 2016. Strategy for reducing the use of highly fluorinated substances, PFASs: Interim report as part of a Government assignment. Report 11/16. [cited 2017 July 12]. https://www.kemi.se/global/rapporter/2016/report-11-16-strategy-for-reducing-the-use-of-higly-fluorinated-substances-pfas.pdf

King MW, Gupta BA, Guidoin R. 2013. Biotextiles as medical implants:15. Vascular prostheses for open surgery. Cambridge (UK): Woodhood. p 434–484.

LaGrega M D. Buckingham PL, Evans JC. 2010. Hazardous waste management. 2nd ed. Long Grove (IL): Waveland. 1232 p.

Liu J, Mejia-Avendaño S. 2013. Microbial degradation of polyfluoroalkyl chemicals in the environment: A review. *Environ Int* 61:98–114.

[NICNAS] National Industrial Chemicals Notification and Assessment Scheme. 2016. NICNAS Handbook - Guide for importers and manufacturers of industrial chemicals in Australia. Sydney (AU). [cited 2017 July 12]. http://webarchive.nla.gov.au/gov/20161017033100/https://www.nicnas.gov.au/regulation-and-compliance/nicnas-handbook

[OECD] Organisation for Economic Co-operation and Development. 1993. OECD Expert Group on Polymers. Third Meeting of the Experts on Polymers: Chairman's Report [ENV/MC/CHEM/RD(93)4]; 1993 Apr; Paris (FR).

[OECD] Organisation for Economic Co-operation and Development. 2009. Data analysis of the identification of correlations between polymer characteristics and potential for health or ecotoxicological concern. OECD Task Force on New Chemicals Notification and Assessment, Expert Group Meeting on Polymers; 2007 Mar; Tokyo, Japan. Paris (FR).

[OECD] Organisation for Economic Co-operation and Development. 2017. OECD portal on per and poly fluorinated chemicals. Country Information section. Paris (FR). [cited 2017 July 12]. http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/

Olabisi O, Adewale K. 2015. Handbook of thermoplastics. 2nd ed. Boca Raton (FL): CRC.

Pianca M, Barchiesi E, Esposto G, Radice S. 1999. End groups in fluoropolymers. *J Fluorine Chem* 95:71–84.

Plunkett RJ. 1987. The history of polytetrafluoroethylene: Discovery and development. In: Seymour RB, Kirshenbaum GS, editors. High performance polymers: Their origin and development. New York (NY): Elsevier. p 261–266.

[PMDA] Japan Pharmaceutical and Medical Device Agency, Ministry of Health, Labour and Welfare. 2003. Basic principles of biological safety evaluation required for application for approval to manufacture (import) medical

devices. Tokyo (JP). PFBS/ELD (Iyakushin) Notification No. 0213001. 2003 Feb 13.

Putnam RE. 1986. Development of thermoplastic fluoropolymers. In: Seymour RB, Kirshenbaum GS, editors. High performance polymers: Their origin and development. New York (NY): Elsevier. p 279–286.

Puts G, Crouse P, Ameduri B. 2014. Thermal degradation and pyrolysis of polytetrafluoroethylene. In: Smith DW, Iacano TS, Iyer SS, editors Handbook of fluoropolymer science and technology. New York (NY): Wiley. p 81–104.

Scheringer M, Trier X, Cousins IT, de Voogt P, Fletcher T, Wang, Z, Webster TF. 2014. Helsingor statement on poly- and perfluorinated alkyl substances (PFAS). *Chemosphere* 114:337–339.

Schlipf M, Schwalm T. 2014. Closing the recyling loop. *Kunststoffe Intl* 2014/06. [cited 2017 July 12]. https://www.kunststoffe.de/en/journal/archive/article/up-cycling-of-end-of-life-fluoroplastics-841786.html

[SPI] Society of the Plastics Industry. 2005. SPIs guide to safe handling of fluoropolymers. Washington (DC).

Starkweather Jr HW, Wu S. 1989. Molecular weight distribution in polymers of tetrafluoroethylene. *Polymer* 30:1669–1674.

Suwa T, Takehisa M, Machi S. 1973. Melting and crystallization behavior of poly(tetrafluoroethylene). New method for molecular weight measurement of poly(tetrafluoroethylene) using a differential scanning calorimeter. *J Appl Polym Sci* 17:3253–3257.

Taylor PH, 2009. ECA incineration testing program: Laboratory-scale incineration testing of fluoropolymers. Dayton (OH): USEPA. USEPA Docket No. OPPT-2003-0071.

Tuminello WH. 1989. Molecular weight distributions of tetrafluoroethylene-hexafluoropropylene copolymers. *Polym Eng Sci* 29:645–653.

Tuminello WH, Buck WH, Kerbow DL. 1993. Rheological molecular weight distribution determination of ethylene/ tetrafluoroethylene copolymers: Implications for long-chain branching. *Macromolecules* 26:499–503.

[UBA] Umweltbundesamt. 2017. Protecting the sources of our drinking water from mobile chemicals. A proposal for implementing criteria and an assessment procedure to identify persistent, mobile and toxic (PM or PMT) substances registered under REACH. Dessau-Roßlau (DE). [cited 2017 July 12]. https://www.umweltbundesamt.de/en/publikationen/protecting-the-sources-of-our-drinking-water-from

[UNEP] United Nations Environment Programme. 2001. Conference of Plenipotentiaries on the Stockholm Convention. UNEP/POPS/CONF/2. [cited 2017 July 12]. http://www.wipo.int/edocs/trtdocs/en/unep-pop/trt_unep_pop_2.pdf

[UNEP] United Nations Environment Programme. 2009: Decision SC-4/17. Geneva (CH). [cited 2017 July 12]. http://chm.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP.4-SC-4-17.English.pdf; also accessible from table of new POPs: http://www.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx

[UNEP] United Nations Environment Programme. 2011. UNEP-POPS-POPRC.11-POPRC-11-4.English.docx. Geneva (CH). [cited 2017 July 12]. http://www.pops.int/TheConvention/POPsReviewCommittee/Meetings/POPRC11/Overview/tabid/4558/mctl/ViewDetails/EventModID/871/EventID/553/xmid/13837/Default.aspx

[UNEP] United Nations Environment Programme. 2017a. Proposal for listing PFHxS (UNEP/POPS/POPRC.13/4). Geneva (CH). [cited 2017 July 12]. http://chm.pops.int/Convention/POPsReviewCommittee/Chemicals/tabid/243/Default.aspx

[UNEP] United Nations Environment Programme. 2017b. Risk management profile for PFOA, (UNEP/POPS/POPRC.13/7/Add.2). Geneva (CH). [cited 2017 July 12]. http://chm.pops.int/Convention/POPsReviewCommittee/Chemicals/tabid/243/Default.aspx

[USC] US Congress. 1976. The Toxic Substance Control Act. 15 U.S.C. 2601-2671. Public Law 94–469.

[USEPA] US Environmental Protection Agency. 1997a. Chemistry assistance manual for premanufacture notification submitters. Washington (DC). EPA 774-R-97-003. March 1997.

[USEPA] US Environmental Protection Agency. 1997b. Polymer exemption guidance manual. Washington (DC). EPA-744-B-97-001. June 1997.

[USEPA] US Environmental Protection Agency. 1999. Category for persistent, bioaccumulative, and toxic new chemical substances. *Fed Regis* 4(213). 1999 Nov 4.

[USEPA] US Environmental Protection Agency. 2010. Reviewing new chemicals under the Toxic Substances Control Act (TSCA) – EPA's Review Process - Chemical categories used to review new chemicals under TSCA: TSCA New Chemicals Program (NCP) Chemical Categories. Washington (DC): USEPA Office of Pollution Prevention and Toxics. [cited 2017 July 12]. http://www.epa.gov/oppt/newchems/pubs/npcchemicalcategories.pdf

[USEPA] US Environmental Protection Agency. 2012. Sustainable futures/P2 framework manual.

[USEPA] US Environmental Protection Agency. 2017a. US Environmental Protection Agency, PFOA Stewardship Program. Washington (DC). [cited 2017 July 12]. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfass#tab-3

[USEPA] US Environmental Protection Agency. 2017b. Using predictive methods to assess hazard under TSCA. Washington (DC). [cited 2017 July 12]. https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-hazard-under-tsca

[USFDA] US Food and Drug Administration. 2017. Guidance for industry: Preparation of premarket submissions for food contact substances: Chemistry recommendations. College Park (MD). [cited 2017 July 12]. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm081818.htm#iid2

[USFDA] US Food and Drug Administration Center for Drug Safety and Radiological Health. 2016. Use of International Standard ISO 10993-1, "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process" Guidance for Industry and Food and Drug Administration Staff Document. Silver Spring (MD). [cited 2017 July 12]. https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-meddev-gen/documents/document/ucm348890.pdf

[USFR] US Federal Register. 1984. Premanufacture Notification Exemptions for polymers; Final rule 49 FR 46066, 1984 Nov 21. FRL-2439-1.

[USFR] US Federal Register. 1995. Premanufacture notification exemptions; Revisions of exemptions for polymers; Final rule. *Fed Regist* 60(60): 16316–16336. Environmental Protection Agency. 60 FR 16316. 1995 Mar 29. FRL–4929–8.

[USFR] US Federal Register. 2006. Premanufacture notification exemption for polymers; Amendment of polymer exemption rule to exclude certain perfluorinated polymers. 40 CFR Part 723. 2006 Mar 7. Fed Reg No 06-02152.

[USFR] US Federal Register. 2010. Premanufacture notification exemption for polymers; Amendment of polymer exemption rule to exclude certain perfluorinated polymers. 40 CFR Part 723. 2010 Jan 27. EPA-HQ-OPPT-2002-0051; FRL-8805-5. RIN 2070-AD58.

[USFR] US Federal Register. 2012. Polymers (b). 40 CFR Sect 723.250.

[USFR] US Federal Register. 2016a. Indirect food additives. 21 CFR, Sect 174–178.

[USFR] US Federal Register. 2016b. Perfluorocarbon resins. 21 CFR, Sect 177.1550.

[USP] US Pharmacopeia 40. 2018. National Formulary 35 (USP), General Chapter 88, Biological Reactivity Tests, In Vivo.

[USP] US Pharmacopeial Convention. 2011. US Pharmacopeia National Formulary 2011: USP 34 NF 29. Rockville (MD). General Notices, Section 5.3.0, p 6.

Wagner PM, Nabholz JV, Kent RJ. 1995. The new chemicals process at the Environmental Protection Agency (EPA): Structure-activity relationships for hazard identification and risk assessment. *Toxicol Lett* 79:67–73.

Williams DF. 1987. Definitions in biomaterials. Amsterdam (NL): Elsevier. 72 p.

W.L. Gore. 2017. Fabrics goal for eliminating PFCs of environmental concern. [cited 2017 July 12]. https://www.gore-tex.com/pfcgoal

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Critical Review

A critical review of the application of polymer of low concern regulatory criteria to fluoropolymers II: Fluoroplastics and fluoroelastomers

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Abstract

Fluoropolymers are a distinct class of per- and polyfluoroalkyl substances (PFAS), high molecular weight (MW) polymers with fluorine attached to their carbon-only backbone. Fluoropolymers possess a unique combination of properties and unmatched functional performance critical to the products and manufacturing processes they enable and are irreplaceable in many uses. Fluoropolymers have documented safety profiles; are thermally, biologically, and chemically stable, negligibly soluble in water, nonmobile, nonbioavailable, nonbioaccumulative, and nontoxic. Although fluoropolymers fit the PFAS structural definition, they have very different physical, chemical, environmental, and toxicological properties when compared with other PFAS. This study describes the composition, uses, performance properties, and functionalities of 14 fluoropolymers, including fluoroplastics and fluoroelastomers, and presents data to demonstrate that they satisfy the widely accepted polymer hazard assessment criteria to be considered polymers of low concern (PLC). The PLC criteria include physicochemical properties, such as molecular weight, which determine bioavailability and warn of potential hazard. Fluoropolymers are insoluble (e.g., water, octanol) solids too large to migrate into the cell membrane making them nonbioavailable, and therefore, of low concern from a human and environmental health standpoint. Further, the study results demonstrate that fluoropolymers are a distinct and different group of PFAS and should not be grouped with other PFAS for hazard assessment or regulatory purposes. When combined with an earlier publication by Henry et al., this study demonstrates that commercial fluoropolymers are available from the seven participating companies that meet the criteria to be considered PLC, which represent approximately 96% of the global commercial fluoropolymer market. *Integr Environ Assess Manag* 2022;00:1–29. © 2022 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Applications, Fluoropolymers, Low concern, PFAS, Property Combinations

INTRODUCTION

"Fluoropolymers are high MW polymers with fluorine atoms directly attached to their carbon-only backbone" (Ebnesajjad, 2017). The carbon–fluorine (C–F) bond is the strongest bond between carbon and another atom and imparts unique, outstanding, and beneficial properties and extraordinary functional performance to fluoropolymers (Ameduri, 2020; Ameduri & Sawada, 2017a, 2017b; Banks et al., 1994; Fluoropolymer Products Group of Plastics Europe [FPG], 2021a; Scheirs, 2007). These properties

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include chemical, biological, and thermal stability, heat and chemical resistance, unique dielectric properties, and durability. Additional fluoropolymer properties include fire resistance, weather resistance, nonwetting, and nonstick. Fluoropolymers are regarded as irreplaceable in many applications because their unique combination of specific properties, which are critical to ensure optimal performance in many applications, cannot be achieved or guaranteed by alternative materials (FPG, 2021a, 2017; Henry et al., 2018; Performance Fluoropolymer Partnership of the American Chemistry Council [PFP], 2020).

Per- and polyfluoroalkyl substances (PFAS), a universe of substances with widely diverse properties that have been used in industrial and consumer applications since the 1950s, include fluoropolymers as a distinct class (Buck et al., 2011; Henry et al., 2018). A single, globally harmonized definition for PFAS has not yet been agreed upon. PFAS have been defined differently based on their structure and atomic composition (Buck et al., 2021; Wallington et al., 2021). For example, the USEPA's working PFAS structure definition is "a structure that contains the unit R-CF_2-CF(R′)(R″), where R, R′, and R″ do not equal "H" and the carbon–carbon bond is saturated (note: branching, heteroatoms, and cyclic structures are included" (USEPA, 2021a). The European Chemicals Agency (ECHA) employed a much broader PFAS structural definition (ECHA, 2020). A recent Organisation for Economic Cooperation and Development (OECD) report, which defined PFAS as fluorinated substances that contain in their structure at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), that is, with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–CF_3) or a perfluorinated methylene group (–CF_2–; OECD, 2021). This report acknowledges that the term "PFAS" is broad, general, and nonspecific, which does not inform whether a compound presents risk or not, but only communicates that the compounds under this term share the same structural trait of having a fully fluorinated methyl or methylene carbon moiety. Further, the report highlights that, among the substances defined as PFAS, there are distinct substances with very different properties: polymers and nonpolymers; solids, liquids and gases; persistent and nonpersistent substances; highly reactive and inert substances; mobile and insoluble (immobile) substances; and (eco) toxic and nontoxic chemicals. In addition, the report recognizes that PFAS have diverse molecular structures (e.g., neutral, anionic, cationic, or zwitterionic; with or without aromatic rings; nonpolymers or polymers; low or high molecular weight (MW), and thus diverse physical, chemical, and biological properties (e.g., involatile or volatile; water soluble or water insoluble; reactive vs. inert; bioaccumulative or nonbioaccumulative) and as such highly recommends that such diversity be properly recognized and communicated in a clear, specific, and descriptive manner when communicating about PFAS.

There is considerable media and public confusion and misunderstanding regarding PFAS, as the many different chemicals and groups are often not clearly differentiated under the broad term PFAS. Per- and polyfluoroalkyl substances, a large, diverse group of substances with vastly different properties, is too broad to allow effective, science-based assessment and regulation of chemical compounds as an entire group. This point has been raised in recent publications that suggest approaches to effectively group PFAS for regulatory assessment (American Chamber of Commerce in Europe [Amcham], 2020a; Buck et al., 2021; Bundesverband der Deutschen Industrie e.V. [BDI], 2021; Fiedler et al., 2020; Miller et al., 2020; Orgalim, 2021; Royal Society of Chemistry [RSC], 2021; Sha et al., 2019; Wallington et al., 2021). A clear understanding of the origin of PFAS found in the environment, the PFAS that are commercially relevant (Buck et al., 2021), and assessment of their properties are needed to be able to determine which classes of PFAS require management action. PFAS must be assessed based on their chemical, physical, thermal, and biological property differences and uses (Amcham, 2020a; BDI, 2021; Buck et al., 2021; RSC, 2021; Wallington et al., 2021). As regulatory frameworks, such as the EU REACH regulation, continue to evolve, more work is needed to distinguish clearly among PFAS based on their properties to assure that regulations are appropriate in scope, proportionate, and are science-based.

Per- and polyfluoroalkyl substances are divided into two primary categories: nonpolymers and polymers (Buck et al., 2011). Polymeric PFAS, generally known as "fluorinated polymers," include fluoropolymers (discussed here), perfluoropolyethers (PFPE), and side-chain fluorinated polymers (SCFP; Buck et al., 2011; Henry et al., 2018 and Supporting Information: Figure 6.1). This article deals strictly with fluoropolymers. Neither PFPE nor SCFP are discussed here.

The nonpolymer category includes perfluoroalkyl substances and polyfluoroalkyl substances. Certain nonpolymer PFAS substances, for example, short- and long-chain per- and polyfluoroalkyl carboxylic acids and sulfonic acids, received regulatory scrutiny recently due to their toxicity, as well as their persistence, potential to bioaccumulate, and/or mobility in the environment. Regulatory processes have been launched worldwide to address these concerns related to specific nonpolymer PFAS. These targeted regulatory measures have evolved increasingly into restrictions on the entire family of PFAS. For example, five Member States of the European Economic Area have initiated a procedure to prepare a joint restriction proposal under the EU REACH Regulation to limit the risks to human health and the environment from the manufacture and use of all substances in the PFAS family based on structure alone (ECHA, 2020). Although fluoropolymers fit the PFAS structural definition, they have vastly different physicochemical, environmental, and toxicological properties than other PFAS in addition to substantial societal benefits and importance (Fluoropolymer Products Group of Plastics Europe [FPG], 2017, 2021a). For

these reasons, fluoropolymers should be considered separately and not aggregated with all other PFAS for regulatory action. Concurrently, the USEPA prepared a PFAS Strategic Roadmap laying out how it plans to evaluate and potentially regulate PFAS (USEPA, 2021a). Recognizing that there are many PFAS very diverse in their physical form, chemical structure and composition, functional characteristics, and toxicity profiles, USEPA "is conducting new research to better understand the similar and different characteristics of specific PFAS and whether and how to address groups and categories of PFAS."

Fluoropolymers have documented safety profiles, are thermally, biologically, and chemically stable, negligibly soluble in water, nonmobile, nonbioavailable, nonbioaccumulative, and nontoxic (Henry et al., 2018). Some fluoropolymers have been demonstrated to meet the "polymers of low concern" (PLC) criteria, and as such do not present notable concern for human health or the environment (Henry et al., 2018). PLC criteria were developed over time within regulatory frameworks around the world as an outcome of chemical hazard assessment processes, which identified physical–chemical properties of polymers that determine polymer bioavailability and thereby report a polymer's potential hazard. For example, many of the physicochemical properties, such as MW, limit the ability of a polymer to cross the cell membrane and therefore limit its bioavailability (Kostal, 2016; Lipinski et al., 2001; USEPA, 2012). The USEPA built on this knowledge to adopt a polymer exemption rule to exempt low-hazard polymers from certain regulatory notification requirements under the Toxic Substances Control Act's (TSCA) new chemicals program (United States Federal Register [USFR], 1984). An OECD expert group on polymers reached consensus on these criteria and their respective metrics, documenting the data required for a polymer to qualify as a PLC to human health and the environment (OECD, 1993). Subsequently, an additional OECD work group concurred that PLC have "insignificant environmental health and human health impacts" (OECD, 2009). In addition, the European Commission commissioned a report (BIO by Deloitte, 2015) wherein several member countries agreed on the polymer properties predictive of adverse human health and environmental hazard. The report outlined eligibility criteria for a polymer to be considered a PLC. In 2019, the industry-led European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) developed a "Conceptual Framework for Polymer Risk Assessment" ("CF4Polymers"; ECETOC, 2019). CF4Polymers provides guiding elements to be considered in assessing potential ecological and human health hazards and risks posed by polymer substances. CF4Polymers also considers specific life-cycle stages of polymer products and their associated routes of exposure. The authors of the CF4Polymers framework support the PLC approach as a means to accomplish polymer risk assessments. They specifically support the findings of Henry et al. (2018) and state that they are "…unaware of scientific evidence to justify generally assigning fluoropolymers the same level of regulatory concern as other PFAS" (ECETOC, 2019). In 2020, the European Commission contracted a study to propose criteria for the identification of polymers requiring registration (PRR) under REACH (Wood, 2020a). The Wood report states that the authors consider that fluoropolymers meet the criteria to be considered PLC, "following the recommendations of Henry et al." Considerable debate and comment on proposals have been put forward as the process and discussion advances (American Chamber of Commerce in Europe [Amcham], 2020b; FPG, 2021a; Hafer, 2021).

Four major fluoropolymers have previously been demonstrated to meet the criteria as PLC (Henry et al., 2018). This 2018 study raised interest in gathering similar data for additional commercial fluoropolymer products, both in scope and polymer type. In this study, seven global fluoropolymer manufacturers from the USA, Europe, and Asia collaborated to gather and present data for 14 additional fluoropolymers. In addition to information describing chemical composition, uses, performance properties, and functionalities of the 14 fluoropolymers, author company data for each of the PLC criteria are presented and discussed. The results demonstrate that each of the 14 commercially manufactured fluoropolymers in this study satisfy the widely accepted assessment criteria to be considered PLC and merit such designation. The study results add further evidence to demonstrate that fluoropolymers are demonstrably different and should not be grouped with other PFAS for hazard assessment or regulatory purposes.

USES, PERFORMANCE PROPERTIES, AND FUNCTIONALITY OF FLUOROPLASTICS AND FLUOROELASTOMERS IN THIS STUDY

The fluoropolymers described and evaluated in this study are high-performance materials used in commercial and industrial applications. Described herein are the industries and sectors (Table 1) and the performance properties and functionalities (Table 2) of the study fluoropolymers. The unparalleled combination of properties makes fluoropolymers critical materials for a broad range of applications and industrial sectors including automotive, aerospace, energy production and storage, and electronics (Table 1). Fluoropolymers are an important driver of the European Green Deal (FPG, 2021a) and UN Sustainability Development Goals (United Nations [UN], 2021), supporting smart mobility, clean energy, and sustainable industry. They are used in various components of renewable energy installations, such as hydrogen and photovoltaic panels and facilitate advanced energy storage and conversion technologies such as lithium-ion batteries (FPG, 2021a). Fluoropolymers are (i) durable, stable, and mechanically strong in harsh conditions; (ii) chemically inert, meeting the requirements for low levels of contaminants and particulates in manufacturing environments that are critical to the food and beverage, pharmaceutical, medical, and semiconductor industries; and (iii) biocompatible, nonwetting, nonstick, and highly resistant to

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TABLE 1 Fluoropolymer end uses and industries

	Transportation		Health care		Chemical		Consumer			Telecommunications			Infra-structure	Renewable energy		
Industries End uses	Automotive	Aerospace	Pharma-ceuticals	Medical devices	Oil and gas	Chemical process industry (CPI)	Production of goods	Protection and packaging	Filtration	Electronics and semicon-ductors	Internet and wireless communica-tions	Textiles Technical textiles	Construc-tion and archi-tecture	Energy production	Hydrogen production	Energy storage
Fluoroplastics																
PVDF homopolymer	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
PVDF copolymer	•	•	•	•		•	•	•	•	•	•	•	•		•	•
ECTFE copolymer	•	•	•		•	•				•	•		•			
ECTFE terpolymer			•		•	•										
PCTFE		•	•							•						
FEVE	•	•								•			•			
EFEP	•			•		•				•						
CPT	•									•						
THV	•	•	•			•	•	•	•	•			•	•		•
Fluoroelastomers																
FEPM	•	•	•	•	•	•	•			•			•	•		
FKM	•	•	•	•	•	•	•	•		•		•	•	•	•	•
FFKM		•	•	•	•	•	•			•						
Specialty																
Amorphous		•	•	•		•				•	•				•	•
Ionomer	•			•		•			•	•				•	•	•

Note: See also Chapter 5 in the Supporting Information.
Abbreviations: CPT, chlorotrifluoroethylene-tetrafluoroethylene; ECTFE, ethylene-chlorotrifluoroethylene; EFEP, ethylene-tetrafluoroethylene-hexafluoropropylene; FEPM, trifluoroethylene-propylene copolymer; FEVE, fluoroethylene-vinyl ether; FKM, HFP-VF2 polymer and HFP-VF2-TFE polymers; FFKM, TFE-PMVE perfluoroelastomer; PCTFE, polychlorotrifluoroethylene; PVDF, polyvinylidene fluoride; THV, TFE-HFP-VF2.

TABLE 2 Fluoropolymer properties and functionality

See Supporting Information: Chapter 2 for property descriptions	Durable				Inert—Stable				
	Mechanical strength	Wear resistance	Flexibility	Low coefficient of friction	Resistance to chemicals	Weatherability	Cryogenic properties (lower than −50 °C)	High operating temperature range	High limiting oxygen index
Fluoroplastics									
PVDF Homopolymer	•	•		•	•	•		•	•
PVDF Copolymer	•	•		•	•	•		•	•
ECTFE Copolymer	•	•		•	•	•	•	•	•
ECTFE Terpolymer		•		•	•	•		•	•
PCTFE	•	•		•	•	•	•	•	•
FEVE	•	•	•	•	•	•		•	•
EFEP	•	•		•	•	•	•	•	•
CPT				•	•	•	•	•	•
THV			•		•	•		•	•
Fluoroelastomers									
FEPM	•	•	•		•	•		•	•
FKM	•	•	•		•	•		•	•
FFKM	•	•	•		•			•	•
Specialty									
Amorphous			•	•	•			•	•
Ionomer			•		•				•

See Supporting Information: Chapter 2 for property descriptions	Functional								
	Electrical insulator—high data transmission rate	Ionic conductivity	Piezo-electrical properties	Barrier properties	Nonstick properties	Ultra high purity grades for clean applications	Optical clarity	Low refractive index—used for optical effects	Polymer processing additive (PPA)[a]
Fluoroplastics									
PVDF Homopolymer			•	•		•		•	•
PVDF Copolymer			•	•				•	•

(*Continued*)

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TABLE 2 (*Continued*)

See Supporting Information: Chapter 2 for property descriptions	Functional								
	Electrical insulator—high data transmission rate	Ionic conductivity	Piezo-electrical properties	Barrier properties	Nonstick properties	Ultra high purity grades for clean applications	Optical clarity	Low refractive index—used for optical effects	Polymer processing additive (PPA)[a]
ECTFE Copolymer	•			•					
ECTFE Terpolymer				•					
PCTFE				•	•	•	•	•	
FEVE				•			•		
EFEP				•	•		•	•	•
CPT				•	•	•	•	•	
THV				•			•	•	•
Fluoroelastomers									
FEPM	•			•					
FKM				•	•				•
FFKM	•			•		•			
Specialty									
Amorphous	•	•		•	•	•	•	•	
Ionomer	•	•		•			•		

Note: See Chapter 5 in the Supporting Information.
Abbreviations: CPT, chlorotrifluoroethylene-tetrafluoroethylene; ECTFE, ethylene-chlorotrifluoroethylene; EFEP, ethylene-tetrafluoroethylene-hexafluoropropylene; FEPM, trifluoroethylene-propylene copolymer; FEVE, fluoroethylene-vinyl ether; FKM, HFP-VF2 polymer and HFP-VF2-TFE polymers; FFKM, TFE-PMVE perfluoroelastomer; PCTFE, polychlorotrifluoroethylene; PVDF, polyvinylidene fluoride; THV, TFE-HFP-VF2.
[a]Polymer Processing Additives (PPA): also known as Polymer Processing Aid, Extrusion Process Aids or Polymer Processing and Recycling Aids.

emperature, fire, and weather (Table 2). Fluoropolymers are the preferred choice of material because of their unique combination of properties that are not achievable from other materials or via other functions. As a result, fluoropolymers have become a critical mainstay for our society providing vital, reliable functionality to a broad range of industrial and consumer products.

Three fluoropolymer types are included in this study: fluoroplastics, fluoroelastomers, and specialty fluoroplastics. Here, we describe briefly each included in this study. Additional details about each polymer are provided in the Supporting Information: Chapter 5.

Fluoroplastics

The fluoroplastics included in this study are: polyvinylidene fluoride (PVDF) homopolymer, PVDF copolymer, ethylene-chlorotrifluoroethylene (ECTFE) copolymer, ECTFE terpolymer, polychlorotrifluoroethylene (PCTFE), fluoroethylene-vinyl ether (FEVE), ethylene-tetrafluoroethylene-hexafluoropropylene (EFEP) terpolymer, chlorotrifluoroethylene-tetrafluoroethylene (CPT) terpolymer, and tetrafluoroethylene, hexafluoropropylene, vinylidene fluoride (TFE-HFP-VF2 [THV]) terpolymer as well as the specialty fluoroplastics, amorphous fluoropolymers, and fluorinated ionomers. Typical monomers used in the manufacture of fluoroplastics include tetrafluoroethylene (TFE), hexafluoropropylene (HFP), vinylidene fluoride (VDF or VF2), chlorotrifluoroethylene (CTFE), vinyl fluoride (VF), trifluoroethylene (TrFE), and perfluoroalkyl vinyl ethers (PAVEs), which include trifluoromethyl trifluorovinyl ether (PMVE), pentafluoroethyl trifluorovinyl ether (PEVE), and heptafluoropropyl trifluorovinyl ether (PPVE). In some copolymers, monomers that do not contain fluorine attached to the olefinic carbons may be used. These include ethylene, propylene, perfluoroalkyl-substituted ethylenes, and others (Ebnesajjad, 2000, 2003; Grot, 2011).

Fluoroelastomers

The fluoroelastomers included in this study are: trifluoroethylene-propylene copolymer (FEPM), HFP-VF2 polymer and HFP-VF2-TFE polymers (FKM), and TFE-PMVE perfluoroelastomer (FFKM). Typical monomers used in the manufacture of fluoroelastomers include VDF, HFP, TFE, CTFE, PAVEs, as well as propylene, 1-hydropentafluoropropene (HPFP), and 2,3,3,3-tetrafluoropropene (HFO-1234yf; FPG, 2021a). Although fluoroelastomers are based on many of the monomers that are also used for the synthesis of fluoroplastics, they are different because of the specific composition, flexibility with subambient glass transition temperatures, as well as their elastomeric properties, resulting from the cross-linking process. Cross-linking, known as curing or vulcanizing, is a hardening process to form chemical bonds between polymer chains that gives polymers their elasticity (Améduri et al., 2001; Drobny, 2016).

PVDF homo- and copolymers

Polyvinylidene fluoride fluoropolymers are specified by end users across the world for their outstanding combination of properties. Because they have high temperature resistance, low permeability, and high mechanical strength, and provide chemical resistance to a wide range of aggressive chemicals, PVDF fluoropolymers are used as a contact surface for the production, storage, and transfer of corrosive fluids (chemically resistant to halogens and acids) in the chemical processing industry, oil and gas transportation, and cables industry (Arkema, 2021a; Gujarat Fluorochemicals Limited, 2018, 2022; Solvay, 2021a). The outstanding resistance to sunlight/UV exposure make PVDF suitable for architectural coatings. The outdoor aging and weathering properties of PVDF resin led to its use in long-lasting paints for coating metal sheet for the past 50 years. PVDF resins can also be used to protect thermoplastics through coextrusion or film lamination techniques to obtain antigrime and antigraffiti surfaces with exceptional weathering properties. PVDF fluoropolymers also exhibit radiation resistance, desirable burn characteristics, flame, and smoke properties, easy processing on industry-standard equipment, and easy postprocessing steps, such as welding and fabrication. PVDF is used as a binder in lithium-ion batteries as well as PVDF film for solar power panels because of its high thermal and electrochemical stability, its stability under harsh environmental conditions, and its strong adhesion properties are critical to achieving environmental goals.

ECTFE (co- and terpolymers)

Ethylene chlorotrifluoroethylene (ECTFE) is a semicrystalline and melt-processable fluoropolymer obtained by the copolymerization of the two monomers, ethylene and chlorotrifluoroethylene, with an essentially 1:1 alternating structure (Ebnesajjad, 2017). Due to its chemical structure, ECTFE offers a unique combination of properties including chemical resistance, high thermal rating, and very good mechanical properties (Solvay, 2021b). ECTFE terpolymer with added hexafluoroisobutylene monomer displays enhanced stress-cracking performances resulting from chain-structure modifications of the polymer. ECTFE is used widely in anticorrosion applications such as coatings or in self-supporting construction (pipes) and architectural films (Solvay, 2021c). One of the principal advantages of ECTFE fluoropolymer is the ease with which it can be processed. It is a true thermoplastic that can be handled by conventional techniques of extrusion as well as by blow, compression, injection, rotational, and transfer molding. Powder coating methods are also applicable. ECTFE embodies an exemplary trade-off among general properties, offering high chemical and mechanical resistance combined with easy processing of the resin.

PCTFE

Polychlorotrifluoroethylene is a homopolymer of chlorotrifluoroethylene. PCTFE is melt processable and can be

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extruded or molded (Satokawa, 1990). PCTFE has outstanding mechanical properties, especially hardness, and chemical resistance compared with PTFE and PFA, although it is slightly inferior to PFA and FEP in heat resistance and chemical resistance (Daikin, 2021a; Satokawa, 1990). PCTFE has been applied widely in the semiconductor industries and aerospace industries (Curbell, 2021; Daikin, 2021a). In addition to distinguished thermal and chemical stability, it has very low moisture absorption and permeation; therefore, PCTFE is used in pharmaceutical packaging (Honeywell, 2021).

FEVE

Fluoroethylene-vinyl ether fluoropolymer resins are manufactured by copolymerization of fluoroethylene monomer and a vinyl ether monomer and consist of alternating fluoroethylene and alkyl vinyl ether segments (AGC Chemicals Company, 2021a; Parker & Blankenship, 2015). They were developed in 1982 as the first solvent-soluble fluoropolymers in the world (Darden & Parker, 2021; Kojima & Yamabe, 1984; Munekata, 1988; Yamabe et al., 1984). The alternating fluorinated segments provide outstanding UV stability, weather resistance, and chemical resistance, while the vinyl ether segments provide solvent compatibility and cross-linking sites (Parker & Blankenship, 2015; Scheirs, 2007). FEVE resins are used to make ultra-weatherable coatings for architectural, aerospace, automotive, bridge, and industrial maintenance markets (Hoshino & Morizawa, 2017).

EFEP

Ethylene-tetrafluoroethylene-hexafluoropropylene is a terpolymer of ethylene, tetrafluoroethylene, and hexafluoropropylene. It was designed to have many of the properties of ETFE. It has a lower processing temperature, which allows it to be coextruded with conventional thermoplastic polymers such as polyamide, ethylene vinyl alcohol (EVOH), and modified polyethylene. EFEP can be extruded, injection molded, and blow molded, and it is used in many applications such as those identified in Supporting Information: Chapter 4.7 (Daikin, 2011a). EFEP is a melt-processable resin with good processability because of its low melting point. It also has excellent mechanical properties, provides chemical resistance, low permeability, exceptional weatherability, and good heat resistance. Other prominent features include inherent flame retardancy as well as good optical properties given that EFEP is highly transparent and has both a low dielectric constant and loss tangent.

CPT

Chlorotrifluoroethylene-tetrafluoroethylene is a terpolymer of chlorotrifluoroethylene, tetrafluoroethylene, and perfluoroalkyl-vinyl-ether. It is a melt-processable polymer and resin, which is readily processed because of its lower melting point. It can be melt-molded as a thermoplastic resin by extrusion, injection, and compression molding. CPT is a modified perfluoroalkoxy fluoropolymer (PFA), which utilizes chlorotrifluoroethylene to provide low permeability to PFA, and it has many outstanding properties as a hybrid polymer of PFA and PCTFE as shown below. It has demonstrated permeation resistance to organic solvent, chemicals, water vapor, and gasoline (Daikin, 2011b). CPT offers superior permeation resistance against gasoline and flexible fuel and can be part of construction meeting the LEV III requirements (US environmental protection regulations in this automotive application). CPT also has notable barrier properties against many kinds of organic solvents and strong acids, especially HF, HCl, and HNO_3. This is very useful for semiconductor applications (Daikin, 2021b). In addition to the features noted above, CPT also provides heat resistance, excellent weatherability, flame retardancy, and good optical properties owing to its high transparency.

THV

THV fluoropolymers are a group of fluorinated thermoplastic polymers composed mainly of tetrafluoroethylene (TFE), hexafluoropropylene (HFP), and vinylidene fluoride (VDF; Domininghaus, 1998; Hintzer & Schwertfeger, 2014; Hull et al., 1997). The melting point of the different grades ranges from approximately 100 °C to nearly 250 °C. THV fluoropolymers are easy to process due to their broad processing windows. Different THV grades exhibit high flexibility, high transparency, bondability to fluorinated and nonfluorinated materials, and very good permeation resistance against fuels and other chemicals. The polymers are used as a barrier layer in fuel hoses, for transparent films and tubing, as matrix materials in composites, and the bonding layer in multilayer construction (Dams & Hintzer, 2017; Hull et al., 1997). The high transparency of the special film makes it an ideal adhesive film for laminated glass and the optimal protective film for surfaces. THV grades compete against other fluorothermoplastic materials for applications that require transparency and low refractive index as well as with fuel barrier materials. Commercial nonfluorinated materials cannot be used as substitutes for THV because of the unique combination of properties. Polymethylmethacrylate (PMMA) is used in conjunction with THV to provide differences in refractive index to create the total reflection needed for polymer optical fibers (Park et al., 2008). Transparent polymers, such as PMMA or polycarbonate, do not have the same chemical resistance or UV resistance to compete directly with THV.

FEPM

Trifluoroethylene-propylene copolymer elastomers, ASTM D1418, are high MW fluoropolymers with alternating tetrafluoroethylene and propylene segments (Kojima et al., 1977). They are also known as TFE-P copolymers. Various articles can be produced by means of compression molding, extrusion, injection molding, and calendering. FEPM elastomers are compounded and cured (cross-linked) to deliver unique and valuable properties by providing exceptional heat resistance with a continuous service temperature higher than

200 °C, outstanding chemical resistance with little or no deterioration even in contact with strong acids, bases, and oxidants at high temperatures, steam resistance, and high electrical resistivity on the order of 10^{15}–10^{16} Ω/cm (bulk resistivity). Formulated FEPM components are now used worldwide in many critical industrial applications where they must function safely in harsh environments, thereby extending the life of critical components and reducing downtime and costly repairs. FEPM elastomers are used in a range of applications including thermal power plants, oil and gas industry, ocean development, chemical and nuclear plants, automotive, aerospace, heavy-duty diesel, electronics, machinery, renewable energy, food processing, and medical. Their noted heat and chemical resistance make them especially valuable in oil and gas extraction (downhole) applications, where reliability is essential to cost effective and environmentally responsible production (Hull, 1983). FEPM elastomers are also used in high-performance wire and cable applications as insulating materials with the highest heat resistance, for example, lightweight, high-voltage automotive cables and motor cables for Japanese high-speed bullet trains (AGC Chemicals Company, 2021b).

Fluoroelastomers (FKM)

FKM are a family of fluoroelastomer materials defined by ASTM international standard D1418 (ASTM, 2021). FKM fluoroelastomers contain vinylidene fluoride (VDF) as a monomer combined with a variety of other fluoromonomers to create a palette of polymers with properties tailored for specific uses (Dams & Hintzer, 2017; Drobny, 2016; Van Cleeff, 1997; Worm & Grootaert, 2001). Cross-linked FKM fluoroelastomers are amorphous polymers designed for demanding service applications in hostile environments characterized by broad operating temperature ranges in contact with industrial chemicals, oils, or fuels (Worm & Grootaert, 2001). FKM fluoroelastomers are used mainly in fabricated parts (e.g., o-rings, gaskets, seals) to provide barriers against a wide range of fluids under severe service conditions (Drobny, 2016). Their design allows stable extrusion and molding processes and fitting in a wide range of processing constraints, reducing the risk of failure and increasing productivity. FKM fluoroelastomers provide high temperature and aggressive fluids resistance and retention of properties over a wide and demanding range of operating use conditions (high and low temperatures) for sealing and fluid transport applications, offering far superior performance than hydrocarbon elastomers. Applications include aerospace, automotive, oil and gas, chemical processing, electrical, office equipment, food, pharmaceuticals, and consumer wearables. Additionally, uncured FKM fluoroelastomers are used as a polymer processing additive (PPA) or polymer extrusion aids in small amounts (50–2000 ppm) dispersed in polyolefins such as high-density polyethylene (HDPE) and linear low-density polyethylene (LLDPE), significantly improving their film extrusion characteristics, reducing melt fracture and die build-up, as well as increasing productivity, minimizing energy and water footprint, and enabling the extrusion of thin films (Lavallée, 2020; Shell, 2020).

FFKM

Perfluoroelastomers, designated by ASTM D1418 as FFKM, are a fully fluorinated class of elastomers that are typically made up of tetrafluoroethylene (TFE), a perfluoro (alkyl vinyl ether; PAVE), and a cure site monomer(s) (Ohkura & Morizawa, 2017). FFKM elastomers offer superior chemical and temperature resistance, excellent resistance to gas and liquid permeation, and resistance to weather and ozone with operating temperatures ranging from −40 °C to 325 °C (Drobny, 2016; Greene-Tweed, 2021a, 2021b). These polymers can also be compounded to meet the special requirements of upstream, midstream, and downstream oil and gas exploration due to their superior properties (Barnwell, 2021; Daemar, 2021). Because of these properties, FFKM elastomers are used in a wide variety of applications such as critical sealing solutions for the aerospace, pharmaceutical, medical, chemical processing, semiconductor, and oilfield industries (Atkinson, 2018; Marshall, 2017).

Amorphous fluoropolymers

Amorphous fluoropolymers are copolymers of TFE and specialty monomers that yield linear, high molar mass noncrystalline polymers (AGC Chemicals Company, 2021c; Gangal & Brothers, 2010; Hintzer et al., 2013; Korinek, 1994; Resnick & Buck, 1997, 1999). Amorphous fluoropolymers have the outstanding chemical and thermal stability and surface properties of semicrystalline perfluoropolymers as well as the unique properties associated with amorphous materials such as optical clarity and high gas permeability. The optical properties are outstanding, with more than 90% transmission, and thereby low dissipation, over a wide range of wavelengths (e.g., 200–2000 nm). TFE/PDD (2,2-bistrifluoromethyl-4,5-difluoro-1,3-dioxole) copolymers have the lowest refractive index known for a solid organic polymer (Groh & Zimmermann, 1991). This unique combination of properties makes amorphous fluoropolymers unmatched for uses in degassing, fiber optics, photolithography, antireflective coatings, passivation and protective coatings for medical, military, and aerospace devices, as well as electronic applications (Gangal & Brothers, 2010; Hintzer et al., 2013).

Fluorinated ionomers

Fluorinated ionomers are copolymers of TFE and a perfluorovinylether monomer containing an ionic group, typically a sulfonic acid or carboxylic acid (Grot, 2011, 2013). Fluorinated ionomers can be extruded or cast into film and converted into ion exchange materials (IXMs). IXMs come in a variety of useful forms offering a broad range of solutions for different applications (AGC Chemicals Company, 2021d; Asahi-Kasei, 2021; Chemours, 2021a). These forms include ion exchange membranes (IEMs), dispersions, and resins. IEMs must possess the required ion transport properties for the electrochemical cell in which they reside to perform well

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and work effectively. Some of these properties include high ionic conductivity, chemical resistance, high operating temperature range, low permeability, and balanced durability and performance (Chemours, 2021b).

Ion exchange membranes (IEMs) stand to play a noteworthy role in today's modern world (Chemours, 2021b) and as such, are utilized in a wide range of applications and end-use industries including electrochemical processing, energy production, and hydrogen production. IEMs revolutionized the chlor-alkali industry (Grot, 2013), the manufacture of primarily caustic soda and chlorine, by eliminating the use of hazardous materials such as mercury and asbestos (Asahi-Kasei, 2021) and, in doing so, reducing energy consumption. Water electrolysis, the process of converting water into hydrogen and oxygen, relies on IEM technology. Although this process requires electricity, renewable energy sources such as solar or wind power can be utilized, allowing the potential for hydrogen to be a "clean" energy source (Science Center, 2021). Hydrogen fuel cells, some of which use a type of IEM known as a proton exchange membrane, can then convert hydrogen to electricity, a crucial technology to reach the stated target of the EU New Green Deal (EC, 2021).

STUDY METHODOLOGY AND DATA

Seven global fluoropolymer manufacturers (AGC Chemicals Americas, Arkema, The Chemours Company, Daikin Industries, Gujarat Fluorochemicals Limited, Solvay Specialty Polymers, and 3M Company) participated in this study and contributed data, writing, critique, and analysis. The companies noted above are members of the US-based Performance Fluoropolymer Partnership (PFP) and/or EU-based Fluoropolymer Product Group (FPG).

This study provides data on 14 fluoropolymers, building on a prior study (Henry et al., 2018). The study was chartered within two global industry groups. Participants put forward candidate fluoropolymers of notable commercial importance for the study and provided company and published data that address the PLC criteria. Thirteen PLC criteria that relate to the polymer structure and properties, including three to physicochemical properties and five to stability, set forth in BIO by Deloitte (2015) and presented in prior work on four fluoropolymers (Henry et al., 2018), are addressed in this study (Figure 1). These criteria are briefly described in Table 3 with further description provided in Supporting Information: Chapter 3 and in the prior work (Henry et al., 2018). Participants provided company and published data and a description of methods and/or public references to demonstrate the origin of the data provided. These methods and references are provided in detail in Supporting Information: Chapter 4. The PLC criteria data were compiled and are presented in Tables 4 and 5.

The data assessment was done in two ways: Companies could self-assess the PLC data if they had the technical resources to do so or they could submit their PLC data to a third-party contractor for an independent technical review. The third-party consultant hired by PFP was GSI Environmental Inc. The objective was to be able to publish the references and methods behind the PLC data provided for each fluoropolymer in the study. In cases where the data and/or methods contained confidential business information, the third-party consultant independently evaluated the information supplied before it was shared in a blinded, aggregate form with the participating project companies. In several cases—FKM, PVDF, and ionomers—several companies submitted data for the same fluoropolymer. The data were combined and are presented in Tables 4 and 5. There is no intentional company attribution for the data presented.

The following describes further how the study data were generated and compiled.

- A third-party consulting company (GSI) was engaged to comment independently on data, methods, and references initially supplied by study participants for their respective fluoropolymers. Several study participants used this third-party consultant.
- Following the initial third-party assessment and assembly of the master data Tables 4 and 5 as well as the FKM data in Supporting Information: Table 4.11, a series of subsequent assessments were conducted (within PFP)

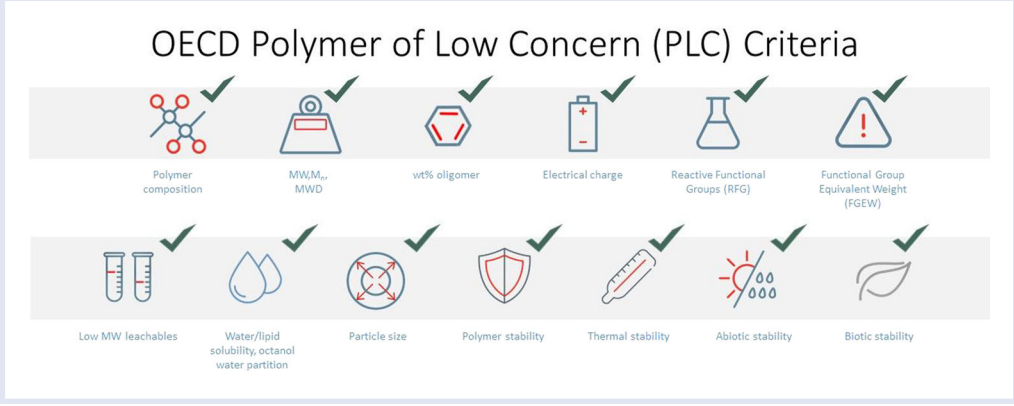

FIGURE 1 OECD polymer of low concern (PLC) criteria add (C) 2021 W.L.Gore & Associates

TABLE 3 Polymer of low concern (PLC) criteria descriptions

(See Supporting Information: Chapter 3 for additional details) Criterion	Description
Polymer composition	The polymer composition criterion requires structure and elemental composition of the polymer be described and identified (e.g., by Chemical Abstracts Service [CAS] number).
Molecular weight, number average molecular weight, MW distribution, and % oligomer <1000 Da	The number average molecular weight (Mn) and oligomer content are the most commonly used criteria for PLC assessment. The EU assessment report (BIO by Deloitte, 2015) states that the "most potential health concern polymers have a number average molecular weight, Mn, <1000 Da and oligomer content >1%." The higher the oligomeric content, the more likely a polymer is to be a health or ecotoxicological (OECD, 2009, p. 9).
	Molecular weight (MW) is an important predictor of biological effect because large molecules (>1000–10 000 Da) are too large to penetrate cell membranes (Supporting Information: in Beyer, 1993, p. 14). Because large molecular weight polymers cannot enter the cell, they cannot react with "target organs," such as the reproductive system, and are not bioavailable. "Therefore, as the Mn of a polymer increases, a reduced incidence of potential health concern effects might be expected" (OECD, 2009, p. 20).
	An additional PLC consideration is the weight percentage of oligomers that are <1000 Da. Oligomers may be composed of, for example, dimers, trimers, and tetramers, meaning they have 2- monomer, 3- monomer, and 4-monomer units, respectively. The EU report (BIO by Deloitte, 2015) concluded that most potential health concern polymers have Mn of <1000 Da and oligomer content of >1%: "…the distribution of potential health concern polymers exhibited an increased incidence of higher oligomer content that began at 5% for <1000 Da and 2% for <500 Da oligomeric content" (OECD, 2009, p. 24).
	Molecular weight distribution (MWD), also known as "polydispersity index," measures the heterogeneity of size of polymer molecules in a polymer. The MWD is an important parameter for predicting potential biological effects of polymers because, although Mn may be a large value, low MW oligomers <1000 Da may be present, which could penetrate the cell.
Ionic character	Electrical charge or ionic character can be anionic, cationic, amphoteric, or nonionic. Specifically, cationic polymers have been associated with aquatic toxicity (Auer et al., 1990; USEPA, 1997a).
Reactive functional groups and RFG ratio to MW	A "reactive functional group" (RFG) is defined as an atom or associated group of atoms in a chemical substance that is intended or can be reasonably expected to undergo facile chemical reaction (USFR, 2012). Some highly reactive functional groups (or a high ratio of RFGs per mole) have been associated with adverse human health and ecotoxicology (e.g., acrylates, methacrylates, isocyanates, anhydrides, aziridines; USEPA, 2010).
	The functional group equivalent weight (FGEW) is used to determine if the RFGs in a polymer are substantially diluted by polymeric material to allow the polymer to be a PLC (USEPA, 1997). The FGEW of a polymer is defined as the ratio of the Mn to the number of functional groups in the polymer. The FGEW is used as an indication of the degree of reactivity of the polymer; the lower the FGEW, the more reactive the polymer and the greater the potential for health and environmental impact (OECD, 2009, p. 10).
Low MW leachables	Low MW leachables are chemical molecules, either inorganic or organic, that migrate (i.e., leach) out of the polymer. These could be residual monomers or oligomers resulting from incomplete polymerization processes, surface residues, or other chemicals used in the manufacturing processes (e.g., initiators, catalysts, chain transfer agents, surfactants).
	Low MW leachables are critically important to the potential for a polymer to affect health and the environment, given that they may be able to migrate out of the polymer and cross cell membranes to potentially react with biomolecules. A report to the EU (BIO by Deloitte, 2015) concluded that "Polymers with <1% MW < 1000 Da and low water extractability are not able to cause systemic effects which are toxicologically or ecotoxicologically relevant."
	Monomers, by nature, are reactive. Unreacted monomers left in a polymer may migrate out of the polymer to react with biomolecules to cause potential adverse effects. Regulatory authorities (BIO by Deloitte, 2015) and the OECD Expert Group on Polymers (OECD, 2009) agree that the residual monomer content of a polymer is critical to determining if it qualifies as a PLC.

(*Continued*)

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TABLE 3 (*Continued*)

(See Supporting Information: Chapter 3 for additional details) Criterion	Description
Particle size	Particle size is also a PLC criterion. Particles that are small enough to reach the deep lung upon inhalation are often associated with adverse health effects. Therefore, to qualify as a PLC, median mass aerodynamic diameter (MMAD) of the polymer particle size should be >5 μm.
Structural and elemental composition	In the US, Chemical Categories of Concern are the result of the review of new chemicals by the USEPA under the TSCA (see https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/chemical-categories-used-review-new). The categories describe the molecular structure, boundary conditions such as MW, equivalent weight, the log of the octanol–water partition coefficient, log *P*, or water solubility, and standard hazard (mammalian and ecological) and (environmental) fate tests to address concerns.
Elemental composition	The elemental composition is a factor in the assessment of the eligibility of polymers for reduced notification requirements. The exclusion of polymers under this step is not a conclusion of hazard but a determination that the elemental composition does not fall within the parameters of the polymer set under which this rule was formulated, and consequently, these polymers would have to follow the standard notification and review process. These elemental requirements differ across jurisdictions as covered in the report to the EU on global regulatory approaches to polymer assessment (BIO by Deloitte, 2015). For example, in the EU under REACH it is proposed that polymers composed from among these elements, covalently bound to C, have reduced hazard: H, N, O, Si, S, F, Cl, Br, or I (BIO by Deloitte, 2015). In contrast, the USEPA Polymer Exemption Rule states that a polymer is eligible for reduced agency review when it has at least two of the following elements: C, H, O, N, S, or Si (USFR, 1995).
Water and lipid solubility and the octanol–water partition coefficient	Water solubility is the extent to which a compound will dissolve in water. According to the OECD (2009) meeting of the Expert Group on Polymers, polymers with "negligible" water solubility, or those described as "hydrophobic" have been represented with a water solubility of 0.000001 mg/L (1×10^{-6} mg/L; assigned arbitrarily; OECD, 2009). That is equivalent to 1 ppt, a very conservative definition.
	Polymers with water solubility <10 mg/L showed generally low health concerns.
	The octanol–water partition coefficient (K_{ow}) is another criterion to assess chemicals and their environmental and health impact. The K_{ow} is a physical–chemical property at equilibrium to represent the lipophilic or hydrophilic nature of a chemical, the distribution of a compound in octanol, representing the lipophilic nature, to its solubility in water, representing the aqueous nature. The higher the K_{ow}, the more lipophilic the compound. Typically, a K_{ow} >5000 or a log K_{ow} >5 means high lipophilicity and, thus, a high potential to bioaccumulate or bioconcentrate. According to the Stockholm Convention, a bioconcentration factor of >5000 and a log K_{ow} >5 is used as a criterion for bioaccumulation.
Stability	Stability is resistance to physical, chemical, or biological transformation. Loss of stability in the polymer breaks it down into smaller pieces, producing low MW species. As was previously described in the Polymer of Low Concern section under the molecular weight, number average molecular weight, MW distribution, and % oligomer <1000 Da heading, molecules with Mn <1000 Da are capable of crossing cell membranes, making unstable polymers potentially hazardous to health and the environment.
Abiotic stability	Polymers are stable; monomers are not. Abiotic degradation may involve sunlight, water, or oxygen. Photochemical transformation is a reaction involving the radiation energy of sunlight (ultraviolet radiation) that may break a bond in a molecule to change it to another chemical entity. Hydrolytic degradation of polymers is another potential way to break the polymer bonds, creating smaller oligomers that may be bioavailable. Chemical oxidation is a reaction involving the loss of electrons from one atom to another.
Biotic stability: aerobic, anaerobic, and in vivo	Biotic stability is assessed by whether the polymer is degraded by microorganisms under oxygenated (aerobic) or anoxic (anaerobic) conditions; in vitro and in vivo stability studies demonstrate this. In vivo biodegradation involves the breaking of the polymer bonds by the action of bacteria, enzymes, and oxidants within the organism.
Thermal stability	Thermal stability of a polymer can be assessed when used as intended under normal, foreseeable use conditions or in extreme temperatures during disposal, such as by incineration. Thermal stability testing may involve Thermogravimetric Analysis (TGA), which determines mass loss over time and temperature of a test substance.

TABLE 4 Fluoroplastics and PLC criteria

	Fluoroplastics				
Supporting Information Data: Chapter	**4.1 PVDF**	**4.2 PVDF-HFP copolymer**	**4.3 ECTFE**	**4.4 ECTFE**	**4.5 PCTFE**
	Polyvinylidene fluoride	Vinylidene fluoride, hexafluoropropene copolymer	Ethylene, chlorotrifluoroethylene copolymer	Ethylene, chlorotrifluorethylene, hexafluoroisobutylene terpolymer	Polychlorotrifluoroethylene
PLC assessment criterion[a]	CAS 24937-79-9	CAS 9011-17-0	CAS 25101-45-5	CAS 54302-04-04	CAS 9002-83-9
Structure	$–(CF_2–CH_2)_n–$	$–(CF_2–CH_2)_n–[CF(CF_3)–CF_2]_m–$	$–(CF_2–CFCl–CH_2–CH_2)_n–$	$–(C_4H_2F_6)_n–(C_2H_4)_m–(C_2ClF_3)_i–$	$–(CF_2–CFCl)_n–$
Polymer composition (must have C, H, Si, S, F, Cl, Br, or I covalently bound to carbon)	Yes	Yes	Yes	Yes	Yes
Molecular weight (Mn)[b] (Mn >1000 Da and oligomer content <1%)	70 000–300 000	80 000–300 000	Mn >50 000	Mn >50 000	70 000–400 000 average based on grade type
Molecular weight distribution Mw[c] ÷ number average M_n	2–3	2–3	1.1	1.7	3
Wt% oligomer (<5% for <1000 Da oligomers, <2% for <500 Da oligomers)	Negligible	Negligible	Negligible	Negligible	Negligible
Ionic character	Neutral	Neutral	Neutral	Neutral	Neutral
Reactive functional groups (RFGs)[d] **and functional group equivalent weight (FGEW)**	None and N/A	None and N/A	None and N/A	None and N/A	None and N/A
Low molecular weight leachables	No active leachables by USP class VI (121 °C)	No active leachables by USP class VI (121 °C)	No active leachables by USP class VI (121 °C)	No active leachables by USP class VI (121 °C)	Negligible
Residual monomers	<50 ppb	<50 ppb	<50 ppb	<50 ppb	<0.1 wt%
Ratio of residual monomers to molecular weight (typical value)	$\sim10^{-12}$–$\sim10^{-13}$	$\sim10^{-12}$–$\sim10^{-14}$	$\sim10^{-13}$	$\sim10^{-13}$	$<10^{-5}$
Structural similarities to RFG of concern	None	None	None	None	None
Reference standard	ASTM D3222-18a	ASTM D5528-18	ASTM D3275-81	ASTM D3275-81	

(*Continued*)

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TABLE 4 (*Continued*)

Supporting Information Data: Chapter	Fluoroplastics 4.1 PVDF	4.2 PVDF-HFP copolymer	4.3 ECTFE	4.4 ECTFE	4.5 PCTFE
Physical–chemical properties					
Water solubility and octanol/water partition coefficient, K_{ow}	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A
Particle size (median mass aerodynamic diameter, MMAD, should be >5 μm)	Powders: 5–300 μm pellets: 2–4 mm	Powders: 5–300 μm pellets: 2–4 mm	D50%: 50–70 μm (typical)	D50%: 50–70 μm (typical)	Pellet: 2-4 μm, flake: 0.54 mm powder: 5–300 micron
Stability					
Hydrolysis, light (hν), Oxidation, biodegradation (aerobic and anaerobic)	Stable	Stable	Stable	Stable	Stable
Thermal stability at normal foreseeable use maximum continuous temp. (°C)	150 °C	150 °C	150 °C	150 °C	120 °C
Meets [a]PLC criteria (Yes or No)	Yes	Yes	Yes	Yes	Yes
Fluorinated polymerization aid (PA) used? (Yes or No)	No	No	No	No	No
Recommended processing/application (use) temperature (T°C)	Processing: 200 °C–250 °C Use max temp: 150 °C	Processing: 180 °C–250 °C Use max temp: 100 °C–140 °C depending on HFP content	Processing: 250 °C–280 °C Use max. Temp: 150 °C	Processing: 250 °C–280 °C Use max. Temp: 150 °C	Molding: 230 °C –330 °C and Use Max same as above at 120 °C

Supporting Information Data: Chapter	Fluoroplastics 4.6 FEVE	4.7 EFEP	4.8 CPT	4.9 THV
	Fluoroethylene-vinyl ether copolymer	1-Propene, 1,1,2,3,3,3-hexafluoro-, polymer with ethylene and 1,1,2,2-tetrafluoroethylene	1,1,1,2,2,3,3-Heptafluoro-3-[(trifluoroethenyl)oxy]propane polymer with chlorotrifluoroethylene and tetrafluoroethylene	1-Propene,1,1,2,3,3,3-hexafluoro-polymer with 1,1-difluoroethylene and tetrafluoroethylene
PLC assessment criterion[a]	cbi	35560-16-8	116018-07-6	25190-89-0

(*Continued*)

TABLE 4 (*Continued*)

	Fluoroplastics			
Supporting Information Data: Chapter	**4.6 FEVE**	**4.7 EFEP**	**4.8 CPT**	**4.9 THV**
Structure	cbi	$-(CH_2-CH_2)_n-(CF_2-CF_2)_m-[CF_2-CF(CF_3)]_l-$	$-(CF_2-CF_2)_n-(CFCl-CF_2)_m-[CF_2-CF(OR_f)]_l-$	$-(CF_2CH_2)_x-(CF_2-CF-CF_3)_y-(CF_2-CF_2)_z$
Polymer composition (must have C, H, Si, S, F, Cl, Br, or I covalently bound to carbon)	Yes	Yes	Yes	Yes
Molecular weight (Mn)[b] (Mn >1000 Da and oligomer content <1%)	7000–46 000	130 000	200 000–300 000	131 000
Molecular weight distribution Mw[c] ÷ number average M_n	2.0–4.0	4	2–5	1.8
Wt% oligomer (<5% for <1000 Da oligomers, <2% for <500 Da oligomers)	Mn <1000 range of <3.5% and Mn <500 is <0.7%	Negligible; <0.1 wt% oligomer content	Negligible; <0.1 wt% oligomer content	wt.% <1000: None
Ionic character	Neutral	Neutral	Neutral	Neutral
Reactive functional groups (RFGs)[d] **and functional group equivalent weight (FGEW)**	None and N/A	None and N/A	None and N/A	None and N/A
Low molecular weight leachables	Negligible; cross-linked as final product	Negligible	Negligible	No active leachables by USP class VI (121 °C)
Residual monomers	0.12%–1.43% non-fluorinated	Negligible	Negligible	None detected
Ratio of residual monomers to molecular weight (typical value)	10^{-7}–10^{-8}	$<10^{-5}$	$<10^{-5}$	$\sim10^{-13}$
Structural similarities to RFG of concern	None	None	None	None
Reference standard		ASTM D7472	ASTM D7471	

(*Continued*)

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TABLE 4 (*Continued*)

	Fluoroplastics			
Supporting Information Data: Chapter	**4.6 FEVE**	**4.7 EFEP**	**4.8 CPT**	**4.9 THV**
Physical–chemical properties				
Water solubility and octanol/water partition coefficient, K_{ow}	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A
Particle size (median mass aerodynamic diameter, MMAD, should be >5 μm)	Solution or flake 150 nm for emulsion	2–4 mm (pellets)	2–4 mm (pellets)	Pellets ~400–750 μm
Stability				
Hydrolysis, light (hν), oxidation, biodegradation (aerobic and anaerobic)	Stable	Stable	Stable	Stable
Thermal stability at normal foreseeable use maximum continuous temp. (°C)	220 °C	130 °C; low melting point 160 °C–190 °C and high decomposiiton temperature of 357 °C–380 °C	200 °C; low melting point 239 °C–251 °C and high decomposition temperature of >400 °C	Continuous use is expected ~room T. (<100 °C as host resin melts at 120 °C); No expected degradation; fluoropolymer degrades >350 °C by TGA
Meets [a]PLC criteria (Yes or No)	**Yes**	**Yes**	**Yes**	**Yes**
Fluorinated polymerization aid (PA) used? (Yes or No)	No	No	No	Yes and No
Recommended processing/application (use) temperature (T°C)	180 °C–200 °C	Molding temperature: 200 °C–280 °C; Use max as noted above	Molding temperature: 310 °C–330 °C; Use max as noted above	Melt processing: <350 °C Application: <100 °C (in LLDPE)

Abbreviations: ECTFE, ethylene-chlorotrifluoroethylene; HFP, hexafluoropropylene; PCTFE, polychlorotrifluoroethylene; PLC, polymer of low concern; PVDF, polyvinylidene fluoride.
[a]See OECD (2009) and BIO by Deloitte (2015) for details on characteristics of a "Polymer of Low Concern" and Supporting Information: Chapter 3.
[b]Molecular Weight is number average molecular weight which is defined as the total weight of the polymer divided by the total number of molecules. It is the mole fraction of molecules in a polymer sample.
[c]Molecular weight is weight average molecular weight which is determined by summing the weights of all the chians and then dividing by the total number of chains. It is the weight fraction of molecules in a polymer sample.
[d]For definition of reactive functional group, lists of low-, moderate-, and high-concern functional groups and FGEW limits, see USEPA polymer exemption guidance manual, BIO by Deloitte (2015, pp. 191–192), and USEPA (2010). See Supporting Information.

TABLE 5 Fluoroelastomers and specialty fluoroplastics—PLC criteria

	Specialty fluoroplastics		Fluoroelastomers		
Supporting Information Data: Chapter	**4.13 Amorphous**	**4.14 Ionomer**	**4.10 FEPM**	**4.11 FKM**	**4.12 FFKM**
	Perfluoro(alkenyl vinyl) ether polymer	Sodium or potassium salts of perfluorosulfonic acid/TFE copolymer or perfluorocarboxylic acid/TFE copolymer	Tetrafluoroethylene-propylene copolymer	1-Propene,1,1,2,3,3,3-hexafluoro-polymer with 1,1-difluoroethylene copolymer and terpolymers	Tetrafluoroethylene-trifluoromethyl trifluorovinyl ether copolymer
PLC assessment criterion[a]	37626-13-4	9002-84-0, 1314-23-4, 409-21-2, 111173-25-2	27029-05-6	9011-17-0, 26425-79-6, 25190-89-0	26425-79-6
Structure	See Supporting Information	See Supporting Information	See Supporting Information	Supporting Information	-(CF2CF2)x -[CF2-CF(OCF3)]y-(Cure Site Monomer)z
Polymer composition (must have C, H, Si, S, F, Cl, Br, or I covalently bound to carbon)	Yes	Yes	Yes	Yes	Yes
Molecular weight[b] (Mn >1000 Da and oligomer content <1%)	150 000–300 000	>100 000	Various grades vary between 146 000–275 000	30 000–340 000	10 000–1 000 000
Molecular weight distribution Mw[c] ÷ number average M_n	1.4–2.5	1.0–2.4	Various grades give various ratios from 1.4 to 3.3	1.2–2.4	1.2–3.5
Wt% oligomer (Figure MWD) (<5% for <1000 Da oligomers, <2% for <500 Da oligomers)	Negligible	Negligible	<0.01%	Negligible to <1%	Negligible
Ionic character	Neutral	Neutral	Neutral	Neutral	Neutral
Reactive functional groups (RFGs)[d]	None and N/A	None and N/A	None and N/A	None and N/A	None and N/A
Functional group equivalent weight (FGEW; typical value)	$>10^5$	$>10^5$	$>10^5$	$>10^4$–10^5	$>10^4$

(*Continued*)

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TABLE 5 (*Continued*)

Supporting Information Data: Chapter	Specialty fluoroplastics 4.13 Amorphous	Specialty fluoroplastics 4.14 Ionomer	Fluoroelastomers 4.10 FEPM	Fluoroelastomers 4.11 FKM	Fluoroelastomers 4.12 FFKM
Low molecular weight leachables	<1 ppm	<1 ppm	No active leachables	<0.4 ppm to <1 ppm	No active leachables
Residual monomers	<1 ppm	<1 ppm	No residual monomers Only cross-linking agent at <1 ppm	<50 ppt to <5 ppm	<50 ppb
Ratio of residual monomers to molecular weight (typical value)	$>10^{-5}$	$>10^{-5}$	10^{-11}–10^{-12}	$>10^{-10}$–10^{-13}	0.25 ppt as Mn = 10^5 (for representative FKM)
Structural similarities to RFG of concern	None	None	None	None	None
Reference standard				ASTM D 1418	
Physical–chemical properties					
Water solubility and octanol/water partition coefficient, K_{ow}	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A	Insoluble/practically insoluble and N/A
Particle size (median mass aerodynamic diameter, aerodynamic diameter, MMAD, should be >5μm)	Solution, sheet or pellets	(1) Aqueous dispersion casting (as a film) followed by annealing or (2) Melt extrusion as a membrane (reinforced)	Sheet or crumb	Sheet or block; powders 300–350 μm stability increased/enhanced when cross-linked	Sheet or block; or "crumb"
Stability					
Hydrolysis, light (hν), oxidation, biodegradation (aerobic and anaerobic)	Stable	Stable	Stable	Stable	Stable

(*Continued*)

TABLE 5 (*Continued*)

Supporting Information Data: Chapter	Specialty fluoroplastics 4.13 Amorphous	Specialty fluoroplastics 4.14 Ionomer	Fluoroelastomers 4.10 FEPM	Fluoroelastomers 4.11 FKM	Fluoroelastomers 4.12 FFKM
Thermal stability at normal foreseeable use maximum continuous Temp (°C)	>250 °C	Sulfonic acid polymer: maximum operating temperature of 175 °C under anhydrous conditions, 220 °C –240 °C in aqueous systems carboxylic acid polymer: use below 120 °C	200 °C	180 °C	200 °C–300 °C
Meets[a] PLC criteria (Yes or No)	Yes	Yes	Yes	Yes	Yes
Fluorinated polymerization aid (PA) used? (Yes or No)	Yes and No	Yes and No	No	Yes and No	Yes and No
Recommended processing/ application (use) temperature (T°C)	<280 °C	Sulfonic acid polymer: maximum operating temperature of 175 °C under anhydrous conditions, 220 °C–240 °C in aqueous systems carboxylic acid polymer: use below 120 °C	–60 °C–204 °C (AFLAS Technical Document)	Melt processing: <300 °C 160 °C–320 °C (cross-linking temperature)	160 °C–320 °C (cross-linking temperature)

Abbreviations: FEPM, trifluoroethylene-propylene copolymer; FKM, HFP-VF2 polymer and HFP-VF2-TFE polymers; FFKM, TFE-PMVE perfluoroelastomer; PLC, polymer of low concern.
[a]See OECD (2009) and BIO by Deloitte (2015) for details on characteristics of a "Polymer of Low Concern."
[b]Molecular weight is *number* average molecular weight.
[c]Molecular weight is weight average molecular weight.
[d]For definition of reactive functional group, lists of low-, moderate-, and high-concern functional groups and FGEW limits, see USEPA polymer exemption guidance manual, BIO by Deloitte (2015, pp. 191–192), and USEPA (2010). See Supporting Information.

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until all data cells in the tables cited above were backed up with a narrative, a testing method, and/or references where publicly available.
- Where several companies have provided data on the same fluoropolymers, the table data presented provide a multicompany compilation and assessment along with appropriate methods and references.
- Individual companies supplying data are identified as authors, but there is no direct attribution regarding which company supplied which data for this study.

PLC ASSESSMENT RESULTS

This study was conducted on commercial fluoropolymer products using the PLC criteria to characterize their potential hazard. Figure 1 illustrates the PLC criteria used (BIO by Deloitte, 2015; Henry et al., 2018). The pictured criteria encompass structure, physicochemical property, and stability criteria evaluated in the study. Data informing structure criteria, MW, Mn, and MW distribution (MWD), physicochemical property criteria, water and lipid solubility and K_{ow}, and stability criteria are presented in Tables 4 and 5. The study also gathered structural data on (a) residual monomers, (b) ratio of residual monomers to MW, (c) structural similarities to reactive functional groups (RFGs) of concern, and (d) thermal stability at normal foreseeable maximum continuous use temperatures. Brief descriptions of PLC criteria are provided in Table 3 with additional details, including references for each criterion in Supporting Information: Chapter 3. An additional data point gathered was whether the fluoropolymer(s) presented utilized a fluorinated polymerization aid (PA) during manufacture. The study results are presented in Tables 4 and 5 and summarized below.

Polymer composition: Each of the fluoroplastics, specialty fluoroplastics, and fluoroelastomers assessed in this study met the criterion of polymer composition whereby either fluorine (F) and/or chlorine (Cl) must be covalently bound to the carbon-only polymer backbone.

MW and MWD: All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the criteria for MW (Mn >1000 Da) and MWD (1–3). The data demonstrate the fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study are high-MW solid polymers with fairly narrow MWD and negligible to low wt% oligomer content. The MW for fluoroplastics in Table 4 and specialty fluoroplastics in Table 5 ranged from 50 000 to 300 000, and the MWD ranged from approximately 1.4 to 3. We note that FEVE was measured in its uncured state and that, upon curing, its MW increased significantly. The MW and MWD were determined in a variety of ways depending on the fluoropolymer and its solubility (or insolubility) in various solvents. The MW and MWD data for fluoroelastomers and specialty fluoroplastics in the study are presented in Table 5. The MW and MWD varied because of the various grades of fluoroelastomers ranging from 100 000 to 250 000 with some less than (down to 10 000) and greater than (up to 500 000). MWD was on the order of 1.4 to 3.5. Fluoroelastomer MW is lower for uncured fluoroelastomer versus cured fluoroelastomer. Cured fluoroelastomer is the form used in many formed-use applications (e.g., gaskets and o-rings). The methods and references for MW and MWD data are presented in the Supporting Information: Chapter 4 with the specific chapter noted in Tables 4 and 5. Methods included size exclusion chromatography (SEC), gel permeation chromatography (GPC) along with osmotic pressure, and parallel plate rheometry methods.

Weight % oligomer: The criteria for wt% oligomer are less than 5% oligomer content for Mn less than 1000 Da, and less than 2% oligomer content for Mn less than 500 Da (BIO by Deloitte, 2015; Henry et al., 2018; see also the Supporting Information). All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the wt% oligomer criteria. Many polymers in the study were reported as "negligible" for oligomers based on analyses conducted. Polymers in the study not cited as negligible have reported numerical data presented in Tables 4 and 5. In addition to SEC and GPC, analytical methods employed included a weight loss upon heating method and the FDA 21 CFR 177.1380 method. The methods and references for wt% oligomer are presented in the Supporting Information: Chapter 4 with the specific chapter noted in Tables 4 and 5.

Ionic character: The fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study are neutral polymers, either containing no ionic groups or may contain anionic at the terminus of their high MW polymer chains as noted in the prior study of fluoropolymers (Henry et al., 2018). Notably different are fluorinated ionomers, which have neutralized (salts) sulfonic acid or carboxylic acid groups pendant to the polymer backbone and as such are neutral and not ionically charged in their polymeric solid form and are low in toxicity and not dermally irritating on skin contact (USEPA, 1997). None of the evaluated polymers in the study have cationic nature. The methods and references for ionic character are presented in the Supporting Information: Chapter 4 with the specific subchapter noted in Tables 4 and 5.

RFG, functional group equivalent weight (FGEW) and structural similarities to RFG of concern: All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the RFG and FGEW criteria. The polymers in this study do not contain the reactive functional groups set forth in the PLC criteria (e.g., acrylates, alkoxysilanes, amines, aziridines, carbodiimides, and so forth; see Supporting Information: Chapter 3). Given that the polymers in this study have no RFGs, the FGEW values in Tables 4 and 5 are very large numbers (such as $>10^4$–10^5) or the value given is not applicable due to the lack of RFGs altogether. Even the polymers with some functional groups present (e.g., fluorinated ionomers) are not reactive. For example, the FEVE polymerization process leads by design to a polymer with neutral and/or anionic end groups. FEVE resins do contain a small amount of hydroxyl and carboxyl functional groups. These functional groups are classified as low concern RFG

by the USEPA (1997) and OECD (2009). There are no RFG structural similarities across the polymers in this study.

Low MW leachables (MW < 1000 Da): All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the low MW leachable PLC criteria, which has been widely discussed (see Supporting Information: Chapter 3 for references). Many of the study polymers report no active leachables, whereas the rest cite values less than 1 ppm (Tables 4 and 5). For FEVE, it is reported that some non-fluorinated polymer PA may well remain in the uncured polymer resin. The methods and references for low MW leachables are presented in the Supporting Information: Chapter 4 with the specific chapter noted in Tables 4 and 5. The data presented in Tables 4 and 5 were determined for each of the respective polymers in this study using techniques such SEC and GPC as the predominant analytical methods along with the use of USP Class VI testing. Additional methods included 21 CFR 177.2600 (USCFR, 2022) and the USEPA's toxicity characteristic leaching procedure (TCLP; SW-846 Test Method 1311; USEPA, 1992).

Residual monomers and ratio of residual monomers to typical MW: PLC criteria of equal interest to the low MW leachables are the residual monomers and the ratio of residual monomers to typical MW (see Supporting Information: Chapter 3 for references). All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the residual monomers and ratio of residual monomers to typical MW PLC criteria. The study data presented in Tables 4 and 5 show the polymers in this study have residual monomers ranging from less than 50 ppb for several fluoropolymers and up to less than 0.1% for PCTFE based on the methods utilized. Fluoroelastomers in this study have residual monomers ranging from less than 50 ppb up to less than 5 ppm. Residual monomers were determined in several ways including dynamic and static headspace gas chromatography/mass spectrometry (GC/MS) at 150 °C. The monomers used in most cases have very low boiling points and are thus readily volatilized (and captured or destroyed) during polymer manufacture processing and drying steps. The methods and references for residual monomer determination are presented in the Supporting Information: Chapter 4 with the specific chapter noted in Tables 4 and 5. Given the very low residual monomer levels reported, the ratio of residual monomers to polymer MW range from 10^{-11} to 10^{-13} for the study polymers.

Water solubility and octanol/water partition coefficient (K_{ow}): The fluoroplastics, specialty fluoroplastics, and fluoroelastomers in this study are solids that are hydro- and oleophobic, practically insoluble in both water and n-octanol. Therefore, a K_{ow} cannot be computed and is not applicable to these substances. It is worth noting that the practical lack of solubility in water (<10 mg/L) and n-octanol indicate the inability for the study fluoropolymers to actively or passively cross cell membranes. This does mean there is no indication that these polymers can bioaccumulate or bioconcentrate in biota (Henry et al., 2018 and this study). The methods and references for solubility are presented in the Supporting Information: Chapter 4 with the specific chapter noted in Tables 4 and 5.

Particle size: To meet the PLC assessment criteria for particle size, a powder must be 5 µm or greater in size (median mass aerodynamic diameter [MMAD]). All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the particle size PLC criterion. As shown in Tables 4 and 5, the fluoroelastomers in this study are provided in sheets, blocks, pellets, or "crumb," and the fluoroplastics and specialty fluoropolymers in this study are provided in the form of powders, pellets, sheets, flake, or in dispersions. References and additional information regarding the form of the study polymers is provided in the Supporting Information: Chapter 4.

Stability: All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the PLC criteria for hydrolysis, light stability, oxidative stability, and aerobic and anaerobic biodegradability (e.g., breakdown into species with Mn <1000 Da). Public literature has abundant thermal, chemical, and biological stability data for the polymers in this study as stability is a hallmark property for these polymers (Ebnesajjad, 2017). For biodegradation, the assessments were largely made based on property data of the study polymers demonstrating they are insoluble and stable in environmental media and thus are not expected to be bioavailable and therefore not biodegrade.

Additionally, published literature reports (Drobny, 2016; Ebnesajjad, 2017; Grot, 2013; Henry et al., 2018; Polymer Industry Association [PIA], 2019) that the study polymers are stable at foreseeable maximum continuous use temperatures presented in Tables 4 and 5. All polymers, including fluoropolymers can degrade when misused or when heated above their recommended use temperatures (Fluoropolymer Products Group of Plastics Europe [FPG], 2012; PIA, 2019). Of course, users are expected to follow guidance for use provided by manufacturers. Hence, the recommended temperatures for reasonably foreseeable use for the study substances are presented in Tables 4 and 5. References and additional information regarding the stability of the study polymers is provided in the Supporting Information: Chapter 4.

Fluorinated PA: If a fluorinated PA was used in the manufacture of the polymer, it was reported for each fluoropolymer in this study. Nine of the 14 fluoropolymers in the study were reported *not* to have used a fluorinated PA in their manufacture. It is industry practice to use fluorinated PAs when it is necessary to obtain specific end-use property or performance requirements generally related to very high-polymer MWs (see also Supporting Information: Chapter 7). For five study polymers, THV, FKM, FFKM, fluorinated ionomers, and amorphous fluoropolymers, a response of "Yes and No" was provided indicating that for some polymer grades a fluorinated PA is used, but not for others. See Supporting Information: Chapter 4 for additional information.

Results summary: This study examined three fluoroelastomers, nine fluoroplastics, and two specialty fluoroplastics:

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ionomers and amorphous. Data for each were gathered from the author companies and assessed by the PLC criteria applicable to the polymer itself "in use" (BIO by Deloitte, 2015; Henry et al., 2018; OECD, 2009). All fluoroplastics, specialty fluoroplastics, and fluoroelastomers in the study met the PLC criteria based on the data presented in Tables 4 and 5 with additional details provided describing methods and references in the Supporting Information: Chapter 4.

Including the four fluoroplastics in the prior study (Henry et al., 2018), data for 18 fluoropolymers have been provided for PLC assessment. These polymers have a wide range of compositions and structures and represent most of the global commercial fluoropolymer market (see additional text in the Discussion). These 18 fluoropolymers represent the major fluoropolymers manufactured and are used worldwide in innumerable critical end-use products and applications. Tables 1 and 2 highlight examples of the end-use markets as well as critical functionality and benefits these polymers provide.

Each of the assessed polymers in this study are insoluble in both water and n-octanol, and thus K_{ow} is not applicable. This lack of solubility in water and octanol confirms that fluoropolymers are not mobile in the environment and are not bioaccumulative and not able to bioconcentrate. The stability studies reported here on each of the study fluoropolymers reveal their stability in terms of light, hydrolysis, heat, oxidation, and biodegradation. When coupled with the lack of solubility, these fluoropolymers are most often characterized as relatively inert materials in the environment. Like any other chemical material or product, it is important to follow the fluoropolymer manufacturer's recommended use and temperature conditions. Tables 4 and 5 describe these recommendations for each fluoropolymer. As reported, the physical forms of the fluoropolymers are largely pellets, blocks, crumb, sheets, some powders (all with MMAD >5 μm). The solid fluoropolymers are not nanoparticles, and concerns related to nanoparticles do not apply during normal product use. Due to the properties described above for the assessed fluoropolymers—large molecules with no water solubility—the fluoropolymers are biologically inert without the practical ability to cross cell membranes.

During the evaluation of the study fluoropolymers, there was a conscious focus on several core PLC parameters: MW, low MW leachables, % oligomers, and residual monomers, which are direct outcomes related to fluoropolymer manufacturing. In addition to what is reported here in Tables 4 and 5 for the fluoropolymers themselves, industry efforts to manage emissions during manufacturing are discussed below.

DISCUSSION

Fluoropolymers have substantial, unique societal value: Fluoropolymers possess a remarkable combination of properties and functional characteristics, as shown in Tables 1 and 2, that make them valued materials of choice in a broad range of industries and applications critical to life and a sustainable environment in the 21st century. Their unparalleled combination of properties and performance characteristics deliver functionality to a wide variety of products and systems critical to achieving important societal goals (Amcham, 2020c; FPG, 2021a; Wood, 2020b). They are strategically important to innovation in vital sectors of the global economy requiring high-speed, high-volume data transmission, miniaturization, or operations in extreme temperatures. Moreover, they are crucial to achieving important societal goals such as decarbonization, renewable energies, and/or competitiveness in the digital transition (FPG, 2021a). Fluoropolymers are indispensable for critical applications in the chemical, electronic, semiconductor, healthcare, and transport sectors and the deployment of 5G networks (FPG, 2021a). For many critical applications, fluoropolymers are the material of choice because alternatives are unable to provide the full complement of performance and functionality required. As such, there are currently no viable commercial alternatives to fluoropolymers in virtually every critical application in which they are used (FPG, 2021a, 2017; PFP, 2020).

Commercial fluoropolymers in this study meet the PLC criteria: Widely used by regulators, PLC criteria have been established around the world and documented by OECD expert groups as an appropriate hazard assessment methodology for polymers in-use and can effectively identify low risk fluoropolymers to help prioritize regulatory action (BIO by Deloitte, 2015; OECD, 1993, 2009). Here, we present PLC data, for hazard assessment, that define a group of fluoropolymers' "in-use" properties. PLC is not a comprehensive life-cycle assessment tool. Full life-cycle assessments consider all phases of product "life" including creation (manufacturing) and end-of-life (disposal). Information on manufacture and end-of-life is provided later in this study. Recently, polymers have been under increased regulatory scrutiny. In 2019, the industry-led European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) developed a Conceptual Framework for Polymer Risk Assessment ("CF4Polymers"; ECETOC, 2019). CF4Polymers provides guiding elements to be considered in assessing potential ecological and human health hazards and risks posed by polymer substances. CF4Polymers also considers specific life-cycle stages of polymer products and their associated routes of exposure. The authors of the CF4Polymers framework support the PLC approach as a means to accomplish polymer risk assessment. They specifically support the findings of Henry et al. (2018) and state that they are "unaware of scientific evidence to justify generally assigning fluoropolymers the same level of regulatory concern as other PFAS" (ECETOC, 2019). In 2020, the European Commission contracted a study to propose criteria to identify PRR under REACH (Wood, 2020a). The report states that the authors consider fluoropolymers meeting the criteria to be considered PLC, "following the recommendations of Henry et al. (2018)."

The properties and characteristics of fluoropolymers are anchored in the strength of the carbon–fluorine bond, which

render them highly stable (thermally, chemically, and biologically), inert, and durable—long lasting in use—under exacting and high-performance conditions. Physical, chemical, thermal, and biological stability are important criteria for a polymer to be considered a PLC. The data presented in Tables 4 and 5 demonstrate that commercial fluoropolymers from the author companies meet the criteria to be considered PLC. The PLC criteria for physicochemical properties reflect the state of the polymers in this study, solids, as well as their inertness and stability. None of the fluoropolymers assessed in this study were soluble in water or octanol. They are biologically inert, insoluble in water and octanol, and not expected to move in or between environmental media. Fluoropolymers are also twice as dense as water. These properties and water insolubility mean fluoropolymers are not mobile in the environment and therefore would not be expected to be found in sources of drinking water. Fluoropolymers are neither bioavailable nor bioaccumulative. These solid polymers cannot be absorbed through a cell membrane via passive or active transport and do not bind or interact with the cell surface (see also Supporting Information: Chapter 8). In addition, whereas aquatic and mammalian toxicology studies of fluoropolymers may be desirable for some, they are technically difficult for insoluble, solid, high-MW polymers. The OECD test guidelines reiterate this in many cases. This is confirmed for example in REACH Annex VII guidance, which repeatedly states toxicity is unlikely to occur "if a substance is highly insoluble in water or the substance is unlikely to cross biological membranes" (see Supporting Information: Chapter 9).

Finally, structure criteria including MW, MWD, residual monomer(s), oligomers, and other synthesis by-products, as represented by low MW extractables and leachables have been determined for the fluoropolymers presented and meet values established for the PLC criteria and regulated uses (e.g., USP). The concentrations in the fluoropolymer that have been evaluated are extremely low, reflective of effective manufacturing processes that minimize these compounds complemented by capture and/or destruction systems for such materials. For additional information, see the section below discussing responsible manufacturing. This study and prior work (Henry et al., 2018) provide a guide for other global fluoropolymer manufacturers to gather and present data on additional commercial fluoropolymers to determine if they too meet the PLC criteria.

Fluoropolymer stability, aka persistence, is not an intrinsic hazard: Fluoropolymers are stable, inert, solid materials. Fluoropolymers resist degradation by acids, bases, oxidants, reductants, photolytic processes, microbes, and metabolic processes; for this reason, they are thermally, chemically, and biologically highly inert. Fluoropolymer stability was presented in the introduction and is further considered in the Supporting Information: Chapters 4 and 5. Fluoropolymers are not expected to degrade under environmental conditions or normal use and processing conditions (Wood, 2020a). They are stable and remarkably durable and are therefore persistent. However, persistence alone does not imply that there is a present or future risk to human health or the environment (Rüdel et al., 2020). Persistence itself is not an intrinsic hazard, as it does not in itself imply or inform the potential for an adverse effect (aka toxicity). There is no language in REACH supporting the notion that persistence alone justifies risk-management measures. REACH has regulated persistence in combination with other properties that do inform potential hazards. In fact, REACH combines persistence with bioaccumulation and toxicity (or "very persistent" with "very bioaccumulative/very mobile" vPvB/vPvM) to justify designation as a substance of very high concern (SVHC) and consideration of potential risk-management measures for uses associated with unacceptable risk. Therefore, persistence on its own does not justify the need for specific risk-management measures. Fluoropolymers themselves are persistent, but they are not bioaccumulative, not mobile, and not toxic and therefore not SVHCs from a regulatory perspective (Ruwona and Henry, 2021).

PFAS grouping and segmentation—Scope of regulatory measures: The OECD definition of PFAS is based only on chemical structure (OECD, 2021). It describes a universe of fluorinated organic substances with vastly different physical, chemical, and biological properties, including polymers and nonpolymers; solids, liquids, and gases; highly reactive and inert substances; soluble and insoluble substances; and volatile and involatile substances and is too broad to allow effective, science-based assessment and regulation of chemical compounds as an entire group (Amcham 2020a; BDI, 2021; Buck et al., 2021; Orgalim, 2021; Wallington et al., 2021). A 2021 OECD report states: "it is highly recommended that such diversity be properly recognized and communicated in a clear, specific and descriptive manner" and "the term 'PFASs' does not inform whether a compound is harmful or not, but only communicates that the compounds under this term share the same trait for having a fully fluorinated methyl or methylene aliphatic carbon moiety" (OECD, 2021).

In this context, the available property data (Tables 4 and 5) reveal that fluoropolymers have distinctly different properties from nonpolymeric PFAS and from SCFPs that have a polymeric backbone that does not contain C–F bonds directly attached to it. The perfluoroalkyl moiety in SCFPs is found in a side-chain connected via a functional group to the polymer backbone and "can potentially lead to the formation of nonpolymer PFAS as a result of degradation" (Fluoropolymer Products Group of Plastics Europe [FPG], 2021b; Wood, 2020a; see Supporting Information: Chapter 6). Segmentation that clearly differentiates the broad PFAS family according to their properties, rather than using a structure-based classification alone (OECD, 2021), is needed for a scientifically sound, risk-based regulatory approach. Regulating all PFAS as one homogenous group (ECHA, 2020) absent consideration of their properties, particularly when the properties are so demonstrably different, neglects basic scientific consideration of these properties,

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which are the foundation of substance differentiation. The USEPA does not consider all PFAS to have similar risk profiles and therefore they are following a categorical grouping approach based on information about similarities in structure, physicochemical properties, and existing test data on the toxicity of PFAS (USEPA, 2021b). Therefore, segmentation based on properties should be conducted before performing any grouping-based risk assessment, placing stable, nonhazardous fluoropolymers that meet the criteria to be considered PLC in a separate category (see also Supporting Information: Chapter 6).

Fluoropolymer market perspective: The commercial fluoropolymer global market sales have been reported to be approximately 230 000 MT (Dams & Hintzer, 2017). Given the expected fluoropolymer market growth, ranging from approximately 4%–5% to 7%–8% (Allied Market Research [AMR], 2022; Future Market Insights [FMI], 2022; FPG, 2021a; Globe Newswire, 2021), a pro forma market table was created for 2021 using a 5% growth rate. Adding ionomers as well as updated amorphous market information (company data) to the above, the total commercial fluoropolymer market sales is estimated to be approximately 330 000 MT in 2021 (see Supporting Information: Chapter 10). Four fluoropolymers: PTFE, FEP, PFA, and ETFE, were the focus of the first fluoropolymer PLC paper (Henry et al., 2018) and account for approximately 64% of fluoropolymers sold globally in 2021 (pro forma basis). The sales volume of these four fluoropolymers is represented by the first four bars in Figure 10.1 in Supporting Information: Chapter 10. This study discusses 14 fluoropolymers representing an additional 32% (pro forma basis) of the global fluoropolymer market. Therefore, this study, in combination with Henry et al. (2018), presents PLC data from the cited manufacturers of commercial fluoropolymers representing approximately 96% of the global commercial fluoropolymer market that meet the criteria to be considered PLC. The projected 2021 sales volume of the major types of commercial fluoropolymers covered in this study (PVDF, FKM, FEPM, amorphous, ionomers, THV, ECTFE, PCTFE, and FFKM, EFEP, CTP, and FEVE) are also represented in Figure 10.1 in Supporting Information: Chapter 10. As noted, estimated market volumes were provided for the sum of FEPM, CPT, EFEP, and FEVE as well as a small "others" category. The fluoropolymer polyvinyl fluoride (PVF) was not covered by these two papers but is also shown in Figure 10.1 in Supporting Information: Chapter 10. Other fluorinated polymers, perfluoropolyethers, and SCFPs are not addressed in this study (see Supporting Information: Chapter 6).

FLUOROPOLYMER LIFE-CYCLE CONSIDERATIONS

This study focuses on the properties of the 14 selected commercial fluoropolymers themselves in-use providing data that demonstrate they meet the criteria to be considered PLC. Additionally, the life-cycle stages of fluoropolymer creation (manufacturing) and disposal at the end of industrial or consumer use (end-of-life) are important to consider. The primary focus in these life-cycle stages is generally nonpolymer PFAS from the manufacturing process or fluoropolymer degradation in end-of-life disposal (ECHA, 2020; FPG, 2021a; Guelfo et al., 2021; Lohmann et al., 2020).

The long-established life-cycle assessment approach to environmental protection and risk management first considers the extent of emissions, their toxicity, and their exposure potential (Guinee et al., 2011). When emissions are sufficiently large in scope, toxicity, and exposure potential, emission-management methods are then considered, including process input changes and emission controls to reduce or eliminate the risk of the emissions. Fluoropolymer manufacturing and disposal life-cycle stages were discussed in the paper that first presented fluoropolymer PLC data (Henry et al., 2018). Here we provide an update and current perspective.

Responsible manufacturing: As corroborated by the data presented here and in prior work (Henry et al., 2018), a large volume percentage and number of commercial fluoropolymers are manufactured that meet the criteria to be considered PLC. Emissions from fluoropolymer manufacture are a key product life-cycle focus. The main focus during the manufacturing phase is not directly related to fluoropolymers but from emissions. Emissions of concern may include nonpolymer PFAS such as fluorinated PAs, unreacted monomers, oligomers, or other unintended by-products formed during manufacturing. It is important to note that, although some high-MW fluoropolymers require use of a fluorinated PA in manufacturing (see also Supporting Information: Chapter 10), it has been reported that at least 50% of commercial fluoropolymers are made without one (Pro-K Fluoropolymer Group, 2021).

Recently, a group of fluoropolymer member companies of FPG voluntarily committed to responsible manufacturing principles through the commissioning of a Regulatory Management Option Analysis, developed by independent consulting firm Chemservices (FPG, 2021a). Member companies of this group are working on individual projects and joint projects at the trade association level with third-party experts. Specifically, companies have committed to continuously improving and/or developing the best available techniques in the manufacturing process, managing environmental emissions, developing R&D programs for the advancement of technologies allowing for the replacement of nonpolymer PFAS PAs and/or working with downstream users to increase the recyclability and reuse of its products in line with the objectives of circular economy (FPG, 2021a). Implementation of this voluntary industry initiative to address concerns relating to fluoropolymers will strengthen already ongoing efforts performed by the fluoropolymer industry promoting responsible manufacturing practices. In addition, member companies are committed to working with EU authorities to establish and implement technical actions to guarantee adequate control of the risks derived from the manufacture and use of fluoropolymers to mitigate such risks wherever possible. This will be done following

transparency principles and agreements to monitor progress. For example, important emission reduction has been demonstrated by major fluoropolymer manufacturers including fluorinated PA recovery for reuse, 99% removal of fluorinated PA in wastewater treatment, and 99.99% capture and destruction efficiency of gaseous emissions routed to a thermal oxidizer (Chemours, 2021c), as well as 99–99.9 plant emission reductions (Daikin, 2021c, 2022). Four other companies have reported replacement of fluorinated PAs with nonfluorinated PAs (Arkema, 2008, 2021b; Chemours, 2022; Gujarat Fluorochemicals Limited, 2022; Solvay, 2022). These are substantial efforts toward mitigating emissions associated with fluoropolymer manufacturing being worked on by fluoropolymer manufacturers. This study and the prior study (Henry et al., 2018) provide a guide for other global fluoropolymer manufacturers to gather and present data on their commercial fluoropolymers in-use demonstrating that they meet the PLC criteria.

End-of-use: At the end of industrial or consumer use, fluoropolymers may be disposed via the following routes: landfill, incineration (e.g., waste-to-energy [WTE] facilities), or reuse/recycling. There is considerable data demonstrating that fluoropolymers such as PTFE do not degrade in the environment or release substances of toxicological or environmental concern (FPG, 2021a; Hintzer & Schwertfeger, 2014). FPG member companies are working with the industry and end users on this subject and are engaged in a research project aimed at identifying conditions required for proper disposal (incineration) of fluoropolymers (FPG, 2021a).

Fluoropolymers are chemically, thermally, and biologically stable (Henry et al., 2018; this study) and therefore are not expected to transform to dispersive nonpolymeric PFAS when disposed of in a landfill. A recent study presented results from OECD guideline biodegradation studies demonstrating that PTFE is stable and does not degrade under environmentally relevant conditions (Ruwona and Henry, 2021). Further, fluoropolymers that meet the criteria to be considered PLC, such as those in this study and prior work (Henry et al., 2018), have negligible leachables, unreacted monomers, and oligomers most likely destroyed in fluoropolymer use processing and would therefore not be expected to significantly contribute to landfill leachate (Ruwona and Henry, 2021).

Available data reveal that fluoropolymers are mineralized (i.e., all C–F bonds broken, hydrofluoric acid generated, and scrubbed to calcium fluoride) under commercial WTE incineration operating conditions (Aleksandrov et al., 2019; Bakker et al., 2021; DEC, 2021; Giraud et al., 2021a, 2021b). In recent pilot scale studies representative of full-scale WTE facilities, the most common form of end-of-life destruction conducted on PTFE found that combustion converted the fluorine into controllable hydrogen fluoride gas and that, of the 31 PFAS studied, no fluorine-containing products of incomplete combustion were produced above background levels (Aleksandrov et al., 2019). Further, a recent study investigating the presence of PFAS in waste incinerator flue gas stated: "based on a literature review, RIVM expects that most of the PFASs will largely degrade during the incineration process and then be removed when the flue gases are cleaned. The remaining PFASs are expected to be removed during the recovery of the carbon dioxide" (Bakker et al., 2021). The RIVM report affirmed that PTFE is the most stable fluorine-containing polymer. For PTFE, the RIVM report concluded that complete thermal decomposition is achieved at a temperature of approximately 800 °C. It was therefore assumed that other fluorine-containing polymers also thermally decompose completely at a temperature of 800 °C. Temperatures at the pyrolysis front and the combustion front in the waste-burning bed range from 900 °C to 1100 °C (Asthana et al., 2006; Ménard et al., 2006), which is well above 800 °C, the temperature at which the complete thermal decomposition of PTFE is achieved (Bakker et al., 2021). Studies for additional fluoropolymers and those with additional pilot and/or full-scale fluoropolymer studies would contribute to this body of data and further affirm their results. The PFP and FPG currently have joint projects working on these potential contributions.

Recycling of fluoropolymer products and articles containing fluoropolymers is difficult because separation of the fluoropolymer from the end products is not always possible (FPG, 2021a; Hintzer & Schwertfeger, 2014; Pro-K Fluoropolymer Group, 2018). This is because fluoropolymers are used predominantly in small components of larger finished articles involving a wide variety of materials. There are several options to recycle fluoropolymer products. In primary recycling, solid fluoropolymer waste is ground and later fed back into the manufacturing cycle of some fluoropolymer products. Recycled fluoropolymers may be used in high-end applications when correctly collected, cleaned, and reprocessed. In secondary recycling, solid fluoropolymer waste is ground, followed by degradation to approximately 1% of the original degree of polymerization by using electron beams, gamma rays, or thermomechanical degradation. The recovered material can be used in the manufacturing of new fluoropolymer products. Lastly, in tertiary recycling or upcycling, solid fluoropolymer is ground, then decomposed into the starting monomers at temperatures higher than 600 °C (pyrolysis) to obtain the same chemical components from which the fluoropolymer was manufactured; monomers, such as tetrafluoroethylene, are purified by distillation, and can then be reused to manufacture new fluoropolymer (3M, 2021; Schlipf & Schwalm, 2014). For the primary and secondary schemes, recycling treatments can be undertaken by the manufacturers of fluoropolymers themselves (onsite), or at a larger scale, mainly by specialist recycling companies. The upcycling needs to be colocated to a fluoropolymer manufacturing plant that can use tetrafluoroethylene.

Primary and secondary recycling is limited because of the presence of fillers, colorants, and other materials in the composition of their final articles. Further, recycling might not work for all end-of-life components, as they are used predominantly in small components of larger

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finished articles involving a wide variety of materials. Therefore, collecting and dismantling for recycling might not be feasible for all products (FPG, 2021a; Hintzer & Schwertfeger, 2014; Pro-K Fluoropolymer Group, 2018). However, it should be noted that upcycling treatment is applicable to some articles containing fluoropolymers, such as pipe liners in chemical plants, as well as other plant components such as pumps, tank liners, seals, hoses, compensators, and many other fluoropolymer components and systems. These are the products for which the high quantities of fluoropolymers are used offering significant recycling potential.

SUMMARY

This study has described the composition, uses, performance properties, and functionalities of 14 commercially available fluoropolymers, including fluoroplastics and fluoroelastomers. Fluoropolymers are the preferred material of choice because of their unique combination of properties, which are not achievable from other materials or via other functions. As a result, fluoropolymers have become a critical mainstay for society and are useful to modern living, as they provide vital, reliable functionality to a broad range of industrial and consumer products. Further, the study has presented data demonstrating the subject fluoropolymers satisfy the widely accepted polymer hazard assessment criteria to be considered PLC. The data presented demonstrate the fluoropolymers in the study are thermally, biologically, and chemically stable, negligibly soluble in water, nonmobile, nonbioavailable, nonbioaccumulative, and nontoxic, and contain low levels of impurities. These results further demonstrate that the fluoropolymer class should be considered distinctly different and should not be grouped with other PFAS for hazard assessment or regulatory purposes. When combined with earlier work (Henry et al., 2018), the study demonstrates that commercial fluoropolymers are available that meet the criteria to be considered PLC, which represent approximately 96% of the global fluoropolymer market. Lastly, emissions from fluoropolymer manufacture and disposal at end-of-use are a product life-cycle focus. Emissions may include nonpolymer PFAS such as fluorinated PAs, unreacted monomers, oligomers, or other unintended by-products formed during manufacturing. Fluoropolymer manufacturers recently committed voluntarily to responsible manufacturing principles by continuously improving and/or developing the best available techniques in the manufacturing process, managing environmental emissions, developing R&D programs for the advancement of technologies allowing for the replacement of fluorinated PAs, and/or increasing recyclability and reusing fluoropolymers in line with the objectives of circular economy.

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CONFLICT OF INTEREST

The authors are employed by companies that commercially manufacture fluoropolymers. SHK is an independent fluorotechnology consultant working on behalf of AGC Chemicals Americas Inc. and principal of BeachEdge Consulting LLC.

DATA AVAILABILITY STATEMENT

Data gathered for this paper is presented in the paper itself and the Supporting Information: Data file provided. Additional data are available upon request from the corresponding author Stephen Korzeniowski (shkorzo@gmail.com).

SUPPORTING INFORMATION

The Supplement contains a glossary of terms as well as additional information on the study of fluoropolymers properties and functionalities, polymer of low concern (PLC) background and criteria, references and methods for the PLC data for the study of fluoropolymers, benefits, features and alternatives assessment for the study of fluoropolymers, the differences between fluoropolymers and side-chain fluorinated polymers, fluoropolymer bioavailability and toxicity studies, fluoropolymer global market information, fluoropolymer socioeconomic analyses and risk-management options analysis (RMOA).

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REFERENCES

3M. (2021). *Up-cycling. Closing the loop.* https://multimedia.3m.com/mws/media/907323O/up-cycling-fluoropolymers-brochure.pdf?fn=Up-Cycling_Brochure_EN.pdf

AGC Chemicals Company. (2021a). *FEVE LUMIFLONbrochure®*. https://www.agcchem.com/wp-admin/admin-ajax.php?juwpfisadmin=false&action=wpfd&task=file.download&wpfd_category_id=170&wpfd_file_id=1927&preview=1&embedded=true

AGC Chemicals Company. (2021b). *Cytop® amorphous fluoropolymers.* https://www.agc-chemicals.com/jp/en/fluorine/products/detail/index.html?pCode=JP-EN-F019

AGC Chemicals Company. (2021c). *Forblue™ Flemion™ fluorinated ion-exchange membranes.* https://www.agc-chemicals.com/jp/en/fluorine/products/category/result.html?c_id=11

AGC Chemicals Company. (2021d). *AFLAS fluoroelastomers.* https://www.agcchem.com/wp-admin/admin-ajax.php?juwpfisadmin=false&action=wpfd&task=file.download&wpfd_category_id=172&wpfd_file_id=1901&preview=1&embedded=true

Aleksandrov, K., Gehrmann, H. J., Hauser, M., Matzing, H., Pigeon, D., Stapf, D., & Wexler, M. (2019). Waste incineration of polytetrafluoroethylene (PTFE) to evaluate potential formation of per- and poly-fluorinated alkyl substances (PFAS) in flue gas. *Chemosphere*, *226*, 898–906. https://doi.org/10.1016/j.chemosphere.2019.03.191

Allied Market Research (AMR). (2022). *Fluoropolymers market by product type.* https://www.alliedmarketresearch.com/fluoropolymers-market

American Chamber of Commerce in Europe (Amcham). (2020a). *Grouping of PFAS: Regulation by distinct PFAS classes is scientifically superior to*

classification by a broad PFAS group. https://www.amchameu.eu/position-papers/grouping-pfas-regulation-distinct-pfas-classes-scientifically-superior

American Chamber of Commerce in Europe (Amcham). (2020b). *A narrow regulatory focus on persistence-only is not justifiable and can undermine innovation to produce materials that support societal sustainability goals*. https://www.amchameu.eu/position-papers/use-persistency-and-mobility-criteria-chemicals-regulation-amcham-eu-comments

American Chamber of Commerce in Europe (Amcham). (2020c). *REACH Restriction: Essential use criteria in the context of socio-economic impact analysis when unacceptable risk is demonstrated*. https://www.amchameu.eu/position-papers/reach-restriction-essential-use-criteria-context-socio-economic-impact-analysis-when

Ameduri, B. (2020). The promising future of fluoropolymers. *Macromolecular Chemistry and Physics*, *221*, 1900573. https://doi.org/10.1002/macp.201900573

Améduri, B., Boutevin, B., & Kostov, G. (2001). Fluoroelastomers: Synthesis, properties and applications. *Progress in Polymer Science (Oxford)*, *26*(1), 105–187. https://doi.org/10.1016/S0079-6700(00)00044-7

Ameduri, B., & Sawada, H. (2017a). *Fluorinated polymers. Volume 1: Synthesis, properties, processing and simulation*. Royal Society of Chemistry.

Ameduri, B., & Sawada, H. (2017b). *Fluorinated polymers. Volume 2: Applications*. Royal Society of Chemistry. https://doi.org/10.1039/9781782629368

Arkema. (2008). *Arkema eliminates fluorosurfactants from Kynar 500PVDF®*. https://www.pcimag.com/articles/88876-arkema-eliminates-fluorosurfactants-from-kynar-500-pvdf

Arkema. (2021a). *KynarPVDF family®*. https://www.extrememateriels-arkema.com/en/product-families/kynar-pvdf-family/chemical-resistance-fuel-resista/download-kynar-pvdf-chemical-res/

Arkema. (2021b). *Fluorosurfactant free KynarPVDF resin®*. https://kynar500.arkema.com/en/product-information/fluorosurfactant-free/

Asahi-Kasei. (2021). *Aciplexion exchange membranes®*. https://www.asahi-kasei.co.jp/salt-electrolysis/en/?_ga=2.192307178.1574253781.1637252713-425530764.1637252713

Asthana, A., Ménard, Y., & Patisson, F. (2006). A 2-D mathematical model of on-grate municipal solid waste combustion. In F. Kongoli & R. G. Reddy (Eds.), *Sohn International Symposium Advanced Processing of Metals and Materials, Volume I—Thermo and physicochemical principles: non-ferrous high-temperature processing* (pp. 465–474). The Minerals, Metals & Materials Society (TMS).

ASTM. (2021). *Standard practice for rubber and rubber latices—Nomenclature* (pp. D1418–D1421). ASTM International. https://doi.org/10.1520/D1418-21

Atkinson, S. (2018). Essential high-purity sealing materials for semiconductor manufacturing. *Sealing Technology*, *7*, 5–7. https://doi.org/10.1016/S1350-4789(18)30284-8

Auer, C. M., Nabholz, J. V., & Baetcke, K. P. (1990). Mode of action and the assessment of chemical hazards in the presence of limited data: Use of structure-activity relationships (SAR) under TSCA, Section 5. *Environ Health Perspect*, *87*, 183–197.

Bakker, J., Bokkers, B., & Broekman, M. (2021). *Per- and polyfluorinated substances in waste incinerator flue gases* (RIVM Report 2021-0143). https://www.rivm.nl/bibliotheek/rapporten/2021-0143.pdf

Banks, R. E., Smart, B. E., & Tatlow, J. C. (1994). *Organofluorine chemistry: Principles and commercial application*s. Plenum.

Barnwell. (2021). *Perfluoroelastomer O rings (FFKM)*. https://www.barnwell.co.uk/products/perfluoroelastomer-ffkm-o-rings/

Beyer, E. C. (1993). Gap junctions. *International Review of Cytology*, *137*, 2.

BIO by Deloitte. (2015). *Technical assistance related to the review of REACH with regard to the registration requirements on polymers* (Final Report). Prepared for the European Commission (DG ENV) in collaboration with PIEP.

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. J. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*, *7*(4), 513–541. https://doi.org/10.1002/ieam.258

Buck, R. C., Korzeniowski, S. H., Laganis, E., & Adamsky, F. (2021). Identification and classification of commercially relevant per- and polyfluoroalkyl substances (PFAS). *Integrated Environmental Assessment and Management*, *17*(5), 1045–1055. https://doi.org/10.1002/ieam.4450

Bundesverband der Deutschen Industrie e.V. (BDI). (2021). *EU chemicals Legislation: Restriction of PFAS (EN)*. english.bdi.eu/publication/news/eu-chemicals-strategy-restriction-of-pfas/

Chemours. (2021a). *Nafion™ ion exchange materials*. https://www.nafion.com/en

Chemours. (2021b). *Economic and environmental improvements with versatile membranes*. https://www.nafion.com/en/support/white-papers/versatility-of-ion-exchange-membranes-white-paper

Chemours. (2021c). *Thermal oxidizer destroying PFAS at greater than 99.99% efficiency*. https://www.chemours.com/en/-/media/files/corporate/fayetteville-works/2020-0320-thermal-oxidizer-efficiency-results-announced.pdf?rev=87dbfd0ebb9c45aeaa475fddd2a899b4&hash=05916EC2AC2B5A3C41135773A3FCDED0

Chemours. (2022). *Chemours announces process innovation with New Viton™ fluoroelastomers advanced polymer architecture (APA) offering*. https://www.chemours.com/en/news-media-center/all-news/press-releases/2022/chemours-announces-process-innovation-with-new-viton-fluoroelastomers-advanced-polymer-architecture

Curbell. (2021). *PCTFE*. https://www.curbellplastics.com/Research-Solutions/Materials/PCTFE

Daemar. (2021). *DuPont Kalrez—Oil and gas product selector guide®*. https://daemar.com/wp-content/uploads/2017/11/OilGasProduct-SelectorGuide_KZE-A40042-00-A0217.pdf

Daikin. (2011a). *EFEP. Fluoropolymer handbook* (pp. 84–93). Daikin Industries.

Daikin. (2011b). *CPT. Fluoropolymer handbook* (pp. 58–62). Daikin Industries.

Daikin. (2021a). *NeoflonPCTFE®*. https://www.daikinchemicals.com/solutions/products/fluoropolymers/neoflon-pctfe.html?_ga=2.84898265.1754525417.1636603879-520575968.1622167042

Daikin. (2021b). *Neoflon CPT*. https://www.daikinchemicals.com/solutions/products/fluoropolymers/neoflon-cpt.html?_ga=2.257391307.1754525417.1636603879-520575968.1622167042

Daikin. (2021c). *Daikin–A responsible steward for PFAS emission reductions and safe alternatives*. https://www.daikinchem.de/sites/default/files/pdf/News/Daikin_Responsible_Steward_PFAS_Emission_Reduction_210601.pdf

Daikin. (2022). *Measures concerning environment emission of PFAS*. https://www.daikinchemicals.com/company/sustainability/pfas.html?_ga=2.233775006.1492271271.1653437409-1591380784.1653437409

Dams, R., & Hintzer, K. (2017). Chapter 1: Industrial aspects of fluorinated oligomers and polymers. In B. Ameduri & H. Sawada (Eds.), *Fluorinated polymers Volume 2: Applications* (pp. 3–31). Royal Society of Chemistry, Cambridge.

Darden, W., & Parker, R. (2021). *Fluoropolymer coatings for plastics*. AGC Chemicals. https://lumiflonusa.com/wp-content/uploads/FluoropolymerCoatingsForPlastics.pdf

Domininghaus, H. (1998). Chapter 2.1.7.4: Tetrafluorethylen/hexafluorpropylen/vinylidenfluorid-terpolymer. In *Kunststoffe, Eigenschaften und Anwendungen* (5th ed., 564f, pp. 525–598). Springer. https://books.google.com/books?id=Ar-oBgAAQBAJ&printsec=frontcover#v=onepage&q&f=false

Drobny, J. G. (2016). *Fluoroelastomers handbook* (2nd ed.). Elsevier. https://www.elsevier.com/books/fluoroelastomers-handbook/drobny/978-0-323-39480-2

Ebnesajjad, S. (2000). *Fluoroplastics Volume 1. Non-melt processible fluoroplastics*. PDL Handbook Series. Plastics Design Laboratory.

Ebnesajjad, S. (2003). *Fluoroplastics Volume 2. Melt processible fluoropolymers*. PDL Handbook Series. Plastics Design Laboratory.

Ebnesajjad, S. (2017). Introduction to fluoropolymers. In *Applied plastics engineering handbook: Processing, materials, and applications* (2nd ed., pp. 55–71). Elsevier. https://doi.org/10.1016/B978-0-323-39040-8.00003-1

ECETOC. (2019). *The ECETOC conceptual framework for polymer risk assessment (CF4POLYMERS)* (Technical Report No. 133-1). May. https://www.ecetoc.org/publication/tr-133-the-ecetoc-conceptual-framework-for-polymer-risk-assessment-cf4polymers/

ECHA. (2020). *The national authorities of Germany, the Netherlands, Norway, Sweden and Denmark invite interested parties to send in evidence and information on the use of per- and polyfluoroalkyl substances (PFAS)*. https://echa.europa.eu/-/five-european-states-call-for-evidence-on-broad-pfas-restriction

Environment & Infrastructure Solutions UK Limited (Wood). (2020a). *Scientific and technical support for the development of criteria to identify and group polymers for Registration/Evaluation under REACH and their impact assessment* (European Commission Report). Brussels. https://circabc.europa.eu/ui/group/a0b483a2-4c05-4058-addf2a4de71b9a98/library/4acda744-777f-473e-b084-76e125430565/details

Environment & Infrastructure Solutions UK Limited (Wood). (2020b). *Socio-economic assessment (SEA) of the US fluoropolymer industry* (Final Report 2020_0225). https://fluoropolymerpartnership.com/wp-content/uploads/2020/03/Socio-Economic-Assessment-of-the-US-Fluoropolymer-Industry-Executive-Summary.pdf

European Commission (EC). (2021). *A European green deal*. https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en

Fiedler, H., Kennedy, T., & Henry, B. J. (2020). A critical review of a recommended analytical and classification approach for organic fluorinated compounds with an emphasis on per- and polyfluoroalkyl substances (PFAS). *Integrated Environmental Assessment and Management*, *17*(2), 331–351. https://doi.org/10.1002/ieam.4352

Fluoropolymer Products Group of Plastics Europe (FPG). (2012). *Guide for the safe handling of fluoropolymer resins*. https://fluoropolymers.plasticseurope.org/index.php/fluoropolymers/irreplaceable-uses-1/reports-policy-documents/tfe-safe-handling-guide

Fluoropolymer Products Group of Plastics Europe (FPG). (2017). *Socio-economic analysis of the European fluoropolymer industry*. https://fluoropolymers.plasticseurope.org/application/files/7816/1167/4026/Final_SEA_Fluoropolymers_summary2017_3.pdf

Fluoropolymer Products Group of Plastics Europe (FPG). (2021a). *Risk management options analysis (RMOA)*. https://fluoropolymers.plasticseurope.org/index.php/fluoropolymers/irreplaceable-uses-1/reports-policy-documents/rmoa

Fluoropolymer Products Group of Plastics Europe (FPG). (2021b). *Fluoropolyers vs. side-chain fluorinated polymers*. https://fluoropolymers.plasticseurope.org/application/files/3516/3913/1778/Fluorpolymers_vs._side_chain_fluorinated_polymers_final.pdf

Future Market Insights (FMI). (2022). *Fluoropolymer market overview*. https://www.futuremarketinsights.com/reports/fluoropolymers-market

Gangal, S. V., & Brothers, P. D. (2010). Perfluorinated polymers, Tetrafluoroethylene-perfluorodioxole copolymers. In *Encyclopedia of polymer science and technology*. John Wiley & Sons Inc. https://doi.org/10.1002/0471440264.pst420.pub2

Giraud, R. J. (2021b). *Municipal waste-to-energy combustion of fluorinated polymers as a potential source of PFOA in the environment*. PhD thesis, University of Delaware.

Giraud, R. J., Taylor, P. H., & Huang, C.-P. (2021a). Combustion operating conditions for municipal waste-to-energy facilities in the U.S. *Waste Management*, *132*, 124–132. https://doi.org/10.1016/j.wasman.2021.07.015

Globe Newswire. (2021). *Global fluoropolymers market is forecasted to grow at a CAGR of 4.3% during the forecast period 2017–2027*. https://www.globenewswire.com/news-release/2021/08/31/2289045/28124/en/Global-Fluoropolymers-Market-is-Forecasted-to-Grow-at-a-CAGR-of-4-3-During-the-Forecast-Period-2017-2027.html

Greene-Tweed. (2021a). *FKM versus FFKM–A comparison of properties*. https://live-greene-tweed.pantheonsite.io/wp-content/uploads/2020/04/FKM-vs-FFKM-Properties-Comparison-R3.pdf

Green-Tweed. (2021b). *FKM vs. FFKM*. https://www.gtweed.com/materials/fkm-vs-ffkm/

Groh, W., & Zimmermann, A. (1991). What is the lowest refractive index of an organic polymer? *Macromolecules*, *24*(25), 6660–6663. https://doi.org/10.1021/ma00025a016

Grot, W. (2011). *Fluorinated ionomers*. PDL Handbook Series. Elsevier Inc. https://doi.org/10.1016/C2010-0-65926-8

Grot, W. (2013). Chapter 12: Fluorinated ionomers: History, properties and applications. In S. Ebenesajjad (Ed.), *Introduction to fluoropolymers* (pp. 277–291). Elsevier. https://doi.org/10.1016/B978-0-323-39040-8.00003-1

Guelfo, J. L., Korzeniowski, S., Mills, M. A., Anderson, J., Anderson, R. H., Arblaster, J. A., Conder, J. M., Cousins, I. T., Dasu, K., Henry, B. J., Lee, L. S., Liu, J., McKenzie, E. R., & Willey, J. (2021). Environmental sources, chemistry, fate and transport of per- and polyfluoroalkyl substances: State of the Science, key knowledge gaps, and recommendations presented at the August 2019 SETAC Focus Topic Meeting. *Environmental Toxicology and Chemistry*, *40*, 3234–3260. https://doi.org/10.1002/etc.5182

Guinee, J. B., Heijungs, R., Huppes, G., Zamagni, A., Masoni, P., Buonamici, R., Ekvall, T., & Rydberg, T. (2011). Life cycle assessment: past, present, and future. *Environmental Science and Technology*, *45*(1), 90–96. https://doi.org/10.1021/es101316v

Gujarat Fluorochemicals Limited. (2018). *InoflarPVDF resins™*. https://www.inoflar.com/

Gujarat Fluorochemicals Limited. (2022). *Company announcement on the development of a non-fluorinated polymerization aid*. https://gfl.co.in/upload/pages/ebce5fed9030753d0ee651bf1f48d0a0.pdf

Hafer, J. (2021). REACH registration of polymers: Identifying polymers of low concern. *ICRL*, *1*(2021), 21–25. https://chemservice-group.com/wp-content/uploads/2021/03/icrl_2021_01.pdf

Henry, B., Carlin, J., Hammerschmidt, J., Buck, R. C., Buxton, L., Fiedler, H., Seed, J., & Hernandez, O. (2018). A critical review of the application of polymers of low concern and regulatory criteria to fluoropolymers. *Integrated Environmental Assessment and Management*, *14*(3), 316–334. https://doi.org/10.1002/ieam.4035

Hintzer, K., & Schwertfeger, W. (2014). Fluoropolymers—Environmental aspects. In D. W. Smith, S. T. Iacono, & S. S. Iyer (Eds.), *Handbook of fluoropolymer science and technology* (pp. 495–520). https://onlinelibrary.wiley.com/doi/10.1002/9781118850220.ch21

Hintzer, K., Zipplies, T., Carlson, D. P., & Schmiegel, W. (2013). Fluoropolymers, Organic. In *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley. https://doi.org/10.1002/14356007.a11_393.pub2

Honeywell. (2021). *Blister packaging*. https://lifesciences.honeywell.com/us/en/applications/healthcare-packaging/blister-packaging

Hoshino, T., & Morizawa, Y. (2017). Chapter 5: Fluorinated specialty chemicals–Fluorinated copolymers for paints and perfluoropolyethers for coatings. In B. Ameduri & H. Sawada (Eds.), *Fluorinated polymers: Volume 2: Applications* (pp. 110–126). The Royal Society of Chemistry. https://pubs.rsc.org.en/content/ebook/978-1-78262-916-0

Hull, D. E. (1983). *Tetrafluoroethylene-propylene copolymer (Aflas): New technology, new uses*. Xenox Inc. http://www.allsealsinc.com/pdf2/Aflas1.pdf

Hull, D. E., Johnson, B. V., Rodricks, I. P., & Staley, J. B. (1997). THV fluoroplastic. In J. Scheirs (Ed.), *Modern fluoropolymers* (257ff.). Wiley & Sons.

Kojima, G., Kojima, H., & Tabata, Y. (1977). A new fluoroelastomer derived from tetrafluoroethylene and propylene. *Rubber Chemistry and Technology*, *50*(2), 403–412. https://doi.org/10.5254/1.3535154

Kojima, G., & Yamabe, M. (1984). A solvent soluble fluororesin for paint. *Journal of Synthetic Organic Chemistry, Japan*, *42*, 841–849.

Korinek, P. M. (1994). Amorphous fluoropolymers—A new generation of products. *Macromolecular Symposia*, *82*(1), 61–65. https://doi.org/10.1002/masy.19940820108

Kostal, J. (2016). Chapter 4: Computational chemistry in predictive toxicology: Status quo et quo vadis? In J. C. Fishbein & J. M. Heilman (Eds.), *Advances in molecular toxicology* (Vol. 10, pp. 136–186). Elsevier. https://doi.org/10.1016/B978-0-12-804700-2.00004-0

Lavallée, C. (2020). Section 4.16 Polymer processing additives (PPA). In J. Macnamara (Ed.), *Film extrusion manual—Process, materials, properties* (3rd ed., pp. 291–302). TAPPI Press. https://imisrise.tappi.org/TAPPI/Products/01/R/0101R360.aspx

Lipinski, C., Lombardo, F., Dominy, B., & Feeny, P. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *46*(1–3), 3–26. https://doi.org/10.1016/s0169-409x(00)00129-0

Lohmann, R., Cousins, I., DeWitt, J., Glüge, J., Goldenman, G., Herzke, D., Lindstrom, A., Miller, M., Ng, C., Patton, S., Scheringer, M., Trier, X., & Wang, Z. (2020). Are fluoropolymers really of low concern for human and environmental health and separate from other PFAS? *Environmental*

Science and Technology, *54*(20), 12820–12828. https://doi.org/10.1021/acs.est.0c03244

Marshall, J. 2017. Kalrez®-type perfluoroelastomers—Synthesis, properties and applications. In J. Scheir (Ed.), *Modern fluoropolymers* (pp. 349–358). John Wiley and Sons.

Ménard, Y., Ashtana, A., Patisson, F., Sesiecq, P., & Ablitzer, D. (2006). Thermodynamic study of heavy metals behaviour during municipal waste incineration. *Process Safety and Environmental Protection*, *84*(B4), 290–296.

Miller, M., Pelch, K., Reade, A., Soehl, A., Trier, X., Venier, M., Wagner, C., Wang, Z., & Blum, A. (2020). Scientific basis for managing PFAS as a chemical class. *Environmental Science & Technology Letters*, *7*(8), 532–543. https://doi.org/10.1021/acs.estlett.0c00255

Munekata, S. (1988). Fluoropolymers as coating materials. *Progress in Organic Coatings*, *16*, 113–134. https://www.sciencedirect.com/science/article/pii/0033065588800104

NY State Department of Environmental Conservation (DEC). (2021). *Norlite environmental sampling report*. https://www.dec.ny.gov/chemical/121118.html

Organisation for Economic Co-operation and Development (OECD). (1993). *OECD expert group on polymers. Third Meeting of the Experts on Polymers: Chairman's Report* (ENV/MC/CHEM/RD(93)4). April.

Organisation for Economic Co-operation and Development (OECD). (2009). *Data analysis of the identification of correlations between polymer characteristics and potential for health or ecotoxicological concern*. OECD Task Force on New Chemicals Notification and Assessment, Expert Group Meeting on polymers, March, 2007, Tokyo, Japan. https://www.oecd.org/env/ehs/risk-assessment/42081261.pdf

Organisation for Economic Co-operation and Development (OECD). (2021). *Reconciling terminology of the universe of per- and polyfluoroalkyl substances: recommendations and practical guidance*. Series on Risk Management No. 61. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/CBC/MONO(2021)25&docLanguage=en

Ohkura, M., & Morizawa, Y. (2017). Fluoroplastics and fluoroelastomers–Basic chemistry and high-performance applications. In B. Ameduri & H. Sawada (Eds.), *Fluorinated polymers Volume 2: Applications*. RSC Polymer Chemistry Series No. 24. RSC. https://doi.org/10.1039/9781782629368

Orgalim. (2021). *Orgalim Position Paper on the restriction of PFAS*. https://orgalim.eu/position-papers/environment-orgalim-position-paper-restriction-pfas?&utm_campaign=Policy%20Brunch%20for%20FPP4EU%207%20February&utm_medium=email&utm_source=Mailjet

Park, E.-J., Park, I.-C., Lee, M. S., & Park, M. (2008). Preparation of cladding polymers for plastical optical fibers—Miscibility and interfacial adhesion of PMMA/THV blends. *Textile Science & Engineering*, *45*(6), 364–369. https://www.koreascience.or.kr/article/JAKO200806135610008.pa1ff8ge

Parker, R., & Blankenship, K. (2015). Fluoroethylene vinyl ether resins for high-performance coatings. In K. B. Tator (Ed.), *ASM handbook Volume 5B, Protective organic coatings*. ASM International. https://doi.org/10.31399/asm.hb.v05b.9781627081726

Performance Fluoropolymer Partnership of the American Chemistry Council (PFP). (2020). *Socio-economic assessment of the US fluoropolymer industry*. https://fluoropolymerpartnership.com/fluoropolymer-facts/socioeconomic-importance/

Polymer Industry Association (PIA). (2019). *Guide to the safe handling of fluoropolymer resins* (5th ed.). https://www.plasticsindustry.org/supply-chain/material-suppliers/fluoropolymers-division-fpd

Pro-K Fluoropolymer Group. (2018). *Recycling of fluoropolymers*. https://www.pro-kunststoff.de/assets/Merkbl%C3%A4tter%20und%20Co/FP%20TM-10-Recycling-of-fluoropolymers.pdf

Pro-K Fluoropolymer Group. (2021). *PFAS restriction—Proposal & request for exemption of fluoropolymers*. https://www.pro-kunststoff.de/fachwissen/pfas-restriction-proposal-und-request-for-exemtion-of-fluoropolymers.html

Resnick, P. R., & Buck, W. (1997). Teflon® AF amorphous fluoropolymers. In J. Scheirs (Ed.), *High performance polymers for diverse applications* (pp. 397–419). John Wiley & Sons.

Resnick, P. R., & Buck, W. H. (1999). Teflon® AF: A family of amorphous fluoeopolymers with extraordinary properties. In W. H. Properties, G. Hougham, P. E. Cassidy, K. Johns, & T. Davidson (Eds.), *Topics in applied chemistry, fluoropolymers* (pp. 25–33). Springer, New York, NY. https://doi.org/10.1007/b114560

Royal Society of Chemistry (RSC). (2021). *Risk-based regulation for per- and poly-fluoroalkyl substances (PFAS)*. https://www.rsc.org/globalassets/22-new-perspectives/sustainability/a-chemicals-strategy-for-a-sustainable-chemicals-revolution/pfas-policy-position-dec-2021.pdf

Rüdel, H., Körner, W., Letzel, T., Neumann, N., Nödler, K., & Reemtsa, T. (2020). Persistent, mobile and toxic substances in the environment: A spotlight on current research and regulatory activities. *Environmental Sciences Europe*, *32*, 5. https://doi.org/10.1186/s12302-019-0286-x

Ruwona and Henry. (2021). *PTFE: Persistence without hazard at environmentally relevant temperatures and durable by design*. Fluoros 2021, Providence, RI.

Satokawa, T. (Ed.) (1990). *Fluoropolymer handbook: PCTFE* (pp. 337–357). Nikkan-Kogyo-Shimbun.

Scheirs, J. (2007). *Modern fluoropolymers—High performance polymers for diverse applications*. John Wiley & Sons.

Schlipf, M., & Schwalm, T. (2014). *Closing the recycling loop, Up-cycling of end-of-life fluoroplastics*. Kunststoffe International. June. https://www.kunststoffe.de/en/journal/archive/article/up-cycling-of-end-of-life-fluoroplastics-841786.html

Science Center. (2021). *Energetic electrolysis: The potentials of hydrogen power*. http://www.sciencenter.org/climatechange/d/cart_activity_guide_energetic_electrolysis.pdf

Sha, B., Schymanski, E., Ruttkies, C., Cousins, I., & Wang, Z. (2019). Exploring open cheminformatics approach for categorizing per- and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts*, *21*, 1835–1851. https://doi.org/10.1039/C9EM00321E

Shell. (2020). *Leveraging maximum value from polymer processing aids in polyethylene blown film* [Educational White Paper]. https://www.shell.us/business-customers/shell-polymers/polyethylene-film.html

Solvay. (2021a). *PVDF design and processing guide*. https://www.solvay.com/sites/g/files/srpend221/files/2018-08/Solef-PVDF-Design-and-Processing-Guide_EN-v2.7_0.pdf)

Solvay. (2021b). *HalarECTFE design and processing guide®*. https://www.solvay.com/sites/g/files/srpend221/files/2018-07/halar-ectfe-design-and-processing-guide-en.pdf)

Solvay. (2021c). *HalarECTFE®*. https://www.solvay.com/en/brands/halar-ectfe

Solvay. (2022). *Innovating with non-fluorosurfactant technologies*. https://www.solvay.com/en/innovation/science-solutions/pfas

United Nations (UN). (2021). *United Nations sustainable development goals*. https://sdgs.un.org/goals

US Code of Federal Regulations (USCFR). (2022). *21 CFR 177.2600 Rubber articles intended for repeated use*. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-177/subpart-C/section-177.2600

USEPA. (1992). *TCLP toxicity characteristic leaching procedure* (SW-846 Test Method 131). https://www.epa.gov/sites/default/files/2015-12/documents/1311.pdf

USEPA. (1997). *Polymer exemption guidance manual* (EPA-744-B-97-001). June.

USEPA. (2010). *Reviewing new chemicals under the Toxic Substances Control Act (TSCA) EPA's Review Process - Chemical categories used to review new chemicals under TSCA: TSCA New Chemicals Program (NCP) Chemical Categories*. Washington (DC): USEPA Office of Pollution Prevention and Toxics. [cited 2017 July 12]. http://www.epa.gov/oppt/newchems/pubs/npcchemicalcategories.pdf

USEPA. (2012). *Sustainable futures/P2 framework manual*. https://www.epa.gov/sustainable-futures/sustainable-futures-p2-framework-manual

USEPA. (2021a). *PFAS strategic roadmap: EPA's commitments to action 2021—2024*. https://www.epa.gov/system/files/documents/2021-10/pfas-roadmap_final-508.pdf

USEPA. (2021b). *PFAS national testing strategy*. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/national-pfas-testing-strategy#:%7E:text=To%20protect%20human%20health%20and,PFAS%20chemicals%20to%20inform%20future

United States Federal Register (USFR). (1984). *Premanufacture notification for polymers* (Final Rule 49 FR 46066, 1984 Nov 21. FR-2439-1).

United States Federal Register (USFR). (1995). *Premanufacture notification exemptions; Revisions of exemptions for polymers* (Final rule 60 FR 16316. 1995 Mar 29. FRL-4929-8). Fed Regist *60*(60), 16316–16336. Environmental Protection Agency.

United States Federal Register (USFR). (2012). Polymers (b). 40 CFR Sect 723.250.

Van Cleeff, A. (1997). Fluoroelastomers. In J. Schiers (Ed.), *Modern fluoropolymers* (pp. 507–614). John Wiley & Sons.

Wallington, T. J., Andersen, M. P. S., & Nielsen, O. J. (2021). The case for a more precise definition of regulated PFAS. *Environmental Science: Processes & Impacts*, *23*(12), 1834–1838. https://doi.org/10.1039/D1EM00296A

Worm, A. T., & Grootaert, W. (2001). Fluorocarbon elastomers. *Encyclopedia of Polymer Science & Engineering*. Wiley. https://doi.org/10.1002/0471440264.pst137

Yamabe, M., Higaki, H., & Kojima, G. (1984). New fluoropolymer coatings. In G. D. Parfitt & A. V. Pastis (Eds.), *Organic coatings science and technology* (Vol. 7, pp. 25–39). Marcel Dekker.

National PFAS Testing Strategy:
Identification of Candidate Per- and Poly-fluoroalkyl Substances (PFAS) for Testing

October 2021

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National PFAS Testing Strategy:
Identification of Candidate Per- and Poly-fluoroalkyl Substances (PFAS) for Testing

Overview

The Environmental Protection Agency (EPA) needs to evaluate a large number of PFAS for potential human and ecological effects. Most of the hundreds of PFAS currently in commerce have limited or no toxicity data, and if EPA attempts to research them one at a time, it will be impossible for EPA to expeditiously understand, let alone address, the risks these substances may pose to human health and the environment. To address this data gap and fundamentally advance our understanding of these substances, EPA has developed this National PFAS Testing Strategy (Strategy) to deepen understanding of the impacts of PFAS, including potential hazards to human health and the environment. This Strategy will help EPA identify and select PFAS for which the Agency will require testing using Toxic Substances Control Act (TSCA) authorities. The Strategy develops categories of PFAS based on information about similarities in structure, physical-chemical properties, and existing test data on the toxicity of PFAS (both publicly available and submitted to EPA under TSCA). Consideration of the existing toxicity data prior to requiring further testing also ensures adherence to the TSCA goal of reducing animal testing. EPA will use the Strategy to identify important gaps in existing data and to select one or more candidate chemicals within identified categories for additional study. EPA expects to exercise its TSCA section 4 order authority to require PFAS manufacturers to conduct and fund the studies. EPA plans to issue the first round of test orders on selected PFAS by the end of 2021 with additional phases thereafter.

1. Introduction

PFAS are a large class of man-made chemicals that have been manufactured and used in a variety of industries since the 1940s. PFAS have been or are currently being synthesized for a variety of different uses ranging from adhesives, coatings for clothes and furniture, fire-fighting foams, and many others. PFAS are also used in industrial applications and processes, and in the manufacturing of countless other chemicals and products. PFAS have been released into the environment during manufacturing and use in industrial, commercial, and consumer settings. In addition, PFAS and products that contain them are regularly disposed of in landfills and incinerators, which can also lead to the further release of these compounds into the soil, water, and air.

Although certain PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been studied extensively, most PFAS lack data for robustly characterizing their potential toxicity. The information developed on certain PFAS provides evidence that exposure to such PFAS can lead to acute and chronic adverse human health outcomes.

Studies in laboratory animals indicate some PFAS can cause reproductive, developmental, liver, kidney, and immunological toxicity. In addition, exposure to some PFAS produce tumors in laboratory animals. In humans, the most consistent findings from epidemiology studies are increased cholesterol levels among exposed populations, with more limited findings related to infant birth weights, effects on the

immune system, cancer (for PFOA), and thyroid hormone disruption (for PFOS). Some PFAS can cause adverse effects on the respiratory system following acute inhalation exposures.[1]

To address the many of the data gaps associated with PFAS, in Congress included in the 2020 National Defense Authorization Act direction to EPA to develop a process for prioritizing which PFAS or classes of PFAS should be subject to additional research efforts based on potential for human exposure to, toxicity of, and other available information. The EPA has also initiated several regulatory activities aimed at collecting exposure- and toxicity-related information. For example, 175 PFAS have been added to the Toxics Release Inventory (TRI), which requires facilities that manufacture, process, and/or otherwise use these PFAS to report release and other waste management information to EPA. This information can be used to better understand human exposures to these chemicals. In addition, in June 2021, EPA proposed a TSCA section 8 rule that would require manufacturers and importers to report the identify of any PFAS manufactured since January 1, 2011, as well as byproducts from the manufacturing process, categories of use, production volumes, disposal information, worker exposures, and any information concerning environmental and human health effects.[2] EPA has identified at least 1,364 PFAS that would potentially be subject to the proposed rule. Finally, EPA is taking steps to address PFAS in drinking water. Under the Safe Drinking Water Act (SDWA), EPA is considering comments on the Fifth Unregulated Contaminant Monitoring Rule (UCMR 5) and preparing a final rule to collect new data on PFAS in drinking water. These data would improve EPA's understanding of the frequency that 29 PFAS are found in the nation's drinking water systems and at what levels. It would also expand the number of drinking water systems participating in the program. EPA's PFAS Strategic Roadmap explains additional actions the Agency plans to take to address PFAS through 2024.[3]

2. Purpose

This document describes EPA's Strategy for identifying candidate PFAS for which EPA plans to require companies to perform testing using its TSCA section 4 authority. The information derived from testing will be used by the Agency to evaluate of toxicity and risks associated with this large class of chemicals, and could further inform the Agency's future research, monitoring, and regulatory efforts. Given the large number of PFAS to which exposures may have occurred or that are currently ongoing, the Strategy is based on an approach that groups similar PFAS into categories. The categories serve as the basis for both identifying PFAS chemicals for testing as well as allowing EPA to establish toxicity levels for PFAS within the identified categories. Thus, rather than seeking data about each of the thousands of individual PFAS, which would require extensive resources in terms of time, costs, and animals, the Strategy aims to identify a representative substance(s) for each chemical category where categories have been constructed to span the landscape of PFAS of interest.

[1] EPA website for Basic Information on PFAS (accessed October 2021)
[2] TSCA Section 8(a)(7) Reporting and Recordkeeping Requirements for Perfluoroalkyl and Polyfluoroalkyl Substances, 86 FR 33926 (web link)
[3] EPA PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024 (2021)

3. Starting List of PFAS

The starting list of PFAS used in developing this Strategy was assembled using the process described below and illustrated in the first two elements in Figure 1.

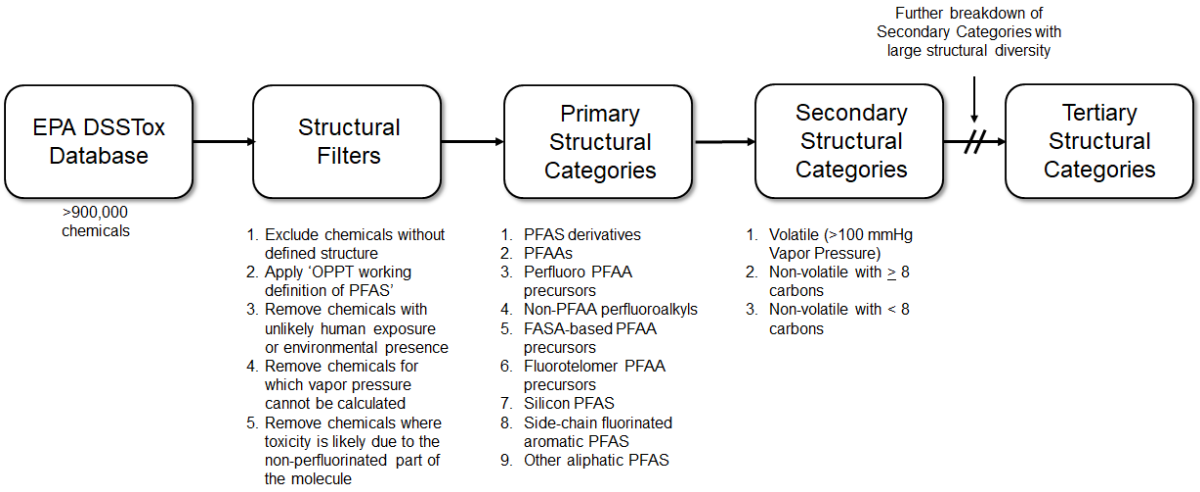

Figure 1: Schematic of Process Used to Create PFAS Categories

In the first step of the process, the EPA DSSTox database was used as the inventory of chemical substances from which the list was drawn (Version – April 2021).[4] The version of the EPA DSSTox database used to assemble the list contains over 900,000 chemical substances.

In the second step of the process, "Structural Filters," EPA used a series of five filters to generate the "starting list" of PFAS considered for the Strategy. First, chemical substances in the database without a defined structure were excluded from consideration because they did not have sufficient information to determine whether they should be considered a PFAS. Second, the resulting chemical substances were filtered for those that met the working definition of a PFAS used by EPA's Office of Pollution Prevention and Toxics (OPPT), which administers TSCA:

> "a structure that contains the unit R-CF_2-CF(R')(R''), where R, R', and R'' do not equal "H" and the carbon-carbon bond is saturated (note: branching, heteroatoms, and cyclic structures are included)."[5]

The working definition identifies chemicals with at least two adjacent carbon atoms, where one carbon is fully fluorinated and the other is at least partially fluorinated. This working definition provides focus on PFAS of concern based on their persistence and potential for presence in the environment and human exposure. For example, chemicals with (-CF2-) that are not (-CF3) are expected to degrade in the environment and most substances with only one terminal carbon (-CF3) are expected to degrade to trifluoroacetic acid, which is a well-studied non-PFAS. Chemicals with such degradation potential and for which vapor pressure could not be calculated were also excluded from the starting list.

[4] Grulke CM, Williams AJ, Thillanadarajah I, Richard AM. EPA's DSSTox database: History of development of a curated chemistry resource supporting computational toxicology research. *Comput Toxicol*. 12:10.1016, 2019.

[5] Ibid TSCA Section 8(a)(7)

In addition, the Strategy focuses on PFAS where the toxicity of the substance is expected to primarily arise from the perfluorinated nature of the compound. As a result, additional filters were applied to develop the starting list. These filters eliminated free radicals and bare anions, while other filters eliminated salt forms where the counterion is expected to exert significant toxicity (e.g., transition metal salts/organometallics) and a variety of ringed structures. Many of the substances removed by the final filter were large multicyclic or macrocyclic structures with a small, fluorinated tail attached at some point.

The five sets of structural filters identified a starting list of 6,504 PFAS used in the development of the Strategy.

4. Dividing PFAS into Categories

Due to the large number and diverse types of PFAS, there have been several efforts to develop systematic terminology for their description and categorization.[6,7] However, the terminology and categories used in these efforts rely on manual assignment by trained chemists using standard criteria, which can be both subjective and time consuming when applied to thousands of chemicals. To overcome these issues, EPA used computer software developed by Su and Rajan[8] to systematically assign the starting list of 6,504 PFAS into the following nine primary categories based on their structure as illustrated in the third element ("Primary Structural Categories") of Figure 1 above:

- PFAS derivatives
- Perfluoroalkyl acids (PFAAs)
- Perfluoro PFAA precursors
- Non-PFAA perfluoroalkyls
- Perfluoroalkane sulfonamide (FASA)-based PFAA precursors
- Fluorotelomer-based PFAA precursors
- Silicon PFAS
- Side-chain Fluorinated Aromatic PFAS
- Other Aliphatic PFAS

PFAS that did not meet the conditions of membership for one of the primary categories listed above based on the structural rules were placed into an additional category denoted as "Others". Substances whose structures could not be resolved by the computer software, such as particular salt forms, were labelled as "Unclassified".

[6] Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P, Jensen, A.A., Kannan, K., Mabury, S.A., and van Leeuwen S.P.J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr Environ Manag.* 7(4):513-541, 2011.

[7] Organization for Economic Cooperation and Development (OECD). Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). 2018. Series on Risk Management, No. 39. ENV/JM/MONO(2018)7.

[8] Su, A., Rajan, K. A database framework for rapid screening of structure-function relationships in PFAS chemistry. *Sci Data* 8:14, 2021.

Each of the primary structural categories were further broken down into one of three secondary categories as illustrated in the fourth element ("Secondary Structural Categories") in Figure 1. The secondary categories include volatiles (>100 mmHg vapor pressure), non-volatiles with ≥8 carbons, and non-volatiles with <8 carbons. These secondary structural categories were employed because historically, changes in the length of the carbon chain have resulted in differences in toxicity and the length of time the chemicals spend in the body. The use of volatility to break down the primary structural categories was important when considering the route of exposure for testing.

Figure 2 below shows a bar graph depicting the number of PFAS within each secondary category that were identified as result of this process. Over 30 percent of the substances in the filtered starting list were assigned to the "Others, gte8" secondary category (gte8 = greater than or equal to 8 carbons). Of the 1,927 PFAS in the "Others, gte8" secondary category, only 29 are "active" in commerce in the United States as determined in recent Active/Inactive reporting required under TSCA at 40 CFR Part 710 (82 FR 37520) (FRL-9964-22).

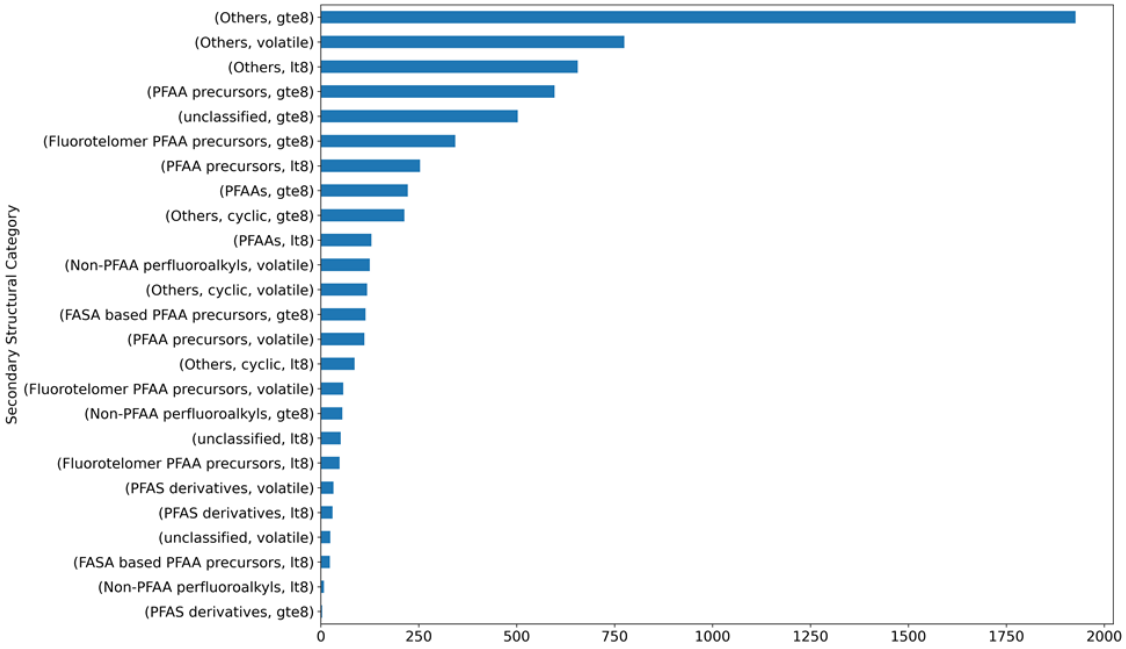

Figure 2: Frequency Plot of Number of Substances by Secondary Category
Key: lt8 = less than 8 carbons; gte8 = greater than or equal to 8 carbons

Since the Strategy is based on an approach that groups similar PFAS into categories based on structure, it is important to evaluate the degree of structural similarity within each category and compare that to similarity across the larger set of PFAS. To achieve this, each PFAS was characterized by a chemical fingerprint[9] [10] that is composed of the various structural features of the molecule. These structural features include the different types and arrangement of elements in the molecule, the bonds that hold

[9] Morgan, H.L. The generation of a unique machine description for chemical structures - A technique developed at Chemical Abstracts Service. *J. Chem. Doc*. 5:107-112, 1965.

[10] Morgan fingerprints are a type of hashed fingerprints. Hashed fingerprints do not require a pre-defined fragment library. Instead, they are generated by enumerating the molecule through all possible fragments that are not larger than a certain size and then converting the fragments into numeric values using a hash function. These numeric values can then be used to indicate bit positions in the hashed fingerprint. Circular fingerprints are generated by considering the 'circular' environment of each atom up to a given radius. The Morgan fingerprints calculated in this study were of length 1024 using a radius of 3.

those elements together, and other features of the chemical. The use of chemical fingerprints allowed for an objective comparison of how similar or different each PFAS is relative to another. When looking at chemical structures chemists often refer to similarity with the concept of structural distance. The smaller the structural distance between two chemicals, the more structurally similar they are. Using the chemical fingerprints, EPA calculated the structural similarity[11] [12] for each possible pair of PFAS on the starting list. This produced a large matrix where the similarity between all PFAS on the starting list could be examined.

To determine which secondary categories needed to be further divided, the structural distances (i.e., the degree of similarity) were calculated both within each secondary category and between categories as illustrated above in the fifth element ("Tertiary Structural Categories") of Figure 1. The rationale behind this approach is that the structural similarity within a category should be greater than the structural similarity between categories. A conceptual schematic of "within" and "between" category distances is provided in Figure 3.

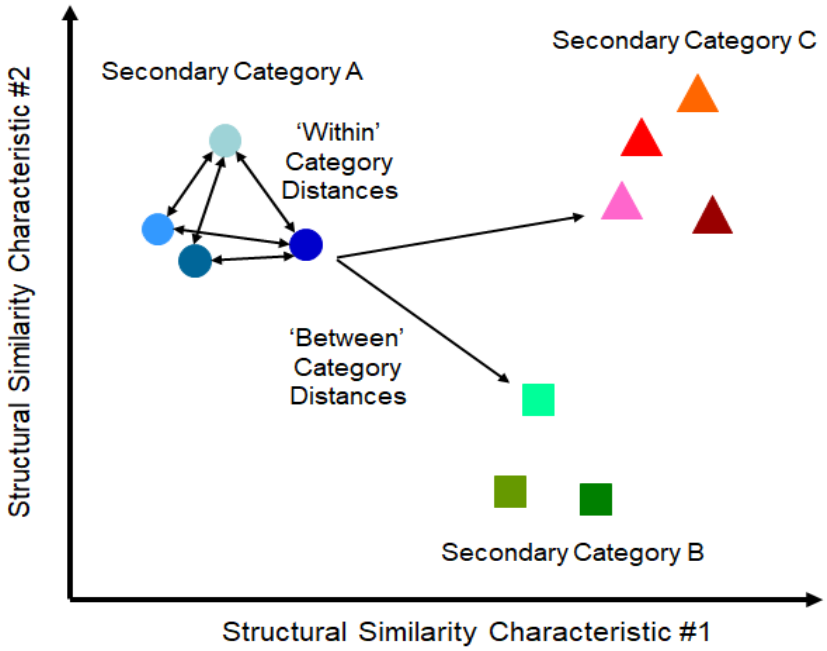

Figure 3. Conceptual Schematic to Illustrate the Within and Between Category Distances for the Secondary Categories

The distributions of the "within" and "between" structural distances among the secondary categories are provided below in Figure 4. A distance threshold for secondary categories that lack adequate structural similarity was set at the lower 5th percentile of the "between" category distribution. Secondary categories exceeding this median distance were further divided into tertiary categories to obtain greater structural similarity. A total of 70 terminal categories were identified (i.e., secondary or tertiary categories with adequate similarity).

[11] Jaccard, P. The distribution of the flora in the alpine zone. *New Phytologist*. 11(2):37–50, 1912.

[12] The Jaccard distance is a unitless number between zero and one that measures how dissimilar two sets (in this case two chemicals) are from one another. A Jaccard distance of zero means the two chemicals are identical, a Jaccard distance of one means the chemicals share nothing in common. In the context of Morgan fingerprints, a Jaccard distance of 0.5 means that half the fingerprint matches between two chemicals while the other half does not match.

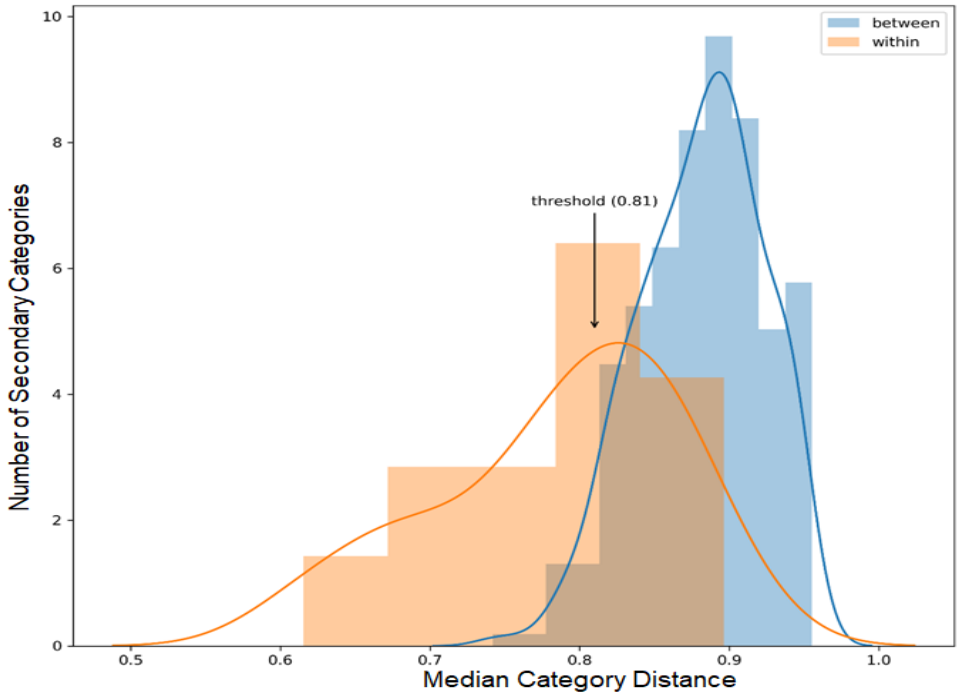

Figure 4: Probability Density Function Plot & Histogram - Within & Between Primary-Secondary Combinations

For each terminal category, EPA calculated the average or "centroid" of all the chemical structural features. The centroid depicts the most representative virtual chemical structure in that category as illustrated below in Figure 5. It may or may not depict an actual PFAS structure. EPA then used the centroids as the conceptual anchor within each terminal category to define a candidate PFAS for testing.

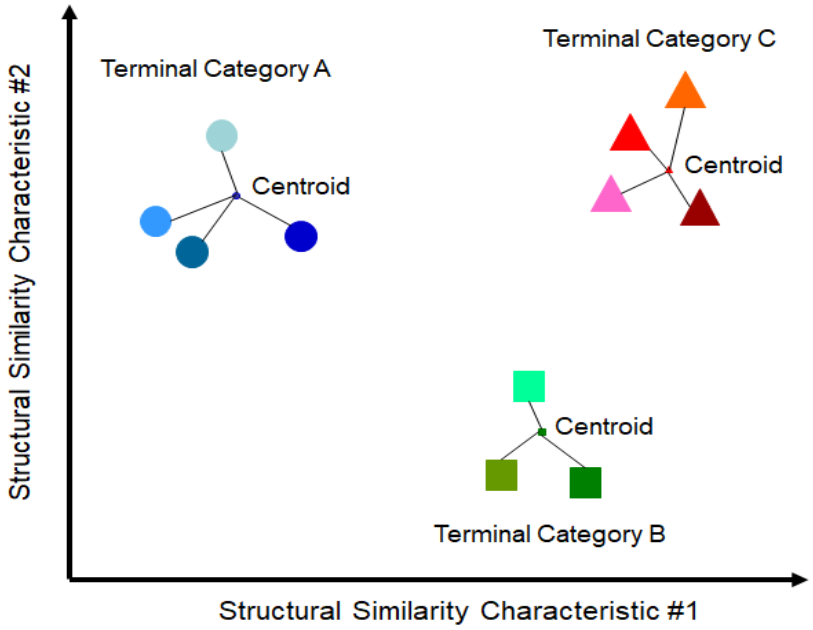

Figure 5: Graphical Illustration of Centroid Concept

5. Assembling Existing Toxicity Data

For each substance on the starting list of PFAS, EPA identified all available, human health-related toxicity studies and divided them into the following study types:

a. Acute
b. Subchronic
c. Chronic including Cancer Bioassays
d. Developmental
e. Reproductive
f. Immunotoxicity
g. Neurotoxicity
h. Toxicokinetics
i. Mutagenicity
j. Sensitization/Irritation

EPA identified toxicity data from two separate sources – the EPA Toxicity Value Database (ToxValDB) and the EPA Chemical Information System (CIS).

The EPA ToxValDB is a compilation of publicly-derived experimental toxicity data on ~34,000 chemicals from 43 distinct sources including US EPA, U.S. Food and Drug Administration (FDA), California Office of Environmental Health Hazard Assessment (OEHHA), Agency for Toxic Substances and Disease Registry (ATSDR), Department of Energy (DOE), California Department of Public Health (DPH), the World Health Organization (WHO), Health Canada, the European Chemicals Agency (ECHA), European Food Standards Agency (EFSA), and the European Commission's Cluster of Systems of Metadata for Official Statistics (COSMOS) database. These sources include toxicity data from the scientific literature, reports, regulatory toxicology study submissions, or government-sponsored studies (e.g., U.S. National Toxicology Program).

The EPA CIS is an internal platform for managing data submissions under TSCA, including toxicity studies. Most of the data within CIS has been provided by industry in conjunction with TSCA submissions and are not publicly available. EPA is working on to make data publicly available to the extent possible under current statutory requirements and given resource constraints.

6. Initial Test Candidate Identification

To identify the initial PFAS candidates for testing, EPA mapped the existing toxicity data from ToxValDB and CIS onto each of the 70 terminal categories. Through this mapping process, EPA identified a total of 56 terminal categories that lack any data about the toxicity of the PFAS in that category. EPA identified PFAS candidates for testing from each of those 56 terminal categories based on the following considerations:

- Whether EPA can identify one or more manufacturer(s) of the PFAS candidate at this time (i.e., EPA can readily and confidently identify recipient(s) for TSCA test orders).[13]

[13] EPA consulted a variety of submissions received pursuant to TSCA (e.g., sections 4, 5 and 8) to identify potential section 4 test order recipients.

- The candidate's structural distance from the centroid of the terminal category (i.e., the closer to the centroid the greater preference for testing).

Of the 56 terminal categories lacking toxicity data, only 24 contained PFAS with an identifiable manufacturer(s) to whom EPA could issue a test order (Appendix A). As a result, EPA will consider the distance from the centroid in selecting PFAS for testing for 24 terminal categories. However, this Strategy is an iterative process and as EPA identifies additional PFAS manufacturers (e.g., through reporting under the future TSCA section 8(a)(7) rule) EPA may expand this initial list of candidate PFAS. Figure 6 below provides an overview of the steps of the process involved in the identification of initial testing candidates.

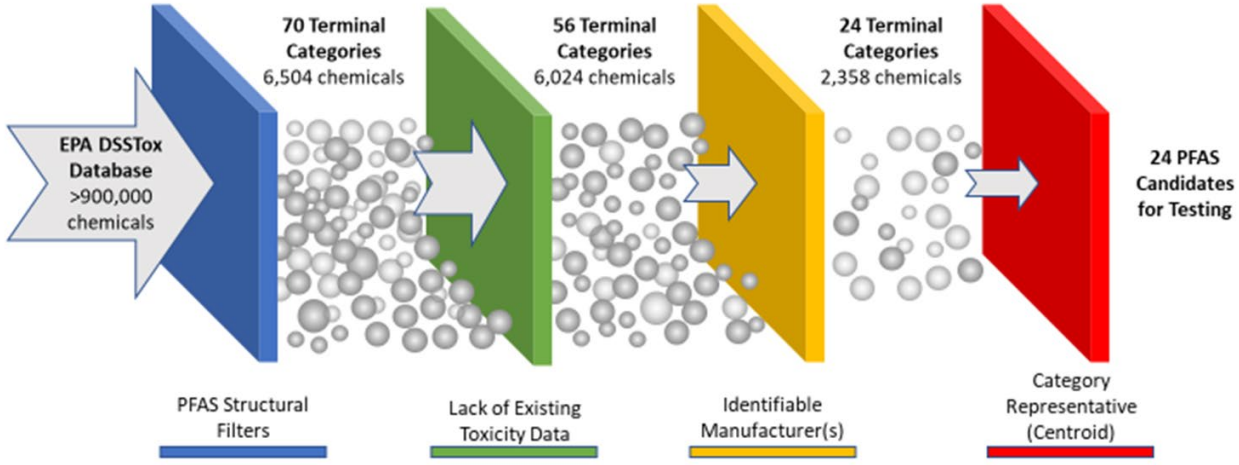

Figure 6: Overview of the Process for Identifying Initial Testing Candidates

7. Potential Tests

EPA's application of the category approach described above is consistent with the statutory mandate to reduce and replace the use of vertebrate animals in the testing of chemicals under section 4(h) of TSCA. The use of a tiered approach to identify specific testing for the candidate PFAS is also consistent with section 4(h) of TSCA.

EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) has developed and uses a variety of test guidelines to support regulatory actions for chemicals under various statutes, including TSCA.[14] These guidelines are extensive and cover a wide array of test endpoints. Other organizations have also developed and utilize similar testing approaches, including the Organization of Economic Cooperation and Development (OECD), which maintains published testing guidelines for evaluating health effects.[15] OECD guidelines are considered routinely by EPA under the OECD mutual acceptance of data (MAD) system.[16] EPA has developed a crosswalk for the OECD guidelines with its own, which also provides a summary of all study types and the organizational codes associated with them.[17] EPA also

[14] EPA web site on Test Guidelines for Pesticides and Toxic Substances (accessed October 2021)

[15] OECD web site on Test Guidelines for Chemicals (accessed October 2021)

[16] OECD web site on Mutual Acceptance of Data (accessed October 2021)

[17] OCSPP list of harmonized test guidelines (last updated September 2019, accessed October 2021)

routinely considers other scientifically relevant information (OSRI) in lieu of testing that is conducted strictly in accordance with test guidelines. OSRI would have to be evaluated by EPA and considered adequate in addressing data needs.

A general overview of the tiered approach is presented below.

Tier I: consists of physical-chemical properties and *in vitro* testing to inform and guide whether additional short-term *in vivo* toxicity and/or toxicokinetic tests should be considered. For instance, PFAS that are gases will generally not be subject to Tier I *in vitro* testing due to methodological limitations and therefore higher tier *in vivo* toxicity testing may be the most logical initial testing approach.

- Physical-chemical property tests: vapor pressure, water solubility, log K_{ow}, particle size and surface tension (measures surfactant properties) to inform the conduct of test guideline protocols (e.g., closed systems for volatile PFAS, relevant route(s) of exposure, etc.).

- *In vitro* metabolism and protein binding studies (e.g., liver metabolism, protein binding and kidney transport protein binding) to inform the need for *in vivo* toxicokinetic studies.

- Some PFAS show positive results for genotoxicity.[18] Therefore, EPA is considering *in vitro* genotoxicity for chromosomal aberrations/gene mutations (e.g., OECD TG 471 and OECD TG 473 or 487) to inform the need for higher-tier *in vivo* toxicity testing for adverse outcomes related to genotoxicity.

- *In vitro* nuclear receptor/activation assays may also be considered because PFAS have been shown to activate multiple nuclear receptors.[19,20] These data can provide insights regarding human relevance (e.g., whether the chemical is active only in the PPARα assay) and inform the need for higher tier *in vivo* toxicity testing (e.g., for cancer and non-cancer endpoints).

Tier II: consists of testing to inform which species and doses to use in Tier III testing. Depending on results of Tier I, and types of toxicities identified for the PFAS categories based on existing available data, Tier II tests may include:

- *In vitro* skin absorption testing (e.g., OECD TG 428) for PFAS that have conditions of use with potential for dermal exposures. Results may also be useful for route-to-route extrapolation, thereby expanding applicability of existing or new higher tier tests.

- *In vivo* genotoxicity testing (e.g., OECD TG 474), depending on the results of Tier I *in vitro* genotoxicity testing.

[18] ATSDR (Agency for Toxic Substances and Disease Registry). 2021. Toxicological Profile for Perfluoroalkyls. U.S. Department of Health and Human Services. May 2021.

[19] Houck, K.A., Patlewicz, G., Richard, A.M., Williams, A.J., Shobair, M.A., Smeltz, M., Clifton, M.S., Wetmore, B.A., Medvedev, A., Makarov, S. Bioactivity profiling of per-and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure. *Toxicology.* 457:152789, 2021.

[20] Ibid, ATSDR.

- Acute *in vivo* inhalation toxicity testing (OECD TG 403), based on Tier I physical-chemical properties testing that indicate potential for surfactant effects.

- *In vivo* toxicokinetic testing in rats and/or mice (OECD TG 417) with evaluation of metabolites. Existing data indicate half-lives and clearance rates may differ significantly among PFAS and species.[21,22] Therefore, this data will inform which species and dosing regimes are most appropriate for higher tier toxicity testing. *In vivo* toxicokinetic testing will be informed by Tier I *in vitro* metabolism and protein binding studies when feasible.

Tier III: consists of testing to identify dose levels (i.e., points of departure) for risk evaluation. Existing data on tested PFAS provide evidence for probable links between PFOA and both kidney and testicular cancer in humans.[23] Other epidemiological studies have identified some associations between PFAS and certain cancers including prostate and breast cancer.[24] Both PFOA and GenX are known to cause tumors in animal studies.[25,26] Based on existing data, PFAS may also cause cancer via a non-genotoxic mechanism. Therefore, EPA will consider systemic toxicity testing that measures adverse endpoints such as liver and kidney disease, immunotoxicity, thyroid function, lipid dysregulation and reproductive and developmental toxicity.[27] The types of effects identified for additional testing may include:

- Testing for cardiac sensitization. Certain terminal categories consisting of short-chain volatile PFAS may be considered for testing for cardiac sensitization[28] because existing data for halogenated hydrocarbons indicate these compounds may lead to cardiac arrythmias and occasionally to sudden death resulting from sensitization of the heart muscle to endogenous compounds in the body (e.g., adrenaline).[29,30]

- 28-day inhalation toxicity test (OECD TG 412). If the Tier II acute inhalation toxicity test shows a toxic dose level (i.e., low observable adverse effect concentration) below the limit dose (< 2,000 mg/m^3), longer duration testing via inhalation route may be considered.

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[21] ibid, ATSDR.

[22] Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M. Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategy for informing future research. *Environ Toxicol Chem*. 40(3):606-630, 2021.

[23] C8 Science Panel web site on the Probable Link Evaluation of Cancer (Created April 2012, accessed October 2021).

[24] ibid ATSDR

[25]United Stated Environmental Protection Agency (EPA). 2016. Health Effects Support Document for Perfluorooctanoic Acid (PFOA). Office of Water. EPA 822-R-16-003. 2016.

[26] United States Environmental Protection Agency (EPA). Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). Public Comment Draft. Office of Water. EPA-823-P-18-001. 2018.

[27] ibid, ATSDR & ibid Fenton

[28] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Evaluation of cardiac sensitization test methods. Technical Report No. 105, 2009.

[29] Brock, W.J., Rusch, G.M., Trochimowicz, H.J. Cardiac sensitization: Methodology and interpretation in risk assessment. *Regul Toxicol Pharmacol.* 38(1):78-90, 2003.

[30] Himmel, H.M. Mechanisms involved in cardiac sensitization of volatile anaesthetics: General applicability to halogenated hydrocarbons? *Crit Rev Toxicol.* 38(9):773-803, 2008.

- 28- or 90-day toxicity testing (OECD TG 407 or 408) may be included in Tier III because some PFAS have shown immunotoxicity, liver and kidney disease, thyroid function, lipid dysregulation in previous studies.[31]

- Prenatal developmental toxicity testing (OECD TG 414) may be included in Tier III because some PFAS have shown delayed ossification and other developmental effects in previous studies.[32]

- Extended one-generation reproductive toxicity testing (OECD TG 443) may be included in Tier III because some PFAS have shown postnatal toxicological effects, including delays in sexual maturation and growth, other developmental delays, and mortality.[33] The extended one-generation reproductive toxicity test can also address concerns related to potential maternal, fetal, and postnatal thyroid hormone disruption as well as includes options for evaluating developmental toxicity and developmental immunotoxicity, which are effects identified in animal and epidemiological studies for some PFAS.[34]

- Carcinogenicity testing (OECD TG 451) may be included in Tier III because some PFAS have produced tumors in animals and have been associated with cancer in humans. The need for carcinogenicity testing will informed by physical-chemical properties, Tier I testing, and existing data. For example, the reactivity, ability to cause glutathione depletion, genotoxicity, *in vitro* nuclear receptor assays, and the results from shorter-duration *in vivo* toxicity studies will be considered holistically in a weight of evidence to inform the need for carcinogenicity testing.

The tiered-testing approach of this Strategy aims to first and foremost collect information for each candidate PFAS that is sufficient to estimate or predict the physical-chemical properties and toxicity of other PFAS in the associated category. EPA anticipates that collecting this information will inform whether refinements to the category may be needed and determine whether testing additional PFAS within a category may be necessary. For example, similarities, differences, or trends in testing results across categories may indicate that further dividing the terminal categories is justified. As EPA obtains data for the candidate PFAS throughout the testing process, the agency may use those results to revise the testing strategy.

8. Phased Implementation

EPA intends to implement the Strategy in Phases (Figure 7). Phase IA is focused on human health data collection. EPA will be initiate Phase IA by the end of 2021 using TSCA Section 4 authorities. Then, in Phase IB, EPA will refine the initial structural categories using mechanistic and toxicokinetic data from EPA Office of Research and Development (ORD) as well as further evaluation of degradation products and exposure data (e.g., environmental monitoring, biomonitoring). The EPA expects to issue further TSCA Test Orders after the categories are refined. The process for refining and issuing Test Orders will be an iterative process as testing data is submitted to the Agency. In the second Phase of the Strategy (Phases IIA and IIB), EPA expects to use the category approach described above to inform ecological toxicity testing needs.

[31] ibid ATSDR & ibid Fenton

[32] ibid ATSDR

[33] ibid ATSDR

[34] ibid ATSDR & ibid Fenton

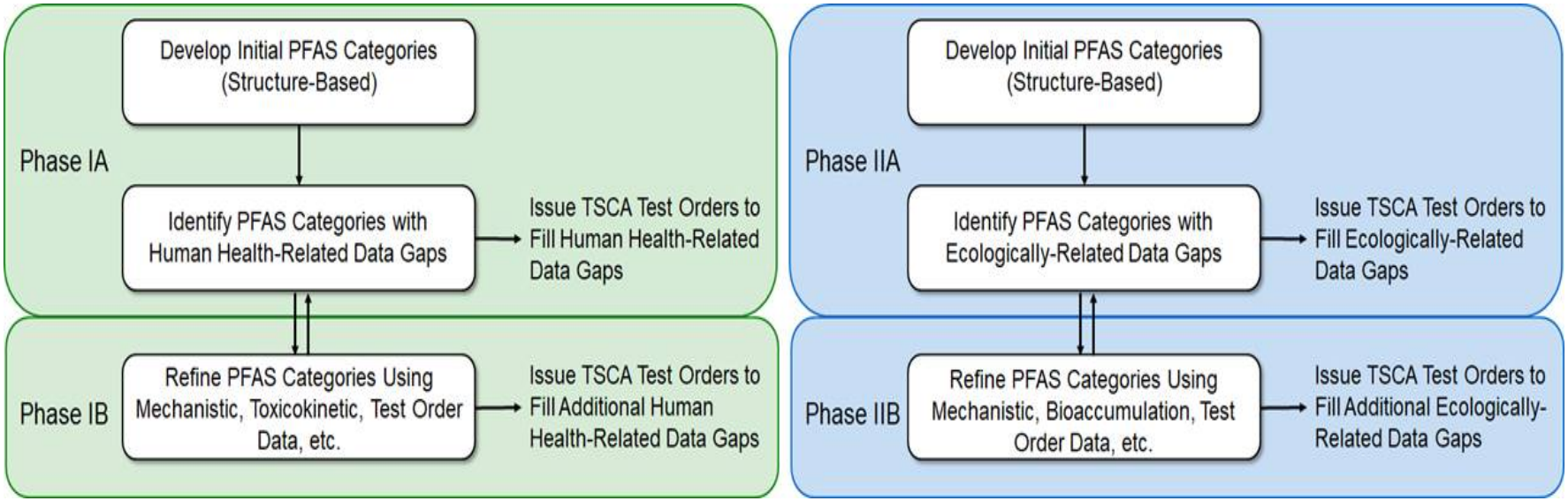

Figure 7: Multi-phase Testing Strategy for Filling Human Health and Ecological Data Gaps. Phases IA and IB are highlighted in green and are focused on human health-related data. Phases IIA and IIB are highlighted in blue and are focused on ecologically-related data.

Appendix A: List of PFAS Candidates for Testing

DTXSID_hyperlink	CASRN	Terminal Category	Candidate PFAS Name
DTXSID4059966	422-05-9	('Fluorotelomer PFAA precursors', 'lt8')	2:1 Fluorotelomer alcohol
DTXSID0046511	306-94-5	('Non-PFAA perfluoroalkyls', 'gte8')	Perflunafene
DTXSID9041811	115-25-3	('Non-PFAA perfluoroalkyls', 'volatile')	Octafluorocyclobutane
DTXSID7046548	355-42-0	('Non-PFAA perfluoroalkyls', 'volatile')	Perfluorohexane
DTXSID50880192	3330-14-1	('Others', 'gte8')	2H-Perfluoro-5-methyl-3,6-dioxanonane
DTXSID60862823	2062-98-8	('Others', 'lt8')	Perfluoro(2-methyl-3-oxahexanoyl) fluoride
DTXSID0059879	355-80-6	('Others', 'lt8')	1H,1H,5H-Perfluoropentanol
DTXSID2067327	27619-88-1	('Others', 'lt8')	3,3,4,4,5,5,6,6,6-Nonafluorohexane-1-sulphonyl chloride
DTXSID3059927	376-90-9	('Others', 'lt8')	Hexafluoroamylene glycol
DTXSID50862736	1682-78-6	('Others', 'volatile')	2,3,3,3-Tetrafluoro-2-(perfluoroethoxy)propanoyl fluoride
DTXSID0061826	1623-05-8	('Others', 'volatile')	Perfluoropropyl trifluorovinyl ether
DTXSID90505110	42532-60-5	('Others', 'volatile')	2,3,3,3-Tetrafluoro-2-(trifluoromethyl)propanenitrile
DTXSID30889183	475678-78-5	('Others, cyclic', 'gte8')	3-Methyl-3-[[(3,3,4,4,5,5,6,6,6-nonafluorohexyl)oxy]methyl]-oxetane
DTXSID30880413	38565-52-5	('Others, cyclic', 'gte8')	3-(Perfluorohexyl)-1,2-epoxypropane
DTXSID7059933	382-28-5	('Others, cyclic', 'lt8')	Perfluoro(N-methylmorpholine)
DTXSID6029177	428-59-1	('Others, cyclic', 'volatile')	Trifluoro(trifluoromethyl)oxirane
DTXSID50880218	15290-77-4	('Others, cyclic', 'volatile')	1H,1H,2H-Perfluorocyclopentane
DTXSID5027140	307-35-7	('PFAA precursors', 'gte8')	Perfluorooctanesulfonyl fluoride
DTXSID70887648	69116-72-9	('PFAA precursors', 'lt8')	Methyl perfluoro-3-[(perfluoro-3-oxopropan-2-yl)oxy]propanoate
DTXSID3044596	16090-14-5	('PFAA precursors', 'lt8')	Perfluoro(4-methyl-3,6-dioxaoct-7-ene)sulfonyl fluoride
DTXSID0047583	423-39-2	('PFAA precursors', 'volatile')	Nonafluoro-1-iodobutane
DTXSID20861913	375-72-4	('PFAA precursors', 'volatile')	Perfluorobutanesulfonyl fluoride
DTXSID6021377	76-13-1	('PFAS derivatives', 'volatile')	1,1,2-Trichloro-1,2,2-trifluoroethane
DTXSID4041284	34455-29-3	('unclassified', 'gte8')	6:2 Fluorotelomer sulfonamide betaine

Title: Application of a Framework for Grouping and Mixtures Toxicity Assessment of PFAS: A Closer Examination of Dose Additivity Approaches

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Running Head: PFAS mixtures risk assessment

Keywords: PFAS, per- and polyfluoroalkyl substances, mixtures, human health, risk assessment

Abstract: Environmental occurrence and biomonitoring data for per- and polyfluoroalkyl substances (PFAS) demonstrate that humans are exposed to mixtures of PFAS. This paper presents a new and systematic analysis of available PFAS toxicity study data using a tiered mixtures risk assessment framework consistent with U.S. and international mixtures guidance. The lines of evidence presented herein include a critique of whole mixture toxicity studies and analysis of dose-response models based on data from subchronic oral toxicity studies in rats. Based on available data to-date, concentration addition and relative potency factor methods are found to be inappropriate due to differences among sensitive effects and target organ potencies and noncongruent dose-response curves for the same effect endpoints from studies using the same species and protocols. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) lack a single mode of action or molecular initiating event and our evaluation herein shows they also have noncongruent dose-response curves. Dose-response curves for long chain perfluoroalkyl sulfonic acids (PFSAs) also significantly differ in shapes of the curves from short chain PFSAs and perfluoroalkyl carboxylic acids (PFCAs) evaluated, and additional differences are apparent when curves are evaluated based on internal or administered dose. Following well-established guidance, the hazard index (HI) method applied to PFCAs and PFSAs grouped separately is the most appropriate approach for conducting a screening level risk assessment for non-polymeric PFAS mixtures, given the current state-of-the science. A clear presentation of assumptions, uncertainties, and data gaps is needed before dose additivity methods, including HI, are used to support risk management decisions. Adverse outcome pathway(s) and mode(s) of action information for PFOA and PFOS and for other non-polymer PFAS are key data gaps precluding more robust mixtures methods. These findings can guide the prioritization of future studies on single chemical and whole mixture toxicity studies.

1. Introduction

The conventional approach to human health risk assessments of chemicals in the environment involves one-at-a-time evaluations of chemicals. For cumulative risk assessments involving co-exposure to chemical mixtures, often simplifying assumptions are made regarding dose additivity, response additivity, and interactions (e.g., synergism or antagonism). In rare cases, comprehensive evaluations of multiple lines of evidence support quantitative estimates of cumulative risks for broad chemical classes such as total petroleum hydrocarbons, polychlorinated biphenyls, organophosphates, and dioxin-like compounds. A variety of mixtures risk assessment methods and decision frameworks have been developed (reviewed in (Rotter *et al.*, 2018; European Food Safety Authority Scientific Committee *et al.*, 2019)). As discussed by Teuschler (2007), several key questions should be addressed prior to using mixtures risk assessment methods, including: 1) When is it appropriate to generalize and assume dose or response additivity?; 2) What information is needed to determine that two or more chemical components of the mixture share a common mode of action (MoA) or have similarly shaped dose-response curves?; 3) What evidence is needed to estimate the toxicity of the mixture if whole-mixture toxicity study data are lacking?; and 4) How should the fraction of unidentified chemicals that may be present in a mixture be addressed? Many of the common chemical mixtures risk assessment methods involve inferences about responses at relatively low doses, using dose-response information from studies with single components often administered at doses higher than environmentally relevant levels. More complete information on low dose responses is needed to refine quantitative approaches and more fully utilize data from studies with component chemicals. Indeed, data either on the exact mixture of concern, or on a "sufficiently similar" whole mixture are frequently critical data gaps (USEPA, 2000). With improved analytical methods and increasing number of chemicals used in commercial application, methods are needed to address the fraction of a mixture that is composed of chemicals lacking toxicity data, or the fraction of the mixture composed of yet unidentified chemicals that may partly contribute to an observed toxicity. Such is the case for non-polymeric per- and polyfluoroalkyl substances (PFAS).

PFAS are a large and diverse group of chemicals whose exact definition is not agreed upon by experts worldwide. Generally speaking, PFAS can be identified by the presence of at least one fully fluorinated carbon-carbon bond (Buck *et al.*, 2011; Wang *et al.*, 2017). PFAS can be subdivided into two broad classes: polymers and non-polymers. Non-polymeric PFAS are either fully fluorinated (perfluorinated) or partially fluorinated (polyfluorinated). Releases of PFAS from specific manufacturing locations or from the use of aqueous film-forming foam (AFFF) has led to

the presence of a large array of non-polymeric PFAS congeners in the environment (Anderson *et al.*, 2016; Backe *et al.*, 2013; Barzen-Hanson *et al.*, 2017; McCord and Strynar, 2019). Drinking water systems in the U.S. that are impacted by PFAS usually have various non-polymeric PFAS present (Guelfo and Adamson, 2018) and serum analysis of the general population consistently detects several of the persistent perfluoroalkyl acids (PFAAs) (Jain, 2018; CDC, 2019). Thus, there is the potential for humans and ecological receptors to be exposed to an uncertain and complex mixture of non-polymeric PFAS.

Exposure to such mixtures poses technical challenges for assessing the potential for health effects, and regulatory and public health agencies worldwide have disparate strategies for addressing this risk. To date, some regulatory environmental guidance values apply to individual PFAAs, while others are based on the sum of concentrations (i.e., concentration-addition) of multiple PFAAs in drinking water or groundwater (see https://pfas-1.itrcweb.org/fact-sheets/ for an up-to-date list of regulatory values). The U.S. Environmental Protection Agency's (USEPA) current lifetime drinking water health advisory for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) of 70 parts per trillion (ppt) for the sum of their concentrations is perhaps the most relevant example of concentration-addition. The USEPA based this concentration-additivity approach on their determination that the two chemicals not only share similar toxic endpoints (developmental effects), but also have equal oral reference doses, when rounded to one significant figure (USEPA, 2016a, 2016b). State agencies in Connecticut, Massachusetts, and Vermont followed suit by applying a similar assumption of concentration additivity but for a broader suite of compounds, including PFOA, PFOS, perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), and perfluoroheptanoic acid (PFHpA); however, the data supporting the assumption of additivity for this range of compounds were not provided by the State agencies.

In 2017, the Australian Environmental Health Standing Committee and the Food Standards Australia and New Zealand (FSANZ) took the position that although there was insufficient information to establish a guidance level for PFHxS, it was reasonable to use the same value for PFHxS as PFOS because the structures of the two compounds are similar, and there was some evidence of similar potency of PFHxS and PFOS in activating peroxisome proliferator-activated receptor alpha (PPARα) (FSANZ, 2017). However, they did not find sufficient similarity between perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) to support an assumption of concentration-additivity across these two classes of PFAAs. Therefore, in Australia

and New Zealand, PFOS and PFHxS concentrations are summed, while PFOA and PFOS concentrations are not.

The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) applies yet a different approach to address human health risks associated with exposure to mixtures of PFAS. As a matter of policy, ATSDR health guideline values (e.g., Environmental Media Evaluation Guides or EMEGs) are applicable to a single substance (ATSDR, 2005). ATSDR's revised draft toxicological profile for PFAAs concluded that "…although there is some evidence of similar health outcomes for some compounds, there is evidence of qualitative and mechanistic differences" that preclude extrapolating findings across PFAS chemicals (ATSDR, 2018b). ATSDR found the available data on interactions among PFAS chemicals, and between PFAS and other chemicals, to be insufficient to quantitatively evaluate mixtures within a toxicity evaluation. Recent site-specific Health Consultations by the Agency show that they address potential risk associated with exposure to a mixture of PFAAs by using the dose-additivity hazard index (HI) approach (described below) of summing the ratio of each chemical's exposure concentration compared to its health-based criteria (ATSDR, 2020).

To date, Health Canada and the National Institute for Public Health and the Environment in the Netherlands (RIVM) appear to be the only regulatory agencies to have explicitly applied some aspect of a mixtures risk assessment framework to PFAS. Health Canada modeled its framework on mixtures guidance from the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) (Meek *et al.*, 2011; Meek, 2013; WHO, 2017) and determined that a dose-additive HI approach for PFOA and PFOS in drinking water is appropriate for the protection of human health. This conclusion was based on the likelihood of co-exposure and a determination of toxicological similarity (e.g., similar MoAs and toxic effects) for PFOA and PFOS. RIVM, however, derived relative potency factors (RPFs) for 19 PFAAs, including PFOA and PFOS, and selected PFOA as the index chemical to extrapolate to other PFAAs (Zeilmaker *et al.*, 2018). RIVM acknowledges numerous simplifying assumptions and limitations, including: 1) focusing on liver hypertrophy as the basis for comparing each PFAA, even though this is not the most sensitive effect across all of the chemicals studied; 2) extrapolating RPFs from chemicals with a similar carbon chain length for 7 PFAAs with data gaps; 3) assuming that the shapes of the dose-response curves are congruent, such that a constant ratio (calculated from benchmark doses) applies across the entire dose-response curve for each chemical; and 4) additivity cannot be fully verified until additional whole mixture toxicity studies are conducted.

In summary, regulatory approaches to addressing risk associated with exposure to a mixture of PFAS are inconsistent. Scientific-based approaches are necessary, including use of established mixtures risk assessment methods and comprehensive evaluations of available data on individual PFAS and PFAS whole mixtures studies.

2. Methods

Following current USEPA mixtures guidance (USEPA, 2000), we examine the existing non-polymeric PFAS database, including dose-response information, and apply established mixtures risk assessment methods to these data to determine what, if any, mixtures effects may occur, and what mixtures risk assessment approach is appropriate given the available data. Dose-response analysis is incorporated as an additional line of evidence to support grouping of component chemicals as well as to assess if relative potency varies (in terms of proportionality in the response mean and variance) across an environmentally relevant range of exposures.

2.1. Mixtures risk assessment framework

Figure 1 illustrates a three-step decision framework that we applied to evaluate mixtures of non-polymeric PFAS. This framework was adapted from similar component-based mixtures frameworks proposed by USEPA (USEPA, 2000; Teuschler, 2007; USEPA, 2007), WHO/IPCS (Meek *et al.*, 2011; Meek, 2013), EFSA (European Food Safety Authority Scientific Committee *et al.*, 2019), and ATSDR (ATSDR, 2018a). Rotter et al. (Rotter *et al.*, 2018) provides a comprehensive review and comparison of these and many other frameworks applied and adapted by regulatory authorities for use in human health risk assessment.

Key elements of the framework are illustrated in Figure 1 and briefly summarized below.

Step 1 informs the initial list of chemicals that are assigned to an assessment group based on the likelihood that co-exposures may occur. The chemicals can be directly measured in potential exposure media and/or estimated based on models that account for environmental degradation, potential for bioavailability, and frequency and duration of exposure relative to pharmacokinetic properties (e.g., serum elimination half-life). Subsequent steps serve to refine the groupings based on additional lines of evidence.

Step 2 involves an assessment of the toxicological similarity based on MoA, most sensitive effect endpoints, likelihood of interactions, and chemical structure. In general, dose addition should apply when component chemicals share a similar adverse outcome pathway, meaning

there is specific evidence of a common mode of action, or more broad evidence of impairment of the same target organ or biological systems. See the discussion in Adams et al. for a proposed standardized target organ and biological systems framework (Adams *et al.*, 2017). Response addition applies when components act on different systems or produce effects that do not influence each other (i.e., "no-interaction" condition such that the response to the first component is the same whether or not a second component chemical is present) (USEPA, 2000). Dose addition and response addition then represent default approaches for toxicologically similar and toxicologically independent chemicals, respectively (USEPA, 2000).

In addition to grouping chemicals based on toxicological similarity, other considerations proposed by mixtures frameworks include physicochemical similarities (European Food Safety Authority Scientific Committee *et al.*, 2019). This evaluation may result in different subgroups of components such that each subgroup is evaluated as a separate mixture. Each evaluation of the framework requires professional judgment when available information is inconsistent or does not clearly point to a single decision path. Under these conditions, USEPA (USEPA, 2000) recommends that if either a dose- or response-addition method is applied (as outlined in Step 3), caveats regarding assumptions and uncertainties should be clearly communicated. Therefore, for mixtures of diverse compounds, such as can be found with non-polymeric PFAS compounds, it is important that Step 2 include an evaluation of the similarity of chemical structures when considering the use of response addition. This evaluation may result in different subgroups of components such that each subgroup is evaluated as a separate mixture. It should be noted that when there is a common apical endpoint, multiple mixtures may be included into one integrated assessment using probabilistic risk estimates or in a qualitative evaluation.

Step 3 involves the selection of an appropriate mixtures method for chemicals that are grouped together. The concepts that distinguish between dose and response additivity help to guide the computational approaches that are applicable. The original USEPA guidelines for mixtures risk assessment released in 1986 referred to dose addition for nongenotoxic toxicants acting by a similar MoA or affecting common organs, whereas response addition was applied to carcinogenic risk, a risk metric that conveys a probability or likelihood of increased incidence of cancer in a population (USEPA, 1986). Dose addition can be thought of as a condition when components of a mixture act as dilutions of one another (USEPA, 2000; Hertzberg *et al.*, 2013; European Food Safety Authority Scientific Committee *et al.*, 2019). Therefore, a distinguishing factor among methods is how the relative potencies inform the weights applied to each component dose. The term response addition should be interpreted with care because the component

responses themselves are not summed, but rather the probabilities of no response are multiplied and subtracted from 1 in order to represent the concept of independent joint action (USEPA, 2000; European Food Safety Authority Scientific Committee *et al.*, 2019; Meek *et al.*, 2011). Concentration-additivity approach is a special case of mixtures additivity methods that requires an assumption of a common mode of action or toxic effect endpoint and requires that the component chemicals be equipotent across a broad range of environmentally relevant doses. This approach is different from dose-addition methods that apply component-specific weights to the concentrations and is generally not well-supported by available data.

Response Addition: Two mixtures methods are possible when response addition is supported and dose-response data on components are available. Because response addition involves the product of the probabilities of no response, the most basic approach is to select representative dose-response models for each component. For each dose-response model, the concentration of the i^{th} component can be converted to a probability of no-response, $1-p_i$. If a physiologically-based pharmacokinetic (PBPK) model or biologically-based dose response (BBDR) is available, then measures of internal dose can be converted to estimates of human equivalent administered dose, from which $1-p_i$ can then be estimated.

Dose Addition: USEPA (USEPA, 2000; Moody and Field, 2000) and WHO/IPCS (Meek *et al.*, 2011; Meek, 2013) present a tiered approach to selecting an appropriate method given the available toxicity data on individual components (or suitable proxy chemicals) and the level of certainty in key assumptions, summarized in Table 1. The intent of the framework is to promote a sequential and transparent evaluation of multiple lines of evidence, such that each consecutive tier applies a refinement, supported by the data, and a progression from a conservative (health protective) to a more realistic (predictive) quantitative analysis of risk. The candidate methods associated with dose addition are organized in a tiered manner, with increasing tiers generally requiring additional data, but affording greater certainty in the toxicity assessment (Table 1).

Perhaps the most common, and often default, method involves the summation of ratios of doses to chemical-specific reference values – the hazard index (HI) method. The HI approach requires chemical component-specific toxicity values, which limits its application. The HI method scales the potency to each chemical's toxicity value, and usually has been applied to non-cancer endpoints:

$$HI = \sum_{i=1}^{n} HQ_i$$

where,

$$HQ_i = \frac{Dose_i}{RfD_i}$$

and,

HI = hazard index (unitless)

HQ_i = hazard quotient for the i^{th} component chemical (unitless)

$Dose_i$ = average daily dose for the i^{th} component chemical (mg/kg-day)

RfD_i = oral reference dose for the i^{th} component chemical (mg/kg-day); can be any relevant toxicity reference value, such as USEPA reference concentration or ATSDR minimal risk level

The HI method for mixtures is most commonly applied under an assumption of dose additivity among chemicals using measured or estimated concentrations. Mixtures frameworks differ on how to address components with different or multiple effect endpoints and target organ systems. Early guidance from the USEPA suggests that grouping component chemicals based on similar target organs is required for dose addition approaches (USEPA, 2000) and the Agency's guidance for conducting risk assessments at national Superfund sites calls for only considering the possible additivity for chemicals with the same critical target organ (USEPA, 1989). However, more recent guidance from the USEPA is more consistent with other U.S. agencies and international authorities. According to USEPA (USEPA, 2007), EFSA (European Food Safety Authority Scientific Committee *et al.*, 2019) and ATSDR (ATSDR, 2018a), for example, component chemicals may be grouped together in a "Tier 0" (see Table 1) mixtures assessment even if the most sensitive effect target organs are dissimilar, as a preliminary and initial screen. If there is a potential for risk based on the preliminary screening assessment (i.e. if HI>1), refinement should then be made using the "Tier 1 or 2" approaches, including evaluating target organ-specific HIs or using the Target Organ Toxicity Dose (TTD) HI approach. The TTD accommodates the assessment of mixtures whose components may produce toxic effects in common target organs of the same species dependent on exposure level (ATSDR, 2018a). Target organ specific toxicity values (TTDs) are used in dose addition methods, if available, in place of the most sensitive effect toxicity value (e.g., RfD or minimal risk level) if the critical target organs or biological systems differ.

Target organ specific TTD-based HIs are calculated as follows:

$$HI_{renal} = \sum_{i=1}^{n} \frac{Dose_i}{TTD_{i,\,renal}}$$

$$HI_{hepatic} = \sum_{i=1}^{n} \frac{Dose_i}{TTD_{i,hepatic}}$$

where,

HI_{renal} = hazard index for endpoints associated with adverse effects on kidney function

$HI_{hepatic}$ = hazard index for endpoints associated with adverse effects on liver function

$Dose_i$ = average daily dose for the i^{th} component chemical (mg/kg-day)

$TTD_{i,renal}$ = target organ specific toxicity value for renal effects for the i^{th} component chemical (mg/kg-day)

$TTD_{i,hepatic}$ = target organ specific toxicity value for hepatic effects for the i^{th} component chemical (mg/kg-day)

Note: the doses and TTDs should all be for the same species.

USEPA and others acknowledge that the application of HI as a default method without consideration of similarity in target organ likely overestimates the risk (USEPA, 2007). The U.S. National Academy of Sciences supports combining chemicals with different initiating events, MoAs, or target organs when there is a common adverse outcome (for example, phthalate exposure may lead to androgen insensitivity syndrome via different MoAs and target organs (National Research Council, 2008)). Consideration of the adverse outcome pathway (AOP) is an alternative means for grouping chemicals and recent studies have suggested a joint impact of chemicals with different MoAs acting on the same AOP (Conley *et al.*, 2018; Kortenkamp, 2020; Lichtenstein *et al.*, 2020). The calculation of a HI based on HQs derived from toxicity values for different target organs or systems is currently a matter of professional judgement. Importantly, HI is considered a "Tier 0" screening method because it generally does not involve a closer examination of toxic similarity or dose-response relationships among component chemicals. Tier 1 and tier 2 evaluations incorporate additional component-specific information.

The summation of ratios of doses to chemical-specific points of departure (POD_i) is referred to as the point of departure index (PODI) method (European Food Safety Authority Scientific Committee *et al.*, 2019). The PODI is given by:

$$PODI = \sum_{i=1}^{n} \frac{Dose_i}{POD_i}$$

where,

PODI = point of departure index (unitless)

$Dose_i$ = average daily dose for the i^{th} component chemical (mg/kg-day)

POD_i = point of departure dose for the i^{th} component chemical (mg/kg-day)

The PODI is included as a Tier 1 method given that additional dose-response analysis may be required to generate comparable POD metrics (e.g., BMDs that correspond to the same benchmark response level). As a metric of risk, the PODI differs from HI in that chemical-specific uncertainty factors, which are built into the RfD or TTDs, may not be accounted for.

The relative potency scaled to an index chemical "A" is referred to as a relative potency factor (RPF) and is given by:

$$RPF_i = \frac{POD_A}{POD_i}$$

where,

RPF_i = relative potency factor for i^{th} component chemical

POD_i = point of departure dose for i^{th} component chemical

POD_A = point of departure dose for the index chemical "A"

The index chemical is typically the chemical in the group for which the most toxicity information is known, and for PFAAs would likely be PFOA or PFOS. The RPF approach typically requires a significant level of effort regarding evaluation of toxicological similarity (USEPA, 2000; Hertzberg *et al.*, 2013). Furthermore, multiple RPF applications may be used to address different exposures (e.g., routes) and different endpoints, resulting in possibly multiple potential chemical groupings depending on the risk assessment scenario and goals (USEPA, 2000). Once stablished, the RPF is then used to scale the concentrations of component chemicals to estimate an equivalent concentration of the index chemical, as if each component chemical is essentially a dilution of the index chemical (Finney, 1942). From here, the sum of the concentrations is compared with the toxicity reference value of the index chemical, either as a HQ or a margin of exposure (the inverse of the HQ; reviewed in more detail in (Benford *et al.*, 2010)):

$$C_{A*} = \sum_{i=1}^{n} RPF_i \times C_i$$

where,

C_{A^*} = mixture's equivalent concentration, *i.e.* the concentration of index chemical after accounting for all components of the mixture, including the concentration of the index chemical (C_A), which has RPF = 1

C_i = concentration of i^{th} component chemical

RPF_i = relative potency factor for i^{th} component chemical

C_A* may then applied in a standard dose- response assessment and compared with the RfD for the index chemical in order to derive a final HQ for the mixture:

$$HQ = \frac{Dose_{A*}}{RfD_A}$$

In a Tier 1 evaluation, dose-response relationships are closely evaluated to examine and verify assumptions regarding relative potency and interactions across a relevant dose range for each component, which can guide the selection of an appropriate index chemical for a group. The supplement to this manuscript illustrates how graphical tools recommended by USEPA (USEPA, 2000), such as isoboles, can be applied using dose-response data on PFAAs (Supplemental Figure S1). A Tier 2 assessment might involve calculating multiple sets of RPFs for a mixture if there are sufficient data to evaluate multiple sensitive effect endpoints. Multiple RPFs can also be used if there are different exposure route-specific potencies (USEPA, 2000). Given that the rank order of component chemicals in terms of potency can vary across endpoints as well as selected benchmark response levels (due to noncongruent dose-response curves), such an analysis can provide a more comprehensive risk characterization.

PBPK or BBDR models and probabilistic methods may also be applicable under the assumption that the mixture exhibits dose additivity. Instead of using these methods to refine estimates of response for each component (as discussed above for use in response additivity), these methods can be used to refine estimates of component-specific PODs, RfDs, and HQs discussed above as part of a "Tier 3" evaluation (Haddad, 2001; Sarigiannis and Gotti, 2008).

Finally, it should be noted that there are also mixtures risk assessment methods that combine dose addition and response addition into a hybrid "integrated addition" approach for multiple component mixtures (Teuschler *et al.*, 2004; Altenburger *et al.*, 2005; Rider and LeBlanc, 2005; USEPA, 2007; Flippin *et al.*, 2009; Rider *et al.*, 2010). This approach applies concepts of both similar and independent MoA in that when there is a common apical endpoint, the multiple similar groups of component chemicals can have separate dose-additive assessments that are then combined via response additional into overall probabilistic risk estimates.

2.2. PFAS Toxicology literature review and data sources

Relevant PFAS toxicological studies were located via searches of public databases including published peer-reviewed literature and online toxicity data curated by regulatory agencies. Key sources of relevant information included the U.S. National Library of Medicine (NLM) and National Institutes of Health (NIH) PubMed, the Registry of Toxic Effects of Chemical Substances, and the NLM Toxicology Data Network. Primary studies were reviewed, and secondary sources (review papers) on mixtures assessment frameworks were also considered. USEPA and NIH have also run selected PFAS through their Tox21 high throughput assays; data are available via the USEPA Chemistry Dashboard (https://comptox.epa.gov/dashboard).

The U.S. National Toxicology Program (NTP) conducted rodent bioassays and kinetic studies of several PFAAs and, in 2018, released data tables for a suite of 28-day oral gavage studies in which male and female Harlan Sprague-Dawley rats were dosed with PFOS, PFHxS, PFBS, PFDA, PFNA, PFOA, and PFHxA (NTP, 2018a, 2018b). These studies were conducted under standardized conditions and, therefore, provide a useful foundation for comparing dose-response relationships attributable to different chemistries, with minimal confounding due to variability in study designs and testing laboratories.

The USEPA ToxCast Chemical Inventory List (EPAPFASINV) was reviewed to identify all PFAS that have been tested for bioactivity in ToxCast/Tox21 high-throughput assays (https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASINVIVO). As of September 2019, ToxCast data were available for 21 unique CASRNs, including several PFSAs (e.g., PFBS, PFHxS, and PFOS), PFCAs (e.g., PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFUnDA), and fluorotelomers (e.g., 8:2 FTOH and 6:2 FTOH). We identified chemicals by CASRN, and sorted the findings by bioactivity outcome (i.e., “Active” vs. “Inactive”) and intended target family.

2.3. Dose-response evaluation

Benchmark dose (BMD) modeling was conducted using USEPA’s BMD software (BMDS version 2.7) in accordance with USEPA guidance (USEPA, 2012a). For dichotomous datasets (e.g., liver hypertrophy), the benchmark response (BMR) was set to 10% extra risk. For continuous datasets, the BMR was set to either 10% (e.g., decreased body weight) or one control standard deviation (e.g., decreased cholesterol and decreased relative kidney weight) when no sufficient biological basis for setting a BMR was available. Dichotomous datasets were modelled via the gamma, logistic, log-logistic, log-probit, probit, Weibull, and quantal-linear models, while continuous datasets were modelled using the exponential (models 2, 3, 4, and 5), Hill, linear,

polynomial (models 2 and 3), and power models. Model fit was assessed based on an evaluation of multiple criteria, including the *p*-value for goodness-of-fit, the Akaike information criterion (AIC), scaled residuals at doses near the BMD, and visual inspection of the dose-response curves, consistent with USEPA guidance (USEPA, 2012a). Examples of model output are given in the Supplemental Materials and referenced herein.

Data tables from the 2018 NTP 28-day oral gavage studies conducted with Harlan Sprague-Dawley (SD) rats exposed to PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS reporting incidence of hepatocellular hypertrophy, serum cholesterol, relative kidney weight, and body weight for male rats were downloaded from the NTP website and were used for BMD modeling (NTP, 2018b, 2018a). Data for PFBA were obtained from a 28-day oral gavage study conducted with male SD rats (Butenhoff *et al.*, 2012). Data for PFUnA and PFDoA were obtained from 42-day oral gavage studies conducted with SD rats (Kato *et al.*, 2015; Takahashi *et al.*, 2014). Data for 8:2 and 6:2 FTOH were obtained from 90-day oral gavage studies conducted with male SD rats (Ladics *et al.*, 2008; Serex *et al.*, 2014).

BMD modeling based on internal serum levels would be preferable for PFAAs because interpretations of dose-response are less likely to be confounded by differences in chemical- and species-specific kinetics (Vogs *et al.*, 2019). However, serum levels have not been consistently reported, and remain a significant data gap in the available literature when comparing relative potencies of PFAAs. Therefore, most of the BMD modeling reported here was conducted with administered dose. The NTP studies demonstrate an approximately linear relationship between administered dose and internal serum level (24 hours after the final dose) for PFNA, PFDA, and PFOS, slight supralinearity (increasing slope with increasing dose) for PFBS and PFHxA, and sublinearity (plateauing for serum levels) for PFHxS and PFOA at the higher administered doses (Supplemental Figure S2) (NTP, 2018a, 2018b). Nonlinearities may contribute uncertainty in inferences regarding the assessment of groupings of mixtures of PFAAs based on these NTP studies, as illustrated in Section 3.4 below using data on hepatocellular hypertrophy as an example.

3. Results

Specific lines of evidence identified in the mixtures framework are summarized below, including studies on pharmacokinetics (PK), nuclear receptor binding activity, and target organ toxicity.

3.1. Review of whole mixtures studies

There are currently less than a dozen published whole mixture toxicity studies with PFAS, which involve dosing mostly binary combinations (pairs) of PFAAs, largely PFOA and PFOS. The available studies used a variety of methods to evaluate potential interactions. Critique of each study's methods is beyond the scope herein; we report only the author's conclusions. Based on stated conclusions from the limited data available to date, it appears that PFOA and PFOS mixtures have complicated toxicological interactions and there is no consistent finding that supports a single assumption regarding mixture effects (Hu and Hu, 2009; Wei *et al.*, 2009; Carr *et al.*, 2013; Hu *et al.*, 2014; Wolf *et al.*, 2014; Hoover *et al.*, 2019; Ojo *et al.*, 2020). *In vitro* whole mixture studies, while more common, are inconclusive and demonstrate that the differences in study design (e.g., choice of *in vitro* model, chemical mixture, and dose) can affect outcomes from exposure to mixtures of PFAAs. The few studies that have evaluated mixture effects *in vivo* demonstrate that findings for similar combinations of PFAAs vary depending on dose, test organism, and endpoint evaluated. Health Canada (Health Canada, 2018a, 2018b) cites results from a conference abstract (Tatum *et al.*, 2010) in which CD-1 mice were administered binary mixtures of PFOA and PFOS. Health Canada determined this study supports dose additivity for some reproductive and developmental parameters, including maternal weight gain, pup body weight, and maternal and neonatal liver weight. However, for the neonatal mortality endpoint, an antagonistic interaction was observed – the mixture of PFOS and PFOA caused *less* mortality than exposure to component PFAAs alone. This is consistent with a recent study of nine non-polymeric PFAS (5 PFCAs, 3 PFSAs, and 6:2 FTOH) on the behavioral effects of zebrafish larvae across multiple concentration ranges that shows that the mixture was less potent than certain PFAAs alone (Menger *et al.*, 2020). Ding et al. (Ding *et al.*, 2013) evaluated binary mixtures of PFOA and PFOS on zebrafish embryonic development and demonstrated that the interactions changed from additive to synergistic to antagonistic depending on the molar ratios. Yang et al. (Yang *et al.*, 2019) assessed binary mixtures of PFOA and PFOS in aquatic invertebrates (*Daphnia magna*) and report synergistic effects on acute mortality and on some, but not all developmental endpoints. Finally, Flynn et al. (Flynn *et al.*, 2019) dosed larval American bullfrogs with binary mixtures of PFOA and PFOS and report additive, synergistic, or no mixture effects, depending upon the endpoint evaluated and mixture dose. The whole mixture toxicity studies

available to date remain inconsistent (i.e. “Yes/Unknown” in Step 2 of Figure 1). Dose addition assumptions for PFAAs are not yet fully characterized by the available whole mixture toxicity data.

A current critical data gap is mixtures studies with non-polymeric PFAS (not just PFOA and PFOS), using environmentally relevant (i.e., part per trillion) doses and a focus on human relevant endpoints. Research currently funded by the Department of Defense Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP) is investigating effects in amphibians and avian species from exposure to whole mixtures of non-polymeric PFAS in AFFF formulations (see: https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs).

3.2. Elimination kinetics by PFAA carbon chain length

Biomonitoring studies report a wide range of elimination half-lives for PFAAs in humans, however, the pattern of differences between short- and long-chain PFAAs is consistent (Table 2). Human serum elimination rates for short-chain PFAAs (defined as less than or equal to 6 fully fluorinated carbons for PFCAs and 5 fully fluorinated carbons for PFSAs) are relatively rapid, ranging from a few days to several months for PFBA, PFHxA, PFHpA, and PFBS. This is in contrast with long-chain PFAAs such as PFHxS, PFOS, and PFOA that have reported serum elimination half-lives ranging 2.3 to 8.5 years. Similar estimates are not available for several long-chain PFCAs (i.e., PFNA, PFDA, or PFUnA) in human serum, but estimates based on measurements of urine (which reflects renal clearance) also indicate a greater potential for biopersistence of long-chain PFCAs, with half-lives ranging from 1.7 to 12 years. PK studies for 8:2 and 6:2 FTOHs are typically unable to report a half-life given the concentration of test material quickly drops below detection limits due to rapid metabolism to terminal carboxylic acids or other compounds.

While PK parameter estimates from animal and human data may provide one line of evidence to support broad grouping strategies based on chain-length (e.g., group short- and long-chain PFAS separately in Step 1 of Figure 1), kinetics information alone may be of limited utility. Given that human biomonitoring data provide a snapshot in time, or preferably multiple measurements over a time period in the same cohort, a critical simplifying assumption is that exposures to co-occurring chemicals have not changed during the interval between measurements. However, if a primary source has been mitigated, or conversely, if a baseline source continues but is unaccounted for, estimates of kinetic parameters from human data can be highly uncertain. This uncertainty is particularly relevant for short-chain PFAAs that are likely

to exhibit more rapid fluctuations in serum and urine following a change in exposure. Moreover, if exposures to short-chain PFAAs are on-going or of sufficient duration compared to long-chain PFAAs, the internal dose metrics that may lead to a toxic effect are most relevant and critical for risk assessment.

3.3. Relevance of complexity in mode of action to mixtures assessment

An important question in mixtures risk assessment is the extent to which knowledge regarding MoA or AOP is needed to support one or more mixtures methods. Meek (Meek, 2013) states that in the context of mixtures assessment, chemicals can reasonably be assigned to the same assessment group if there is a *biologically plausible* (emphasis added) sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data, a more tractable decision criteria than requiring a full understanding of MoA at the molecular level. For example, the current target lipid model for dose additivity of mixtures of polyaromatic hydrocarbons (PAHs) is based on the idea that PAHs can cause narcosis (disruption of cellular function) through a shared site of action (target lipids) in aquatic organisms (Di Toro *et al.*, 2000; French-McCay, 2002). Similar to the HI approach discussed previously, an assessment of a mixture of PAHs is evaluated by summing the toxic units – chemical-specific ratios of the molar concentration in water divided by the molar concentration that yields 50% mortality (LC_{50}). For PFAAs, Peters and Gonzalez (Peters and Gonzalez, 2011) previously argued that there is compelling evidence that the mechanism for toxicity induced by PFAA exposure is complex, likely mediated by more than one nuclear receptor, and variable for different PFAA compounds.

Although, numerous *in vivo* gene-expression and *in vitro* reporter assay studies have demonstrated that activation of PPARα may be involved in many of the toxicities associated with PFAAs (Rosen, Lee, *et al.*, 2008; Rosen, Abbott, *et al.*, 2008; Wolf *et al.*, 2008, 2012), PPARα does not appear to mediate all of the effects associated with PFAA exposure (see Supplemental Tables S1 and S2). Studies suggest that multiple nuclear receptors likely play a role in mediating the toxicities observed in a single target organ (See also (Elcombe *et al.*, 2010), and reviewed in (Health Canada, 2018b, 2018a). Rosen and colleagues (Rosen, Lee, *et al.*, 2008; Rosen, Abbott, *et al.*, 2008) exposed wild-type and PPARα knockout mice to PFOA and the PPARα agonist WY-14,643 (WY) and measured transcriptional changes in the liver. Although gene expression changes were found to be primarily mediated by PPARα, PFOA also induced a subset of genes involved in xenobiotic metabolism through the nuclear receptor CAR (constitutive activated/androstane receptor). Similarly, NTP (NTP, 2019) also found that a broad suite of PFAAs (i.e., PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS) could induce the

expression of PPARα- and CAR-related genes in the liver, indicating that hepatotoxic effects of PFAAs may be mediated through multiple nuclear receptors. Further demonstrating the complexity of the MoA, experiments conducted with wild-type and PPARα-knockout mice indicate that PFOA, PFNA, and PFOS induce developmental toxicity through different MoAs. For example, PFNA-induced developmental toxicity in wild-type mice is not observed in PPARα-knockout mice, indicating that PFNA may primarily induce developmental toxicity through PPARα (Wolf *et al.*, 2010). Alternatively, PPARα appears to only mediate some of the developmental and reproductive effects associated with PFOA, as gestational exposure to PFOA induces full litter resorptions in wild-type and knockout mice, while other developmental effects are only observed in wild-type mice (Abbott *et al.*, 2007). Finally, gestational exposure to PFOS induced neonatal lethality and delayed eye opening in both wild-type and PPARα-knockout mice, indicating that many developmental effects associated with PFOS are likely mediated through a MoA independent of PPARα (Abbott *et al.*, 2009).

While the aforementioned studies highlight the roles for PPARα and CAR, they do not capture the full suite of nuclear receptors that have been identified as potentially contributing to toxicity associated with PFAAs. In addition to PPARα and CAR, *in vitro* reporter gene studies have demonstrated that PFAAs can bind to and activate the thyroid receptor (Ren *et al.*, 2015), the human pregnane x receptor (PXR) (Zhang *et al.*, 2017), and PPAR gamma (PPARɣ) (Zhang *et al.*, 2014). USEPA's high-throughput Tox21 *in vitro* dataset indicates short- and long-chain PFCAs, PFSAs, and FTOHs can interact with around two dozen different nuclear receptors (Supplemental Table S1). Intriguingly, there are clear chain-length dependent effects. Short-chain PFBS and PFHxA demonstrate relatively low activity, interacting with just 0 to 2 nuclear receptors, while long-chain PFAS can interact with as many as 6 to 16 different nuclear receptors. In addition to interacting with fewer nuclear receptors, short-chain PFCAs and PFSAs also tend to have weaker binding affinity toward many nuclear receptors and proteins (Figure 2 and Supplemental Table S2). For example, PFBS and PFHxA exhibit relatively weak potency to induce thyroid receptor activity with IC_{50} values of >1,000 and >500 μM, respectively, whereas PFOA and PFOS exhibit order-of-magnitude higher potencies with IC_{50} values of 42 and 16 μM, respectively (Ren *et al.*, 2015). This difference in relative potency suggests that the MoA for toxicity of long-chain PFAAs may be different and more complicated. However, generalizations regarding MoA may not apply to chemicals grouped by chain-length alone. For example, short-chain PFHpA (C7) interacts with a similar number of nuclear receptors as PFOA, indicating that PFHpA may have a MoA more like that of PFOA than PFHxA.

Collectively, these results provide a compelling line of evidence to guide a mixtures approach for PFAAs away from the use of the relative potency factor approach (Figure 1, Step 3). No single nuclear receptor or molecular initiating event is likely to be responsible for all of the observed toxicities associated with short- and long-chain PFCAs, PFSAs, and FTOHs. Therefore, it is unlikely that grouping strategies and mixtures methods that focus on a specific nuclear receptor (e.g., PPARα) will be predictive of human risk. Consistent with the mixtures framework (Figure 1, Step 2), given uncertainty in grouping chemistries based on a common MoA, we explored dose-response information for chemicals that share the same effect endpoint (e.g., target organ toxicity).

3.4. Dose-response for hepatocellular hypertrophy

The liver is a well-established target organ for many PFCAs and PFSAs. According to the diagnostic criteria outlined in Hall et al. (Hall *et al.*, 2012), hepatocellular hypertrophy and hepatomegaly is a common non-adverse, adaptive response following activation of nuclear receptors such as PPARα or constitutive androstane receptor. This response should only be considered adverse if it coincides with histopathology (i.e., necrosis or inflammation) or clinical chemistry (e.g., biologically relevant changes in aspartate transaminase, alanine aminotransferase, alkaline phosphatase) that indicates organ damage, both of which sometimes, but not always have been observed following PFAA administration to rodents. Regardless, RIVM recently proposed an RPF approach for assessing PFAA mixture toxicity based upon hepatocellular hypertrophy. To build upon RIVM's work and to investigate the appropriateness of this mixtures approach using a different dataset, we performed BMD modeling on the incidence of hepatocellular hypertrophy observed in male rats orally exposed for 28-days to PFBA, PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS (NTP, 2018a, 2018b; Butenhoff *et al.*, 2012), PFUnDA and PFDoDA for 42-days (Kato *et al.*, 2015; Takahashi *et al.*, 2014) or 6:2 FTOH and 8:2 FTOH for 90-days (Ladics *et al.*, 2008; Serex *et al.*, 2014). The log-logistic model provides an adequate fit for all modelled datasets and results are summarized in Supplemental Table S3. BMDs are lowest for long-chain PFAAs (ranging 0.281 mg/kg-day for PFUnDA to 1.96 mg/kg-day for PFDoDA), compared to short-chain PFAAs (i.e., 121, 97.9, and 392 mg/kg-day for PFBA, PFBS, and PFHxA, respectively) and FTOHs (i.e., 48.9 and 228 mg/kg-day for 8:2 FTOH and 6:2 FTOH, respectively[1]).

[1] For 8:2 FTOH, the incidence of hepatocellular hypertrophy increased from 0% at 25 mg/kg-day (NOEL) to 100% at 125 mg/kg-day (LOEL). Therefore, due to dose spacing there is uncertainty around the shape of the dose-response curve and the BMD estimate.

These results clearly demonstrate short-chain PFAAs (e.g., PFBA, PFHxA, and PFBS) are less potent inducers of hepatocellular hypertrophy than long-chain PFAAs (e.g., PFOA and PFOS), and are approximately equipotent as FTOHs. In partial agreement with RIVM's analysis, shapes of the dose-response curves were similar for short- and long-chain PFCAs, and PFBS (slopes ranged from 12.0 to 18.0; Figure 3A). Geometrically congruent curves may indicate that it is appropriate to group these specific PFAAs for purposes of hepatotoxic risk assessment or if assuming the dose-response curves for liver toxicity are representative for other endpoints (Figure 1, Step 2 – leading to dose addition for selected component chemicals and endpoints). However, a contradictory finding to RIVM is evident from the dose-response curves for PFOS (slope = 4.1) and PFHxS (slope = 4.6), which are approximately congruent with each other, but not with the dose-response curves for PFCAs and PFBS (Figure 3A). This finding indicates that long-chain PFSAs (i.e., PFOS and PFHxS) should not be grouped with short-chain PFSAs (i.e., PFBS) or any PFCAs. This finding contradicts an assumption of dose additivity of PFOA and PFOS currently applied by USEPA and other agencies, since proportional benchmark doses do not occur across the dose ranges associated with adverse effects for either chemical in NTP's 28-day study.

Interestingly, dose-response curves for long-chain PFCAs and PFSAs based upon internal dosimetry (i.e., plasma PFAA level 24 hours after the final administered dose) support a slightly different grouping strategy (see Supplemental Table S3). As can be seen from Figure 3B, the slopes of the internal serum liver-toxicity dose-response curves for PFDA, PFNA, PFOA, and PFHxS are similar and range from 13 to 18, while the slope of the dose-response curve for PFOS is 4.6. Again, this result indicates that PFOS should not be grouped with long-chain PFCAs, while it may be appropriate to group PFHxS with long chain PFCAs based upon internal dosimetry. This also further highlights the need to correlate internal serum levels with a broader range of non-polymeric PFAS and toxicity outcomes.

3.5. Dose-response for effects on serum cholesterol

To date, a biologically plausible MoA has not yet been established to explain how increased exposure to PFAAs could cause an elevation in serum cholesterol levels in humans. The role of PPARα in lipid metabolism is well established and suggests that an inverse relationship with serum cholesterol is more likely. Prolonged activation of PPARα leads to increased lipid metabolism, thereby reducing serum cholesterol levels. This hypothesis is supported by a recent phase I clinical trial with PFOA, which demonstrated that when human serum levels of PFOA are comparable to the relatively high levels achieved in rodent studies, cholesterol levels *decline*

rather than increase (Convertino *et al.*, 2018). In rodent models, exposure to PFAAs tends to reduce total serum cholesterol levels (Supplemental Figure S3; (Kennedy *et al.*, 2004). However, some epidemiology studies suggest individuals with higher serum levels of PFOA, PFOS, PFNA, and PFDA also tend to have higher serum total cholesterol and low-density lipoprotein (LDL)-cholesterol (reviewed in (ATSDR, 2018b)). An explanation for the inconsistency in human and animal data is uncertain.

Regardless of the limitations and uncertainties surrounding serum cholesterol specifically, there is evidence that PFAAs can alter lipid metabolism in humans and animal models. Therefore, we conducted BMD modeling on administered dose and serum total cholesterol levels to understand the potency and dose-response relationship for short- and long-chain PFCAs, PFSAs, and FTOHs. Oral exposure to both short- and long-chain PFCAs for 28-days had a weak effect on total serum cholesterol levels in male rats, and the majority of the data was not amenable to BMD modeling (Supplemental Figure S3, Panels A and B). Alternatively, strong dose-response relationships were observed for all PFSAs, with BMDs of 54.4 mg/kg-day PFBS (exponential model 2/3 with modelled variance), 1.71 mg/kg-day PFHxS (Hill model with constant variance), and 0.0972 mg/kg-day PFOS (exponential model 4 with constant variance) (Supplemental Figure S3, Panel C). Similar to the PFCAs, oral exposure to 8:2 FTOH and 6:2 FTOH for 90-days had minimal impact on total serum cholesterol levels in male rats and the datasets were not amenable to BMD modelling (Supplemental Figure S3, Panel D).

Disparate responses in serum cholesterol following exposure to PFCAs and PFSAs may support separate groupings for PFCAs and PFSAs (Figure 1, Step 3 – chemical groups informed by dose-response analysis). However, in humans, both PFCAs (PFOA, PFNA, PFDA) and PFSAs (PFOS) have been associated with similar impacts on serum cholesterol levels, which conflicts with the modelled rodent dataset. Clearly, additional data are required to better understand the MoA underlying a potential increase in total cholesterol in humans before any conclusions regarding grouping for this endpoint can be made.

3.6. Analysis of chemical structure similarity

Non-polymeric PFAS comprise a large set of chemicals and chemical structures (Buck *et al.*, 2011; Wang *et al.*, 2017; Chelcea *et al.*, 2020). These PFAS encompass a broad range of Markush structures, with a wide array of chemical and physical properties. Moreover, non-polymeric PFAS comprise cationic, anionic, and zwitterionic forms, among others. USEPA has attempted to speciate non-polymeric PFAS based, primarily, on overarching chemical identifiers;

for example, USEPA has reported their speciation efforts for perfluoroalkyl sulfonamides in the ToxPrint chemotype (CT) database (Patlewicz, 2019). Even within a specific group like perfluoroalkyl sulfonamides, there can be a large number of compounds with diverse additional Markush groups, which furthers adds a level of complexity when assessing toxicity and conducting a mixtures risk assessment.

Non-polymeric PFAS can adopt a wide array of different 3-D structures – depending upon chain length – but can also adopt different conformations *in vivo*. This is largely an overlooked area of research for non-polymeric PFAS but could be important when attempting any quantitative structure activity relationship (QSAR) analysis and molecular modeling analysis. Furthermore, the binding affinity, hydrogen bonding, structure orientation, binding kinetics (or lack thereof), can be important when assessing the toxicokinetic/toxicodynamic properties of non-polymeric PFAS. Moreover, even with USEPA's attempt at speciation of non-polymeric PFAS, and grouping PFAS based on a chemical identifier, these specific groups can also encompass a wide array of physical and chemical properties. The speciation into Markush groups, varying chemical and physical properties with each group, and the number of unknowns, further supports the notion that there is currently no support for a simplifying assumption that all non-polymeric PFAS can grouped for purposes of mixtures assessment (Figure 1, Steps 1 and 2).

3.7. Summary

In summary, whole mixtures or binary component mixture studies suggest that dose-additivity assumptions for PFAAs are not yet supported by the available whole mixture toxicity data. Although some non-polymeric PFAS may share similar target organs dependent upon exposure level, the most sensitive effects, as defined by regulatory agencies in the U.S., including developmental endpoints and immune endpoints, are not amenable to in-depth mixtures assessment (Tier 1 or higher in Figure 1, Step 3) and adverse outcome pathways have not been clearly elucidated for multiple PFAAs and the same apical endpoint. Only liver data from animal bioassays are amenable to comparing the shape of dose-response curves across a range of non-polymeric PFAS. For most of the PFAAs, the available data for increased relative kidney weight are not amenable to dose-response modeling (see Supplemental Table S4 for examples of Hill dose-response model parameters for PFNA, PFDoDA, PFBs, PFHxS, and PFOS). Similarly, the NTP datasets for body weight are also not amenable to dose-response analysis for PFBA, PFUnDA, PFDoDA, and PFHxS (see Supplemental Table S5 for examples of power dose-response model parameters for remaining PFAAs). For total cholesterol, with the exception of PFBA, data on PFCAs were not amenable to dose-response modeling; however, differences in

the shapes of the dose-response data presented graphically is illustrative (see Supplemental Figure S3). Developmental and reproductive endpoints have either not been tested across a large enough range of non-polymeric PFAS in similar study designs, have inconsistent endpoints (e.g., reduced body weight versus delayed eye opening) or are actually not appropriate endpoints of concern for some PFAAs such as PFHxA (Iwai *et al.*, 2019). Based on the liver data alone, however, different approaches for grouping for mixtures risk assessment are apparent whether the evaluation is based on internal serum dose (the preferred approach) or based on administered dose. The available data currently suggest that PFOS should not be grouped with long-chain PFCAs for mixtures risk assessment, and some PFCAs (PFOA, PFNA, PFDA) may be grouped together based on similar toxicities and similar dose-response slopes. It is not clear, however, that those same conclusions would hold for different toxicity endpoints, such as effects on development or the immune system.

4. Discussion

Regulatory and public health agencies around the globe are developing and implementing guidance and regulations to address the environmental risks associated with non-polymeric PFAS. Just as the chemical-specific action levels vary greatly, agencies have also addressed the issue of mixtures quite differently. A fundamental data gap is that toxicity values (e.g., oral RfDs) have only been derived for a handful of non-polymeric PFAS (e.g., PFHxA, PFHxS, PFOA, PFOS, PFBS) and clear MoAs or AOPs have not been defined. Furthermore, the best information to support assumptions about mixture toxicity of chemicals with toxicity values would be data on the whole mixture or sufficiently similar mixtures. However, to date, there are currently less than a dozen published whole mixture toxicity studies with PFAS, most of which involve dosing binary combinations of only a few PFAAs, and these data reveal no consistent finding that supports a single interpretation of these data. Therefore, it is yet unclear if mixture effects (dose or response addition) are of concern for exposure to non-polymeric PFAS. Herein, we applied well-established frameworks for assessing risk to a mixture of PFAS when only individual chemical data are available.

The initial step in any mixtures assessment framework involves identifying the subset of chemicals for which co-exposure may be occurring and for which the mixtures risk assessment may be appropriate. For PFAS, this is challenged by the currently available analytical limitations. Nonetheless, empirical environmental sampling data and/or information on non-polymeric PFAS of concern in commercial products can be used to estimate exposure groups. It should be noted

that exposure to polymeric PFAS is unlikely to present a significant human health risk due to their high molecular weight, low absorbance, and low reactivity, which contributes to a general lack of bioavailability (USEPA, 2012b; Henry *et al.*, 2018). Although the clearance or elimination rate of PFAAs with different chain-lengths (i.e., "long-chain" versus "short-chain") has shown to vary dramatically, use of half-life alone is not likely a sufficient discriminator to determine the mixture of concern without additional information about the magnitude and frequency of exposures relative to the half-lives. Together, the relative half-lives and the exposure scenario will determine the internal dose profile of a mixture. If on-going exposures have been mitigated and the purpose of the risk assessment is forward-looking, then grouping non-polymeric PFAS based on their elimination kinetics may be appropriate. However, if exposures are on-going and occur potentially on a daily basis, or if the risk assessment's purpose is to evaluate past risk during on-going co-exposure, kinetic half-life differences are of little relevance given that the various non-polymeric PFAS will likely co-exist *in vivo*.

Secondly, one should assess the toxicological similarity based on MoA and most sensitive effect endpoints (and related AOPs) of the identified components in the mixture. Available data continue to demonstrate that toxicity induced by PFAA exposure may occur across several biological systems and is not mediated by a single nuclear receptor. Our evaluation of USEPA's *in vitro* dataset shows that short- and long-chain PFCAs, PFSAs, and FTOHs can interact with around two dozen different nuclear receptors (Table S1). Therefore, it is unlikely that grouping strategies and mixtures methods that focus on a specific nuclear receptor (e.g., PPARα) will be predictive of human risk to a mixture of non-polymeric PFAS. However, there are clear toxicological similarities based on chain-length, because short-chain PFAAs demonstrate relatively low activity, interacting with 0 to 2 nuclear receptors with weaker binding affinity, while long-chain PFAAs can interact with as many as 6 to 16 different nuclear receptors (Table S1). Thus, if the relevant mixture of concern includes both long- and short-chain PFAS, subdividing the components based on chain-length may make sense given that there are differences in sensitive target organs and PFAS do not appear to act via a similar MoA. Another consideration when assessing candidates for grouping based on similarities in dose-response relationships is to evaluate concentrations expressed on a molar basis (e.g., mol/L), essentially normalizing mass-per-volume (e.g., g/L) by molecular weight (MW) (g/mole). For example, Vogs et al. (Vogs *et al.*, 2019) examined relative potencies of PFOS, PFHxS, PFOA, and PFBS using the zebrafish embryo model and compared POD ratios expressed in terms of molar concentrations of internal and external dose. Normalizing by MW may reduce a source of variability when evaluating the support for dose-additivity assumptions and deriving toxicity weighting factors used to generate a

weighted summation of dose or concentration, as has been effectively demonstrated for PAHs with the toxic unit approach (Di Toro *et al.*, 2000; French-McCay, 2002).

With an unknown MoA and lack of appropriate single molecular target, it is clear that relative potency factor (and toxic equivalency factors) approaches that would group short- and long-chain PFCAS, PFSAs, and FOTHs are not supported by the data. We next explored the dose-response relationships of various non-polymeric PFAS across similar endpoints to assess the applicability of dose- or concentration-additivity or HI methods. In general, dose addition most directly applies when component chemicals act on similar biological systems (e.g., target organs, such as the liver or systems such as the reproductive system) and elicit a common response (USEPA, 2000). To date, hepatocellular hypertrophy and kidney effects remain the only endpoints for which there are similar toxicity data from similar study designs, for multiple non-polymeric PFAS. We conducted BMD modeling on the incidence of hepatocellular hypertrophy observed in male rats orally exposed for 28-days to PFBA, PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS (NTP, 2018a, 2018b; Butenhoff *et al.*, 2012), PFUnDA and PFDoDA for 42-days (Kato *et al.*, 2015; Takahashi *et al.*, 2014) or 6:2 FTOH and 8:2 FTOH for 90-days (Ladics *et al.*, 2008; Serex *et al.*, 2014) to evaluate the potency and dose-response relationships across these non-polymeric PFAS. Our analyses demonstrate that for the PFAS for which we have applicable data, it is evident that these non-polymeric PFAS are not equipotent across a range of doses. Short-chain PFAAs and the FTOHs evaluated are less potent inducers of hepatocellular hypertrophy than long-chain PFAAs. The slopes of the dose-response curves were approximately the same for PFCAs (short- and long-chain) and PFBS. PFOS and PFHxS also exhibited congruent shapes with each other, but not with PFCAs (Figure 3). This finding indicates that long-chain PFSAs (i.e., PFOS and PFHxS) should not be grouped with short-chain PFSAs (i.e., PFBS) or any PFCAs, suggesting that the concentration-addition method used by the USEPA and several state agencies, is not supported by the currently available data. It is unknown how well these conclusions, based on analysis of hepatoxicity in the rat, are applicable across different target organs or in humans. However, EPA and NTP have developed a structurally diverse library of 150 PFAS, which they are testing for hepatotoxicity, immunotoxicity, developmental toxicity, mitochondrial toxicity, developmental neurotoxicity, hepatic clearance, and toxicokinetics in a suite of high-throughput *in vitro* assays (Patlewicz, 2019; Thomas, 2019). By maximizing structural diversity, this research may inform read-across efforts and PFAS grouping strategies to support human health risk assessment.

Until additional data become available, the use of a default screening-level HI method applied to non-cancer endpoints may be the only option for a preliminary mixtures assessment for non-polymeric PFAS for chemicals in the same assessment group, consistent with USEPA, ATSDR, EFSA and WHO guidance and the Health Canada and ATSDR approaches. For demonstration purposes, we developed a hypothetical site mixtures risk assessment for a dataset consisting of a variety of short- and long-chain PFCAs and PFSAs (Tables 3 and 4). Under this hypothetical scenario, most of the individual PFAS concentrations would exceed most of the drinking water screening levels reported by U.S. federal and state agencies. We can compare this outcome with alternative approaches by applying the default risk equation for residential exposure to non-carcinogens in groundwater and the exposure factors for drinking water ingestion rate and body weight to reflect values recommended by USEPA for infant receptors, as the most sensitive receptor for noncarcinogens. In this example, the HQ is less than or equal to 1 for each chemical; however, the HI for all components combined is 1.6 (equal to 2 when rounded to one significant figure), which exceeds a target risk threshold of HI≤1, indicating a need to conduct a refined assessment and the potential for risk (Table 3). If PFCAs and PFSAs are summed separately, neither group would yield a HI greater than 1, suggesting no unacceptable risk. Therefore, the choice of how to combine chemical-specific risk estimates may change the interpretation of risk in this example.

Additionally, a rudimentary example of an RPF calculation is shown (Table 4), using PODs calculated from the 28-day rat study results reported by NTP for liver hypertrophy (NTP, 2018b, 2018a). In this example, the PODs are taken directly from the animal studies (based on a 10% response level), rather than converted to a human-equivalent dose (HED). The RPF method is not fully demonstrated in this example because the predicted mixture response is not estimated from the dose-response curves of the index chemicals (PFOA and PFOS) (USEPA, 2000). Such an approach would require a different method of derivation of the HED than used to calculate the current oral RfDs (i.e., multiplying clearance rate by the average serum level corresponding to a POD) (USEPA, 2016a; 2016b). Note how the RPFs for the short-chain PFAAs (i.e., PFHxA and PFBS) are orders of magnitude lower than their respective index chemicals – PFOA for the PFCAs, and PFOS for the PFSAs. In this example, the final HQs, after summing equivalent concentrations of the index chemicals, are less than or equal to 1 separately and when added together. Each approach has significant limitations, and moreover, the example shows how the decision outcome varies depending on the method selected. Critical data gaps remain, including whole-mixture toxicity tests, evaluations of toxicity across an expanded suite of non-polymeric PFAS and endpoints (including developmental outcomes), and better defined MoAs or AOPs.

Different decisions regarding aggregation of component chemicals of a mixture can lead to different risk assessment conclusions; therefore, transparent discussion of key assumptions, supporting lines of evidence, and their quantitative impacts are necessary if a mixture approach is utilized for PFAS risk assessment.

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References

Abbott, B.D. *et al.* (2009) Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPARα) in the mouse. *Reproductive Toxicology*, **27**, 258–265.

Abbott, B.D. *et al.* (2007) Perfluorooctanoic Acid Induced Developmental Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated Receptor-alpha. *Toxicological Sciences*, **98**, 571–581.

Adams, V.H. *et al.* (2017) Implementation of the basic hazard index screening for health risks associated with simultaneous exposure to multiple chemicals using a standardized target organ and systems framework: Hazard Index Screening Using Standardized TTOS Framework. *Integr Environ Assess Manag*, **13**, 852–860.

Altenburger, R. *et al.* (2005) ALGAL TOXICITY OF NITROBENZENES: COMBINED EFFECT ANALYSIS AS A PHARMACOLOGICAL PROBE FOR SIMILAR MODES OF INTERACTION. *Environ Toxicol Chem*, **24**, 324.

Anderson, R.H. *et al.* (2016) Occurrence of select perfluoroalkyl substances at U.S. Air Force aqueous film-forming foam release sites other than fire-training areas: Field-validation of critical fate and transport properties. *Chemosphere*, **150**, 678–685.

ATSDR (2018a) Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors (Update) Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Helath Service, Atlanta, Georgia.

ATSDR (2020) Health Consultation: Per- and Polyfluoroalkyl Substances (PFAS) in the Pease Tradeport Public Water System EPA PWS ID: 1951020; Pease Air Force Base; Portsmouth, Newington, and Greenland, New Hampshire. EPA Facility ID: NH7570024847 Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Helath Service, Atlanta, Georgia.

ATSDR (2005) Public Health Assessment Guidance Manual (Update) Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Helath Service, Atlanta, Georgia.

ATSDR (2018b) Toxicological Profile for Perfluoroalkyls, Draft for Public Comment Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.

Backe, W.J. *et al.* (2013) Zwitterionic, Cationic, and Anionic Fluorinated Chemicals in Aqueous Film Forming Foam Formulations and Groundwater from U.S. Military Bases by Nonaqueous Large-Volume Injection HPLC-MS/MS. *Environ. Sci. Technol.*, **47**, 5226–5234.

Bartell, S.M. *et al.* (2010) Rate of Decline in Serum PFOA Concentrations after Granular Activated Carbon Filtration at Two Public Water Systems in Ohio and West Virginia. *Environmental Health Perspectives*, **118**, 222–228.

Barzen-Hanson, K.A. *et al.* (2017) Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environ. Sci. Technol.*, **51**, 2047–2057.

Benford, D. *et al.* (2010) Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food and Chemical Toxicology*, **48**, S2–S24.

Buck, R.C. *et al.* (2011) Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr Environ Assess Manag*, **7**, 513–541.

Butenhoff, J.L. *et al.* (2012) Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. *Reproductive Toxicology*, **33**, 513–530.

Carr, C.K. *et al.* (2013) Testing for departures from additivity in mixtures of perfluoroalkyl acids (PFAAs). *Toxicology*, **306**, 169–175.

CDC (2019) Fourth National Report on Human Exposure to Environmental Chemicals Update U.S Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, Georgia.

Chang, S.-C. *et al.* (2008) Comparative Pharmacokinetics of Perfluorobutyrate in Rats, Mice, Monkeys, and Humans and Relevance to Human Exposure via Drinking Water. *Toxicological Sciences*, **104**, 40–53.

Chelcea, I.C. *et al.* (2020) Investigating the OECD database of per- and polyfluoroalkyl substances – chemical variation and applicability of current fate models. *Environ. Chem.*

Conley, J.M. *et al.* (2018) Mixed “Antiandrogenic” Chemicals at Low Individual Doses Produce Reproductive Tract Malformations in the Male Rat. *Toxicological Sciences*, **164**, 166–178.

Convertino, M. *et al.* (2018) Stochastic Pharmacokinetic-Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate (PFOA). *Toxicological Sciences*, **163**, 293–306.

Di Toro, D.M. *et al.* (2000) Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environ Toxicol Chem*, **19**, 1951–1970.

Ding, G. *et al.* (2013) Combined Effects of PFOS and PFOA on Zebrafish (Danio rerio) Embryos. *Arch Environ Contam Toxicol*, **64**, 668–675.

Elcombe, C.R. *et al.* (2010) Hepatocellular hypertrophy and cell proliferation in Sprague–Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. *Arch Toxicol*, **84**, 787–798.

European Food Safety Authority Scientific Committee *et al.* (2019) Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. *17*, **3**, 77.

Finney, D.J. (1942) THE ANALYSIS OF TOXICITY TESTS ON MIXTURES OF POISONS. *Ann Applied Biology*, **29**, 82–94.

Flippin, J.L. *et al.* (2009) Predictive Modeling of a Mixture of Thyroid Hormone Disrupting Chemicals That Affect Production and Clearance of Thyroxine. *Int J Toxicol*, **28**, 368–381.

Flynn, R.W. *et al.* (2019) Acute and chronic effects of perfluoroalkyl substance mixtures on larval American bullfrogs (Rana catesbeiana). *Chemosphere*, **236**, 124350.

French-McCay, D.P. (2002) Development and application of an oil toxicity and exposure model, OilToxEx. *Environ Toxicol Chem*, **21**, 2080–2094.

FSANZ (2017) Hazard assessment report – Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorohexane sulfonate (PFHxS) Food Standards Australia New Zealand.

Guelfo, J.L. and Adamson, D.T. (2018) Evaluation of a national data set for insights into sources, composition, and concentrations of per- and polyfluoroalkyl substances (PFASs) in U.S. drinking water. *Environmental Pollution*, **236**, 505–513.

Haddad, S. (2001) A PBPK Modeling-Based Approach to Account for Interactions in the Health Risk Assessment of Chemical Mixtures. *Toxicological Sciences*, **63**, 125–131.

Hall, A.P. *et al.* (2012) Liver Hypertrophy: A Review of Adaptive (Adverse and Non-adverse) Changes—Conclusions from the 3rd International ESTP Expert Workshop. *Toxicol Pathol*, **40**, 971–994.

Health Canada (2018a) Guidelines for Canadian drinking water quality: guideline technical document : perfluorooctane sulfonate (PFOS). Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Health Canada (2018b) Guidelines for Canadian drinking water quality: guideline technical document : perfluorooctanoic acid (PFOA). Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Health Canada (2019) Summary Table: Health Canada Draft Guidelines, Screening Values and Toxicological Reference Values (TRVs) for Perfluoroalkyl Substances (PFAS) Health Canada.

Henry, B.J. *et al.* (2018) A critical review of the application of polymer of low concern and regulatory criteria to fluoropolymers: Fluoropolymers PLC. *Integr Environ Assess Manag*, **14**, 316–334.

Hertzberg, R.C. *et al.* (2013) A four-step approach to evaluate mixtures for consistency with dose addition. *Toxicology*, **313**, 134–144.

Hoover, G. *et al.* (2019) In vitro and in silico modeling of perfluoroalkyl substances mixture toxicity in an amphibian fibroblast cell line. *Chemosphere*, **233**, 25–33.

Hu, J. *et al.* (2014) Synergistic effects of perfluoroalkyl acids mixtures with J-shaped concentration–responses on viability of a human liver cell line. *Chemosphere*, **96**, 81–88.

Hu, X.-Z. and Hu, D.-C. (2009) Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. *Arch Toxicol*, **83**, 851–861.

Iwai, H. *et al.* (2019) Addendum to Iwai and Hoberman (2014)—Reassessment of Developmental Toxicity of PFHxA in Mice. *Int J Toxicol*, **38**, 183–191.

Jain, R.B. (2018) Time trends over 2003–2014 in the concentrations of selected perfluoroalkyl substances among US adults aged ≥20 years: Interpretational issues. *Science of The Total Environment*, **645**, 946–957.

Kato, H. *et al.* (2015) Repeated dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. *Environmental Toxicology*, **30**, 1244–1263.

Kennedy, G.L. *et al.* (2004) The Toxicology of Perfluorooctanoate. *Critical Reviews in Toxicology*, **34**, 351–384.

Kortenkamp, A. (2020) Which chemicals should be grouped together for mixture risk assessments of male reproductive disorders? *Molecular and Cellular Endocrinology*, **499**, 110581.

Ladics, G.S. *et al.* (2008) 90-Day Oral Gavage Toxicity Study of 8-2 Fluorotelomer Alcohol in Rats. *Drug and Chemical Toxicology*, **31**, 189–216.

Li, Y. *et al.* (2018) Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med*, **75**, 46–51.

Lichtenstein, D. *et al.* (2020) An adverse outcome pathway-based approach to assess steatotic mixture effects of hepatotoxic pesticides in vitro. *Food and Chemical Toxicology*, **139**, 111283.

Luz, A.L. *et al.* (2019) Perfluorohexanoic acid toxicity, part I: Development of a chronic human health toxicity value for use in risk assessment. *Regulatory Toxicology and Pharmacology*, **103**, 41–55.

McCord, J. and Strynar, M. (2019) Identification of Per- and Polyfluoroalkyl Substances in the Cape Fear River by High Resolution Mass Spectrometry and Nontargeted Screening. *Environ. Sci. Technol.*, **53**, 4717–4727.

Meek, M.E. (Bette) (2013) International experience in addressing combined exposures: Increasing the efficiency of assessment. *Toxicology*, **313**, 185–189.

Meek, M.E. (Bette) *et al.* (2011) Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regulatory Toxicology and Pharmacology*, **60**, S1–S14.

Menger, F. *et al.* (2020) Behavioural effects and bioconcentration of per- and polyfluoroalkyl substances (PFASs) in zebrafish (Danio rerio) embryos. *Chemosphere*, **245**, 125573.

Moody, C.A. and Field, J.A. (2000) Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environ. Sci. Technol.*, **34**, 3864–3870.

National Research Council (2008) Phthalates and Cumulative Risk Assessment: The Task Ahead National Academies Press, Washington, D.C.

NTP (2019) NTP Technical Report on the Toxicity Studies of Perfluoroalkyl Carboxylates (Perfluorohexanoic Acid, Perfluorooctanoic Acid, Perfluorononanoic Acid, and Perfluorodecanoic Acid) Administered by Gavage to Sprague Dawley (Hsd:Sprague Dawley SD) Rats National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, North Carolina.

NTP (2018a) TOX-96:Toxicity Report Tables & Curves. Pathology Tables, Survival and Growth Curves from NTP Short-Term Studies U.S Department of Health and Human Services, National Institute of Environmental Health, National Toxicology Program.

NTP (2018b) TOX-97: Toxicity Report Tables & Curves. Pathology Tables, Survival and Growth Curves from NTP Short-Term Studies U.S Department of Health and Human Services, National Institute of Environmental Health, National Toxicology Program.

Ojo, A.F. *et al.* (2020) Combined effects and toxicological interactions of perfluoroalkyl and polyfluoroalkyl substances mixtures in human liver cells (HepG2). *Environmental Pollution*, **263**, 114182.

Olsen, G.W. *et al.* (2009) A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. *Toxicology*, **256**, 65–74.

Olsen, G.W. *et al.* (2007) Half-Life of Serum Elimination of Perfluorooctanesulfonate,Perfluorohexanesulfonate, and Perfluorooctanoate in Retired Fluorochemical Production Workers. *Environmental Health Perspectives*, **115**, 1298–1305.

Patlewicz, G. (2019) Perspectives on the development, evaluation and application of in silico approaches for predicting toxicity (PANWAT).

Peters, J.M. and Gonzalez, F.J. (2011) Why Toxic Equivalency Factors Are Not Suitable for Perfluoroalkyl Chemicals. *Chem. Res. Toxicol.*, **24**, 1601–1609.

Ren, X.-M. *et al.* (2015) Structure–activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Arch Toxicol*, **89**, 233–242.

Rider, C.V. *et al.* (2010) Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. *International Journal of Andrology*, **33**, 443–462.

Rider, C.V. and LeBlanc, G.A. (2005) An Integrated Addition and Interaction Model for Assessing Toxicity of Chemical Mixtures. *Toxicological Sciences*, **87**, 520–528.

Rosen, M.B., Abbott, B.D., *et al.* (2008) Gene Profiling in the Livers of Wild-type and PPARα-Null Mice Exposed to Perfluorooctanoic Acid. *Toxicol Pathol*, **36**, 592–607.

Rosen, M.B., Lee, J.S., *et al.* (2008) Toxicogenomic Dissection of the Perfluorooctanoic Acid Transcript Profile in Mouse Liver: Evidence for the Involvement of Nuclear Receptors PPARα and CAR. *Toxicological Sciences*, **103**, 46–56.

Rotter, S. *et al.* (2018) Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution. *Critical Reviews in Toxicology*, **48**, 796–814.

Russell, M.H. *et al.* (2015) Inhalation and oral toxicokinetics of 6:2 FTOH and its metabolites in mammals. *Chemosphere*, **120**, 328–335.

Sarigiannis, D. and Gotti, A. (2008) Biology-Based Dose-Response Models for Health Risk Assessment of Chemical Mixtures. *Fresenius Environmental Bulletin*, **17**, 1439–1451.

Serex, T. *et al.* (2014) Toxicological evaluation of 6:2 fluorotelomer alcohol. *Toxicology*, **319**, 1–9.

Takahashi, M. *et al.* (2014) Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. *J. Toxicol. Sci.*, **39**, 97–108.

Tatum, K.R. *et al.* (2010) Developmental toxicity of perfluoroalkyl acid mixtures in CD-1 mice.

Teuschler, L. (2007) Deciding which chemical mixtures risk assessment methods work best for what mixtures☆. *Toxicology and Applied Pharmacology*, **223**, 139–147.

Teuschler, L.K. *et al.* (2004) A Feasibility Study of Cumulative Risk Assessment Methods for Drinking Water Disinfection By-Product Mixtures. *Journal of Toxicology and Environmental Health, Part A*, **67**, 755–777.

Thomas, R.S. (2019) A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing. U.S. EPA / ORD.

USEPA (2012a) Benchmark Dose Technical Guidance Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2007) Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document. National Center for Environmental Assessment, United States Environmental Protection Agency, Cincinnati, OH.

USEPA (1986) Guidelines for the Health Risk Assessment of Chemical Mixtures Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2016a) Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) Health and Ecological Criteria Division, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2016b) Health Effects Support Document for Perfluorooctanoic Acid (PFOA) Health and Ecological Criteria Division, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2014) Provisional Peer-Reviewed Toxicty Vlaues for Perfluorobutane Sulfonate (CASRN 375-73-5) and Related COmpound Potassium Perfluorobutane Sulvonate (CASRN 29420-49-3 Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

USEPA (1989) Risk assessment guidance for superfund. Volume I. Human health evaluation manual (Part A). Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

USEPA (2012b) TSCA Work Plan Chemicals: Methods Document Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC.

Vogs, C. *et al.* (2019) Toxicokinetics of Perfluorinated Alkyl Acids Influences Their Toxic Potency in the Zebrafish Embryo (Danio rerio). *Environ. Sci. Technol.*, **53**, 3898–3907.

Wang, Z. *et al.* (2017) A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environ. Sci. Technol.*, **51**, 2508–2518.

Wei, Y. *et al.* (2009) Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (Gobiocypris rarus) using toxicogenomic analysis. *Aquatic Toxicology*, **95**, 27–36.

WHO (2017) Chemical Mixtures in Source Water and Drinking-Water World Health Organization, Geneva.

Wolf, C.J. *et al.* (2008) Activation of Mouse and Human Peroxisome Proliferator–Activated Receptor Alpha by Perfluoroalkyl Acids of Different Functional Groups and Chain Lengths. *Toxicol Sci*, **106**, 162–171.

Wolf, C.J. *et al.* (2012) Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): Further investigation of C4–C12 compounds. *Reproductive Toxicology*, **33**, 546–551.

Wolf, C.J. *et al.* (2010) Developmental Effects of Perfluorononanoic Acid in the Mouse Are Dependent on Peroxisome Proliferator-Activated Receptor-Alpha. *PPAR Research*, **2010**, 1–11.

Wolf, C.J. *et al.* (2014) Evaluating the additivity of perfluoroalkyl acids in binary combinations on peroxisome proliferator-activated receptor-α activation. *Toxicology*, **316**, 43–54.

Yang, H.-B. *et al.* (2019) Antioxidant defence system is responsible for the toxicological interactions of mixtures: A case study on PFOS and PFOA in Daphnia magna. *Science of The Total Environment*, **667**, 435–443.

Zeilmaker, M.J. *et al.* (2018) Mixture exposure to PFAS: A Relative Potency Factor approach. *Gecombineerde blootstelling aan PFAS: een benadering met factoren voor relatieve potentie*.

Zhang, L. *et al.* (2014) Structure-dependent binding and activation of perfluorinated compounds on human peroxisome proliferator-activated receptor γ. *Toxicology and Applied Pharmacology*, **279**, 275–283.

Zhang, Y. *et al.* (2013) Biomonitoring of Perfluoroalkyl Acids in Human Urine and Estimates of Biological Half-Life. *Environ. Sci. Technol.*, **47**, 10619–10627.

Zhang, Y.-M. *et al.* (2017) Poly- and perfluorinated compounds activate human pregnane X receptor. *Toxicology*, **380**, 23–29.

Table 1. Key elements of various tiered methods for mixtures risk assessment.

	Definition	Exposure Assessment	Hazard Assessment	Risk Characterization	Example Mixture Methods
Tier 0	• Minimal data • Simple, semi-quantitative • Conservative point estimates	• Sum concentrations or doses of components • High uncertainty in extrapolation from surrogate(s)	• Dose addition of all components, without refinement • Assume similar MoA, target organ, and/or effect endpoint	• Chemical-specific hazard quotients • To address toxicity data gap, extrapolate from compound with greatest toxicity	• Hazard Index • Target Toxicity Dose – based Hazard Index
Tier 1-2	• Some data gaps for selected chemicals and/or mixture • Quantitative, but with assumptions	• Valid measured and modeled estimates • Real world levels and environmental conditions • Point estimates, some actual data	• Refined potency based on individual PODs (BMDs, NOAELs) • Amenable to grouping by target organ or effect endpoint • Dose-response analysis, evaluation of slopes	• Margin of exposure assessment for individual chemicals and/or mixture by group • Sum RPF-adjusted exposure or dose and divide by toxicity value for index chemical	• POD Index • RPFs
Tier 3	• Reliable data and models to characterize chemicals and mixture • Probabilistic • Multiple lines of evidence for interaction	• Plausible ranges and probability distributions • Data on key constituents of mixture • External and internal dose	• PODs, amenable to grouping by MoA or target organ • PBPK and/or BBDR models predictive of internal dose at relevant exposure levels	• Group by MoA and/or common critical effect • Probabilistic; likelihood that RPF-adjusted exposure or dose exceeds level of concern	• PBPK or BBDR model for constituents and/or mixture • Integration of distributions of exposure and dose response

Notes:

BBDR = biologically based dose-response model; BMD = benchmark dose; MoA = mode of action; NOAEL = no-observed-adverse-effect level; PBPK=physiologically based pharmacokinetic model; POD=point of departure; RPF=relative potency factor

Table 2. Estimates of human serum and urine elimination half-lives of PFAAs.

Half-Life Type	PFAA Group	PFAS	Chain Length	Elimination Half-Life	References
Serum	PFCA	PFBA	C4	2.9 days[1]	(Chang *et al.*, 2008)
		PFHxA	C6	32 days[2]	(Russell *et al.*, 2015)
		PFHpA	C7	70 days[2]	(Russell *et al.*, 2015)
		PFOA	C8	3.5 years[2] to 3.8 years[1]	(Olsen *et al.*, 2007)
				2.3 years[4]	(Bartell *et al.*, 2010)
				2.7 years[3]	(Li *et al.*, 2018)
	PFSA	PFBS	C4	25.8 days[2]	(Olsen *et al.*, 2009)
		PFHxS	C6	7.3 years[2] to 8.5 years[1]	(Olsen *et al.*, 2007)
				5.3 years[3]	(Li *et al.*, 2018)
		PFOS	C8	3.4 years[3]	(Olsen *et al.*, 2007)
				4.8 years[2] to 5.4 years[1]	(Li *et al.*, 2018)
Urinary	PFCA	PFHpA	C7	1.2 - 1.5 years[1]; 0.82 - 1.0 years[2]	(Zhang *et al.*, 2013)
		PFOA	C8	2.1 - 2.6 years[1]; 1.2 - 1.5 years[2]	
		PFNA	C9	2.5 - 4.3 years[1]; 1.7 - 3.2 years[2]	
		PFDA	C10	4.5 - 12 years[1]; 4.0 - 7.1 years[2]	
		PFUnA	C11	4.5 - 12 years[1]; 4.0 - 7.4 years[2]	
	PFSA	PFHxS	C6	7.7 - 35 years[1]; 7.1 - 25 years[2]	
		PFOS	C8	6.2 - 27 years[1]; 5.8 - 18 years[2]	

Notes:

[1]Arithmetic mean; [2]Geometric mean; [3]Assumed to be arithmetic mean, but not stated; [4]Median

Table 3. Hypothetical example illustrating application of the hazard index approach for infants consuming drinking water.

Chemical	**C** (ng/L)	**DW** (L/day)	**BW** (kg)	**EF** (days/year)	Dose[1] (mg/kg-day)	Oral RfD (mg/kg-day)	Critical Effect Target Organ	HQ[2]	Source for RfD
PFNA	11	0.78	15	350	5.5E-07	2E-06	liver	0.3	(Health Canada, 2019)
PFOA	43	0.78	15	350	2.1E-06	2E-05	development	0.1	(USEPA, 2016b)
PFHxA	87	0.78	15	350	4.3E-06	0.25	kidney	0.00002	(Luz *et al.*, 2019)
PFOS	446	0.78	15	350	2.2E-05	2E-05	development	1	(USEPA, 2016a)
PFHxS	92	0.78	15	350	4.6E-06	6E-05	liver	0.1	(Health Canada, 2019)
PFBS	21	0.78	15	350	1.0E-06	2E-03	kidney	0.0007	(USEPA, 2014)
Sum:	700						Sum (Hazard Index):	1.6	

Notes

BW = infant body weight; C = concentration; DW = infant drinking water ingestion rate; EF = exposure frequency; HQ = hazard quotient

[1] Dose = $(C/1x10^6)$ x DW x (EF/365) / BW

[2] HQ = dose / RfD

Table 4. Hypothetical example illustrating application of the RPF approach.

Chemical	**POD Value** (mg/kg-day)[1]	**POD Ratio**[2]	**RPF** (unitless)	**C** (ng/L)	**Equiv. Conc.**[3] (ng/L)	% of Mixture
PFNA	0.528	PFOA/PFNA	2	11	23	34.23%
PFOA	1.08	PFOA/PFOA	1	43	43	65.42%
PFHxA	392	PFOA/PFHxA	0.003	87	0.23	0.35%
			Sum:	141	66	100%
PFOS	0.957	PFOS/PFOS	1	446	446	90.12%
PFHxS	1.77	PFOS/PFHxS	0.5	92	49	9.84%
PFBS	97.9	PFOS/PFBS	0.01	21	0.21	0.04%
			Sum:	559	495	100%

Chemical	**C** (ng/L)	**DW** (L/day)	**BW** (kg)	**EF** (days/year)	**Dose**[4] (mg/kg-day)	**Oral RfD** (mg/kg-day)	**HQ**[5]
$PFOA_{equiv}$	66	0.78	15	350	3.3E-06	2E-05	0.2
$PFOS_{equiv}$	495	0.78	15	350	2.5E-05	2E-05	1

Notes:
BMD = benchmark dose; BW = infant body weight; C = concentration; DW = infant drinking water ingestion rate; EF = exposure frequency; Equiv. Conc. = concentration equivalent to the index chemical; HQ = hazard quotient; POD = point of departure; RPF = relative potency factor

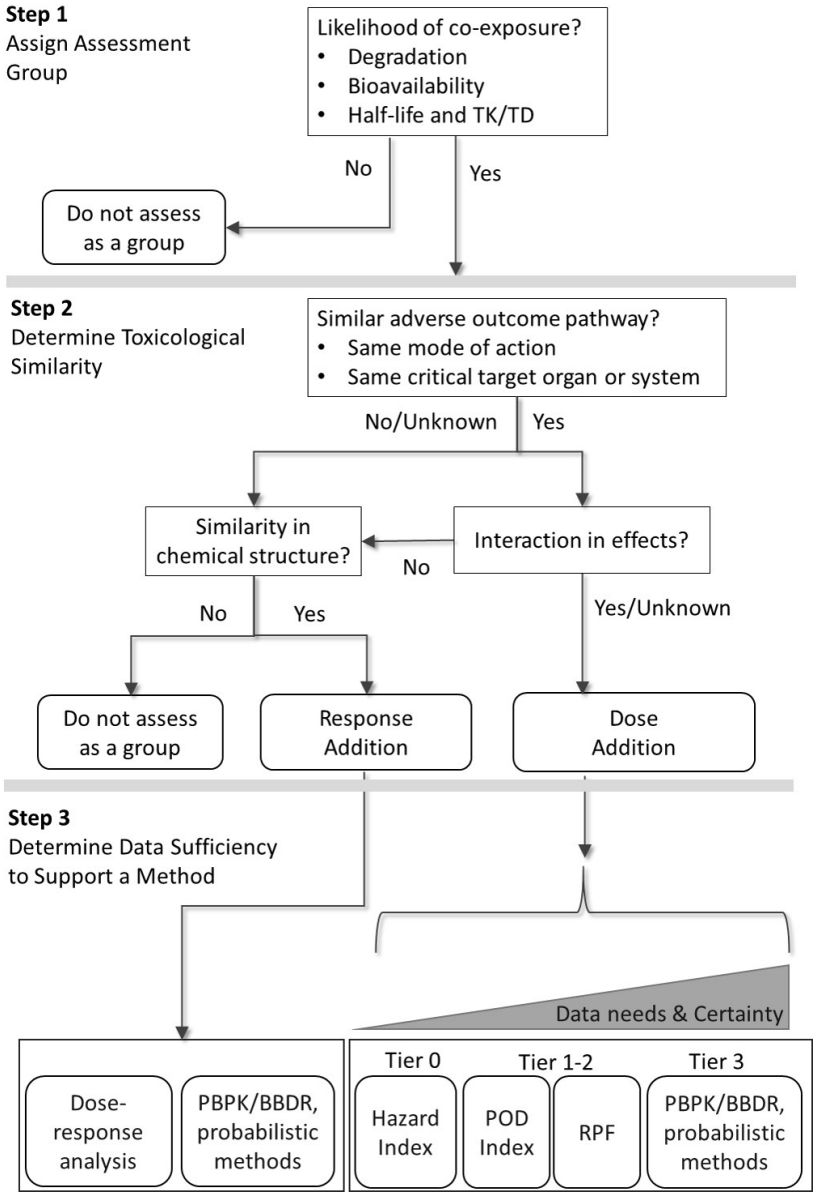
[1] The POD is the BMD calculated for a benchmark response (BMR) of 10% change using the best-fit dose-response model calculated with BMDS. NTP (NTP, 2018a, 2018b) 28-day oral gavage study with rats; liver hypertrophy.
[2] The POD ratio is the BMD of the index chemical (either PFOA or PFOS) divided by the BMD of the chemical of interest.
[3] Equivalent concentration of the index chemical, after adjusting for relative potency. Equiv. Conc. = RPF x C.
[4] Dose = $(C/1x10^6)$ x DW x (EF/365) / BW
[5] HQ = dose / RfD

Figure 1. Decision flow chart illustrating a component-based mixtures risk assessment framework for PFAS. Refer to Table 1 for a summary of key elements of the tiered approach. BBDR = biologically-based dose-response model; PBPK = physiologically-based pharmacokinetic model; POD = point of departure; RPF = relative potency factor; TK/TD = toxicokinetic/toxicodynamic.

Figure 2. Relative potency of PFAAs and FTOHs based on reactivity with various human nuclear receptors, using PFOA as the index chemical. See Supplemental Table S-2 for corresponding tabular summary of binding activity metrics and values.

PPAR-alpha, C_{20max} = human peroxisome proliferator-activated receptor alpha, concentration that produces 20% of the maximal response; PPAR-alpha, AC_{50} = human peroxisome proliferator-activated receptor alpha, half-maximal activity concentration; FABP = human liver fatty acid binding protein; PXR = human pregnane X receptor; TR = human thyroid receptor

Figure 3. Log-logistic dose response curves for incidence of hepatocellular hypertrophy based upon (A) administered dose and (B) internal dose (plasma levels).



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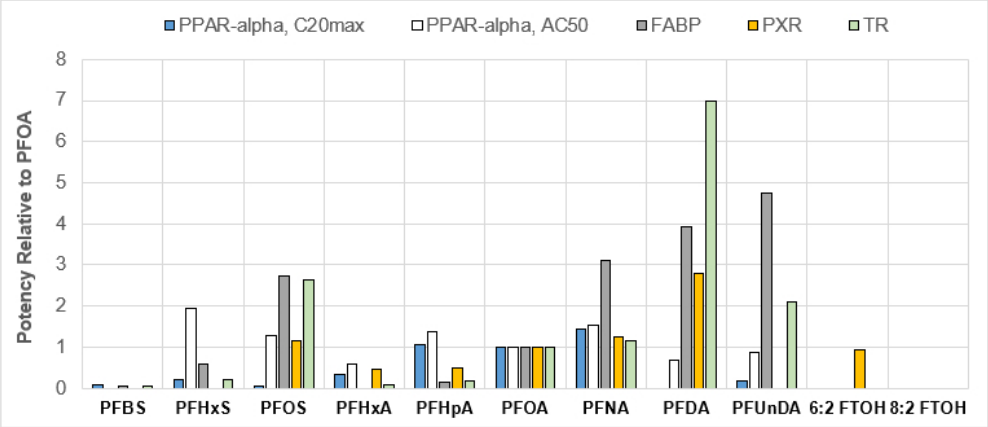

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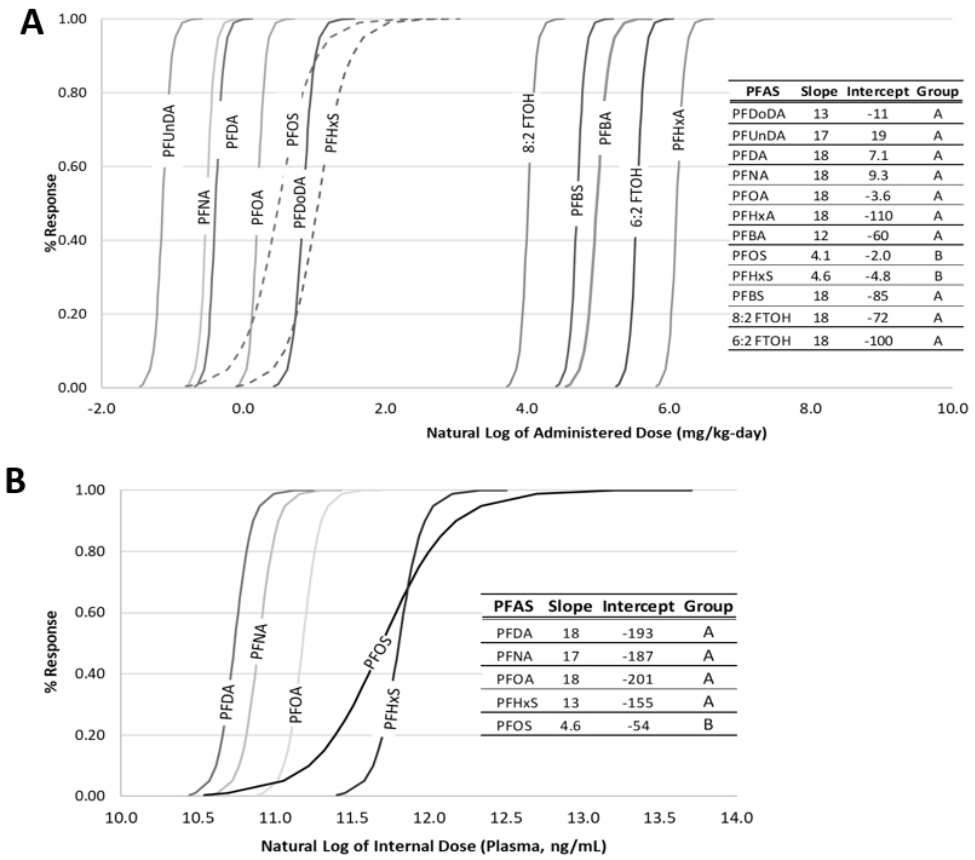

Figure 3. Log-logistic dose response curves for incidence of hepatocellular hypertrophy based upon (A) administered dose and (B) internal dose (plasma levels).

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FINAL
Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts

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Prepared by:

U.S. Environmental Protection Agency
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Acronyms and Abbreviations

3D	Three-dimensional
8-NO2Gua	8-nitroguanine
8-OHdG	8-hydroxydeoxy-guanosine
AASLD	American Association for the Study of Liver Diseases
ABC	ATP Binding Casette
ACG	American College of Gastroenterology
AChE	Acetylcholinesterase
Acot	Acyl-CoA thioesterase
ACOX	Acyl-CoA oxidase
Acsl1	Acyl-CoA synthetase
ADME	Absorption, distribution, metabolism, excretion
AFFF	Aqueous film forming foam
AL	Human-hamster hybrid cells
ALP	Alkaline phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	Alanine aminotransferase
Ap1s1	Adaptor related protein complex 1 subunit sigma 1
APC	Antigen presenting cell
APFO	Ammonium perfluorooctanoate
APOA4	Apolipoprotein A4
apoB	Apolipoprotein B
ApoC-III	Apolipoprotein C-III
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BAFF	B cell activating factor
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
BCRP	Breast cancer resistance protein
BK	Bradykinin
BMD	Benchmark dose
BMD_{10}	Dose corresponding to a 10% change in response
BMDL	Benchmark dose lower limit
$BMDL_{10}$	Dose level corresponding to the 95% lower confidence limit of a 10% change
BMDS	Benchmark Dose Software
BMI	Body mass index
BMR	Benchmark response
BTB	Blood testes barrier
BWT	Birth weight
C3a	Complement 3
C_{last7}	Average concentration over final week of study
CAD	Coronary artery disease
CalEPA	California Environmental Protection Agency
CAR	Constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CAT	Catalase
C_{avg}	Average blood concentration
$C_{avg,pup,gest}$	area under the curve normalized per day during gestation
$C_{avg,pup,gest,lact}$	area under the curve normalized dose per day during gestation/lactation

$C_{avg,pup,lact}$	area under the curve normalized per day during lactation
$C_{avg,pup,total}$	area under the curve in gestation/lactation added to the area under the curve from diet (post-weaning) divided by two years
CCL	Contaminant Candidate List
CCK	Cholecystokinin
CCK-8	Cell Counting Kit-8
CD	Circular dichronism
CDC	Centers for Disease Control and Prevention
cDNA	complementary DNA
Ces	Carboxylesterases
CETP	Cholesteryl ester transfer protein
C-F	Carbon-fluorine
c-fos	Transcription factor complex
CHD	Coronary heart disease
CHF	Congestive heart failure
CHO	Chinese hamster ovary
CHOP	C/EBP homologous protein
CI	Confidence interval
CIMT	Carotid intima-media thickness test
CL_R	Renal clearance
C_{max}	Maximum blood concentration
$C_{max\_pup\_gest}$	Maximum fetal concentration during gestation
$C_{max\_pup\_lact}$	Maximum fetal concentration during lactation
CNS	Central nervous system
Cpt1a	Carnitine palmitoyltransferase 1a
CS	collagen sandwich
CSF	cancer slope factor
CSM	Cholestyramine
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCF	2′,7′-dichlorofluorescein
DCF-DA	Dichlorodihydro-fluorescein diacetate
DDE	Dichlorodiphenyl dichloroethane
DMP	3,5-dimethyl pyrazole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DNMT	Deoxyribonucleic acid methyltransferases
DNP	Dinitrophenyl
dpf	Days post fertilization
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program and Outcomes Study
DWI-BW	Body weight-based drinking water intake
E2	Estradiol
eGFR	Estimated glomerular filtration rate
EPA	Environmental Protection Agency
ER	Endoplasmic reticulum
ER-	Estrogen receptor negative
ETC	Electron transport chain
F_1	First generation
F_2	Second generation
Fabp	Fatty acid binding protein
FACS	Fluorescence activated cell sorting
FeNO	Fractional exhaled nitric oxide
FFA	Free fatty acids
FT4	Free thyroxine
FXR	Farnesoid X receptor

GBCA	Genetic and Biomarker Study for Childhood Asthma
GCL	Glutamate-cysteine ligase
GD	Gestation day
GFR	Glomerular filtration rate
GGT	γ-glutamyltransferase
GM	Geometric mean
GO	Gene Ontology
GSH	Glutathione
GSPE	Grape seed proanthocyandidn extract
GSSG	Glutathione disulfide
GST	Glutathione S-transferases
HAT	Histone acetylase
HAWC	Health Assessment Workplace Collaborative
HDAC	Histone deacetylase
HDL	High-density-lipoprotein
HED	Human equivalent dose
HEK-293	Human embryonic kidney
HERO	Health and Environmental Research Online
HESD	Health Effects Support Document
HFC	7-hydroxytrifluoro-methylcoumarin
HFD	High-fat diet
HFMD	Hand, foot, and mouth disease
HFPO	Hexafluoropropylene oxide
Hib	*Haemophilus influenza* type b
HK	High-molecular-weight kininogen
hL-FABP	Human liver fatty acid binding protein
HMOX	Heme oxygenase
HNF	Hepatocyte nuclear factor
HOME	Health Outcomes and Measures of the Environment
HPA	Hypothalamic-pituitary-adrenal
HR	Hazard ratio
HRL	Health reference level
HSA	Human serum albumin
IARC	International Agency for Research on Cancer
IDL	Intermediate-density lipoprotein
IFN	Interferon
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor 1
IHD	Ischemic heart diseases
IHIC	Hepatic immune cell
IL	Inflammatory cytokine
INMA	Spanish Environment and Childhood (Infancia y Medio Ambiente)
IP	Intraperitoneal
IPCS	International Programme on Chemical Safety
IQR	Interquartile range
IRIS	Integrated Risk Information System
IV	Intravenous
k_{12}	Intercompartment transfer rate
k_a	Absorption rate
K_d	Disassociation constant
K_H	Henry's Law Constant
KK	Kallikrein-kinn system
KLH	Keyhole limpet hemocyanin
$K_{mem/w}$	Membrane/water partition coefficients
K_{oc}	Organic carbon-water partitioning coefficient
K_{ow}	Octanol-water partition coefficient
LBW	Low birth weight

LCM	Liver capsular macrophage
LCT	Leydig cell tumors
LD	Lactation day
LDL	Low-density lipoprotein
L-FABP	Liver fatty acid binding protein
LH	Luteinizing hormone
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
Lpl	Lipoprotein lipase
LTRI	Lower respiratory tract infection
LXR	Liver X receptor
LYZ	Lysozyme
M/P	Milk/plasma
MAIT	Mucosal associated invariant T
MCLG	Maximum Contaminant Level Goal
Me-PFOSA-AcOH or MeFOSAA	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
MDA	Malondialdehyde
MFC	7-methoxy-4-trifluoromethylcoumarin
miRNA or miRs	Microribonucleic acids
MMP	Mitochondrial membrane potential
MMR	Measles, mumps, and rubella
MOA	Mode of action
MOBA	Norwegian Mother, Father, and Child Cohort Study
MRL	Minimum reporting level
mRNA	Messenger ribonucleic acid
MRPs	Multidrug resistance-associated proteins
MS	Multiple sclerosis
MyD	Myeloid differentiation
NAFLD	Non-alcoholic fatty liver disease
NCI	National Cancer Institute
NF- κB	Nuclear factor kappa B
NHANES	National Health and Nutrition Examination Survey
NK	Natural killer
NO	Nitric oxide
NOAEL	No-observed-adverse-effect level
NOD	Nucleotide-binding and oligomerization domain
NOS	Nitric oxide synthase
NP	Niemann-Pick disease
NPDWR	National Primary Drinking Water Regulation
Nrf2	Nuclear factor erythroid 2–related factor 2
NTCP	Sodium-taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OATPs	Organic anion transporting polypeptides
OATs	Organic anion transporters
OCM	Organotypic culture models
OECD	Organisation for Economic Co-operation and Development
OPR	Opioid Receptor
OR	Odds Ratio
ORD	Office of Research and Development
OST	Office of Science and Technology
P_0	Parental generation
p0AL	Mitochondrial deficient cell line
PACT	Pancreatic acinar cell tumors

PAD	Peripheral artery disease
PanIN	Pancreatic intraepithelial neoplasia
PBMC	Peripheral blood mononuclear cells
PBPK	Physiologically-based pharmacokinetic
PC	Partition coefficient
PDCD	Programmed cell death protein
PECO	Populations, Exposures, Comparator, and Outcome
PERK	Protein kinase-like endoplasmic reticulum kinase
PFAA	Perfluoroalkyl acids
PFAS	Per- and polyfluoroalkyl Substances
PFBA	Perfluorobutanoic acid
PFCAs	Perfluoroalkyl carboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane-sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PG	Prostaglandin
P_{ion}	Passive anionic permeability
PK	Pharmacokinetic
pKa	Negative base-10 logarithm of acid dissociation constant
PLCO	Prostate, Lung, Colorectal, and Ovarian Screening Trial
P_{milk}	Maternal milk: blood partition coefficient
PND	Postnatal day
PNW	Postnatal week
POD	Point of departure
POD_{HED}	Point of departure human equivalent dose
POUNDS-Lost	Prevention of Obesity Using Novel Dietary Strategies-Lost
PP2A	Protein phosphatase 2A
PPAR	Peroxisome proliferator activated receptor
PPK	Plasma prekallikrein
ppm	Parts per million
PR-	Progesterone receptor negative
PSA	Prostate-specific antigen
PTB	Preterm birth
PWS	Public water system
PXR	Pregnane X receptor
Q1	Quartile one
Q2	Quartile two
Q3	Quartile three
Q4	Quartile four
QA	Quality assurance
R_0	Baseline risk
r^0_{milk}	Starting milk consumption rate
r^1_{milk}	Week 1 milk consumption rate
r^2_{milk}	Week 2 milk consumption rate
r^3_{milk}	Week 3 milk consumption rate
RARα	Retinoic acid receptor α
RASA3	RAS P21 protein Activator 3
RCC	Renal cell carcinoma
RD	Regular diet
RfD	Reference dose
R_{fm}	Fetus:mother concentration ratio
r^i_{milk}	Milk consumption rate for the i^{th} week of lactation

RNA	Ribonucleic acid
RNS	Reaction nitrogen species
ROS	Reactive oxygen species
RR	Rate ratio
RRBS	Reduced representation bisulfite sequencing
RSC	Relative source contribution
SAB	Science Advisory Board
SBP	Systolic blood pressure
SDWA	Safe Drinking Water Act
SES	Socioeconomic status
SGA	Small for gestational age
SIRT	Sirtuin
slco1d	Solute carrier organic anion transporter
SMR	Standardized mortality ratios
SOD	Superoxide dismutase
SRBC	Sheep red blood cells
SREBP	Sterol regulatory element-binding protein
T1D	Type 1 diabetes
T4	Thyroxine
TC	Total cholesterol
TET	Methylcytosine dioxygenases
tfc	Transcription factor
tgf	Transforming growth factor
TLDA	Taqman low density arrays
TLR	Toll-like receptor
T_{max}	Time to C_{max}
TNF	Tumor necrosis factor
TNP	Trinitrophenyl
TReg	Regulatory T cell
TSCATS	Toxic Substance Control Act Test Submissions
TTEs	Transplacental efficiencies
TTR	Transthyretin
TXB	Thromboxane
UCMR3	Third Unregulated Contaminant Monitoring Rule
UF	Uncertainty factors
UF_A	Interspecies UF
UF_D	Database UF
UF_H	Intraspecies UF
UF_L	LOAEL-to-NOAEL extrapolation UF
UF_S	UF for extrapolation from a subchronic to a chronic exposure duration
UF_C	Composite uncertainty factor
µM	Micromolar
UPR	Unfolded protein response
UV-vis	Ultraviolet-visible
V_d	Volume of distribution
vtg1	Vitellogenin 1
VLDL	Very low-density lipoproteins
Vldlr	Very low-density lipoproteins receptor
WHO	World Health Organization
WoS	Web of Science
WTC	World Trade Center
XBP1	Spliced X box-binding protein 1
ZFL	Zebrafish liver cell line

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final toxicity values for *perfluorooctanoic acid (PFOA), including all isomers and nonmetal salts.* The toxicity assessment for PFOA is a scientific report that describes the evaluation of the available animal toxicity and human epidemiology data in order to characterize noncancer and cancer human health hazards. This assessment also includes f*inal toxicity values* associated with noncancer health effects (i.e., oral reference doses, or RfDs) and cancer effects (i.e., cancer slope factors, or CSFs) following oral PFOA exposure. It is not a risk assessment, as it does not include an exposure assessment or an overall risk characterization nor does it address the legal, policy, social, economic, or technical considerations involved in risk management. The PFOA toxicity assessment can be used by EPA, states, Tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, the potential risk associated with human exposures to PFOA, its isomers, and its nonmetal salts.

This final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) per- and polyfluoroalkyl substances (PFAS) Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 (U.S. EPA, 2022e) as well as feedback from the public comment period (U.S. EPA, 2024c). This final assessment builds upon the literature review presented in the 2016 *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (hereafter referred to as the 2016 PFOA HESD) (U.S. EPA, 2016c) and is an update of the SAB review draft, *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water (U.S. EPA, 2021c)*, and the subsequent *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2022e).

PFOA and its related salts are members of the PFAS group. These manufactured chemicals have a history of industrial and consumer use in the United States and are considered persistent chemicals based on their physicochemical properties. Some of the human health concerns about exposure to PFOA and other PFAS stem from their resistance to hydrolysis, photolysis, metabolism, and microbial degradation in the environment and in the human body. PFAS are not naturally occurring; they are man-made compounds that have been used widely over the past several decades in industrial applications and consumer products since many PFAS have repellant and surfactant properties. Frequently used as emulsifiers and as stain-, oil-, or water-repellents, PFAS are found in a variety of environmental media and in tissues of organisms, including humans.

Under the EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, longer-chain perfluoroalkyl carboxylic acids (PFCAs) by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (U.S. EPA, 2021a). However, PFOA remains persistent in environmental media because it is resistant to environmental degradation processes.

The purpose of this human health toxicity assessment is to derive toxicity values pertaining to oral exposure for PFOA. The development of this toxicity assessment relied on a robust systematic review process, based on the EPA peer-reviewed human health risk assessment methodology outlined in the EPA ORD Staff Handbook for Developing IRIS Assessments (U.S. EPA, 2022d), to identify human epidemiological, animal toxicological, mechanistic, and toxicokinetic data relevant to oral exposure. The PFOA systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) was developed prior to the initiation of this assessment and largely mirrors the Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments (U.S. EPA, 2020b). The protocol outlines the scoping and problem-formulation efforts and describes the systematic review, including study quality evaluation, and the dose-response methods used to conduct this assessment. The final assessment incorporates peer-reviewed studies captured from: EPA's 2016 PFOA HESD (U.S. EPA, 2016c), literature searches of scientific databases and gray literature from 2013 through February 2023, the SAB PFAS Review Panel recommendations, and public comment. Consistent with the analysis provided in the peer-reviewed draft assessment (U.S. EPA, 2021c) and with recommendations from external peer review (i.e., the SAB PFAS Review Panel; (U.S. EPA, 2022e)), this final assessment focused on qualitative and quantitative assessment of five "priority" health outcome categories based on those with the strongest weight of evidence. These five priority health outcomes are cancer, hepatic, developmental, cardiovascular, and immune. The results of the systematic literature reviews and qualitative assessments for the remaining "nonpriority" health outcomes are presented in the Appendix accompanying this final assessment (U.S. EPA, 2024a).

Qualitative Assessment of Noncancer Effects

Overall, the available evidence indicates that PFOA exposure is likely to cause hepatic, immunological, cardiovascular, and developmental effects in humans, given sufficient exposure conditions (e.g., at measured levels in humans as low as 1.1 to 5.2 ng/mL and at administered doses in animals as low as 0.3 to 1.0 mg/kg/day). These judgments are based on data from epidemiological studies of infants, children, adolescents, pregnant individuals, and nonpregnant adults, as well as short-term (28-day), subchronic (90-day), developmental (gestational), and chronic (2-year) oral-exposure studies in rodents. For hepatic effects, the primary support is evidence of increased serum liver enzyme levels (i.e., alanine transaminase (ALT)) in humans and coherent evidence of hepatotoxicity in animals, including increased liver weights and hepatocellular hypertrophy accompanied by necrosis, inflammation, or increased liver enzyme levels that indicate liver injury. For immunological effects, the primary support is evidence of developmental immunosuppression in humans, specifically decreased antibody response to vaccination against tetanus and diphtheria in children, and evidence of immunosuppression and other types of immunotoxicity in studies of adult animals, including decreased IgM response to sheep red blood cells, reduced spleen and thymus weights, changes in immune cell populations, and decreased splenic and thymic cellularity. For cardiovascular effects, the primary support is evidence of increased serum lipid levels in humans and alterations to lipid homeostasis in animals. For developmental effects, the primary evidence is decreased birth weight in human infants and decreased offspring survival, decreased fetal and pup weight, delayed time to eye opening, and related pre- and postnatal effects in animal studies. According to the protocol described in Appendix A (U.S. EPA, 2024a) and aligned with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d), selected quantitative data in medium and high

confidence studies from these identified hazards were used to derive toxicity values (see Table ES-1). Specific criteria for data and study selection are provided in Appendix A (U.S. EPA, 2024a) and Section 4.1.

Quantitative Assessment of Noncancer Effects and Oral RfD Derivation

EPA followed agency guidelines and methodologies for risk assessment in determining points of departure (PODs) for the derivation of the RfDs for PFOA (U.S. EPA, 2022d, 2014, 2012a, 2011b, 2002b) and performed modeling following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For data from epidemiological studies, the dose-response modeling approach was selected based on the health outcome and available data. A hybrid modeling approach, which estimated the probability of responses at specified exposure levels above the control, was conducted when clinically adverse outcome levels could be defined (i.e., for developmental, hepatic, and cardiovascular effects) following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For other outcomes (i.e., immune effects), study results from multivariate models were used to define a benchmark response (BMR). For data from animal toxicological studies, EPA conducted benchmark dose modeling, when possible, to empirically model the dose-response relationship in the range of observed data. When BMDLs could not be derived, EPA used a no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) approach.

PODs were converted to external POD human equivalent doses (POD_{HEDS}) using pharmacokinetic modeling (see Section 4.1.3). Consistent with the recommendations presented in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), EPA considered the database of information to inform the application of uncertainty factors (UFs) to POD_{HEDS} to address intraspecies variability, interspecies variability, extrapolation from a LOAEL to NOAEL, extrapolation from a subchronic to a chronic exposure duration, and database deficiencies. EPA derived and considered multiple candidate RfDs from both human epidemiological and animal toxicological studies across the four priority noncancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental) (see Figure ES-1 for candidate RfD values). Additional details on candidate RfD derivation for PFOA are available in Section 4.1.

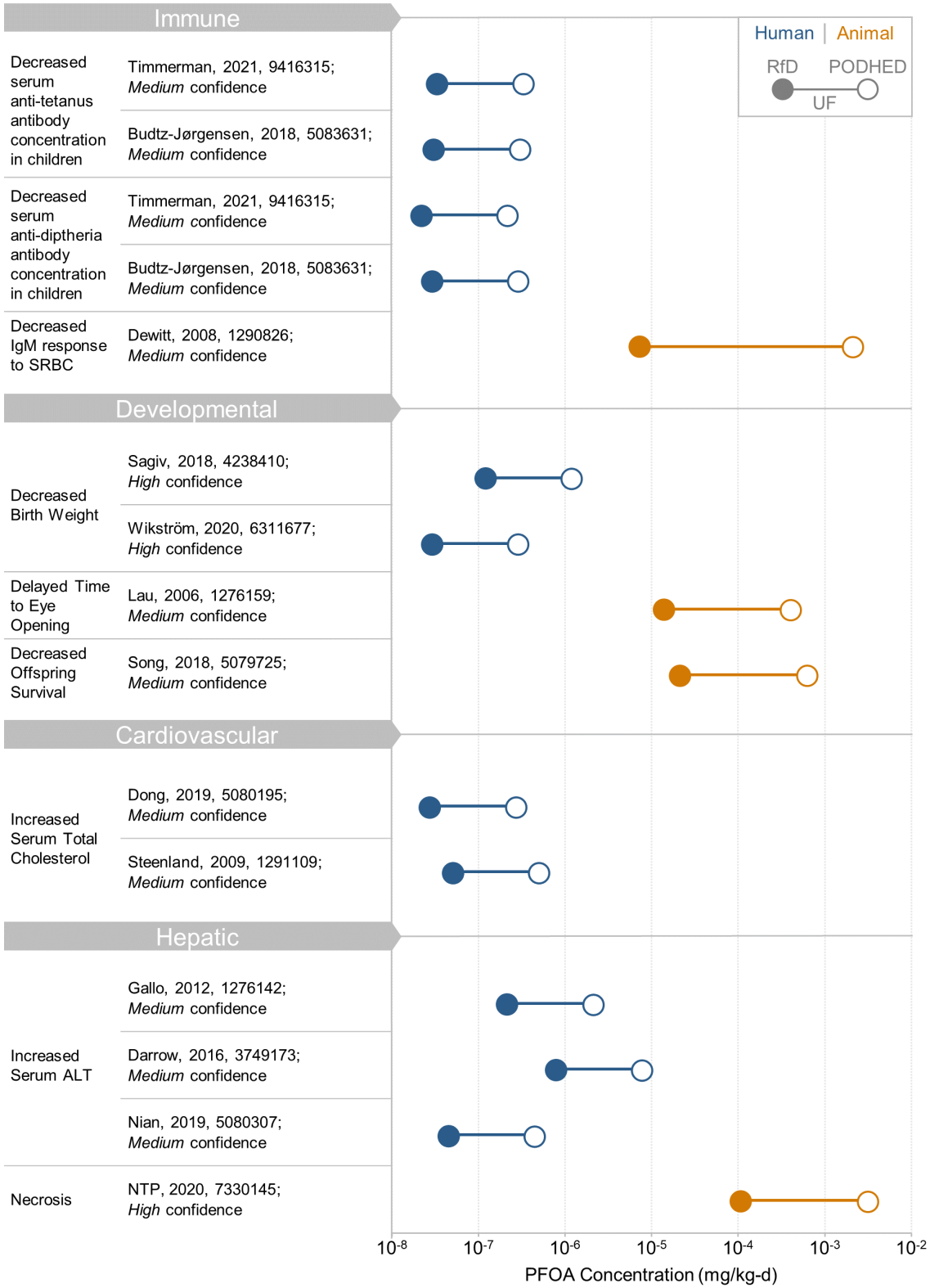


Figure ES-1. Schematic Depicting Candidate RfDs Derived From Epidemiological and Animal Toxicological Studies of PFOA

See text and Figure 4-4 in Section 4.1 for additional detail on dose-response modeling for PFOA studies.

The co-critical effects for the oral RfD of 3 x 10^{-8} mg/kg/day were decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children (Budtz-Jørgensen and Grandjean, 2018), decreased infant birth weight (Wikström et al., 2020), and increased total cholesterol in adults (Dong et al., 2019) (see Table ES-1). These co-critical effects were selected based on the procedures outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and consistent with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d). The RfD was derived by using a total UF of 10 to account for intraspecies variability (UF_H). Notably, the RfD is protective of effects that may occur in sensitive populations (i.e., embryo and fetus, infants, and young children), as well as hepatic effects in adults that may result from PFOA exposure. As two of the co-critical effects identified for PFOA are developmental endpoints and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOA is applicable to both short-term and chronic risk assessment scenarios.

Qualitative Carcinogenicity Assessment

Consistent with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), EPA reviewed the available data and conducted a weight of the evidence evaluation across the human epidemiological, animal toxicological, and mechanistic studies and concluded that PFOA is *Likely to Be Carcinogenic to Humans* via the oral route of exposure (see Section 3.5). Epidemiological studies provided evidence of kidney and testicular cancer in humans and some evidence of breast cancer in a study of one susceptible subpopulation. Animal toxicological studies in Sprague-Dawley rats reported Leydig cell tumors (LCT), pancreatic acinar cell tumors (PACT), and hepatocellular tumors after chronic oral exposure. Available mechanistic data suggest that multiple modes of action (MOAs) play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in humans and animal models. A full MOA analysis, including in-depth discussions on the potential MOAs for kidney and testicular tumors, as well as discussions on the potential MOAs and human relevance for pancreatic and liver tumors observed in rats, is presented in Section 3.5.4.2.

Quantitative Cancer Assessment and Cancer Slope Factor Derivation

EPA followed agency guidelines for risk assessment in deriving CSFs for PFOA (U.S. EPA, 2022d, 2012a, 2005a). EPA selected *medium* and *high* confidence studies for derivation that met criteria outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and Section 4.1.1, conducted benchmark dose modeling (U.S. EPA, 2012a), and used the same pharmacokinetic modeling approach as described for the derivation of noncancer RfDs above (see Section 4.2.2). From the studies that met the criteria, EPA derived and considered multiple candidate CSFs from both epidemiological and animal toxicological studies across multiple tissue and organ types (i.e., kidney, liver, pancreas, testes). Candidate CSFs were derived for epidemiological data on renal cell carcinoma (RCC) and kidney cancer using weighted linear regressions to calculate quartile-specific relative kidney cancer risks. Relative risks were then converted to the absolute risk scale, yielding an internal CSF, which represents the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was then divided by the selected clearance value and converted to an external dose CSF. For animal toxicological studies, multistage cancer models were used to predict the doses at which the selected BMR for tumor

incidence would occur. BMDLs for each tumor type (LCTs, hepatocellular adenoma or carcinoma, and pancreatic acinar cell adenoma or adenocarcinoma) served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type.

The oral slope factor of 0.0293 $(ng/kg/day)^{-1}$ for RCC in human males from Shearer et al. (2021) was selected as the basis of the overall CSF for PFOA (see Table ES-1; rationale in Section 4.2). Per EPA's *Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005a, b), age-dependent adjustment factors were not applied during CSF derivation because there was a lack of information to support a mutagenic MOA for PFOA, and the available evidence did not report an increased susceptibility to cancer following PFOA exposure during early life. Additional detail on candidate CSF derivation and CSF selection is provided in Table 4-12 and Table 4-13 in Section 4.2.

Final Toxicity Values for PFOA

Table ES-1. Final Toxicity Values for PFOA

Toxicity Value Type	Critical Effect(s)	Study, Confidence	Species, Sex, Age	Toxicity Value[a,b]
Reference Dose	Co-critical effects: decreased serum anti-tetanus and anti-diphtheria antibody concentration in children; decreased birth weight in infants; Increased serum total cholesterol in adults	Budtz-Jørgensen (2018), *Medium*; Wikström et al. (2020), *High*; Dong et al. (2019), *Medium*	Human, male and female, PFOA concentrations at age five years and anti-tetanus antibody serum concentrations at age seven years; human, male and female, PFOA serum concentrations in first and second trimesters; human, male and female, 20–80 years	3×10^{-8} (mg/kg/d)
Cancer Slope Factor	Renal cell carcinoma	Shearer et al. (2021), *Medium*	Human, male and female, 55–74 years	0.0293 (ng/kg/d)−1

Notes:
[a] Reference doses were rounded to one significant figure.
[b] Increase in cancer risk per 1 ng/(kg*d) increase in dose.

1 Background

1.1 Purpose of This Document

The primary purpose of this toxicity assessment for perfluorooctanoic acid (PFOA) is to describe the best available science on the human health effects associated with PFOA exposure and the derivation of toxicity values (i.e., noncancer reference doses (RfDs) and cancer slope factors (CSFs)). The latest health science on PFOA was identified, evaluated using systematic review methods, and described, and subsequently, a cancer classification was assigned and toxicity values were developed. The final cancer classification and cancer and noncancer toxicity values in this assessment build on the work described in the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a), *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c), and the *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2016c). This final toxicity assessment for PFOA reflects expert scientific recommendations from the U.S. Environmental Protection Agency (EPA) Science Advisory Board (SAB) (U.S. EPA, 2022e) and public comments received on the draft assessment (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114; U.S. EPA (2024c)).

In addition to documenting EPA's basis for the cancer classification and toxicity values, this document serves to:

- Describe and document transparently the literature searches conducted and systematic review methods used to identify health effects information (epidemiological and animal toxicological studies and physiologically based pharmacokinetic models) in the literature (Sections 2 and 3; Appendices A and B, (U.S. EPA, 2024a)).
- Describe and document literature screening methods, including use of the Populations, Exposures, Comparators, and Outcomes (PECO) criteria and the process for tracking studies throughout the literature screening (Section 2; Appendix A, (U.S. EPA, 2024a)).
- Identify epidemiological and animal toxicological literature that reports health effects after exposure to PFOA (and its related salts) as outlined in the PECO criteria (Section 3).
- Describe and document the study quality evaluations conducted on epidemiological and animal toxicological studies considered potentially useful for point-of-departure (POD) derivation (Section 3).
- Describe and document the data from all epidemiological studies and animal toxicological studies that were considered for POD derivation (Section 3).
- Synthesize and document the adverse health effects evidence across studies. The assessment focuses on synthesizing the available evidence for five main health outcomes that were found to have the strongest weight of evidence, as recommended by the SAB – developmental, hepatic, immune, and cardiovascular effects, and cancer (Section 3) –and also provides supplemental syntheses of evidence for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects, reproductive effects in males or females, and general toxicity (Appendix C, (U.S. EPA, 2024a)).

- Evaluate and document the available mechanistic information (including toxicokinetic understanding) associated with PFOA exposure to inform interpretation of findings related to potential health effects in studies of humans and animals, with a focus on five main health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer) (Section 3).
- Develop and document strength of evidence judgments across studies (or subsets of studies) separately for epidemiological, animal toxicological, and mechanistic lines of evidence for the five main health outcomes (Section 3).
- Develop and document integrated expert judgments across evidence streams (i.e., epidemiological, animal toxicological, and mechanistic streams) as to whether and to what extent the evidence supports that exposure to PFOA has the potential to be hazardous to humans (Section 3).
- Determine the cancer classification for PFOA using a weight-of-evidence approach (Section 3.5.5).
- Describe and document the attributes used to evaluate and select studies for derivation of toxicity values. These attributes are considered in addition to the study confidence evaluation domains and enable extrapolation to relevant exposure levels (e.g., studies with exposure levels near the range of typical environmental human exposures, broad exposure range, or multiple exposure levels) (Section 4).
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation (Section 4).
- Derive candidate RfDs (Section 4.1) and CSFs (Section 4.2), select the final RfD (Section 4.1.6) and CSF (Section 4.2.3) for PFOA, and describe the rationale.
- Characterize hazards (e.g., uncertainties, data gaps) (Sections 3, 4, and 5).

1.2 Background on Per-and Polyfluoroalkyl Substances

Per-and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals that share a common structure of a chain of linked carbon and fluorine atoms. The PFAS group includes PFOA, perfluorooctane sulfonic acid (PFOS), and thousands of other chemicals. There is no consensus definition of PFAS as a class of chemicals (OSTP, 2023). Consistent with three related structural definitions associated with EPA's identification of PFAS included in the fifth Contaminant Candidate List[1] (CCL 5), the universe of environmentally relevant PFAS – including parent chemicals, metabolites, and degradants – is approximately 15,000 compounds.[2] The 2018 Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)* includes over 4,700 PFAS (OECD, 2018).

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States, since the 1950's. PFAS have strong, stable carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Buck et al., 2011; Beach et al., 2006). The chemical structures of PFAS enable

[1] The CCL is a list, published every 5 years, of unregulated contaminants that are not subject to any current proposed or promulgated NPDWRs, are known or anticipated to occur in public water systems, and might require regulation under SDWA.
[2] See the EPA List of PFAS Structures available at: https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCT.

them to repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties. These properties make PFAS useful for commercial and industrial applications and make many PFAS extremely persistent in the human body and the environment (Kwiatkowski et al., 2020; Calafat et al., 2019; Calafat et al., 2007). Because of their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many different PFAS co-occur in environmental media (e.g., air, water, ice, sediment) and in tissues and blood of aquatic and terrestrial organisms, including humans.

With regard to structure, there are many families or classes of PFAS, each containing many individual structural homologues that can exist as either branched-chain or straight-chain isomers (Buck et al., 2011). These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl acids (PFAAs), fluorotelomer-based substances, and per- and polyfluoroalkyl ethers. PFOA belong to the PFAA family of the non-polymer PFAS category and is among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review, see Podder et al. (2021)).

1.3 Chemical Identity

PFOA is a perfluorinated aliphatic carboxylic acid. It is a fully fluorinated organic synthetic acid that was used in the United States primarily as an aqueous dispersion agent and emulsifier in the manufacture of fluoropolymers and in a variety of water-, oil-, and stain-repellent products (e.g., adhesives, cosmetics, fire-fighting foams, greases and lubricants, paints, polishes) (NLM, 2022b). It can exist in linear- or branched-chain isomeric form. PFOA is a strong acid that is generally present in solution as the perfluorooctanoate anion. Therefore, this assessment applies to all isomers of PFOA, as well as nonmetal salts of PFOA that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body).

PFOA is water soluble and mobile in water, with an estimated log organic carbon-water partition coefficient (log K_{oc}) of 2.06 (Zareitalabad et al., 2013). PFOA is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and it dissipates by advection, dispersion, and sorption to particulate matter. PFOA has low volatility in its ionized form but can adsorb to particles and be deposited on the ground and into water bodies. Because of its persistence, it can be transported long distances in air or water, as evidenced by detections of PFOA in arctic media and biota, including polar bears, oceangoing birds, and fish found in remote areas (Lindstrom et al., 2011; Smithwick et al., 2006).

Physical and chemical properties and other reference information for PFOA are provided in Table 1-1. There is uncertainty in the estimation, measurement, and/or applicability of certain physical/chemical properties of PFOA in drinking water, including the K_{oc} (Nguyen et al., 2020; Li et al., 2018d), octanol-water partition coefficient (K_{ow}), and Henry's Law Constant (K_H) (NCBI, 2022; ATSDR, 2021). For example, for K_{ow}, the Agency for Toxic Substances and Disease Registry (ATSDR) (2021) and Lange et al. (2006) reported that a value could not be measured because PFOA is expected to form multiple layers in octanol-water mixtures.

For a more detailed discussion of the chemical and physical properties and environmental fate of PFOA, please see the *PFAS Occurrence and Contaminant Background Support Document for the Final PFAS National Primary Drinking Water Regulation* (U.S. EPA, 2024e), the 2016

Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (U.S. EPA, 2016c), and the *Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2022a).

Table 1-1. Chemical and Physical Properties of PFOA

Property	Perfluorooctanoic Acid; Experimental Average	Source
Chemical Abstracts Service Registry Number (CASRN)[a]	335-67-1	NLM (2022a)
Chemical Abstracts Index Name	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctanoic acid	
Synonyms	PFOA; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; octanoic acid, pentadecafluoro-; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid	EPA CompTox Chemicals Dashboard
Chemical Formula	$C_8HF_{15}O_2$	NLM (2022a)
Molecular Weight	414.069 g/mol	NLM (2022a)
Color/Physical State	White to off-white powder (ammonium salt)	NLM (2022a)
Boiling Point	192°C	NLM (2022a)
Melting Point	54.3°C	NLM (2022a)
Vapor Pressure	0.0316 mm Hg at 19°C 0.017 mm Hg at 20°C	NLM (2022a); ATSDR (2021) (extrapolated)
Henry's Law Constant (K_H)	0.362 Pa-m^3/mol (converts to 3.57E-06 atm-m^3/mol)	ATSDR (2021)
pK_a	1.30, 2.80, −0.5–4.2, 0.5, 0.5	NLM (2022a); ATSDR (2021)
K_{oc}	631 ± 7.9 L/kg (mean ± 1 standard deviation of selected values)	Zareitalabad et al. (2013) (converted from log K_{oc} to K_{oc})
Solubility in Water	2,290 mg/L at 24°C (estimated); 3,300 mg/L at 25°C; 4,340 mg/L at 24.1°C 9,500 mg/L at 25°C; 3,300 mg/L at 25°C	NLM (2022b) ATSDR (2021)

Notes: CASRN = Chemical Abstracts Service Registry Number; K_{oc} = organic carbon-water partitioning coefficient; K_{ow} = octanol-water partition coefficient; pK_a: negative base-10 logarithm of acid dissociation constant.
[a] The CASRN given is for linear PFOA, but the toxicity studies are based on both linear and branched; thus, this assessment applies to all isomers of PFOA.

1.4 Occurrence Summary

1.4.1 Biomonitoring

The U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOA has been detected in up to 98% of serum samples taken in biomonitoring studies that are representative of the U.S. general population. Blood levels of PFOA declined by >70% between 1999 and 2018, presumably due to restrictions on its

commercial usage in the United States (CDC, 2017). However, studies of residents in locations of suspected PFAS contamination show higher serum levels of PFAS, including PFOA, compared with the general U.S. population as reported by NHANES (ATSDR, 2022; Table 17-6 in ITRC, 2020; Kotlarz et al., 2020; Yu et al., 2020).

Under EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, including perfluorononanoic acid (PFNA) and longer-chain perfluorocarboxylic acids (PFCAs), by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (U.S. EPA, 2021a). Manufacturers have since shifted to alternative short-chain PFAS, such as hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (two "GenX" chemicals). Additionally, other PFAS were found in human blood samples from recent (2011–2016) NHANES surveys (e.g., perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoDA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), PFNA, and 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH or MeFOSAA)). There is less publicly available information on the occurrence and health effects of these replacement PFAS than for PFOA, PFOS, and other members of the carboxylic acid and sulfonate PFAS categories.

1.4.2 Ambient Water

Among the PFAS with established analytical methods for detection, PFOA is one of the dominant PFAS compounds detected in ambient water both in the United States and worldwide (Remucal, 2019; Dinglasan-Panlilio et al., 2014; Zareitalabad et al., 2013; Benskin et al., 2012; Ahrens, 2011; Nakayama et al., 2007). Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions in the United States, often targeting sites with known manufacturing or industrial uses of PFAS such as the Great Lakes, the Cape Fear River, and waterbodies near Decatur, Alabama (Cochran, 2015; Konwick et al., 2008; Nakayama et al., 2007; Boulanger et al., 2004; Hansen et al., 2002; 3M Company, 2000). PFOA concentrations in global surface waters range over seven orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels (Jarvis et al., 2021; Zareitalabad et al., 2013).

PFOA concentrations in surface water tend to increase with increasing levels of urbanization. Across the Great Lakes region, PFOA was higher in the downstream lakes (Lake Erie and Lake Ontario), which are more heavily impacted by urbanization, and lower in the upstream lakes (Lakes Superior, Michigan, and Huron), which are located in a relatively rural and forested area (Remucal, 2019). Similarly, Zhang et al. (2016b) found measured surface water PFOA concentrations in urban areas (urban average PFOA concentration = 10.17 ng/L; n = 20) to be more than three times greater than concentrations in rural areas (rural average PFOA concentration = 2.95 ng/L; n = 17) within New Jersey, New York, and Rhode Island. Seasonal variations in PFOA levels in U.S. surface waters remain largely unknown because of a lack of experimental evidence examining alterations in PFOA concentrations across time.

1.4.3 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOA. Serum PFOA concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges.

EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are suspected to be present in drinking water and do not have health-based standards set under the Safe Drinking Water Act (SDWA). Under the UCMR, drinking water is monitored from public water systems (PWSs), specifically community water systems and non-transient, non-community water systems. The UCMR improves EPA's understanding of the frequency and concentrations of contaminants of concern occurring in the nation's drinking water systems. The first four UCMRs collected data from a census of large water systems (serving more than 10,000 people) and from a statistically representative sample of small water systems (serving 10,000 or fewer people). UCMR 3 monitoring occurred between 2013 and 2015 and is currently the most comprehensive nationally representative finished water dataset for PFOA (U.S. EPA, 2024d, e). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed. PFOA was found above the UCMR 3 minimum reporting level (20 ng/L) in 379 samples at 117 systems serving a population of approximately 7.6 million people located in 28 states, Tribes, or U.S. territories (U.S. EPA, 2024d, e).

More recent state data were collected using newer EPA-approved analytical methods and some state results reflect lower reporting limits than those in the UCMR 3. State data are available from 32 states: Alabama, Arizona, California, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin (U.S. EPA, 2024d, e). State results show continued occurrence of PFOA in multiple geographic locations. These data also show PFOA occurrence at lower concentrations and significantly greater frequencies than were measured under the UCMR 3, likely because the more recent monitoring was able to rely on more sensitive analytical methods (U.S. EPA, 2024d, e). More than one-third of states that conducted nontargeted monitoring detected PFOA and/or PFOS at more than 25% of systems (U.S. EPA, 2024d, e). Among the detections, PFOA concentrations ranged from 0.21 to 650 ng/L with a range of median concentrations from 1.27 to 5.61 ng/L (U.S. EPA, 2024d, e). Monitoring data for PFOA and PFOS from states that conducted targeted monitoring efforts, including 15 states, demonstrate results consistent with the nontargeted state monitoring. Within the 20 states that conducted nontargeted monitoring, there are 1,260 systems with results above 4.0 ng/L and 1,577 systems with results above 4.0 ng/L (U.S. EPA, 2024d, e). These systems serve populations of 12.5 and 14.4 million people, respectively. Monitoring data for PFOA from states that conducted targeted sampling efforts showed additional systems exceeding 4 ng/L (U.S. EPA, 2024d, e).

Finally, the fifth UCMR (UCMR 5) was published in December 2021 and requires sample collection and analysis for 29 PFAS, including PFOA, between January 2023 and December 2025 using drinking water analytical methods developed by EPA (U.S. EPA, 2021g). The UCMR 5 defined the minimum reporting level at 4 ng/L for PFOA using EPA Method 533, which is lower than the 20 ng/L used in the UCMR 3 with EPA Method 537 (U.S. EPA, 2021g). Therefore, UCMR 5 will be able to provide nationally representative occurrence data for PFOA

at lower detection concentrations. While the complete UCMR 5 dataset is not currently available, the small subset of data released (7% of the total results that EPA expects to receive) as of July 2023 is consistent with the results of UCMR 3 and the state data described above (U.S. EPA, 2024d, e).

Likewise, Glassmeyer et al. (2017) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging chemical and microbial contaminants, including 11 PFAS. In this study, PFOA was reported in source water at 76% of systems, at a median concentration of 6.32 ng/L and maximum concentration of 112 ng/L. Similarly, in treated drinking water, PFOA was detected in 76% of systems, with a median concentration of 4.15 ng/L and maximum concentration of 104 ng/L.

1.5 History of EPA's Human Health Assessment of PFOA

EPA developed an HESD for PFOA after it was listed on the third CCL (CCL 3) in 2009 (U.S. EPA, 2009). An HESD is synonymous with a toxicity assessment in that they both describe the assessment of cancer and noncancer health effects and derive toxicity values. The 2016 PFOA HESD was peer reviewed in 2014 and revised based on consideration of peer reviewers' comments, public comments, and additional studies published through December 2015. The resulting *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)*(U.S. EPA, 2016c) was published in 2016 and described the assessment of cancer and noncancer health effects and the derivation of a CSF and noncancer RfD for PFOA.

EPA initiated an update to the 2016 PFOA HESD in 2021 when the agency made a determination to regulate PFOA with a national primary drinking water regulation (NPDWR) (U.S. EPA, 2021d). The initial update of the 2016 PFOA HESD was the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). This assessment described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, a relative source contribution (RSC), and cancer classification, which would subsequently be used to prepare draft and final toxicity assessments for PFOA. The agency sought peer review from the EPA SAB PFAS Review Panel on key scientific issues, including the systematic review approach for evaluating health effects studies, the derivation of oral toxicity values, the RSC, and the cancer classification for PFOA.

The SAB provided draft recommendations on June 3, 2022, and final recommendations on August 23, 2022 (U.S. EPA, 2022e). To be responsive to the SAB recommendations, EPA developed a detailed response to comments document (U.S. EPA OW, 2023) and addressed every recommendation from the SAB in the development of the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a). Briefly, EPA:

- updated and expanded the scope of the studies included in the assessment;
- expanded the systematic review steps beyond study quality evaluation to include evidence integration to ensure consistent hazard decisions across health outcomes;
- separated hazard identification and dose-response assessment;
- added protocols for all steps of the systematic review and more transparently described the protocols;

- evaluated alternative pharmacokinetic models and further validated the selected model;
- conducted additional dose-response analyses using additional studies and endpoints;
- evaluated and integrated mechanistic information;
- strengthened the weight-of-evidence discussion for cancer effects and rationale for the cancer classification;
- strengthened the rationales for selection of PODs for the noncancer health outcomes; and
- clarified language related to the RSC determination, including the relevance of drinking water exposures and the relationship between the RfD and the RSC.

EPA then released the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* for a 60-day public comment period. This assessment described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, an RSC, and cancer classification for PFOA.

EPA incorporated feedback from public comment into this final assessment and developed a detailed response to public comment document (U.S. EPA, 2024c). Briefly, EPA has improved descriptions of rationale and added clarifications related to the systematic review protocol used for this assessment, study and endpoint selection for POD derivation, and the modeling choices related to toxicity value derivation. Therefore, this *Final Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts* incorporates feedback from external peer review and public comment and supersedes all other health effects documents produced by the EPA Office of Water for PFOA.

2 Summary of Assessment Methods

This section summarizes the methods used for the systematic review of the health effects literature for all isomers of perfluorooctanoic acid (PFOA), as well as nonmetal salts of PFOA, that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body). The purposes of this systematic review were to identify the best available and most relevant health effects literature, to evaluate studies for quality, and to subsequently identify health effects and studies for dose-response assessment. A detailed description of these methods is provided as a protocol in Appendix A (U.S. EPA, 2024a).

2.1 Introduction to the Systematic Review Assessment Methods

The methods used to conduct the systematic review for PFOA are consistent with the methods described in the draft and final *EPA ORD Staff Handbook for Developing IRIS Assessments* (U.S. EPA, 2022d, 2020a) (hereafter referred to as the Integrated Risk Information System (IRIS) Handbook) and a companion publication (Thayer et al., 2022). EPA's IRIS Handbook has incorporated feedback from the National Academy of Sciences (NAS) at workshops held in 2018 and 2019 and was well regarded by the NAS review panel for reflecting "significant improvements made by EPA to the IRIS assessment process, including systematic review methods for identifying chemical hazards" (NASEM, 2021). Furthermore, EPA's IRIS program has used the IRIS Handbook to develop toxicological reviews for numerous chemicals, including some PFAS (U.S. EPA, 2023b, 2022c). Although the IRIS Handbook was finalized concurrently with the development of this assessment, the revisions in the final IRIS Handbook compared with the draft version do not conflict with the methods used in this assessment. The assessment team concluded that implementing minor changes in study quality evaluation between the draft and final IRIS Handbook versions would not change the assessment conclusions. Therefore, EPA considers the methods described herein to be consistent with the final IRIS Handbook and cites this version accordingly. Additionally, the methods used to conduct the systematic review are also consistent with and largely mirror the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

For this updated PFOA toxicity assessment, systematic review methods were consistent with those in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). for the steps of literature search; screening; study quality evaluation; data extraction; display of study evaluation results; synthesis of human and experimental animal data; and evidence integration for all health outcomes through the 2020 literature searches, as presented in the preliminary analyses of the 2021 *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* draft document that was reviewed by the Science Advisory Board (SAB) (U.S. EPA, 2022e, 2021c). The EPA then focused the remaining steps of the systematic review process (synthesis and integration of mechanistic data; derivation of toxicity values) on health outcomes with the strongest weight of evidence based on the conclusions presented in the 2021 draft documents, and consistent with the recommendations of the SAB (U.S. EPA, 2022e). These five "priority" health outcomes are developmental, hepatic, immune, cardiovascular, and cancer. The updated systematic review focused on the priority health outcomes was published in 2023 as

the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a).

The following subsections provide a summary of methods used to search for and screen identified literature, evaluate the identified studies to characterize study quality, extract data, and select studies for dose-response analysis. Extracted data are available in interactive visual formats (see Section 3) and can be downloaded in open access, interactive formats. The full systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) provides a detailed description of the systematic review methods that were used. The protocol also includes the description of the problem formulation and key science issues guiding this assessment.

2.1.1 Literature Database

The EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this PFOA toxicity assessment based on three main data streams: 1) literature published from 2013 through February 6, 2023 identified via literature searches conducted in 2019, 2020, 2022 and 2023 of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g., searches of the gray literature, studies shared with EPA by the SAB, studies submitted through public comment), and 3) literature identified in EPA's 2016 *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2016c). All of these streams are described in detail below.

For the literature searches, the search strings focused on the chemical name (PFOA and its related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, in vitro, in silico) or health outcomes. The EPA conducted a literature search in 2019 (covering January 2013 through April 11, 2019), which was subsequently updated by a search covering April 2019 through September 3, 2020 prior to SAB review of the draft assessment (2020 literature search), a third search covering September 2020 through February 3, 2022 prior to release of the draft assessment for public comment (2022 literature search), and a final supplemental search covering February 4, 2022 through February 6, 2023.

The publicly available databases listed below were searched for literature containing the chemical search terms outlined in Appendix A (U.S. EPA, 2024a):

- Web of Science™ (WoS) (Thomson Reuters),
- PubMed® (National Library of Medicine),
- ToxLine (incorporated into PubMed post 2019), and
- TSCATS (Toxic Substances Control Act Test Submissions).

The search strings and literature sources searched are described in Appendix A (U.S. EPA, 2024a)).

For the second data stream, other review efforts and searches of publicly available sources were used to identify relevant studies (see Appendix A, (U.S. EPA, 2024a)), as listed below:

- Studies cited in assessments published by other U.S. federal, international, and/or U.S. state agencies (this included assessments by ATSDR (ATSDR, 2021) and California Environmental Protection Agency (CalEPA, 2021)),

- Studies identified during mechanistic or toxicokinetic evidence synthesis (i.e., during manual review of reference lists of relevant mechanistic and toxicokinetic studies deemed relevant after screening against mechanistic- and ADME-specific PECO criteria),
- Studies identified by the SAB in their final report dated August 23, 2022 (U.S. EPA, 2022e), and
- Studies submitted through public comment by May 2023 (https://www.regulations.gov/docket/EPA-HQ-OW-2022–0114).

For the third data stream, EPA relied on epidemiological and animal toxicological literature synthesized in the 2016 PFOA HESD to identify studies relevant to the five priority health outcomes, as recommended by SAB and consistent with preliminary conclusions from EPA's analysis in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). The 2016 PFOA HESD contained a summary of all relevant literature identified in searches conducted through 2013. EPA's 2016 PFOA HESD relied on animal toxicological studies for quantitative analyses whereas epidemiology studies were considered qualitatively, as a supporting line of evidence. This updated assessment includes epidemiological studies that were identified and presented in the 2016 PFOA HESD for the five priority health outcomes. It also includes "key" animal toxicological studies from the 2016 PFOA HESD, which includes studies that were selected in 2016 for dose-response modeling. The details of the studies included from the 2016 PFOA HESD are described in Appendix A (U.S. EPA, 2024a).

All studies identified through the data streams outlined above were uploaded into the publicly available Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608).

EPA has continued to monitor the literature published since February 2023 for other potentially relevant studies. Potentially relevant studies identified after February 2023 that were not recommended by the SAB in their final report or via public comment are not included as part of the evidence base for this updated assessment but are provided in a repository detailing the results and potential impacts of new literature on the assessment (see Appendix A, (U.S. EPA, 2024a)).

2.1.2 Literature Screening

This section summarizes the methods used to screen the identified health effects, mechanistic, and absorption, distribution, metabolism, excretion (ADME) literature. Briefly, the EPA used populations, exposures, comparators, and outcomes (PECO) criteria to screen the literature identified from the literature sources outlined above in order to prioritize studies for dose-response assessment and to identify studies containing supplemental information such as mechanistic studies that could inform the mode of action analyses. The PECO criteria used for screening the health effects, toxicokinetic, and mechanistic literature are provided in Appendix A (U.S. EPA, 2024a).

Consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b), studies identified in the literature searches and stored in HERO were imported into the SWIFT Review software platform and the software was used to identify those studies most

likely to be relevant to human health risk assessment. Studies captured then underwent title and abstract screening by at least two independent reviewers using screening tools consistent with the IRIS Handbook ((U.S. EPA, 2022d); DistillerSR or SWIFT ActiveScreener software), and studies that passed this initial screening underwent full-text review by at least two independent reviewers. Health effects studies that met PECO inclusion criteria following both title and abstract screening and full-text review underwent study quality evaluation as described below (Section 2.1.3). Studies that were tagged as containing relevant PBPK models were sent to the modeling technical experts for scientific and technical review. Studies tagged as supplemental and containing potentially relevant mechanistic or ADME (or toxicokinetic) data following title and abstract and full-text level screening underwent further screening using mechanistic- or ADME-specific PECO criteria, and those deemed relevant underwent light data extraction of key study elements (e.g., extraction of information about the tested species or population, mechanistic or ADME endpoints evaluated, dose levels tested; see Appendix A, (U.S. EPA, 2024a)). Supplemental studies that were identified as mechanistic or ADME during screening did not undergo study quality evaluation.

For the supplemental literature search conducted in 2023 and literature received through public comment, studies were screened for relevancy and considered for potential impact on the toxicity assessments for PFOA. Consistent with the IRIS Handbook (U.S. EPA, 2022d), the studies identified after February 3, 2022, including studies recommended via public comment, were "considered for inclusion only if they [were] directly relevant to the assessment PECO criteria and [were] expected to potentially impact assessment conclusions or address key uncertainties" (U.S. EPA, 2022d). For the purposes of this assessment, the EPA defined impacts on the assessment conclusions as data from a study (or studies) that, if incorporated into the assessment, have the potential to significantly affect (i.e., by an order of magnitude or more) the final toxicity values (i.e., RfDs and CSFs) or alter the cancer classification for PFOA (see Appendix A, (U.S. EPA, 2024a)).

2.1.3 Study Quality Evaluation for Epidemiological Studies and Animal Toxicological Studies

Study quality evaluations were performed consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). For study quality evaluation of the PECO-relevant human epidemiological and animal toxicological studies (i.e., studies identified in the four literature searches (all health outcomes for the 2019 and 2020 searches; the five priority health outcomes for the 2022 search; studies impacting assessment conclusions within the five priority health outcomes for the 2023 search (see Appendix A, (U.S. EPA, 2024a))), studies recommended by the SAB, studies recommended via public comment that reported potentially significant results on one or more of the five priority health outcomes, epidemiological studies from the 2016 PFOA HESD that reported results on one or more of the five priority health outcomes, and key animal toxicological studies from the 2016 PFOA HESD), two independent primary reviewers followed by a quality assurance (QA) reviewer assigned ratings about the reliability of study results (*good, adequate, deficient* (or *"not reported"*), or *critically deficient*) for different evaluation domains as described in the IRIS Handbook (U.S. EPA, 2022d) (see Appendix A, (U.S. EPA, 2024a)). These study quality evaluation domains are listed below and

details about the domains, including prompting questions and suggested considerations, are described in Appendix A (U.S. EPA, 2024a).

- Epidemiological study quality evaluation domains: participant selection; exposure measurement criteria; outcome ascertainment; potential confounding; analysis; selective reporting; and study sensitivity.
- Animal toxicological study quality evaluation domains: reporting quality; allocation; observational bias/blinding; confounding/variable control; reporting and attrition bias; chemical administration and characterization; exposure timing, frequency, and duration; endpoint sensitivity and specificity; and results presentation.

The independent reviewers performed study quality evaluations using a structured platform housed within EPA's Health Assessment Workplace Collaboration (HAWC; https://hawcproject.org/). Once the individual domains were rated, reviewers independently evaluated the identified strengths and limitations of each study to reach an overall classification on study confidence of *high, medium, low*, or *uninformative* for each PECO-relevant endpoint evaluated in the study consistent with the IRIS Handbook (U.S. EPA, 2022d). A study can be given an overall *mixed* confidence rating if different PECO-relevant endpoints within the study receive different confidence ratings (e.g., *medium* and *low* confidence ratings).

2.1.4 Data Extraction

Data extraction was conducted for all relevant human epidemiological and animal toxicological studies determined to be of *medium* and *high* confidence after study quality evaluation. Because of the abundance of *medium* and *high* confidence studies in this database, data were only extracted from *low* confidence epidemiological studies when data were limited for a health outcome or when there was a notable effect, consistent with the IRIS Handbook (U.S. EPA, 2022d). Studies evaluated as being *uninformative* for an endpoint were not considered further when characterizing that endpoint and therefore did not undergo data extraction. All health endpoints were considered for extraction, regardless of the magnitude of effect or statistical significance of the response relative to the control group. The level of detail in data extractions for different endpoints within a study could differ based on how the data were presented for each outcome (i.e., ranging from a narrative summary to a full extraction of dose-response effect size information).

Extractions were conducted using DistillerSR for epidemiological studies and HAWC for animal toxicological studies. An initial reviewer conducted the extraction, followed by a second reviewer conducting an independent QA who confirmed accuracy and edited/corrected the extraction as needed. Discrepancies in data extraction were resolved by discussion and confirmation within the extraction team.

Data extracted from epidemiology studies included population, study design, year of data collection, exposure measurement, and quantitative data from statistical models. Data extracted from statistical models reported in the studies included the health effect category, endpoint measured, sample size, description of effect estimate, covariates, and model comments. Data extracted from animal toxicological studies included information on the experimental design and exposure duration, species and number of animals tested, dosing regime, and endpoints

measured. Further information about data extraction can be found in Appendix A (U.S. EPA, 2024a).

2.1.5 Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes. Evidence synthesis refers to the process of analyzing the results of the available studies (including their strengths and weaknesses) for consistency and coherence, often by evidence stream (e.g., human or animal) and health outcome (i.e., an organ- or organ system-level category of related health effects and endpoints). In evidence integration, the evidence across streams is considered together and integrated to develop judgments (for each health outcome) about whether the chemical in question poses a hazard to human health. Consistent with the IRIS Handbook, groups of related outcomes within a health outcome category were considered together as a unit of analysis during evidence synthesis and evidence integration (U.S. EPA, 2022d). For example, birth weight, birth length, and head circumference were all considered under the unit of analysis of the fetal growth restriction.

Evidence syntheses are summary discussions of the body of evidence for each evidence stream (i.e., human and animal) for each health outcome analyzed. The available human and animal health effects evidence were synthesized separately, with each synthesis resulting in a summary discussion of the available evidence. For the animal toxicological evidence stream, evidence synthesis included consideration of studies rated *high* and *medium* confidence. For the epidemiological evidence stream, evidence synthesis was based primarily on studies of *high* and *medium* confidence, including discussion of study quality considerations, according to the recommendations of the SAB (U.S. EPA, 2022e). Consistent with the IRIS Handbook (U.S. EPA, 2022d), *low* confidence epidemiological studies and results were used only in a supporting role and given less weight during evidence synthesis and integration compared to *high* or *medium* confidence studies. *Low* confidence epidemiological studies were included in evidence syntheses in order to capture all of the available data for PFOA in the weight-of-evidence analyses. As described above, *uninformative* studies were not extracted or included in the evidence syntheses. Results from epidemiological studies were discussed within sections organized by population type, including children, general population adults, pregnant women, and occupational populations. Childhood was defined as the effect of environmental exposure during early life: from conception, infancy, early childhood and through adolescence until 21 years of age (U.S. EPA, 2021b). Epidemiological studies were excluded from the evidence synthesis narrative if they included data that were reported in multiple studies (e.g., overlapping NHANES studies). Studies reporting results from the same cohort and on the same health outcome as another study were considered overlapping evidence, and to avoid duplication or overrepresentation of results from the same group of participants, these additional studies were not discussed in the evidence synthesis narrative. In cases of overlapping studies, the study with the largest number of participants and/or the most accurate outcome measures was given preference. For the five priority health outcomes, EPA also developed mechanistic syntheses.

For evidence integration, conclusions regarding the strength of evidence were drawn for each health outcome across human and animal evidence streams. For the five priority health outcomes, this included consideration of epidemiological studies identified in the 2016 PFOA HESD, as well as mechanistic evidence. The evidence integration provides a summary of the causal interpretations between PFOA exposure and health effects based on results of the

available epidemiological and animal toxicological studies, in addition to the available mechanistic evidence. Considerations when evaluating the available studies included risk of bias, sensitivity, consistency, strength (effect magnitude) and precision, biological gradient/dose-response, coherence, and mechanistic evidence related to biological plausibility. The judgments were directly informed by the evidence syntheses and based on structured review of an adapted set of considerations for causality first introduced by Austin Bradford Hill (Hill, 1965).

The evidence integration was conducted according to guidance outlined in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments* (U.S. EPA, 2020b). The evidence integration included evidence stream evaluation, in which the qualitative summaries on the strength of evidence from studies in animals and humans were evaluated, and subsequent inference across all evidence streams. Human relevance of animal models as well as mechanistic evidence to inform mode of action were considered. Evidence integration produced an overall judgment about whether sufficient or insufficient evidence of an association with PFOA exposure exists for each human health outcome, as well as the rationale for each judgment. The potential evidence integration judgments for characterizing human health effects are ***evidence demonstrates, evidence indicates (likely), evidence suggests, evidence inadequate,*** and ***strong evidence supports no effect***. Considerations for each evidence integration judgment are summarized within corresponding evidence integration sections in an evidence profile table (EPT). EPTs were organized by evidence stream (i.e., human, animal, and mechanistic, respectively), and, within evidence streams, units of analysis with the strongest evidence were presented first.

Additional details about evidence synthesis and integration are summarized in Appendix A (U.S. EPA, 2024a).

2.2 Dose-Response Assessment

Evidence synthesis and integration enabled identification of the health outcomes with the strongest weight of evidence supporting causal relationships between PFOA exposure and adverse health effects, as well as the most sensitive cancer and noncancer endpoints within those health outcomes. Dose-response modeling was performed for endpoints within health outcomes with data warranting evidence integration conclusions of *evidence demonstrates* and *evidence indicates (likely)* for noncancer endpoints and carcinogenicity descriptors of *Carcinogenic to Humans* and *Likely to be Carcinogenic to Humans*. EPA identified specific studies for dose-response modeling and POD derivation following attributes described in Table 7-2 of the IRIS Handbook (U.S. EPA, 2022d). Examples of study attributes evaluated included study design characteristics, study confidence, and data availability, among others (see Appendix A, (U.S. EPA, 2024a)). Human epidemiological and animal toxicological studies that were consistent with the overall weight of evidence for a specific endpoint were considered for dose-response. Additionally, for human evidence, all *high* or *medium* confidence studies pertaining to a specific endpoint were considered; for animal evidence, only animal toxicological studies with at least two PFOA exposure groups that were of *high* or *medium* confidence were considered. Relevance of the endpoint or species reported by animal toxicological studies to human health effects was also considered. Additional information on study selection is provided in Appendix A (U.S. EPA, 2024a).

2.2.1 Approach to POD and Candidate RfD Derivation for Noncancer Health Outcomes

The current recommended EPA human health risk assessment approach for noncancer POD derivation described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* includes selection of a benchmark response (BMR), analysis of dose and response within the observed dose range, followed by extrapolation to lower exposure levels (U.S. EPA, 2002b). For noncancer health outcomes, EPA performed dose-response assessments to define PODs, including low-dose extrapolation, when feasible, and applied uncertainty factors (UFs) to those PODs to derive candidate RfDs. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002b). For PFOA, multiple candidate RfDs were derived within a health outcome as described in Section 4.

For PFOA animal toxicological studies, EPA attempted benchmark dose (BMD) modeling on all studies considered for dose response to refine the POD. BMD modeling was performed after converting the administered dose reported by the study to an internal dose using a pharmacokinetic model (see Section 4.1.3 for additional details). This approach resulted in dose levels corresponding to specific response levels near the low end of the observable range of the data and identified the lower limits of the BMDs (BMDLs) which serve as potential PODs (U.S. EPA, 2012a). EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (i.e., BMD) that corresponds to a predetermined level of response (i.e., benchmark response or BMR). For dichotomous data, the BMR is typically set at either 5% or 10% above the background or the response of the control group. For continuous data, a BMR of one-half or one standard deviation from the control mean is typically used when there are no outcome-specific data to indicate what level of response is biologically significant (U.S. EPA, 2012a). For dose-response data for which BMD modeling did not produce an adequate model fit, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD. However, a POD derived using a BMD approach typically provides a higher level of confidence in the conclusions for any individual case, as the BMDL takes into account all the data from the dose-response curve, incorporates the evaluation of the uncertainty in the BMD, and is related to a known and predefined potential effect size (i.e., the BMR) (U.S. EPA, 2022d, 2012a). For noncancer endpoints, there were several factors considered when selecting the final model and BMD/BMDL, including the type of measured response variable (i.e., dichotomous or continuous), experimental design, and covariates (U.S. EPA, 2012a). However, as there is currently no prescriptive hierarchy, selection of model types was often based on the goodness-of-fit and judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. The *Benchmark Dose Technical Guidance* provides a "BMD Decision Tree" to assist in model selection (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

For the epidemiological studies considered for dose-response assessment, EPA used multiple modeling approaches to determine PODs, depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies,

EPA used a hybrid modeling approach that involves estimating the incidence of individuals above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control (U.S. EPA, 2012a) because the EPA was able to define a level considered clinically adverse for these outcomes (see Appendix E, (U.S. EPA, 2024a)). As sensitivity analyses for comparison purposes, EPA also performed BMD modeling and provided study LOAELs/NOAELs as PODs for the epidemiological hepatic and serum lipid dose-response studies. For the immune studies, for which a clinically defined adverse level is not well established, EPA used multivariate models provided in the studies and determined a BMR according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

After POD derivation, EPA used a pharmacokinetic model for human dosimetry to estimate human equivalent doses (HEDs) from both animal and epidemiological studies. A pharmacokinetic model for human dosimetry is used to simulate the HED from the animal PODs and is also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal dose POD obtained from dose-response modeling (Section 4.1.3). Based on the available data, a serum PFOA concentration was identified as a suitable internal dosimetry target for the human and animal endpoints of interest. Next, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HEDS}) to account for five possible areas of uncertainty and variability: human variation, extrapolation from animals to humans, extrapolation to chronic exposure duration, the type of POD being used for reference value derivation, and extrapolation to a minimal level of risk (if not observed in the data set). UFs used in this assessment were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). For additional detail on UFs see Appendix A (U.S. EPA, 2024a). The POD_{HED} for a particular candidate RfD is divided by the composite UFs.

The general steps for deriving an RfD for PFOA are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints affected by exposure to PFOA. This step involves selecting the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study confidence, and characterized for adverse health outcomes, the risk assessor selects health endpoints/outcomes judged to be relevant to human health and among the most sensitive, defined as effects observed in the lower exposure range. Considerations that might influence selection of endpoints include whether data have dose-response information, magnitude of response, adversity of effect, and consistency across studies.

Step 1a (for dose-response data from a study in an animal model): Convert administered dose to an internal dose. A pharmacokinetic model is used to predict the internal dose (in the animals used in the toxicity studies) that would correspond to the administered dose used in the study (see 4.1.3 for additional detail). A number of dose-metrics across lifestages are selected for simulation in a mouse, rat, or monkey. Concentrations of PFOA in blood are considered for all the internal dose-metrics.

Step 2: Conduct dose-response modeling. See above and Appendix E (U.S. EPA, 2024a) for study-specific details.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (e.g., BMDL, NOAEL) is converted to an HED following the method described in Section 4.1.3.

Step 4: Select appropriate UFs and provide rationale for UF selection. UFs are applied in accordance with EPA methodology considering variations in sensitivity among humans, differences between animals and humans (if applicable), the duration of exposure in the critical study compared with the lifetime of the species studied, and the completeness of the epidemiological or animal toxicological database (U.S. EPA, 2002b).

Step 5: Calculate the chronic RfD. The RfD is calculated by dividing the POD_{HED} by the composite (total) UF (UF_c) specific to that POD_{HED}.

$$RfD = \left(\frac{POD_{HED}}{UF_C}\right)$$

where:

POD_{HED} = calculated from the internal dose POD using the human pharmacokinetic (PK) model presented in Section 4.1.3.2.

UF_C = Composite (total) UF calculated by multiplying the selected individual UFs for variations in sensitivity among humans, differences between animals and humans, duration of exposure in the critical study compared with the lifetime of the species studied, and completeness of the toxicology database, in accordance with EPA methodology (U.S. EPA, 2002b).

2.2.2 Cancer Assessment

2.2.2.1 Approach for Cancer Classification

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, a descriptive weight-of-evidence expert judgment is made, based on all available animal, human, and mechanistic data, as to the likelihood that a contaminant is a human carcinogen and the conditions under which the carcinogenic effects may be expressed (U.S. EPA, 2005a). A narrative is developed to provide a complete description of the weight of evidence and conditions of carcinogenicity. The potential carcinogenicity descriptors presented in the 2005 guidelines are:

- Carcinogenic to Humans
- Likely to Be Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not Likely to Be Carcinogenic to Humans

More than one carcinogenicity descriptor can be applied if a chemical's carcinogenic effects differ by dose, exposure route, or mode of action (MOA) [3]. For example, a chemical may be

[3]MOA is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. It is contrasted with "mechanism of action," which implies a more detailed understanding and description of events.

carcinogenic to humans above but not below a specific dose level if a key event in tumor formation does not occur below that dose. MOA information informs both the qualitative and quantitative aspects of the assessment, including the human relevance of tumors observed in animals. The MOA analysis must be conducted separately for each target organ/tissue type (U.S. EPA, 2005a).

2.2.2.2 Derivation of a Cancer Slope Factor

EPA's 2005 *Guidelines for Carcinogen Risk Assessment* recommends a two-step process for the quantitation of cancer risk as a CSF. A CSF is a plausible upper bound lifetime cancer risk from chronic ingestion of a chemical per unit of mass consumed per unit body weight per day (mg/kg-day) (U.S. EPA, 2005a). This process varied slightly depending on whether the CSF was based on an animal toxicological or epidemiological study, as described below.

The first step in the process is using a model to fit a dose-response curve to the data, based on the doses and associated tumors observed (U.S. EPA, 2005a). In the second step of quantitation, the POD is extrapolated to the low-dose region of interest for environmental exposures. The approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). When evidence indicates that a chemical causes cancer through a mutagenic MOA (i.e., mutation of deoxyribonucleic acid (DNA)) or the MOA for carcinogenicity is not known, the linear approach is used and the extrapolation is performed by drawing a line (on a graph of dose vs. response) from the POD to the origin (zero dose, zero tumors). The slope of the line (Δresponse/Δdose) gives rise to the CSF, which can be interpreted as the risk per mg/kg/day (U.S. EPA, 2005a).

For animal toxicological studies, EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). First, a PK model converted the administered dose reported by the study to an internal dose (see Section 4.1.3 for additional details). Then, BMDS fits multistage models, the preferred model type (U.S. EPA, 2012a), to the data and the model is used to identify a POD for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD_{10} (the dose corresponding to a 10% increase in tumors) and the $BMDL_{10}$ (the 95% lower confidence limit for that dose) are also reported and are often used as the POD. Similar to noncancer PODs, selection of model types is often based on the goodness-of-fit (U.S. EPA, 2012a). For PFOA, after a POD was determined, a PK model was used to calculate the HED for animal oral exposures (POD_{HED}). The CSF is derived by dividing the BMR by the POD_{HED}. See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

For epidemiological data, EPA used linear regression between PFOA exposure and cancer relative risk to estimate dose response as well as the generalized least-squares for trend (glst) modeling (Greenland and Longnecker, 1992) using STATA v17.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC). The CSF was then calculated as the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/kg-day increase in dose. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/(kg-day) increase in

dose. This was done by dividing the internal serum CSF by the selected clearance value, which is equivalent to dividing by the change in external exposure that results in a 1 ng/mL increase in serum concentration at steady-state. EPA also considered evaluating the dose-response data using the BMDS; however, categorical data from case-control studies cannot be used with the BMDS since these models are based on cancer risk, and the data needed to calculate risks (i.e., the denominators) were not available. See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

In addition, according to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), affirmative determination of a mutagenic MOA (as opposed to defaulting to a mutagenic MOA based on insufficient data or limited data indicating potential mutagenicity) indicates the potential for higher cancer risks from an early-life exposure compared with the same exposure during adulthood, and so requires that the application of age-dependent adjustment factors (ADAFs) be considered in the quantification of risk to account for additional sensitivity of children. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposure to a mutagenic chemical.

In cases for which a chemical is shown to cause cancer via an MOA that is not linear at low doses, and the chemical does not demonstrate mutagenic or other activity consistent with linearity at low doses, a nonlinear extrapolation is conducted. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* state that "where tumors arise through a nonlinear MOA, an oral RfD or inhalation reference concentration, or both, should be developed in accordance with EPA's established practice of developing such values, taking into consideration the factors summarized in the characterization of the POD" (U.S. EPA, 2005a). In these cases, an RfD-like value is calculated based on the key event[4] for carcinogenesis or the tumor response.

2.2.3 Selecting Health Outcome-Specific and Overall Toxicity Values

Once all of the candidate toxicity values were derived, EPA then selected a health outcome-specific toxicity value for each hazard (cancer and noncancer) identified in the assessment. This selection can be based on the study confidence considerations, the most sensitive outcome, a clustering of values, or a combination of such factors; the rationale for the selection is presented in the assessment. Key considerations for candidate value selection are described in the IRIS Handbook (U.S. EPA, 2022e) and include: 1) the weight of evidence for the specific effect or health outcome; 2) study confidence; 3) sensitivity and basis of the POD; and 4) uncertainties in modeling or extrapolations. The value selected as the organ/system-specific toxicity value is discussed in the assessment.

The selection of overall toxicity values for noncancer and cancer effects involves the study preferences described above, consideration of overall toxicity, study confidence, and confidence in each value, including the strength of various dose-response analyses and the possibility of

[4]The key event is defined as an empirically observed precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element.

basing a more robust result on multiple data sets. The values selected as the overall RfD and CSF are discussed in Section 4.

3 Results of the Health Effects Systematic Review and Toxicokinetics Methods

3.1 Literature Search and Screening Results

Studies referenced in this assessment are cited as "Author Last Name, Publication Year, HERO ID" and are available in EPA HERO: A Database of Scientific Studies and References. The HERO ID is a unique identifier for studies available in HERO. Additional study metadata are publicly available and can be obtained by searching for the HERO ID on the public facing webpage available here: https://hero.epa.gov/.

The three database searches yielded 7,160 unique records (combined for PFOA and PFOS) prior to running SWIFT Review. Table 3-1 shows the results from database searches conducted in April 2019, September 2020, February 2022, and February 2023.

Table 3-1. Database Literature Search Results

Database	Date Run: Results
WoS	4/10/2019: 3,081 results 9/3/2020: 1,286 results 2/2/2022: 1,021 results 2/6/2023: 966 results
PubMed	4/10/2019: 2,191 results 9/3/2020: 811 results 2/2/2022: 1,728 results 2/6/2023: 719 results
TOXLINE	4/10/2019: 60 results
TSCATS	4/11/2019: 0 results
Total number of references from all databases for all searches[a]	4/2019: 3,382 results 9/2020: 1,153 results 2/2022: 1,858 results 2/2023: 1,153 results
Total number of references after running SWIFT Review[a]	4/2019: 1,977 results 9/2020: 867 results 2/2022: 1,370 results 2/2023: 881 results
Total number of unique references moved to screening[b]	**4,802**

Notes:
[a] The number of studies includes duplicate references across search dates due to overlap between search years.
[b] Duplicates across search dates removed.

The additional sources of literature outlined in Section 2.1.1 (i.e., assessments published by other agencies, studies identified during epidemiological, mechanistic, or toxicokinetic syntheses, studies identified by the Science Advisory Board (SAB), and EPA's 2016 Health Effects Support Documents (HESDs) for perfluorooctanoic acid (PFOA) (U.S. EPA, 2016c) and perfluorooctane sulfonate (PFOS) (U.S. EPA, 2016b)) yielded 238 unique records (combined for PFOA and PFOS).

The 4,802 studies captured with the SWIFT Review evidence streams filters and the 238 records identified from additional sources yielded a total of 5,011 unique studies. These 5,011 studies were moved to the next stage of screening (title and abstract screening using either DistillerSR or SWIFT ActiveScreener). Of the 5,011 unique studies, 1,062 moved on to full-text level review, 1,697 were excluded during title and abstract screening, and 2,252 were tagged as containing potentially relevant supplemental material. Of the 1,062 screened at the full-text level, 784 were considered to meet population, exposure, comparison, outcome (PECO) eligibility criteria (see Appendix A, (U.S. EPA, 2024a)) and included relevant information on PFOA. The 784 studies that were determined to meet PECO criteria after full-text level screening included 451 epidemiological (human) studies, 40 animal toxicological studies, 15 physiologically based pharmacokinetic (PBPK) studies (2 of which were also relevant epidemiological studies), and 280 studies that were not extracted (e.g., *low* confidence studies, meta-analyses, studies from the 2022 and 2023 searches that did not evaluate effects on one of the priority health outcomes). An additional 20 PBPK studies were identified during the toxicokinetic screening for a total of 35 PBPK studies. Details of the literature search and screening process are shown in Figure 3-1.

The 451 epidemiological studies and 40 animal toxicological studies relevant to PFOA underwent study quality evaluation and were subsequently considered for data extraction as outlined in Sections 2.1.3 and 2.1.4 (see Appendix A, (U.S. EPA, 2024a)). The results of the health outcome-specific study quality evaluations and data extractions are described in Sections 3.4 and 3.5.

Additionally, the 35 studies tagged as containing relevant PBPK models relevant to PFOA were reviewed by pharmacokinetic (PK) subject matter experts for inclusion consideration. The included studies are summarized in Section 3.3.2 and parameters described in these studies were considered for incorporation into the animal and human PK models, which are summarized in Section 4.1.3.

Finally, the 129 toxicokinetic and 273 mechanistic studies identified as relevant for PFOA moved on to a limited data extraction as described in the Appendix (U.S. EPA, 2024a). The toxicokinetic studies pertaining to ADME are synthesized in Section 3.3.1. The mechanistic studies relevant to the five priority health outcomes are synthesized in Sections 3.4 and 3.5 and were considered as part of the evidence integration.

In addition to the studies identified through database searches and the other sources outlined above, public comments submitted in response to the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a) included 944 references relevant to PFOA and/or PFOS, which were reviewed for relevance to the toxicity assessment. Of the 944 studies, 297 were duplicates of studies included in the toxicity assessment and 31 were duplicates of studies included in the 2016 PFOA or PFOS HESD assessment. The 599 studies that were not identified in the HESDs and were not included in the toxicity assessments underwent additional review to identify studies with that could impact assessment conclusions as outlined in Appendix A.3 (U.S. EPA, 2024a). Ultimately, none of the 599 studies were incorporated in the toxicity assessments upon further screening. The submitted references were either deemed not relevant after secondary review, were supplemental studies (e.g., PFOA or PFOS assessments published by other scientific bodies, mechanistic, ADME, etc), or were already included in the PFOA or PFOS toxicity assessments. Additionally, several references reported information on PFOA or PFOS

and non- priority health outcomes and were therefore not included. The results of this screening can be found in the docket ("Review of Public Comment References Related to PFOA and PFOS Health Effects;" https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

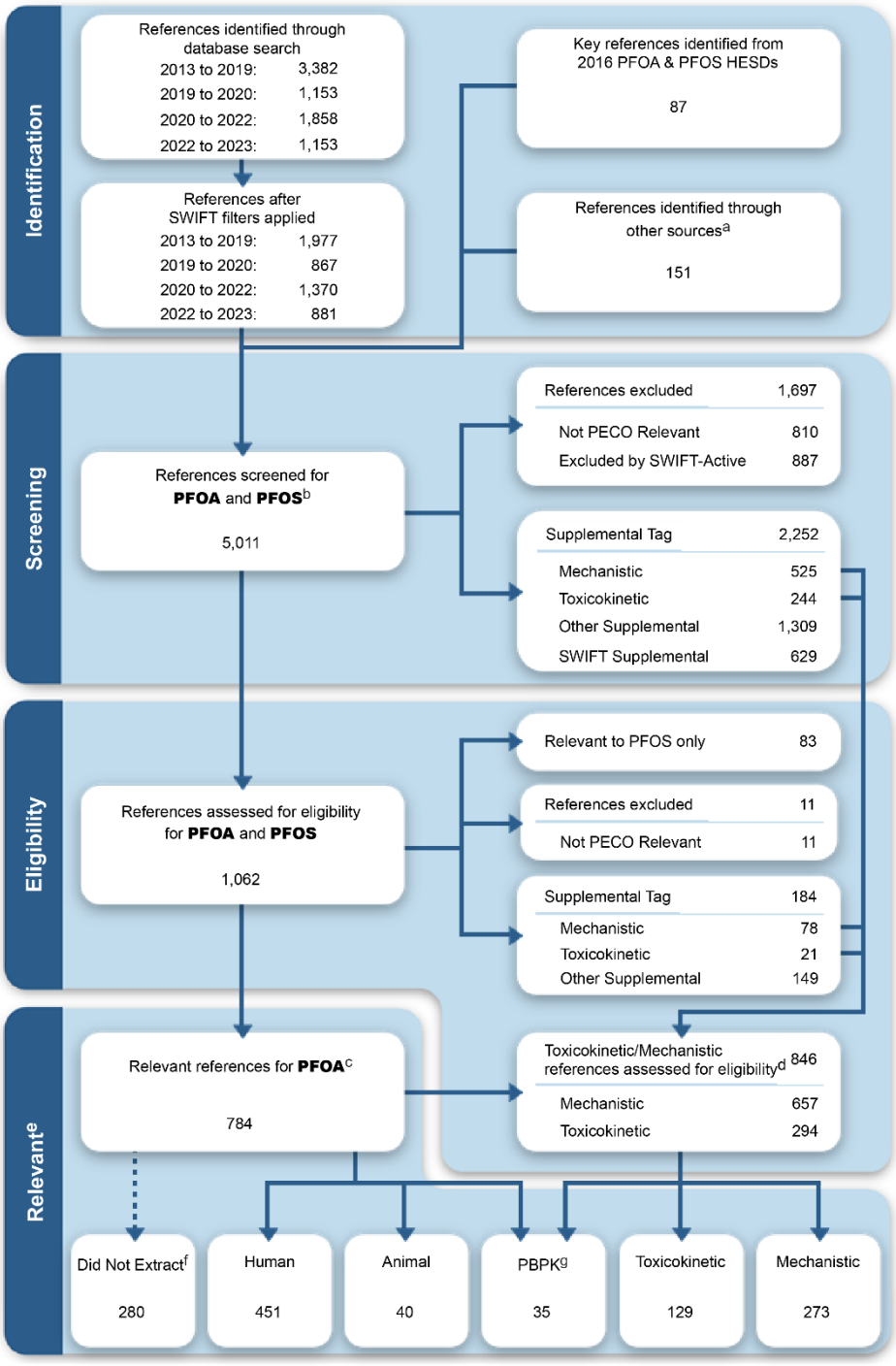


Figure 3-1. Summary of Literature Search and Screening Process for PFOA

Interactive figure and additional study details available on HAWC.
Interactive figure based on work by Magnuson et al. (2022).

"Other sources" include assessments published by other agencies, studies identified during epidemiological, mechanistic, or toxicokinetic syntheses, and studies identified by the SAB.
[a] References identified by SAB and through database searches were counted as identified through database search only.
[b] Includes number of unique references after deduplication of studies captured with the SWIFT Review evidence streams filters and records identified from additional sources.
[c] Includes number of unique references considered to meet PECO eligibility criteria at the full-text level and include relevant information on PFOA.
[d] Includes number of unique references identified during title/abstract screening, full-text screening, and data extraction assessed for toxicokinetic and/or mechanistic eligibility.
[e] Only includes references with relevant information on PFOA.
[f] References tagged to 'Not a priority human health system' include those identified in the 2019 search that overlap with 2016 PFOA HESD references or those identified in 2022 and 2023 searches.
[g] Includes 15 PBPK references (2 of which were also relevant epidemiological references) determined to meet PECO criteria plus an additional 20 PBPK references identified during the toxicokinetic screening.

3.1.1 Results for Epidemiology Studies of PFOA by Health Outcome

Of the 451 epidemiological studies that met the inclusion criteria and underwent extraction, 193 had a cohort study design, 177 had a cross-sectional design, 42 had a case-control design, and 39 had other study designs (e.g., nested case-control). Epidemiological studies were categorized into 18 health outcomes. Most studies reported on the cardiovascular (n = 96), developmental (n = 92), metabolic (n = 78), or immune systems (n = 68). Studies that reported outcomes spanning multiple health outcomes were not counted more than once in the grand totals shown in Figure 3-2.

	Study Design				
Health System	Case-control	Cohort	Cross-sectional	Other	**Grand Total**
Cancer	8	6	3	5	22
Cardiovascular	5	24	60	7	96
Dermal	0	1	0	0	1
Developmental	4	61	20	7	92
Endocrine	1	8	18	8	35
Gastrointestinal	1	6	0	0	7
Hematologic	0	0	7	1	8
Hepatic	1	7	20	4	32
Immune	5	35	19	9	68
Metabolic	7	36	30	5	78
Musculoskeletal	0	1	6	2	9
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	0	6	18	2	26
Reproductive, Male	0	7	14	1	22
Reproductive, Female	9	24	22	4	59
Respiratory	1	4	1	0	6
Other	0	3	3	0	6
Grand Total	42	193	177	39	451

Figure 3-2. Summary of Epidemiology Studies of PFOA Exposure by Health System and Study Design[a]

Interactive figure and additional study details available on HAWC.

[a] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.1.2 Results for Animal Toxicological Studies of PFOA by Health Outcome

Of the 40 animal toxicological studies that met the inclusion criteria and underwent extraction, most studies had either short-term (n = 16) or developmental (n = 16) study designs and most were conducted in mice (n = 33). The mouse studies had developmental (n = 16), short-term (n = 15), and subchronic (n = 2) study designs. The remaining studies reported results for rats (n = 7) using chronic (n = 3), short-term (n = 2), subchronic (n = 1), or reproductive (n = 1) study designs, or monkeys (n = 1) using a chronic study design. Animal toxicological studies were categorized into 15 health outcomes. Most studies reported results for the hepatic (n = 30), whole-body (n = 25; i.e., systemic effects such as bodyweight), reproductive (n = 19), or developmental (n = 15) systems. Studies that reported outcomes spanning multiple health outcomes, study designs, or species were not counted more than once in the grand totals shown in Figure 3-3.

	Study Design & Species								
	Short-term		Subchronic		Chronic		Developmental	Reproductive	Grand Total
Health System	Mouse	Rat	Mouse	Rat	Monkey	Rat	Mouse	Rat	
Cancer	0	0	0	0	0	3	1	0	4
Cardiovascular	2	2	0	0	0	2	3	0	8
Developmental	0	0	0	0	0	1	13	1	15
Endocrine	3	2	0	0	0	3	3	1	11
Gastrointestinal	0	0	0	0	1	2	0	0	3
Hematologic	1	1	0	0	0	1	0	0	3
Hepatic	11	2	2	1	0	3	11	1	30
Immune	5	2	2	0	0	2	2	1	13
Metabolic	0	1	0	0	0	2	3	0	6
Musculoskeletal	1	0	0	0	0	0	0	0	1
Nervous	2	0	0	0	0	1	2	1	6
Renal	1	1	1	0	0	2	1	1	7
Reproductive	3	1	1	1	0	3	9	1	19
Respiratory	0	1	0	0	0	1	0	0	2
Whole Body	10	2	2	1	0	3	7	1	25
Grand Total	15	2	2	1	1	3	16	1	40

Figure 3-3. Summary of Animal Toxicological Studies of PFOA Exposure by Health System, Study Design, and Species[a,b]

Interactive figure and additional study details available on HAWC.

[a] A study can report on more than one study design and species. Row grand totals represent the number of unique studies and are not a sum of study design and species tags.

[b] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.2 Data Extraction Results

All data from this project are available in the public HAWC site (https://hawc.epa.gov/assessment/100500248/) displayed as exposure-response arrays, forest plots, and evidence maps. Data extracted from the 451 epidemiological studies are available here. Data extracted from the 40 animal toxicological studies are available here. See Sections 3.4 and 3.5 for health outcome-specific data extracted for synthesis development. Additionally, the limited data extractions from the ADME and mechanistic studies are also available in HAWC.

3.3 Toxicokinetic Synthesis

As described in Section 3.1, EPA identified 129 and 35 studies containing information relevant to the toxicokinetics and PBPK modeling of PFOA, respectively. The results of these studies are described in the subsections below and additional information related to toxicokinetic characteristics of PFOA can be found in Appendix B (U.S. EPA, 2024a).

3.3.1 ADME

PFOA is resistant to metabolic and environmental degradation due to its strong carbon-fluorine bonds. It also is resistant to metabolic biotransformation. Thus, the toxicity and pharmacodynamics of the parent compound (the anion when dissociated in water or the body) are the concern. Because of its impacts on cellular receptors and proteins, PFOA can influence the biotransformation of dietary constituents, intermediate metabolites, and other xenobiotic chemicals by altering enzyme activities and transport kinetics. PFOA is known to activate peroxisome proliferator-activated receptor (PPAR) pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Findings of transcriptional activation of many genes in peroxisome proliferator-activated receptor alpha (PPARα)-null mice after PFOA exposure, however, indicate that the effects of PFOA are mediated by other modes of action (MOAs) in addition to PPAR activation and consequent peroxisome proliferation (Wen et al., 2019c; Rosen et al., 2017; U.S. EPA, 2016c; Oshida et al., 2015a; Oshida et al., 2015b). The available data indicate that PFOA exposure can also activate the constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and pregnane X receptor (PXR), and can affect metabolic activities linked to these nuclear receptors (Rosen et al., 2017; U.S. EPA, 2016c; Oshida et al., 2015a; Oshida et al., 2015b). Activation of these receptors resulting from PFOA exposure could in turn impact the toxicokinetics of PFOA itself (Andersen et al., 2008).

PFOA is not readily eliminated from humans and other primates. Toxicokinetic profiles and the underlying mechanism for half-life differences between species and sexes are not completely understood, although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. Thus far, three transport families appear to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs) (Klaassen and Aleksunes, 2010; Launay-Vacher et al., 2006). These transporters are critical for gastrointestinal absorption, uptake by the tissues, and excretion via bile and the kidney. These transport systems are located at the membrane surfaces of the kidney tubules, intestines, liver, lungs, heart, blood brain barrier (BBB), blood placental barrier, blood testes barrier (BTB), and mammary glands where they function to protect the organs, tissues, and fetus through active removal of foreign compounds (Klaassen and Aleksunes, 2010 Zaïr, 2008, 9641805; Ito and Alcorn, 2003). However, luminal transporters in the kidney may cause reuptake of PFOA from the proximal tubule resulting in decreased excretion from the body (Weaver et al., 2010). This reuptake would lead to PFOA persisting in the body over time. Transporters involved in enterohepatic circulation have also been identified that may facilitate uptake and reuptake of PFOA from the gut (Ruggiero et al., 2021).

There are differences in transporters across species, sexes, and individuals. In addition, more PFOA-specific information is available for the OAT and OATP families than for the MRPs.

These data limitations have hindered the development of PK models for use in predicting effects in humans based on the data from animal toxicological studies.

3.3.1.1 Absorption

PFOA absorption data are available in laboratory animals for oral, inhalation, and dermal exposures, and extensive data are available from humans demonstrating the presence of PFOA in serum (descriptions of available studies are provided in the Appendix, (U.S. EPA, 2024a)). In vitro absorption data indicate that uptake is influenced by pH, temperature, and concentration as well as OATP activity (see Appendix B, (U.S. EPA, 2024a)).

3.3.1.1.1 Cellular Uptake

The available information indicates that the absorption process requires transport from the external environment across the interface of the gut, lung, or skin. Uptake in cells cultured in vitro is fast and saturable, consistent with the role of transporters. Cellular transfection of cells with vectors coding for organic ion transporters have confirmed their role in uptake of PFOA (Kimura et al., 2017; Yang et al., 2010; Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). Several studies suggest involvement of OATs, OATPs, and MRPs in enterocytes in the uptake of PFOA (Klaassen and Aleksunes, 2010; Zaïr et al., 2008). Few studies have been conducted on the intestinal transporters for PFOA in humans or laboratory animals, although one study supports a role for OATPs in PFOA uptake by immortalized intestinal cells (Kimura et al., 2017). Most of the research has focused on transporters in the kidney that are relevant to excretion and were carried out using cultured cells transfected with the transporter proteins.

In addition to facilitated transport, there is evidence supporting passive diffusion in cells cultured in vitro (Yang et al., 2009b) and in placenta in vivo (Zhang et al., 2013b). Since PFOA is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across interface membranes was thought to be dominated by transporters or mechanisms other than simple diffusion across the lipid bilayer. Recent mechanistic studies, however, support transporter-independent uptake through passive diffusion processes. Ebert et al. (2020) determined membrane/water partition coefficients ($K_{mem/w}$) for PFOA and examined passible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. In this system, the partition coefficients (PCs) were considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes.

Uptake by cells may be influenced by interactions with lipids and serum proteins. PFOA exhibited lower levels of binding to lipids and phospholipids relative to PFOS, which correlated with uptake into lung epithelial cells (Sanchez Garcia et al., 2018). Phospholipophilicity correlated to cellular accumulation better than other lipophilicity measures. The extent to which PFOA phospholipophilicity influences absorption through the gastrointestinal tract, lungs, or skin is unknown.

3.3.1.1.2 Absorption and Bioavailability in Humans and Animals

In vivo, PFOA is well-absorbed following oral exposure, as evidenced by the presence of PFOA in serum of humans following exposure to contaminated drinking water (Xu et al., 2020c; Worley et al., 2017a). Studies on male rats administered PFOA by gavage using a single or multiple dose regimen estimated dose absorption of at least 92.3% (Cui et al., 2010; Gibson and

Johnson, 1979). In rats, the time to reach the maximum PFOA plasma concentration (T_{max}) following oral exposure is very fast and varies by sex (Dzierlenga et al., 2019a; Kim et al., 2016). For example, the study by Kim and colleagues estimated T_{max} after a single oral dose of 1 mg/kg to be 1.44 hours in female rats versus 2.07 days in males.

Recent studies confirm that bioavailability of PFOA after oral exposure is very high in rats. Serum concentrations after oral dosing ranged from 82%–140% of levels measured after intravenous (IV) dosing, which may reflect increased reabsorption by intestinal transporters by the oral route relative to the IV route of exposure (Dzierlenga et al., 2019a; Kim et al., 2016). Bioavailability of PFOA appears to be modified by diet. Using in vitro and in vivo (BALB/c mice) systems, Li et al. (2015) found that PFOA bioavailability is strongly influenced by diet, with high fat diets associated with reduced absorption. The authors suggest that colloidal stability in intestinal solutions may be an important factor influencing PFOA bioaccessibility.

The available data, although limited, also support PFOA absorption through both inhalation (Hinderliter et al., 2006a) and dermal routes (Fasano et al., 2005; Kennedy, 1985; O'Malley and Ebbins, 1981).

3.3.1.2 Distribution

3.3.1.2.1 PFOA Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature regarding the distribution of PFOA in humans and animals are provided in Appendix B (U.S. EPA, 2024a). Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. Distribution of PFAS to plasma has been reported to be chain length-dependent (Jin et al., 2016). Increasing chain length (from C6 to C11) correlated with an increased mass fraction in human plasma. Within the blood cell constituents, PFOA preferentially accumulates in platelets over red blood cells and leukocytes (De Toni et al., 2020). Among different kinds of human blood samples, PFOA accumulates to highest levels in plasma, followed by whole blood and serum (Forsthuber et al., 2020; Poothong et al., 2017; Jin et al., 2016). Poothong et al. (2017) found that median PFOA concentrations in plasma, serum, and whole blood were 1.90, 1.60, and 0.93 ng/mL, respectively. These findings suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum (Ehresman et al., 2007) will not provide accurate estimates for PFOA.

PFOA is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOA interactions with human serum albumin (HSA) (Gao et al., 2019; Cheng and Ng, 2018; Yue et al., 2016; Zhang et al., 2013a; Macmanus-Spencer et al., 2010; Qin et al., 2010; Salvalaglio et al., 2010; Weiss et al., 2009; Wu et al., 2009; Luebker et al., 2002). In vitro analyses found that plasma proteins can bind 97%–100% of the PFOA in plasma from humans, cynomolgus monkeys, and rats (Kerstner-Wood et al., 2003). HSA is the primary PFOA binding protein in plasma (Han et al., 2003) and intermolecular interactions are mediated through van der Waals forces and hydrogen bonds (Chen et al., 2020; Macmanus-Spencer et al., 2010). Beesoon and Martin (2015) determined that linear PFOA molecules bound more strongly to calf serum albumin than the branched-chain isomers in the order of 4m < 3m < 5m < 6m (iso) < linear. PFOA-mediated conformational changes may interfere with albumin's ability to transport its natural ligands and pharmaceuticals (Wu et al., 2009) such as fatty acids, thyroxine (T4), warfarin, indole, and benzodiazepine.

Binding to albumin and other serum proteins may affect transfer of PFOA from maternal blood to the fetus (Gao et al., 2019). Since there is effectively a competition between PFOA binding in maternal serum versus cord blood, lower cord blood albumin levels compared with maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017) found that high concentration of cord serum albumin was associated with higher PFOA transfer efficiencies, whereas high maternal serum albumin concentration was associated with reduced transfer efficiency.

Other plasma proteins that bind PFOA, albeit with lower affinity than HSA, include low-density lipoproteins (LDLs), alpha-globulins (alpha-2-macroglobulin), gamma-globulins, transferrin, and fibrinogen (Kerstner-Wood et al., 2003). PFOA also binds the serum thyroid hormone transport protein, transthyretin (TTR), causing up to a 50% inhibition of T4 binding to TTR (Weiss et al., 2009). In contrast to serum proteins, little is known regarding PFOA binding to proteins in the gut. One study found that PFOA can bind to and cause a conformational change in pepsin (Yue et al., 2016), though it is unclear whether PFOA-pepsin interactions impact absorption from the gut or distribution to other compartments in the body.

3.3.1.2.2 PFOA Binding to Subcellular Fractions, Intracellular Proteins, and Transporters

Han et al. (2005) observed a sex-dependent subcellular distribution of PFOA in the liver and kidney of male and female adult rats necropsied 2 hours after oral gavage dosing. The proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats. They hypothesized that females might have a greater amount than males of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. In the kidney, the subcellular distribution did not show a sex difference comparable to the one seen for liver; however, the protein-bound fraction in males (42%) was about twice that of females (17%), which differs from the sex differences found for the liver.

In a study of human cells (Zhang et al., 2020a), PFOA preferentially distributed to cytosol followed by nuclei and mitochondria in human colorectal cancer cells, human lung epithelial cells, and human normal liver cells. In liver cells, PFOA binds to the liver fatty acid binding protein (L-FABP) through polar and hydrophobic interactions (Yang et al., 2020a; Zhang et al., 2013a; Luebker et al., 2002). L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators (Erol et al., 2004) and constitutes 2%–5% of the cytosolic protein in hepatocytes.

PFOA interactions with various protein transporters play a role in the tissue uptake of orally ingested PFOA. The transporters are located at the interface between serum and a variety of tissues (e.g., liver, kidneys, lungs, heart, brain, testes, ovaries, placenta, uterus) (Klaassen and Aleksunes, 2010). The liver is an important uptake site for PFOA. OATPs and MRPs, at least one OAT, and the sodium-taurocholate cotransporting polypeptide (NTCP) – a hepatic bile uptake transporter – have been identified at the boundary of the liver at the portal blood and/or the canalicular membranes within the liver (Kusuhara and Sugiyama, 2009; Zaïr et al., 2008; Kim, 2003). Transporters responsible for PFOA transport across the placenta are not well understood, though preliminary studies examining transporter expression identified OAT4 as a candidate receptor (Kummu et al., 2015). The expression of nine transporter genes was found to

vary at different stages of gestation (Li et al., 2020a), though direct experimental evidence for these transporters in mediating transfer of PFOA to the fetus is lacking.

3.3.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOA distributes to a wide range of tissues, organs, and matrices throughout the body. Although blood and liver are major sites of PFOA accumulation (Olsen et al., 2001c), recent findings confirm PFOA accumulation in other tissues and fluids including brain and cerebral spinal fluid (Wang et al., 2018; Fujii et al., 2015; Maestri et al., 2006), major organs including lung and kidney (Maestri et al., 2006), endocrine tissues including the thyroid gland, pituitary gland, and pancreas (Pirali et al., 2009; Maestri et al., 2006), and gonads and follicular fluid (Kang et al., 2020; Maestri et al., 2006). Pérez et al. (2013) measured PFOA levels in autopsy tissue samples (liver, kidney, brain, lung, and bone) collected within 24 hours of death and found PFOA in bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below the limit of detection (LOD) in the brain. Maestri et al. (2006) measured pooled post-mortem tissue samples and found the highest levels in lung (3.8 ng/g), kidney (3.5 ng/g), and liver (3.1 ng/g). It should be noted, however, that autopsy and surgical tissues may not necessarily accurately reflect PFAS tissue distribution in the living body (Cao and Ng, 2021). Several studies examined a limited number of tissues in primates and observed higher levels in serum compared with liver (Butenhoff et al., 2004b; Butenhoff et al., 2002; Griffith and Long, 1980).

Most whole animal toxicological studies that measured PFOA distribution were conducted in rats and mice by oral dosing. Studies in primates measured PFOA in blood and liver following oral administration (Butenhoff et al., 2004b; Butenhoff et al., 2002). PFOA primarily distributes to serum, liver, lungs, and kidney across a range of dosing regimens and durations (NTP, 2020, 2019; Kemper, 2003; Ylinen et al., 1990) in rats and in mice (Guo et al., 2019; Burkemper et al., 2017; Li et al., 2017b; Lou et al., 2009; Lau et al., 2006). Sex-specific differences in PFOA levels were observed in several rat studies. For example, in a 28-day study (NTP, 2019), PFOA plasma concentrations were higher in males than in females across all dose groups even though females were administered a 10-fold higher dose of PFOA, suggesting that female rats excrete PFOA more efficiently than males. Sex-specific differences were less striking in studies conducted in mice compared with rats (Lou et al., 2009; Lau et al., 2006).

Liver PFOA levels are regulated in part by PPARα. In human and rodent hepatocytes, PPARα activation induces expression of genes involved in lipid metabolism and cholesterol homeostasis. PFOS and PFOA structurally resemble fatty acids and are well-established ligands of PPARα in the rat and mouse liver. As PPARα agonists, PFOS and PFOA can induce ß-oxidation of fatty acids, induce fatty acid transport across the mitochondrial membrane, decrease hepatic very low-density lipoprotein (VLDL)-triglyceride and apolipoprotein B (apoB) production, and promote lipolysis of triglyceride-rich plasma lipoproteins (Fragki et al., 2021). The liver can transport PFOA from hepatocytes to bile ducts, which is mediated at least partly by PPARα (Minata et al., 2010). PFOA levels were significantly lower in PPARα-null mice than in wild-type mice exposed to doses of 25 and 50 µmol/kg, supporting a role for PPARα in PFOA clearance in the liver (Minata et al., 2010) but not excluding other factors regulating PFOA levels. It is unclear what role PPARα plays in PFOA clearance in the liver of humans.

Studies administering radiolabeled PFOA to whole animals demonstrate the range of tissue distribution in rats (Kemper, 2003) and mice (Bogdanska et al., 2020; Burkemper et al., 2017) that includes the central nervous system (CNS), cardiovascular, gastrointestinal, renal, immune, reproductive, endocrine, and musculoskeletal systems. PFOA crossed the BBB in males an order of magnitude more efficiently than in females (Ylinen et al., 1990). Fujii and colleagues (2015) found that PFOA can cross the BBB in mice, although a relatively small amount of administered PFOA was measured in the brains (0.1%). Also in mice, Burkemper et al. (2017) observed the highest PFOA levels in bone, liver, and lungs. Bogdanska et al. (2020) also observed PFOA in testes of C57BL/6 mice at levels similar to those observed in epididymal fat and in intestines. In BALB/c mice exposed to PFOA for 28 days, PFOA levels in the testes increased with increasing dose (Zhang et al., 2014b), and PFOA accumulated in the epididymis of BALB/c mice in a dose-dependent manner (Lu et al., 2016).

Fujii and colleagues (2015) observed that perfluoroalkyl carboxylic acids (PFCAs) (C6 and C7) were excreted relatively rapidly through urine in mice, whereas longer-chained PFCAs (≥C8) accumulated in the liver. Moreover, PFAS with longer chain lengths were found to exhibit increasing affinity for serum and L-FABPs. The authors suggest that differential lipophilicity driven by chain length may account for the distribution patterns of PFAS, which is consistent with the findings of high levels of PFOA accumulation in serum and liver. These large sequestration volumes of PFOA observed in the liver seem to be attributable to the liver's large binding capacity in mice.

3.3.1.2.4 Distribution During Reproduction and Development

Many recent human studies have quantified the distribution of PFOA from pregnant mothers to their fetuses and from mothers to their infants. Distribution from pregnant mother to fetus has been confirmed by measuring PFOA levels in placenta, cord blood, and amniotic fluid during gestation and at birth. The ratio of PFOA in placenta relative to maternal serum during pregnancy (R_{PM}) ranged from 0.326 to 0.460 (Chen et al., 2017a; Zhang et al., 2013b). Gestational age and PFOA branching characteristics influence transport across the placenta. PFOA concentrations within the placenta increase during gestation from the first to third trimester (Mamsen et al., 2019). Linear PFOA is detected at a higher frequency and at higher concentrations in maternal serum than branched PFOA isomers. However, branched PFOA is more efficiently transported into the placenta than linear PFOA (Cai et al., 2020; Chen et al., 2017a).

Several studies reported a strong positive correlation between maternal and cord serum PFOA levels in humans (Kato et al., 2014; Porpora et al., 2013). The ratio of PFOA in cord serum relative to maternal serum ranged from 0.55 to 1.33 (see Appendix, (U.S. EPA, 2024a)) and generally increased with gestational age (Li et al., 2020a). Factors such as exposure sources, parity, and other maternal demographics are postulated to influence variations in maternal serum PFAS concentrations and cord:maternal serum ratios (Brochot et al., 2019; Kato et al., 2014). Cord:maternal serum ratios represent transplacental efficiencies (TTEs), which exhibit a U-shaped curve with PFAS chain length (Zhang et al., 2013b) and generally increase as the PFAS branching point moves closer to the carboxyl or sulfonate moiety (Zhao et al., 2017a).

Lower levels of PFOA were measured in amniotic fluid compared with the placenta and cord blood (all collected at delivery) (Zhang et al., 2013b). The mean concentration ratio between

amniotic fluid and maternal blood (collected no more than one hour before delivery) was higher for PFOA (0.13) than for PFOS (0.0014). The mean concentration ratio between amniotic fluid and cord blood was higher for PFOA (0.023) than for PFOS (0.0065). Authors attributed the differences in ratios between the two compartments to the solubilities of PFOS and PFOA and their respective protein binding capacities in the two matrices.

PFOA also distributes widely in human fetal tissues. Mamsen et al. (2017) measured the concentrations of five PFAS in fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark. PFOA was detected in placenta and fetal liver, extremities, heart, intestines, lungs, connective tissues, spinal cord, and ribs, and concentrations were highest in the placenta and lung. Different patterns of PFOA distribution were observed in fetal tissues depending on fetal age (Mamsen et al., 2019). Fetal tissue:maternal serum ratios of PFAS were calculated by dividing the fetal tissue concentration by the maternal serum concentration. In general, fetal tissue:maternal serum ratios of PFOA increased from the first trimester to the third trimester, except for the liver and heart, which showed the highest fetal tissue:maternal serum ratios in the second trimester compared with the third trimester.

Studies in humans also confirm that the distribution of PFOA from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding (Cariou et al., 2015 Mogensen, 2015, 3859839, Gyllenhammar, 2018, 4778766; Mondal et al., 2014). Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching. In the Mondal study (Mondal et al., 2014), the mean maternal serum PFOA concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOA than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding (Mogensen et al., 2015b; Mondal et al., 2014). Cariou et al. (2015) reported that PFOA levels in breastmilk were approximately 30-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOA was 0.038 ± 0.013. The authors noted that the transfer rates of PFAS from serum to breastmilk were lower compared with other lipophilic persistent organic pollutants such as polychlorinated biphenyls.

Several studies have confirmed PFOA distribution from rat and mouse dams to fetuses and pups, as well as variable PFOA levels across many fetal tissues (Blake et al., 2020; Macon et al., 2011; White et al., 2011; Fenton et al., 2009; Hinderliter et al., 2006b; Butenhoff et al., 2004a; Han et al., 2003; Mylchreest, 2003). Interestingly, Fujii et al. (2020) found that the milk/plasma (M/P) concentration ratio for PFOA also exhibited a U-shaped curve with increasing chain length but it did not correlate to lipophilicity of PFAS in FVB/NJcl mice. These findings suggest that the amount transferred from mother to pup during lactation may also relate to chain length-dependent clearance.

3.3.1.2.5 Volume of Distribution in Humans and Animals

In humans, the volume of distribution (V_d) for PFOA has been assigned values between 170 and 200 mL/kg (see Appendix B, (U.S. EPA, 2024a)). V_d values may be influenced by differences in distribution between males and females, between pregnant and nonpregnant females, and across serum, plasma, and whole blood.

V_d estimates derived in mice and rats vary by species, age, sex, and dosing regimen. For example, Dzierlenga et al. (2019a) calculated the apparent volume of central and peripheral

distribution in male and female adult rats after oral dosing. A one-compartment model for males and a two-compartment model for females was used to characterize PFOA levels. Peripheral V_d values were dramatically lower than central V_d values at all doses after oral administration and, interestingly, also after IV administration. While peak tissue levels were reached readily in both males and females, tissue levels in males were steady over the course of several days whereas tissue levels in females dropped quickly, in the span of hours. Further discussion on the V_d for PFOA can be found in Section 5.6.2.

3.3.1.3 Metabolism

Consistent with other peer-reviewed, published reports and reviews (ATSDR, 2021; Pizzurro et al., 2019; U.S. EPA, 2016c), the literature reviewed for this assessment do not provide evidence that PFOA is metabolized in humans, primates, or rodents.

3.3.1.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Most studies have investigated the elimination of PFOA in humans, cynomolgus monkeys, and rats. Fewer studies measured elimination in mice, hamsters, and rabbits. Available evidence supports urine as the primary route of excretion in most species, though fecal elimination is prominent in rats. In rats, hair is another route of elimination in both males and females. In female humans and animals, elimination pathways include menstruation, pregnancy (cord blood, placenta, amniotic fluid, and fetal tissues) and lactation (breast milk) (see Appendix B, (U.S. EPA, 2024a)). Results of elimination half-life determination studies in mammals suggest that PFOA elimination time is longest in humans (years), intermediate in monkeys (days to weeks), and shortest in rodents (hours to days).

3.3.1.4.1 Urinary and Fecal Excretion

Studies in laboratory animals provide evidence that urine is typically the primary route of excretion but that sex impacts excretion by both routes, and these sex differences appear to be species-specific. Limited evidence supports excretion via the fecal route in laboratory animals and humans and via hair in animals. Most studies in all species indicate that excretion by the fecal route is substantially lower than that observed by the urinary route. Excretion through the fecal route appears to be more prominent in males compared with females and in rodents compared with humans. Nevertheless, a comprehensive set of principles governing resorption by renal, hepatic, and enteric routes and how these impact excretion and retention of PFOA has not been established in either humans or animals.

Human studies examined PFOA excretion after oral exposure, primarily through the urinary route. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the sex and age of the individual. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, indicating that renal resorption is less prevalent for the branched-chain isomers (Fu et al., 2016; Zhang et al., 2015).

Fujii et al. (2015) measured PFOA clearance in mice and humans. Male and female FVB/NJcl mice were administered PFOA by IV (0.31 μmol/kg) or gavage (3.13 μmol/kg) and serum concentration data were analyzed using a two-compartment model. Mouse

urinary clearance was analyzed by dividing the total amount excreted in the urine during a 24-hour period with the area under the curve (AUC) of the serum concentration. Human data were analyzed from paired (bile-serum) archived samples from patients undergoing nasobiliary drainage, percutaneous transhepatic biliary drainage, or percutaneous transhepatic gallbladder drainage for 24 hours. Urine-serum pairs were collected from healthy donors. Urinary and biliary clearance was determined by dividing the cumulative urine or bile excretion in a 24-h period with the serum concentration. Fecal clearance was calculated using the estimated biliary resorption rate.

The authors estimated that the total human clearance for PFOA was 0.096 mL/kg/day; PFOA clearance rates via urinary, biliary, and fecal routes were estimated to be 0.044, 2.62, and 0.052 mL/kg/day, respectively. The reabsorption rate of bile excreting PFOA was estimated to be 0.98 (derived by assigning a V_d of 200 mL/kg, a serum half-life of 3.8 years, and the presumption that PFOA could only be excreted into the urine and feces via the bile). The authors also noted that estimated total human clearance was 50–100 times lower than the estimated PFOA clearances in mice after oral gavage dosing.

In rats, urine PFOA concentrations differed with age, dose, and sex (Hinderliter et al., 2006b). For all rats dosed between 3 and 8 weeks of age, urinary excretion of PFOA was substantially higher in females than in males, and this difference increased with age. Several additional studies in rats found that females excreted much higher levels in urine compared with males and compared with feces (Kim et al., 2016; Cui et al., 2010; Benskin et al., 2009).

3.3.1.4.2 Renal and Enterohepatic Resorption

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats (Cheng et al., 2006; Hinderliter et al., 2006b; Kudo et al., 2002). Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs and OATPs located in the proximal portion of the descending tubule (Yang et al., 2010; Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). The results of in vitro studies were consistent with an in vivo analysis of OATPs gene and protein expression in rat kidneys (Yang et al., 2009b). Organic anion transporters polypeptide 1a1 (OATP1a1) is located on the apical side of proximal tubule cells and is a potential mechanism for renal reabsorption of PFOA in rats. The level of messenger ribonucleic acid (mRNA) of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney and is regulated by sex hormones. Thus, higher expression of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate which is consistent with reduced excretion in males.

Fewer studies have investigated enterohepatic resorption of PFOA. Gastrointestinal elimination of PFOA was reported in a case report of a single human male with high serum levels of perfluorinated chemicals who was treated with a bile acid sequestrant (cholestyramine (CSM)) (Genuis et al., 2010). Before treatment, PFOA was detected in urine (3.72 ng/mL) but not in stool (LOD = 0.5 ng/g) or sweat samples. After treatment with CSM for 1 week, the serum PFOA concentration decreased from 5.9 ng/g to 4.1 ng/g, and stool PFOA levels increased to 0.96 ng/g. This observation suggests that PFOA is excreted in bile and that enterohepatic resorption via intestinal transporters limits the loss of PFOA via feces. Studies in mice (Cheng and Klaassen, 2008a; Maher et al., 2008) suggest that increased expression of MRP3 and MRP4,

coupled with decreased OATP levels, leads to increased biliary excretion of bile acids, bilirubin, and potentially toxic exogenous substances, including PFOA.

Zhao et al. (2017b) demonstrated that PFOA was a substrate for human OATP1B1, OATP1B3, and OATP2B1 transporters expressed in liver using in vitro studies of Chinese hamster ovary (CHO) and human embryonic kidney (HEK-293) cells transfected with transporter complementary DNA (cDNA). Under these conditions, the three OATPs expressed in human hepatocytes can transport the longer chain PFOA (C8) and perfluorononanoate (C9), but not the shorter chain perfluoroheptanoate (C7). Preliminary evidence suggests that enterohepatic resorption could limit elimination of PFOA by the fecal route, including the recent observation that PFOA binds to NTCP, a transporter that mediates the uptake of conjugated bile acids (Ruggiero et al., 2021). The extent to which this pathway operates in vivo and whether enterohepatic resorption plays a substantial role in the retention of PFOA in humans and animals is still unknown.

3.3.1.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

In humans, PFOA can readily pass from mothers to their fetuses during gestation and through breast milk during lactation. In conjunction with elimination through menstruation, discussed in Section 3.3.1.4.4, human females clearly eliminate PFOA through routes not available to males. The total daily elimination of PFOA in pregnant human females was estimated to be 11.4 ng/day, lower than the 30.1 ng/day estimated for PFOS (Zhang and Qin, 2014). Mamsen et al. (2019) estimated a placenta PFOA accumulation rate of 0.11% increase per day during gestation and observed that the magnitude of elimination may be influenced by the sex of the fetus. A human study by Zhang et al. (2013b) observed that the mean levels in the cord blood, placenta, and amniotic fluid were 58%, 47%, and 1.3%, respectively, of those in the mother's blood, demonstrating that cord blood, placenta, and amniotic fluid are additional routes of elimination in pregnant females. Blood loss during childbirth could be another source of excretion. Underscoring the importance of pregnancy as a lifestage when excretion is altered, Zhang et al. (2015) observed that the partitioning ratio of PFOA concentrations between urine and whole blood in pregnant women (0.0011) was lower than the ratios found in nonpregnant women (0.0028). The rate and extent of elimination through these routes are affected by parity (Lee et al., 2017b; Jusko et al., 2016) and may be affected by the increase in blood volume during pregnancy (Pritchard, 1965).

Women can also eliminate PFOA via lactation (Kang et al., 2016a; Thomsen et al., 2010; Tao et al., 2008). Cariou et al. (2015) measured PFOA in maternal serum, cord serum, and breast milk from females with planned Cesarean births. The observed mean ratio of cord serum to maternal serum PFOA was 0.78 in this study. However, the mean ratio between breast milk and maternal serum was 0.038, suggesting transfer from maternal blood to breast milk is lower than transfer from maternal blood to cord blood.

Studies in laboratory animals support elimination through pregnancy and lactation similar to what has been observed in humans. Fujii et al. (2020) used the M/P concentration ratio as a measure of chemical transferability in FVB/NJcl mice. Maternal plasma PFOA concentrations were significantly higher than in milk (M/P ratio was 0.32). The M/P ratios were similar for C8, C9, C12, and C13, arguing against a direct relationship with lipophilicity. Potential roles for binding proteins in breast milk or transporters in breast tissue have not been investigated.

In summary, partitioning to the placenta, amniotic fluid, fetus, and breast milk represent important routes of elimination in humans, and may account for some of the sex differences observed for blood and urinary levels of PFOA by sex and lifestage.

3.3.1.4.4 Other Routes of Elimination

Menstruation may be an important factor in the sex-specific differences observed in PFOA elimination. Zhang et al. (2013c) estimated a menstrual serum PFOA clearance rate of 0.029 mL/day/kg. The link between menstruation and PFOA elimination is based on several observations. First, postmenopausal females and adult males have longer PFOA elimination half-lives than premenopausal adult females (Zhang et al., 2013c). Challenging the assumption that this is due to menstruation, Singer et al. (2018) failed to find evidence of associations between menstrual cycle length and PFAS concentrations. Second, several studies reported on an association between increased serum concentrations of PFOA and PFOS and early menopause (Taylor et al., 2014; Knox et al., 2011). However, a reanalysis of these data (Ruark et al., 2017) suggested that the association between increased serum PFAS and early menopause could be explained by reversed causality, and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Ruark et al. (2017) thus highlight the importance of considering menstrual blood loss as a PFAS elimination pathway. Additional studies may be needed to clarify the significance of menstruation in PFOA elimination.

One study, Gao et al. (2015a), found that hair is a potential route of PFAS elimination in rats. A dose-dependent increase in hair PFOA concentration was observed in all exposed animals. Interestingly, hair PFOA concentrations for all treatment doses were significantly higher in males than in females. The sexually dimorphic difference in hair concentrations may be attributed to the sex differences observed in PFOA elimination rate and the transfer from serum to hair.

3.3.1.4.5 Half-Life Data

Because there is no evidence that PFOA is metabolized in mammals, half-life determinations are governed by excretion. There have been several studies of half-lives in humans all supporting a long residence time for serum PFOA with estimates measured in years rather than months or weeks (see Appendix B, (U.S. EPA, 2024a)). The calculated PFOA half-lives reported in the literature vary considerably, which poses challenges in predicting both the routes and rates of excretion. Half-life estimates vary considerably by species, being most rapid in rodents (measured in hours to days), followed by primates (measured in days to weeks) and humans (measured in years). Half-life estimates were shorter in human and rodent females relative to males. In the single primate study discussed below, half-lives were shorter in males compared with females.

PFOA half-life values in humans ranged from 0.53 years for a branched PFOA in young adult females (Zhang et al., 2013c) to 22 years in a study of primiparous women in Sweden (Glynn et al., 2012) and varied by geographical region (Gomis et al., 2017) (see Appendix B, (U.S. EPA, 2024a)). Age, lifestage, and sex differences in PFOA half-lives have not been rigorously evaluated, though estimates in males are generally longer than those in females (Li et al., 2018c; Gomis et al., 2017; Fu et al., 2016) and exhibit an age-related increase in adults (Genuis et al., 2014 Zhang, 2013, 3859849). While most studies were conducted in adults and/or adolescents,

one study in newborns (Spliethoff et al., 2008) calculated a half-life for PFOA of 4.4 years. Linear isomers exhibit longer half-lives than branched isomers (Zhang et al., 2013c).

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOA half-lives along with measured intake and serum and urine PFOA concentrations (Xu et al., 2020c; Worley et al., 2017a; Fu et al., 2016; Zhang et al., 2013d) (see Appendix B, (U.S. EPA, 2024a)). PFOA half-life values among these 4 studies varied from 1.7 years in Xu et al. (2020c) to 4.7 years in Fu et al. (2016). These comparisons support principles suggested by the broader literature. First, sex related differences with males exhibiting somewhat longer half-lives compared with females (especially females of reproductive age) may relate, at least in part, to menstruation as a route of elimination (Zhang et al., 2013c). Second, blood and urine concentrations varied by several orders of magnitude across these four studies. While blood and urine PFOA concentrations varied by two orders of magnitude across these studies, half-life estimates were similar, ranging from 1.77 to 4.70 years. This variability in serum and urine concentrations may reflect the role of nonurinary routes of PFOA excretion; the variability in concentrations may also reflect the difficulty in measuring renal resorption. Finally, only two studies estimated PFOA intake in subjects (Xu et al., 2020c; Worley et al., 2017a). The multiple routes of exposure to PFOA and the need to understand historical exposure levels to estimate PFOA intake is an ongoing challenge for many studies that examine PFOA elimination. These factors, as well as age and health status of subjects, likely contribute to the reported variability in PFOA half-life estimates in humans.

In experimental animals, half-life values are reported in days rather than in years. Values in cynomolgus monkeys ranged from 13.6 to 41.7 days (Butenhoff et al., 2004b) and were generally longer than those observed in rodents, but much shorter than values observed in humans. Depending on the experimental conditions, half-lives in rats ranged from 0.03 days in females exposed to a high dose of 40 mg/kg (Dzierlenga et al., 2019a) to 13.4 days in males exposed to a relatively low dose of 0.4 mg/kg (Benskin et al., 2009). Rats exposed by the IV route exhibited shorter half-lives than rats administered the same dose by the oral gavage route (Dzierlenga et al., 2019a; Kim et al., 2016). Similar to humans and mice, half-life estimates were shorter in adult female rats compared with male rats. In contrast, female half-life values exceeded male values in cynomolgus monkeys, suggesting that species-specific factors impact elimination across sexes. Similar to findings in humans, PFOA branched isomers exhibited shorter half-lives compared with linear forms.

3.3.2 Pharmacokinetic Models

Pharmacokinetic (PK) models are tools for quantifying the relationship between external measures of exposure and internal measures of dose. For this assessment, PK models were evaluated for their ability to allow for 1) cross-species PK extrapolation of animal studies of both cancer and noncancer effects and 2) the estimation of the external dose associated with an internal dose metric that represents the POD calculated from either animal toxicological or epidemiological studies. The following sections first describe and evaluate published PK modeling efforts and then present conclusions from analyses that assessed the utility of the models to predict internal doses for use in dose-response assessment.

Numerous PK models for PFOA have been developed and published over the years to characterize the unique ADME described in Section 3.3.1. These approaches can be classified

into three categories: classical compartmental models, modified compartmental models, and PBPK models. With classical compartmental modeling, the body is defined as either a one- or two-compartment system with volumes and intercompartmental transfer explicitly fit to the available PFAS PK dataset. Modified compartmental models are more physiologically based in that they attempt to characterize unique aspects of in vivo ADME through protein binding, cardiac output, and known renal elimination from the published literature. However, these models still rely on explicit fitting of data to the non-physiological parameters. Finally, PBPK models describe the tissues and organs of the body as discrete, physiologically based compartments with transport between compartments informed by the available data on physiologically relevant quantifications of blood flow and tissue perfusion. Determining additional, non-physiological parameters typically requires explicitly fitting the PBPK model to time-course concentration data. However, the number of parameters estimated through data fitting is generally fewer than for classical PK or modified compartmental models. A review of the available PK models regarding their ability to predict PFOA ADME is provided below.

3.3.2.1 Classical Compartmental Analysis

The most common approach for the prediction of serum levels of PFOA is to apply a relatively simple one-compartment model. This type of model describes the toxicokinetics of the substance with a single differential equation that describes the rate of change in the amount or concentration of the substance over time as a function of the exposure rate and the clearance rate. This type of model describes the relationship between exposure, serum concentration, and clearance and can be used to predict one of these values when the other two values are set. Additionally, because the model can produce predictions of changes in exposure and serum concentration over time, these models can be applied to fill the temporal gaps around or between measured serum concentrations or exposures.

The most common use for these models in human populations is to predict serum concentrations from estimated exposures. Some examples of this include the work by Shin et al. (2011) who evaluated the exposure predictions from an environmental fate and transport model by comparing the predicted serum PFOA concentrations to observed values and by Avanasi et al. (2016) who extended the work of Shin et al. (2013) by applying a population model to investigate how variability and uncertainty in model parameters affect the prediction of serum concentrations.

Some examples of one-compartment models used to predict human exposure from serum concentrations include the work of Dassuncao et al. (2018) who used a model to describe historical changes in exposure in seafood and consumer products, Hu et al. (2019) who used paired tap water and serum concentration to estimate the proportion of total exposure that originates from drinking water, and Balk et al. (2019) who used measured concentrations in drinking water, dust and air samples, and serum concentrations in developing children (measured at several time points) to assess the relative proportion of exposure that originates from dietary exposure. Zhang et al. (2019) performed a similar study using community tap water measurements and serum concentrations to estimate the proportion of PFOA exposure in humans that originates from drinking water.

Other applications are used to better understand the toxicokinetics of PFOA in humans by combining estimated exposure values and serum values to estimate clearance and half-life in a

population of interest. One example of this type of model application was presented by Gomis et al. (2016) who used measurements of serum and exposure, in the form of air concentrations during occupational exposure, to estimate an elimination half-life for PFOA. Those authors were also able to identify the relative contributions of direct occupational exposure to PFOA, indirect occupational exposure to PFOA precursors, and background, non-occupational PFOA exposure. Another example was presented by Worley et al. (2017a) who estimated the half-life of PFOA using exposure predicted from drinking water PFAS concentrations in a community with contaminated drinking water. Fu et al. (2016) used paired serum and urine samples from an occupational cohort to estimate the half-life separately from renal clearance (CL_R) (in urine) and in the whole-body (in serum). One challenge in the estimation of half-life is the problem of estimating exposure to PFOA. Russell et al. (2015) addressed this problem by estimating the amount of bias in elimination half-life that is introduced when the ongoing background exposure is not taken into account, with application to PFOA as an example.

One common modification of the one-compartment model is to perform a "steady-state approximation" (i.e., to assume that the rate of change of the serum concentration is zero). This scenario occurs when an individual experiences constant exposure, constant body habitus, and constant clearance over a timespan of several half-lives. Because of the long half-life of PFOA, steady state is a reasonable assumption for adults starting from the age of 25 and above. However, the steady state approximation cannot be applied for ages younger than 21 years of age (EPA defines childhood as <21 years of age; (U.S. EPA, 2021b)) due to ongoing development during childhood and adolescence. This growth dilutes the concentration of the chemical in the body and results in lower levels than would be seen in its absence. Even though pubertal development including skeletal growth typically ends several years prior to the age of 25, there is a period after growth ceases during which PFOA levels increase until the adult steady-state level is reached. The general acceptability of the steady-state assumption in adults has the caveat that pregnancy or breastfeeding will result in changes in serum concentration and will not be accounted for in the steady-state approximation.

When adopting a steady-state assumption, the rate of change in serum levels over time is zero. It follows that the ratio between exposure to the substance and clearance determines the serum concentration. This is the approach used in the 2016 PFOA HESD to determine the constant exposure associated with a serum concentration (U.S. EPA, 2016c). A similar approach was used in the recent toxicity assessment performed by CalEPA (CalEPA, 2021). Publications reporting applications of similar models include the work of Zhang et al. (2015) who used paired human urine and serum data to estimate the total intake of PFOA and compared it to the rate of urinary elimination, and Lorber et al. (2015) who examined the effects of regular blood loss due to phlebotomy on PFOA levels and extrapolated that finding to clearance via menstruation.

In animals, three classical PK models for PFOA have been published since the 2016 PFOA HESD. In Dzierlenga et al. (2019a), male Sprague-Dawley rats were dosed with PFOA via oral gavage at 6, 12, and 48 mg/kg, or intravenously at 6 mg/kg. Female Sprague-Dawley rats were dosed with PFOA via oral gavage at 40, 80, 320 mg/kg or intravenously at 40 mg/kg. Following the administration of PFOA, rats were sacrificed from five minutes to 50 days post-dosing for males and from five minutes to 12 days post-dosing in females. Differences in length of study for each sex represent the sex-dependent difference in half-lives for which adult female rats eliminate PFOA more rapidly than adult males. For both sexes, measured plasma concentrations

characterized the biphasic PK curve. From these exposure scenarios, Dzierlenga et al. (2019a) developed a two-compartment model to characterize PK parameters of interest such as the alpha- and beta-phase half-life, central and peripheral compartment volumes, and total PFOA clearance. For each dosing scenario, a single set of PK parameters were fit, making extrapolation to other dosing scenarios difficult. However, the authors demonstrate a significant difference between males and females in beta-phase half-life and overall clearance. This difference in half-life is critical when considering internal dosimetry for a pregnant dam during developmental PK studies.

Fujii et al. (2015) conducted a PK analysis in mice by dosing male and female mice either intravenously with 0.313 μmol/kg or through oval gavage with 3.13 μmol/kg. Following administration of PFOA, blood concentrations were collected through tail veins beginning immediately following dosing up to 24 hours post-dosing. Fujii et al. (2015) used these data to develop a two-compartment model to describe sex-dependent PK in mice. Unfortunately, the follow-up time of 24 hours post-dosing is not long enough to accurately characterize the beta-phase elimination of PFOA, which the authors predicted was 627 days. The small amount of change in PFOA levels within a 24-hour timespan will make the estimated terminal half-life from a two-compartment model unreliable because PFOA will still be in the distribution phase. In addition, the functional form fit for the oral gavage data in Fujii et al. (2015) reflects a one-compartment model with gavage dosing making it not possible to compare the predicted half-lives between the two routes of exposure. While the reported data could be used for characterizing absorption and distribution of PFOA, it cannot be used for characterizing the elimination phase. Additionally, a study with a much longer follow-up time of 80 days post-dosing reported a half-life of 15.6–21.7 days (Lou et al., 2009).

Finally, Gomis et al. (2016) utilized the functional form of a two-compartment model with oral gavage to predict internal dosimetry of PFOA in rats using PK data from Perkins et al. (2004). However, because the scope of the Gomis et al. (2017) study involved predicting internal dose points-of-departure, PK parameters are not presented.

3.3.2.2 Modified Compartmental Models

In addition to the common one-compartment models described above, several models for humans have been developed to extend the simple one-compartment model to describe the PK during pregnancy and lactation. The key factors that must be introduced into the model are the changes in body habitus that occur during pregnancy (e.g., increases in blood plasma volume and body weight), the distribution and transfer of the substance between the maternal and fetal tissues, the transfer from the mother to the infant during nursing, and postnatal development, including growth of the infant during the early period of life. The mathematical formulation of this type of model requires two differential equations, one describing the rate of change in amount or concentration in the mother and one describing the rate of change in infants. One such developmental model with a lactational component was used to predict the maternal serum concentrations and exposure from measurements of PFOA concentrations in breast milk (Abdallah et al., 2020). Verner et al. (2016) presented another developmental model to predict PFOA serum concentrations in the mother and child and predict previous exposure using mother/child paired serum measurements at different times. This model included all the key aspects previously mentioned for developmental PK models. Another developmental model was developed by Goeden et al. (2019) to evaluate the relationship between drinking water

concentrations and infant serum levels during breastfeeding resulting from gestational and lactational transfer of PFOA that had accumulated within the mother. A distinguishing feature of the Goeden et al. (2019) model is that it incorporates an adjustment for the increased intracellular water in infants and young children compared with adults, under the assumption that PFAS distribution into tissues, quantified by the V_d, will increase proportionally to intracellular water content. This lifestage difference in intracellular water content may explain why the ratio of PFOA in cord blood versus maternal blood at childbirth tends to be less than one. Monroy et al. (2008) reported median cord blood PFOA concentration to be 87% of maternal serum, while the median ratio of fetal tissue to placenta PFOA concentration was found to be generally greater than one (Mamsen et al., 2019). One oversight of this model is that the rate equations take concentration into account, but they do not account for decreases in concentration due to increasing body weight (growth dilution). This is a significant factor for infants who grow quickly.

Other unique analyses that extended the one-compartment framework were publications by Shan et al. (2016), who estimated the exposure to specific isomers of PFOA using measurements in food, tap water, and dust to estimate the isomeric profiles of the substances in human serum, and Convertino et al. (2018) who used a two-compartment PK-pharmacodynamic model to describe changes in serum concentration during a dose-escalation, phase one clinical trial with PFOA and describe how those serum changes are correlated with changes in serum total cholesterol (TC) and free thyroxine (FT4).

Pharmacokinetic models that can accommodate longer half-life values than would be predicted based on standard ADME concepts and allow for dose-dependent changes in excretion rate compared with the classic one- or two-compartment approaches have been published as tools to estimate internal doses for humans, monkeys, mice, and rats (Loccisano et al., 2013; Wambaugh et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011; Andersen et al., 2006). The underlying assumption for all the models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

One of the earliest PK models (Andersen et al., 2006) was created using the post-dosing plasma data from the Butenhoff et al. (2004b) study in cynomolgus monkeys. In this study, groups of six monkeys (three per sex per group) were dosed for 26 weeks with 0, 3, 10, or 20 mg/kg PFOA (and also a high dose of 30 mg/kg PFOA for only the first 12 days) and followed for more than 160 days after dosing. Metabolism cages were used for overnight urine collection. Since urine specimens could only account for overnight PFOA excretion, total volume and total PFOA were extrapolated to 24-hour values based on the excretion rate (volume per hour) for the volume collected and the hours of collection.

The Andersen et al. (2006) model was based on the hypothesis that saturable resorption capacity in the kidney would possibly account for the unique half-life properties of PFOA across species and sexes. The model structure was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells (Andersen et al., 2006).

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption with

transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOA concentrations than at low concentrations. In addition to decreased renal excretion due to the renal resorption, excretion is also reduced in the model by implementing a constant proportion of PFOA that is bound to protein in plasma and is not available for renal filtration.

The model was parameterized using the body weight and urine output of cynomolgus monkeys (Butenhoff et al., 2004b) and a cardiac output of 15 L/h-kg from the literature (Corley et al., 1990). A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were optimized to fit the data for plasma and urine at lower concentrations and then applied for the 20 mg/kg/day dose, which was assumed to represent a concentration at which renal resorption was saturated. On the basis of the data for the dose of 20 mg/kg/day, the model was able to predict the decline in plasma levels after the cessation of dosing. The predictions were adequate for one of the three modeled monkeys; for the other two monkeys, the model predicted higher serum concentrations than were observed. That discrepancy between model prediction and observations could have occurred because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells. The authors also observed that three of the monkeys had faster CL_R of PFOA than the other three monkeys, indicating interindividual variability in clearance.

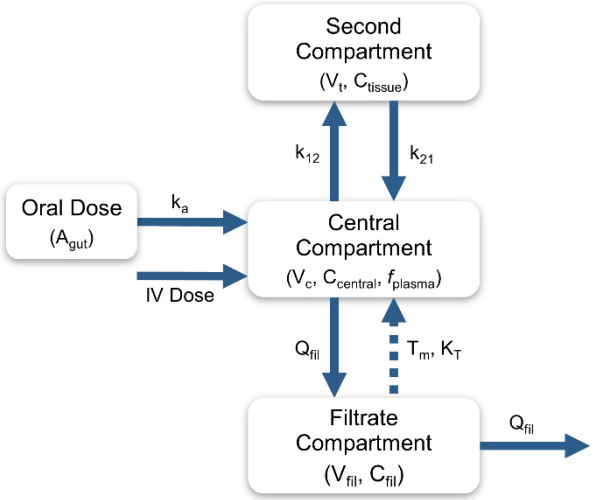


Figure 3-4. Schematic for a Physiologically Motivated Renal-Resorption PK Model for PFOA

Adapted from Wambaugh et al. (2013).

Building on the work of other researchers, Wambaugh et al. (2013) developed and published a PK model to support the development of an EPA RfD for PFOA (U.S. EPA, 2016c). The model was applied to data from studies conducted in monkeys, rats, or mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal-resorption term was used. This concept has played a fundamental role in the design of all of the published PFOA models summarized in this section. The model structure is depicted in Figure 3-4 (adapted from Wambaugh et al. (2013)).

Wambaugh et al. (2013) placed bounds on the estimated values for some parameters of the Andersen et al. (2006) model to support the assumption that serum carries a significant portion of the total PFOA body load. The Andersen et al. (2006) model is a modified two-compartment model in which a primary compartment describes the serum and a secondary deep tissue compartment acts as a specified tissue reservoir. Wambaugh et al. (2013) constrained the total V_d such that the amount in the tissue compartment was not greater than 100 times that in the serum. As a result, the ratio of the two volumes (serum versus total) was estimated in place of establishing a rate of transfer from the tissue to serum, but the rate of transfer from serum to tissue was also estimated from the data. A nonhierarchical model for parameter values was also assumed. Under this assumption, a single numeric value represents all individuals of the same species, sex, and strain. This sex assumption was applied to male and female rats to determine sex-specific parameters because of established sex-specific toxicokinetic differences. Conversely, monkeys and mice were only grouped by species and strain with only female parameters available for mice and male/female monkey data pooled together for a single set of parameters. Body weight, the number of doses, and magnitude of the doses were the only parameters varied for different studies. Measurement errors were assumed to be log-normally distributed. Table 4-3 in Section 4.1.3.1.1 provides the estimated and assumed PK parameters applied in the Wambaugh et al. (2013) model for each of the species evaluated.

The PK data that supported the Wambaugh et al. (2013) analysis were derived from two in vivo PFOA PK studies. The monkey PK data were derived from Butenhoff et al. (2004b), and the data for the rats (M/F) were from Kemper et al. (2003). Two strains of female mice were analyzed separately, with CD1 information derived from Lou et al. (2009) and C57BL/6 information derived from DeWitt et al. (2008). The data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and sexes and to identify serum levels associated with the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) external doses. Prior distributions for the parameters were chosen to be broad, log-normal distributions, allowing the fitted parameters to be positive and for the posterior distribution to be primarily informed by the data likelihood rather than by the priors.

3.3.2.3 PBPK Models

An alternative approach to the use of a classical or modified compartmental model is a PBPK model, which describes the changes in substance amount or concentration in a number of discrete tissues. One of the main advantages of a PBPK model is the ability to define many parameters based on physiological data, rather than having to estimate them from chemical-specific data. Such physiological parameters include, for example, organ volumes and the blood flow to different organs; they can be measured relatively easily and are chemical independent. Another advantage is that the amount and concentration of the substance can be predicted in specific tissues, in addition to blood. This can be valuable for certain endpoints for which it is expected that a tissue concentration would better reflect the relevant dosimetry compared with blood concentration.

The first PBPK model developed for PFOA was reported in a series of publications by Loccisano et al., which together describe the PK of PFOA in rats, monkeys, and humans, in both adult and developmental (for rat and human) scenarios (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011). These models were developed based on an earlier “biologically

motivated" model that served as a bridge between a one-compartment model and PBPK by implementing a tissue compartment (similar to a two-compartment model), an absorption compartment, and a renal filtrate compartment with saturable renal resorption (Tan et al., 2008). The work of Tan et al. (2008) was a development of the earlier work of Andersen et al. (2006) previously discussed. The PBPK model of Loccisano and colleagues then extended this "biologically motivated" model by the addition of discrete tissue compartments, rather than a single compartment representing all tissues.

A series of follow-up studies applied the Loccisano and coauthors' model structure, with extensions, to address how PK variation in human populations could bias the result of the study. This consisted of the work of Wu et al. (2015) who developed a detailed model of adolescent female development during puberty and menstrual clearance of PFOA to investigate the interaction between chemical levels and the timing of menarche, Ruark et al. (2017) who added a detailed description of menopause to evaluate how that affects serum levels and the epidemiological association between early menopause and PFOA levels, Ngueta et al. (2017) who implemented a reduction in menstrual clearance in individuals using oral contraceptives and the interaction between oral contraceptive use, endometriosis, and serum PFOA levels, and Dzierlenga et al. (2020b; 2020c) who applied a model of thyroid disease (Dzierlenga et al., 2019b) to describe changes in PFOA urinary clearance due to disease state.

In addition to this set of studies, Fabrega et al. (2014) updated the model of Loccisano et al. (2013) for humans by modeling a human population using regional food and drinking water measurements and human tissue data collected from cadavers in a region of Spain. The use of human tissue data is relatively rare due to the challenges in sourcing human tissue but may prove preferable to the assumption that human distribution is similar to distribution in an animal model. However, Fabrega et al. (2014) estimated their tissue to blood partition coefficients from the ratio of tissue concentrations in the cadavers to the average serum concentrations in live volunteers who lived in the same region but were sampled several years earlier (Ericson et al., 2007) and they provided no details on how their renal-resorption parameters were estimated from the human blood concentrations. This model was further applied to a population in Norway and extended to other PFAS (Fàbrega et al., 2015).

Brochot et al. (2019) presented the application of a PBPK model for PFOA with gestation and lactation lifestages to describe development and predicted maternal, infant, and breastmilk concentrations over a variety of scenarios including the prediction of maternal levels across multiple pregnancies.

One of the major challenges in the parameterization of PBPK models for PFOA is the estimation of the chemical-dependent parameters such as those involved in protein binding and renal clearance. One way to investigate this issue is to perform in vitro experiments to help inform the parameters. Worley et al. (2017b) used in vitro measurements of renal transporter activity to describe in detail the various steps involved in the renal filtration, resorption, and excretion of PFOA. Cheng et al. (2017) went farther in their use of in vitro data and used measurements of PFOA interactions with binding proteins, as well the measured rates of several transporters, to parameterize a rat PBPK model.

No new animal PBPK models for PFOA have been published since the 2016 PFOA HESD (U.S. EPA, 2016c). See the 2016 PFOA HESD (U.S. EPA, 2016c) for a more in-depth review of PFOA PBPK models.

3.4 Noncancer Health Effects Evidence Synthesis and Integration

3.4.1 Hepatic

EPA identified 33 epidemiological studies (reported in 39 publications)[5,6] and 31 animal toxicological studies that investigated the association between PFOA and hepatic effects. Of the epidemiological studies, 21 were classified as *medium* confidence, 8 as *low* confidence, 1 as *mixed* (*medium/low*) confidence, and 9 were considered *uninformative* (Section 3.4.1.1). Of the 31 animal toxicological studies, 5 were classified as *high* confidence, 22 as *medium* confidence, 2 as *low* confidence, and 2 were considered *mixed* (*medium/uninformative* and *medium/low/uninformative*) (Section 3.4.1.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence epidemiology and animal toxicological studies are considered qualitatively in this section (e.g., to inform the weight of the evidence for hazard assessment), they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.1.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.1.1.1 Introduction and Summary of Evidence From the 2016 PFOA HESD

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Bilirubin and γ-glutamyltransferase (GGT) are also routinely used to evaluate potential hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Elevated liver serum biomarkers are frequently an indication of liver injury, though not as specific as structural or functional analyses such as histology findings and liver disease.

There are 13 epidemiological studies (14 publications)[6] from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA exposure and hepatic effects, and study quality evaluations are shown in Figure 3-5. Emmett et al. (2006) and Jain et al. (2014) were rated as *uninformative* and will not be further discussed. Nine out of the 12 remaining studies were rated as *medium* quality and all investigated changes in serum liver enzymes. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-2 and below.

[5] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all used NHANES data from overlapping years.
[6] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b); however, data for PFOA and hepatic outcomes is reported in Olsen (2001a).

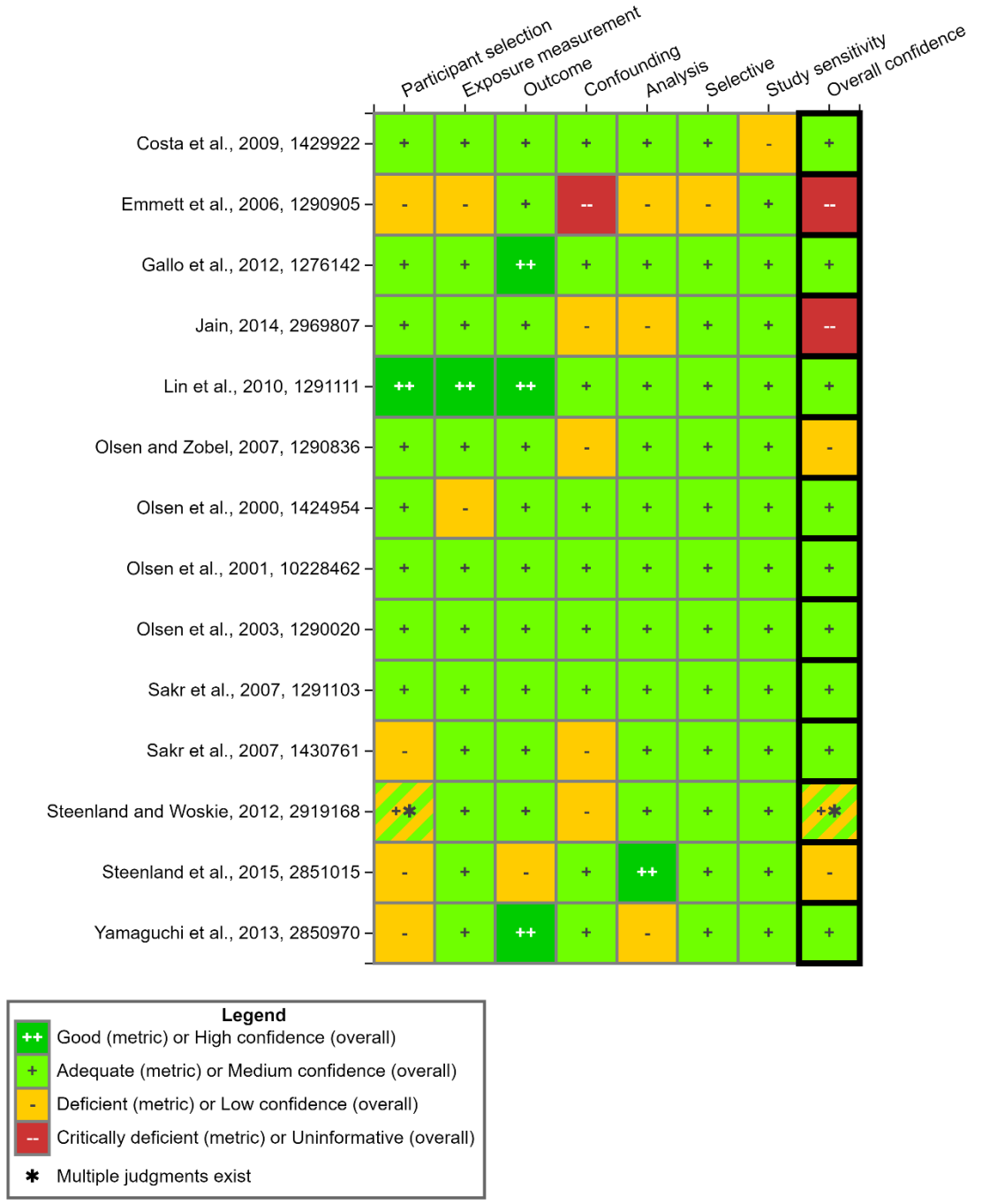


Figure 3-5. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects Published Before 2016 (References in the 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Lin et al. (2010) is a *medium* confidence study that examined 2,216 adults in the NHANES study (1999–2000, and 2003–2004) and observed that higher serum concentrations of PFOA were associated with abnormal liver enzymes increases in the U.S. general population. For each

increase in log-PFOA, the serum ALT and GGT concentrations (U/L) increased by 1.86 units (95% CI: 1.24, 2.48), and 0.08 units (95% CI: 0.05, 0.11), respectively (Lin et al., 2010). Importantly, when PFOS, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, the associations remained and were slightly larger; one unit increase in serum log-PFOA concentration was associated with a 2.19 unit (95% CI: 1.4, 2.98) increase in serum ALT concentration (U/L), and a 0.15 unit (95% CI: 0.11, 0.19) increase in serum log-GGT concentration (U/L). Another *medium* confidence cross-sectional study (Yamaguchi et al., 2013) conducted in Japan reported a positive correlation between PFOA and ALT.

A *medium* confidence study in a highly exposed community provides further support for the positive association between PFOA exposure and ALT findings in the U.S. general population. One of the largest studies of PFOA exposure and ALT in adults, Gallo et al. (2012), evaluated 47,092 adults from the C8 Health Project living in communities in Ohio and West Virginia impacted by a manufacturing-related PFOA-contaminated drinking water supply. Natural-log transformed serum PFOA concentrations were associated with ln-ALT in linear regression models (regression coefficient: 0.022; 95% CI: 0.018, 0.025) and with elevated ALT in logistic regression models across deciles of PFOA (OR = 1.10; 95% CI: 1.07, 1.13). The evidence of an association between PFOA and GGT or bilirubin was less consistent. The level of bilirubin increased with increasing PFOA at low PFOA concentrations and decreased with increasing PFOA levels at higher PFOA concentrations, producing an inverse roughly U-shaped curve of the relationship between PFOA and bilirubin.

Several *medium* confidence cross-sectional occupational studies reported that higher concentrations of PFOA were associated with higher liver enzyme levels, such as ALT, AST, GGT, and total bilirubin (Costa et al., 2009; Sakr et al., 2007a; Sakr et al., 2007b). However, other *medium* confidence cross-sectional occupational studies in PFOA production workers reported mostly null findings, with some positive associations with ALT in specific locations or specific years (Olsen and Zobel, 2007; Olsen et al., 2003; Olsen et al., 2001a; Olsen et al., 2000).

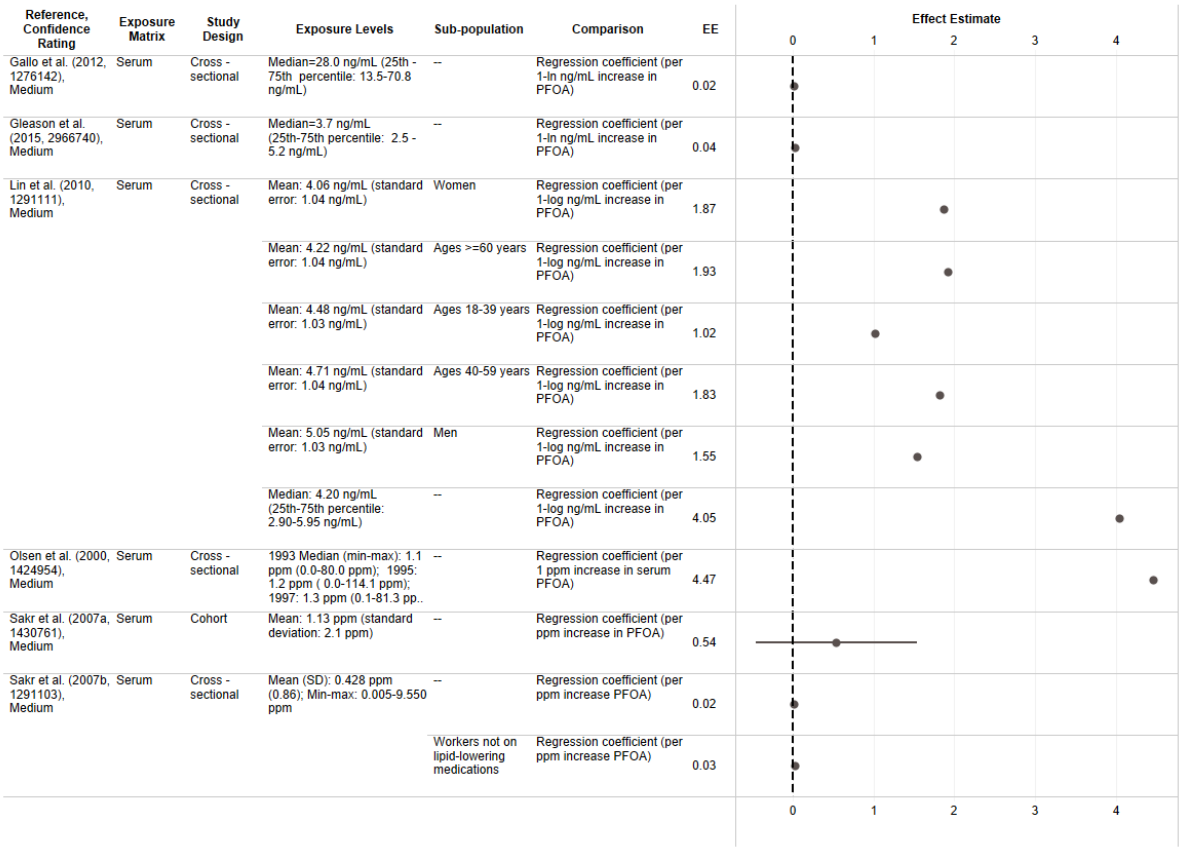


Figure 3-6. Overall ALT Levels from 2016 PFOA HESD Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

The associations with ALT indicate the potential for PFOA to affect liver function; however, studies of functional hepatic endpoints were limited to two studies in an occupational cohort. The first study was a *low* confidence study that observed no association between PFOA and hepatitis or fatty liver disease; however, there was a positive association with non-hepatitis liver disease with a 10-year lag time (Steenland et al., 2015). A *medium* confidence cohort mortality study of workers exposed to PFOA at a DuPont chemical plant in West Virginia observed no association between PFOA exposure levels and nonmalignant chronic liver disease deaths (Steenland and Woskie, 2012).

In conclusion, the majority of the *medium* confidence studies support an association between PFOA exposure and increases in serum ALT in multiple populations, including occupational and highly exposed communities as well as the general population (see Figure 3-6). Multiple studies demonstrated statistically significant increases in ALT (Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010; Olsen et al., 2000) or elevated ALT (Gallo et al., 2012) after PFOA exposure. Increases were also observed for AST and GGT, though less consistently across the available studies.

Table 3-2. Associations Between Elevated Exposure to PFOA and Hepatic Outcomes from Studies Identified in the 2016 PFOA HESD

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]	Serum Protein[a]	Albumin[a]
Costa, 2009, 1429922 *Medium*	Cross-sectional	Occupational	↑↑	↑	↑↑	↑↑	NA	↓	↓
Gallo, 2012, 1276142 *Medium*	Cross-sectional	Adults	↑↑	NA	↑↑	NA	NA	NA	NA
Lin, 2010, 1291111 *Medium*	Cohort	Adults	↑↑	NA	↑↑	NA	NA	NA	NA
Olsen and Zobel, 2007, 1290836 *Low*	Cross-sectional	Occupational	↑↑	↓	↑↑	↑↑	NA	NA	NA
Olsen, 2003, 1290020 *Medium*	Cross-sectional	Occupational	↑↑	–	↑	NA	NA	NA	NA
Olsen, 2001, 10228462 *Medium*	Cohort	Occupational	↑	↑	↓	↑	NA	NA	NA
Olsen, 2000, 1424954 *Medium*	Cross-sectional	Occupational	↑↑	NA	NA	NA	NA	NA	NA
Sakr, 2007, 1291103 *Medium*	Cross-sectional	Occupational	↑	↑	↑↑	NA	NA	NA	NA
Sakr, 2007, 1430761 *Medium*	Cohort	Occupational	↑	↑↑	↑	NA	NA	NA	NA
Steenland and Woskie, 2012, 2919168 *Mixed c*	Cohort	Occupational	NA	NA	NA	NA	–	NA	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	NA	NA	↑	NA	NA
Yamaguchi, 2013, 2850970 *Medium*	Cross-sectional	Adults and adolescents	↑↑	↑↑	↑	NA	NA	NA	NA

Notes: ALP = alkaline phosphatase; ALT = alanine transferase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Emmett et al., 2006, 1290905 was not included in the table due to their *uninformative* overall study confidence ratings.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Steenland and Woskie, 2012, 2919168 was rated *medium* confidence for comparisons with the DuPont referent group and *low* confidence for comparisons with the U.S. population.

3.4.1.1.2 Study Quality Evaluation Results for the Relevant Epidemiology Studies Identified from the Updated Literature Review

There are 20 epidemiological studies (25 publications)[7] that were identified from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and hepatic effects. Study quality evaluations for these 25 publications are shown in Figure 3-7 and Figure 3-8. Of these 25 publications, 12 were classified as *medium* confidence, 6 as *low* confidence, and 7 were considered *uninformative*.

The following informative studies examined liver enzymes in adults: two cross-sectional studies (Nian et al., 2019; Wang et al., 2012); multiple publications of data from NHANES (Omoike et al., 2020; Jain, 2019; Jain and Ducatman, 2019a, c; Liu et al., 2018d; Gleason et al., 2015); one cohort with retrospective exposure assessment (Darrow et al., 2016); one prospective cohort (Salihovic et al., 2018); one open-label controlled trial (Convertino et al., 2018); and one occupational cohort (Olsen et al., 2012). Most of these studies were in general population adults, but some assessed specific populations such as the elderly (Salihovic et al., 2018) and fluorochemical plant workers (Olsen et al., 2012; Wang et al., 2012). In addition, one occupational cohort (Girardi and Merler, 2019) and three cross-sectional studies (Liu et al., 2018b; Darrow et al., 2016; Rantakokko et al., 2015) examined functional liver endpoints in adults (histology, liver disease, hepatic fat mass). In children and adolescents, four studies were available, including one cohort (Mora et al., 2018) and three cross-sectional studies (Jin et al., 2020; Attanasio, 2019; Khalil et al., 2018), with one examining histology endpoints (Jin et al., 2020).

All of the studies of adults and children in the general population, except for Darrow et al. (2016), and one of the two occupational cohorts (Olsen et al., 2012) measured exposure to PFOA using biomarkers in blood. Darrow et al. (2016) modeled exposure based on residential history, drinking water sources, and water consumption rates. The other occupational cohort study estimated PFOA exposure based on job duties (Girardi and Merler, 2019). The *uninformative* studies were excluded due to potential confounding (Abraham et al., 2020; Sinisalu et al., 2020; Predieri et al., 2015; Jiang et al., 2014), lack of information on participant selection (Sinisalu et al., 2021), use of PFAS as the dependent variable (Jain, 2020a), or in cases for which the independent variable is a genetic variant and thus not affected by PFAS exposure (Fan et al., 2014).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies (e.g., AST serum levels, functional assays), the evidence synthesis below included details on any *low* confidence studies available in addition to *high* and *medium* confidence studies. Studies considered *uninformative* were not considered further in the evidence synthesis.

[7] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); and Gleason et al. (2015) all used NHANES data from overlapping years.

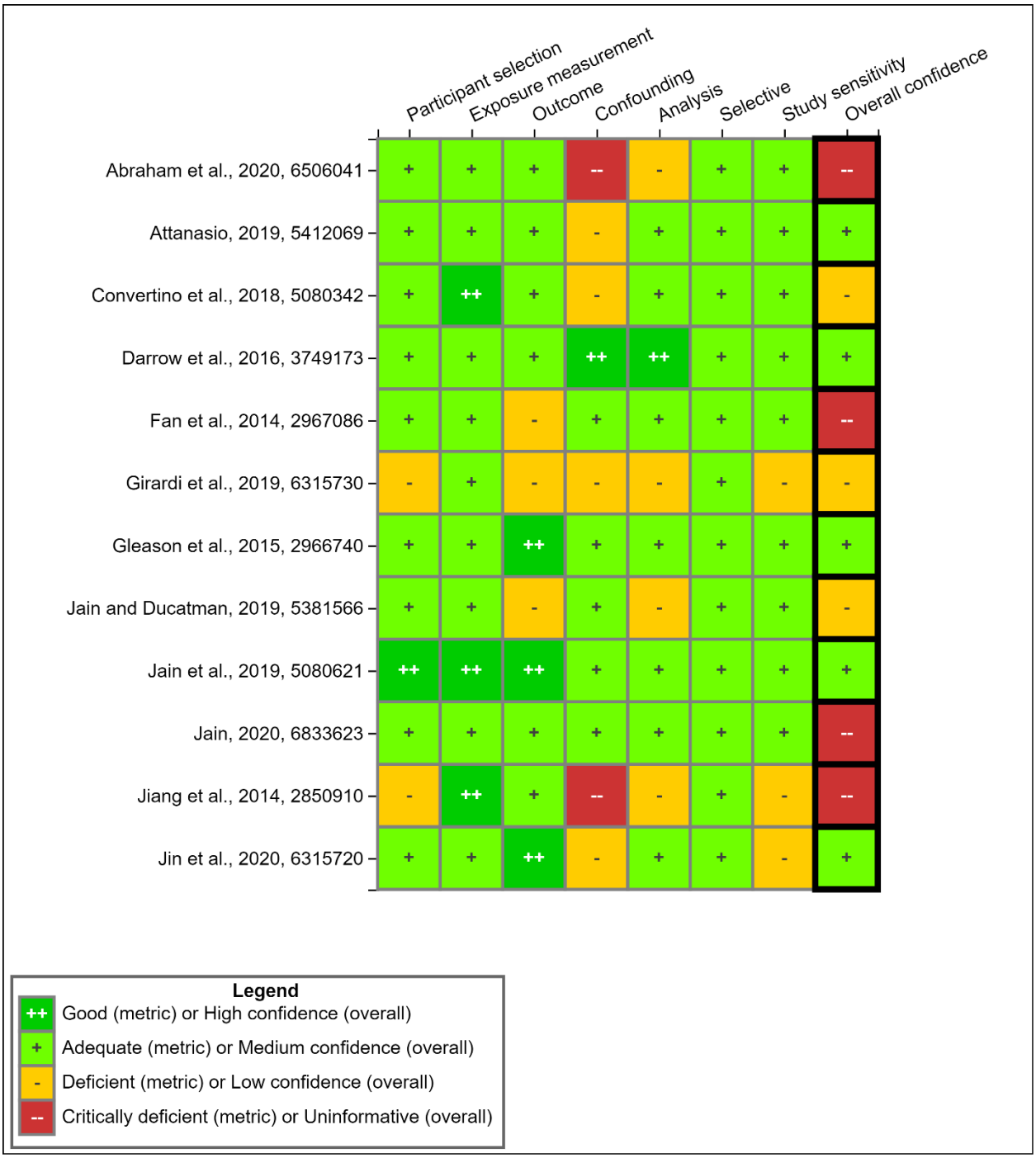


Figure 3-7. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects[a]

Interactive figure and additional study details available on HAWC.

[a]Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

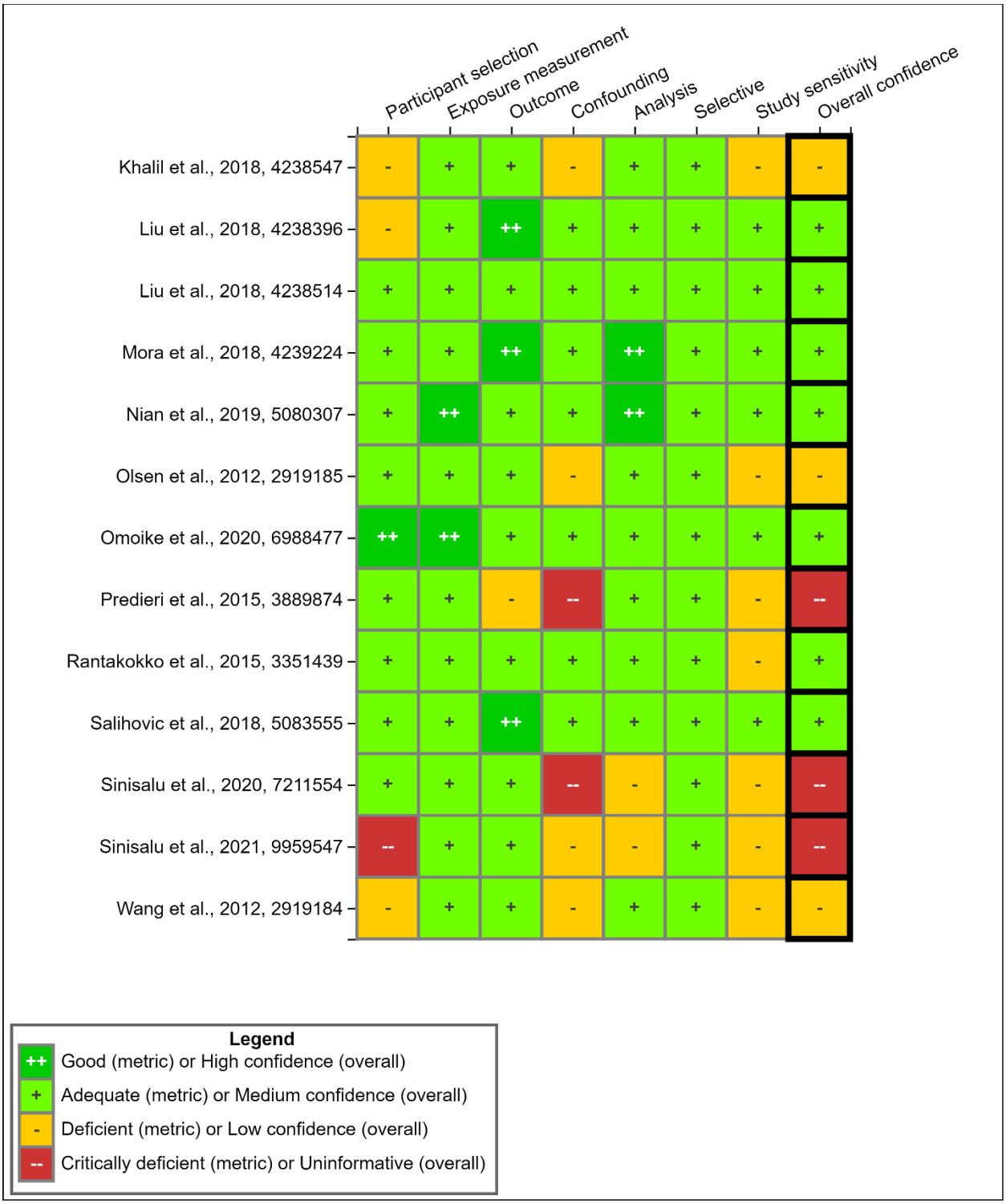


Figure 3-8. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

[a] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

3.4.1.1.3 Synthesis of Hepatic Injury From the Updated Literature Review

Results for the studies that examined ALT are presented in the Appendix (U.S. EPA, 2024a). As shown in Figure 3-9 and Figure 3-10, of the available informative studies that measured ALT in adults, statistically significant positive associations between ALT and PFOA (i.e., increased ALT as a continuous measure with higher PFOA exposure levels) were observed in all of the *medium* confidence studies, which consisted of one cross-sectional study (Nian et al., 2019), two cohort studies (Salihovic et al., 2018; Darrow et al., 2016), and two NHANES publications (Jain, 2019; Gleason et al., 2015).

In addition, an exposure-response gradient was observed in the single study that examined quintiles of exposure (Darrow et al., 2016). This study additionally examined elevated ALT as a dichotomous outcome and reported an OR of 1.16 (95% CI: 1.02, 1.33) in the highest versus lowest quintiles of exposure (Figure 3-9). The positive associations in Jain (2019) were observed only in certain sub-groups (e.g., by renal function (i.e., glomerular filtration stage), obesity status) and according to no clear pattern across sub-groups (NHANES 2003–2014), but in Gleason et al. (2015), the positive association was observed in the entire study population (NHANES 2007–2010). Results of the *low* confidence studies of ALT in adults are presented in Appendix D (U.S. EPA, 2024a) and not described further in this section because there are numerous *medium* confidence studies describing ALT measures in adults that were included in the 2016 PFOA HESD or identified in the updated literature search.

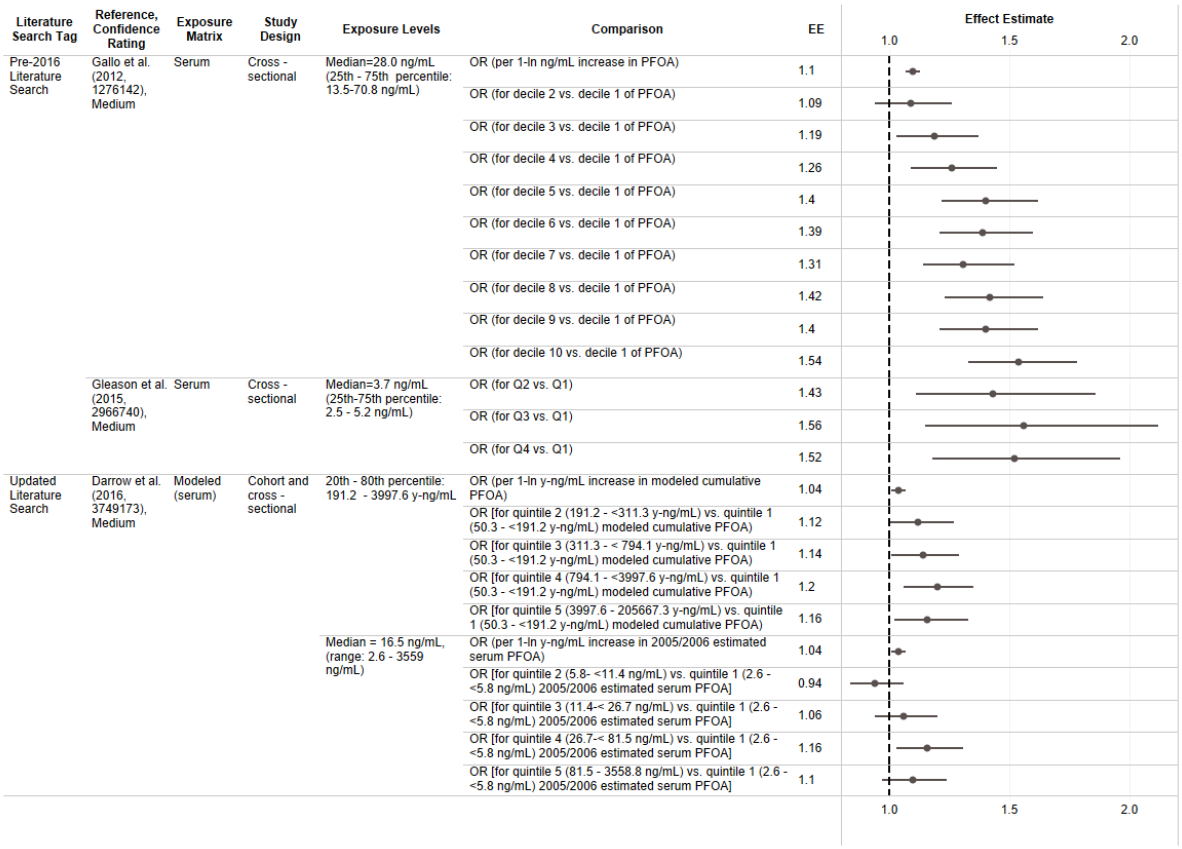


Figure 3-9. Odds of Elevated ALT Levels from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

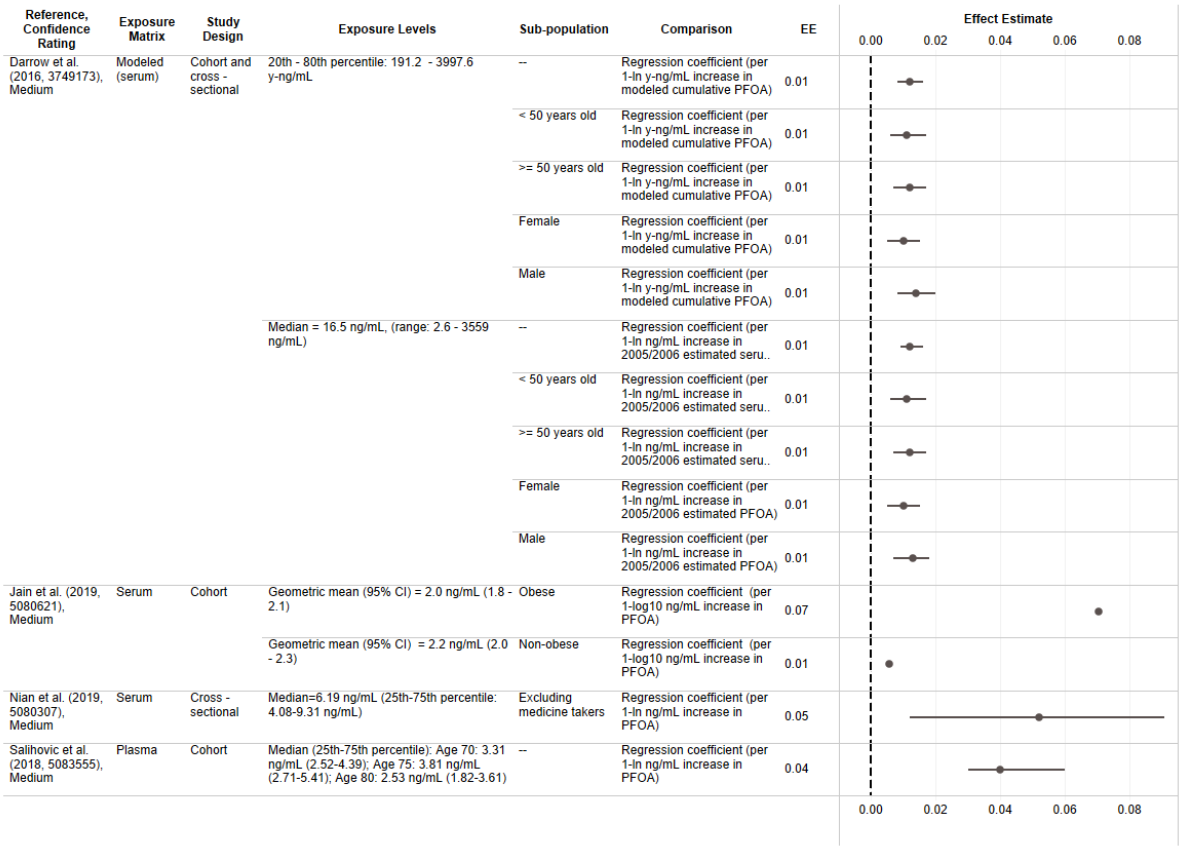


Figure 3-10. ALT Levels from *Medium* Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

In children and adolescents, positive associations were observed in girls (with exposure-response gradient across quartiles) in the *medium* confidence study by Attanasio et al. (2019) and in the *low* confidence study of obese children (Khalil et al., 2018). However, inverse associations were observed in boys in Attanasio et al. (2019) and Mora et al. (2018), which may indicate that the associations in children are less consistent than in adults or that there are sex differences in children. Insufficient data were available to assess the potential for effect modification by sex.

The studies that examined AST are presented in Appendix D (U.S. EPA, 2024a). In adults in the general population, positive associations were observed in the two *medium* confidence studies (Jain, 2019; Nian et al., 2019). In the two *low* confidence studies of fluorochemical plant workers (Olsen et al., 2012; Wang et al., 2012), no associations were observed. In children including adolescents, the *medium* confidence study (Attanasio, 2019) reported a positive association in girls but an inverse association in boys. In the *low* confidence study (Khalil et al., 2018), the direction of association was inverse, but the result was extremely imprecise. For the other liver enzymes (bilirubin, GGT), results were generally consistent with those of ALT and AST, with the exception that inverse associations for bilirubin were observed in some studies (Salihovic et al., 2018; Darrow et al., 2016).

For functional measures of liver injury, two *medium* confidence studies (one in adults and one in children including adolescents) examined histology endpoints. Both studies examined lobular inflammation. Rantakokko et al. (2015) reported that higher PFOA exposure levels were associated with extremely reduced odds of lobular inflammation (OR = 0.02, $p < 0.05$), whereas Jin et al. (2020) reported the opposite direction of association, though the results in the latter study were nonmonotonic and not statistically significant. Jin et al. (2020) additionally reported lower odds of ballooning and portal inflammation, but higher odds of steatosis (association nonmonotonic) and nonalcoholic steatohepatitis. Three additional studies examined some form of liver disease. In a *medium* confidence study, Darrow et al. (2016) reported no increases in any liver disease or specifically enlarged liver, fatty liver, or cirrhosis. In contrast, in a *low* confidence study, Girardi and Merler (2019) reported that workers at a PFAS production plant had higher mortality from liver cancer or cirrhosis when compared with regional mortality statistics and a control group of nonchemical workers ($p < 0.05$ for some comparisons). Lastly, a second *low* confidence study by Liu et al. (2018b) examined hepatic fat mass and found no correlation with PFOA exposure.

3.4.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 12 animal toxicological studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 19 studies identified from recent systematic literature searches and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and hepatic effects. Study quality evaluations for these 31 studies are shown in Figure 3-11 and Figure 3-12.

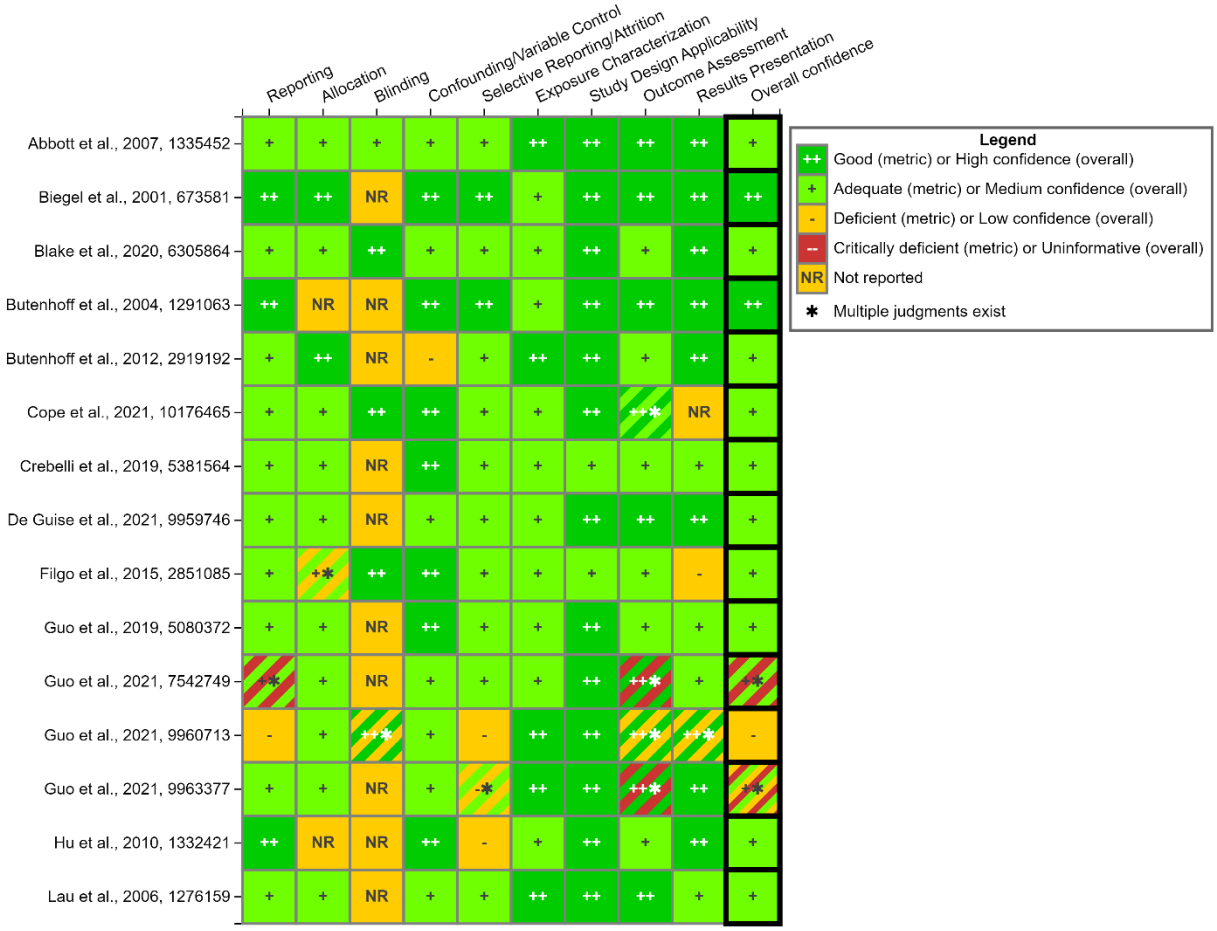


Figure 3-11. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hepatic Effects

Interactive figure and additional study details available on HAWC.

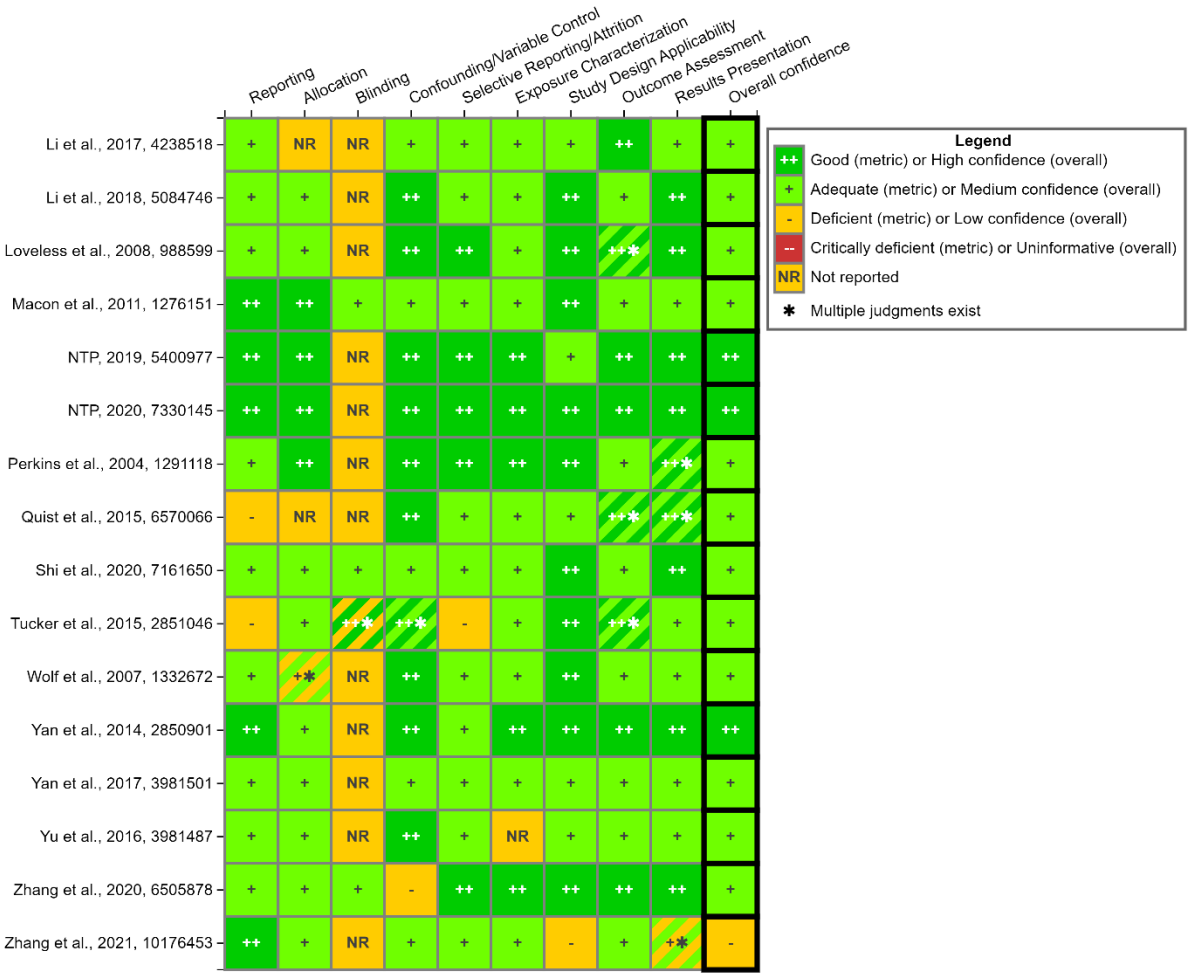


Figure 3-12. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hepatic Effects (Continued)

Interactive figure and additional study details available on HAWC.

Hepatic effects (e.g., increased absolute and relative liver weight, altered clinical parameters indicating potential liver injury, and histopathological alterations of liver tissue) were observed in male and female mice, rats, and monkeys after oral PFOA exposures of different durations. Data from numerous studies provide evidence confirming that the liver is a target of PFOA toxicity.

3.4.1.2.1 Liver Weight

Generally, increases in absolute and/or relative liver weight were observed in all available PFOA animal toxicological studies, regardless of species, sex, lifestage, and exposure paradigm (Figure 3-13 and Figure 3-14). Significant increases in absolute and relative liver weight were reported at doses as low as 0.05 mg/kg/day and 0.31 mg/kg/day, respectively (Li et al., 2017b; Yan et al.,

2014), and were often observed at the lowest dose administered in each study. In male mice, significant increases in absolute and/or relative liver weights were observed at doses ranging from 0.31 to 30 mg/kg/day after 4–5 weeks of exposure (Guo et al., 2021a; Shi et al., 2020; Crebelli et al., 2019; Guo et al., 2019; Li et al., 2017b; Yu et al., 2016; Yan et al., 2014; Minata et al., 2010; Loveless et al., 2008). Similarly, significant increases in absolute and relative liver weights were reported in male rat short-term/subchronic studies at doses of 0.625–30 mg/kg/day (NTP, 2019; Cui et al., 2009; Loveless et al., 2008; Perkins et al., 2004). Two subchronic dietary studies in adult male rats with exposures lasting 13–16 weeks reported significantly increased absolute and relative liver weights at doses as low as 1 mg/kg/day (NTP, 2020; Perkins et al., 2004). In one chronic study in male Crl:CD BR (CD) rats, relative liver weight was significantly increased after 15 months of exposure to 13.6 mg/kg/day via the diet (Biegel et al., 2001). Similar results were observed at the 1-year interim sacrifice of a 2-year dietary study in male Sprague-Dawley rats exposed to 14.2 mg/kg/day PFOA, but the effect was not statistically significant at the 2-year timepoint (Butenhoff et al., 2012). Male cynomolgus monkeys orally administered PFOA capsules daily for 26 weeks also had significantly increased absolute liver weights at doses ≥3 mg/kg/day, though the increase in relative liver weight was only statistically significant in the highest dose group (30/20 mg/kg/day) (Butenhoff et al., 2002).

Several systemic toxicity studies evaluating liver weight in female mice and rats after short-term, subchronic, or chronic PFOA exposures are also available (De Guise and Levin, 2021; NTP, 2020; Zhang et al., 2020b; NTP, 2019; Li et al., 2017b; Butenhoff et al., 2012). Two 28-day studies in female mice reported significant increases in absolute liver weight at doses ranging from 0.05 to 5 mg/kg/day (relative liver weight not reported) (Zhang et al., 2020b; Li et al., 2017b). A third 28-day study in female B6C3F1 mice reported significant increases in absolute and relative liver weights at both doses tested (1.88 and 7.5 mg/kg/day) (De Guise and Levin, 2021). NTP (2019) conducted a 28-day gavage study in female Sprague-Dawley rats and reported significant increases in both absolute and relative liver weights at doses ≥25 mg/kg/day. In a chronic feeding study (see study design details in Section 3.4.4.2.1.2), NTP (2020) reported significant increases in absolute and relative liver weight in female Sprague-Dawley rats after 16 weeks of exposure to 63.4 but not 18.2 mg/kg/day PFOA. A 2-year feeding study in female Sprague-Dawley rats similarly found no significant difference in absolute or relative liver weight at doses of 1.6 or 16.1 mg/kg/day PFOA (Butenhoff et al., 2012).

There are also multiple reproductive and developmental toxicity studies that report maternal and/or offspring liver weight in rodents after gestational PFOA exposures. Blake et al. (2020) reported significant increases in absolute and relative liver weights in CD-1 mouse dams exposed to PFOA at doses of 1 or 5 mg/kg/day from GD 1.5 to GD 11.5 or GD 1.5 to GD 17.5. Yahia et al. (2010) similarly reported significant increases in maternal ICR mouse absolute liver weights at doses ≥5 mg/kg/day and relative liver weights at doses ≥1 mg/kg/day. Quist et al. (2015) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17. At PND 21, significantly increased relative liver weights in offspring were observed as low as 0.3 mg/kg/day. In a 2-generation reproductive toxicity study in Sprague-Dawley rats (Butenhoff et al., 2004a), P_0 dams dosed with 1, 3, 10, or 30 mg/kg/day PFOA at least 70 days prior to mating through lactation did not show consistent alterations in absolute or relative liver weights at the time of sacrifice on PND 22. However, significantly increased absolute and relative liver weights were observed in P_0 males and male F_1 offspring starting at the lowest dose of 1 mg/kg/day, whereas no statistically significant differences in absolute or relative liver weights were reported for female F_1 offspring.

Several other developmental toxicity studies reported significantly increased maternal, fetal, and/or pup liver weights associated with gestational PFOA exposure, but the authors did not further examine tissue or serum samples for hepatic effects (Cope et al., 2021; Li et al., 2018a; Tucker et al., 2014; Macon et al., 2011; White et al., 2011; White et al., 2009; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006). For example, White et al. (2011) orally dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day PFOA from GD 1 to GD 17. F_1 offspring liver-to-body weight ratios were significantly increased at 1 mg/kg/day on PND 22 and at 5 mg/kg/day on PND 22 and PND 42. Macon et al. (2011) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17 (full gestation) or GD 10 to GD 17 (late gestation). At PND 7, significantly increased absolute and relative liver weights in offspring were observed as low as 0.3 mg/kg/day after full-gestation exposure; significantly increased absolute and relative liver weights were also observed at the high dose of 1 mg/kg/day PFOA after late-gestation exposure (PND 4 and PND 7; relative liver weights were also significantly increased at PND 14). Wolf et al. (2007) reported that offspring of pregnant CD-1 mice orally dosed with 0 and 5 mg/kg/day on GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, and GD 15–GD 17 or with 20 mg/kg/day on GD 15–GD 17 had significantly increased liver-to-body weight ratios at PND 22. White et al. (2009) reported that offspring of CD-1 mice exposed to 5 mg/kg/day PFOA during gestation or during gestation plus lactation had significantly increased liver-to-body weight ratios on PND 1. Inconsistent results were observed on PND 22 and PND 128 in male and female CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day PFOA from GD 1.5 to GD 17.5 and then given either a high- or low-fat diet starting on PND 22 (Cope et al., 2021). Specifically, increased relative liver weights were observed at PND 22 for both males and females exposed to 1 mg/kg/day (statistically significant in males only), but not at PND 128 (Cope et al., 2021). One study reported no significant change in relative liver weights, which were only measured on PND 48 in the female offspring of C57BL/6N mouse dams exposed to 0.5 or 1 mg/kg/day PFOA in drinking water from GD 6 to GD 17 (Hu et al., 2010).

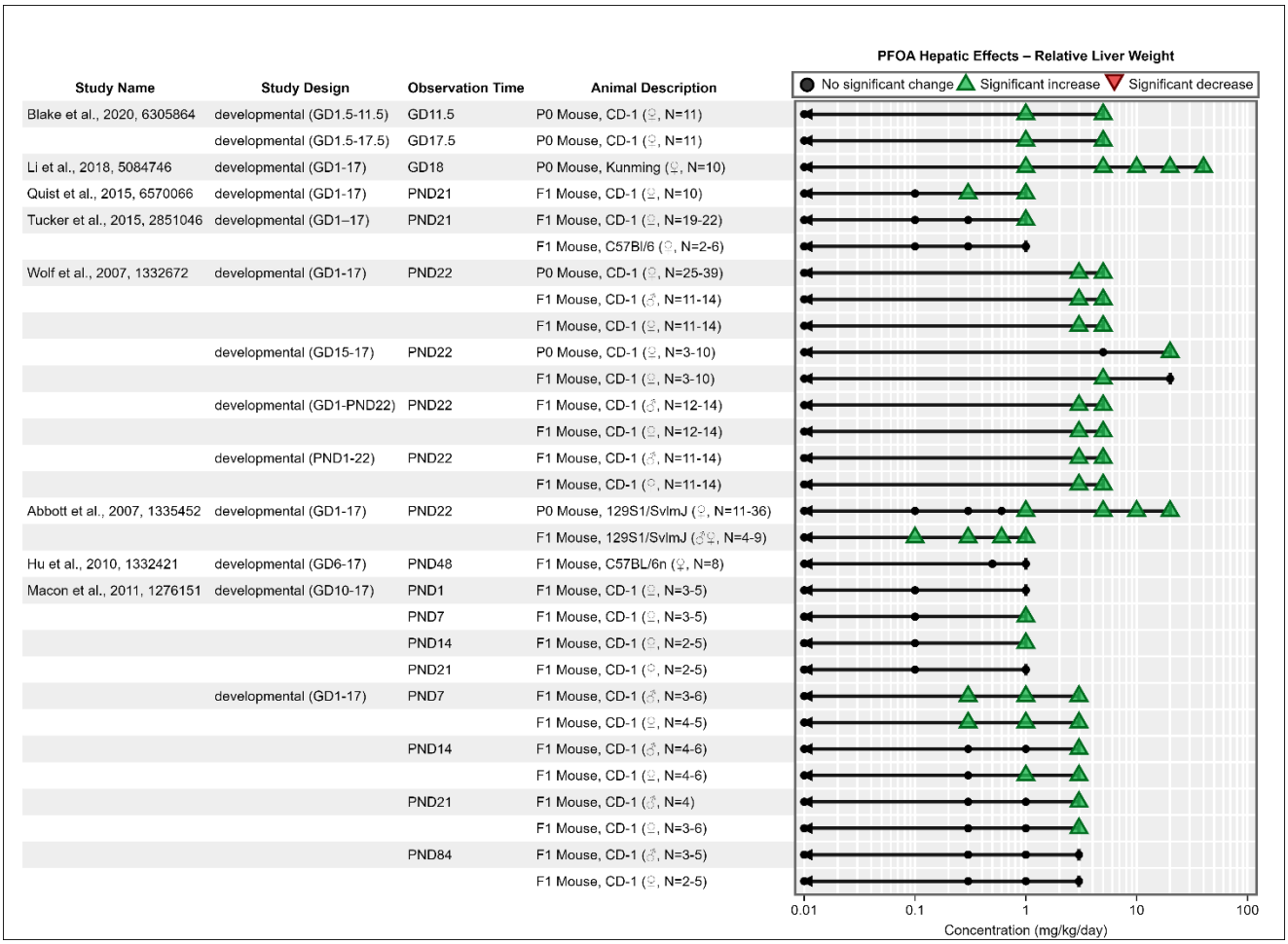


Figure 3-13. Relative Liver Weight in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; PNW = postnatal week; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day; wk = week; y = year.

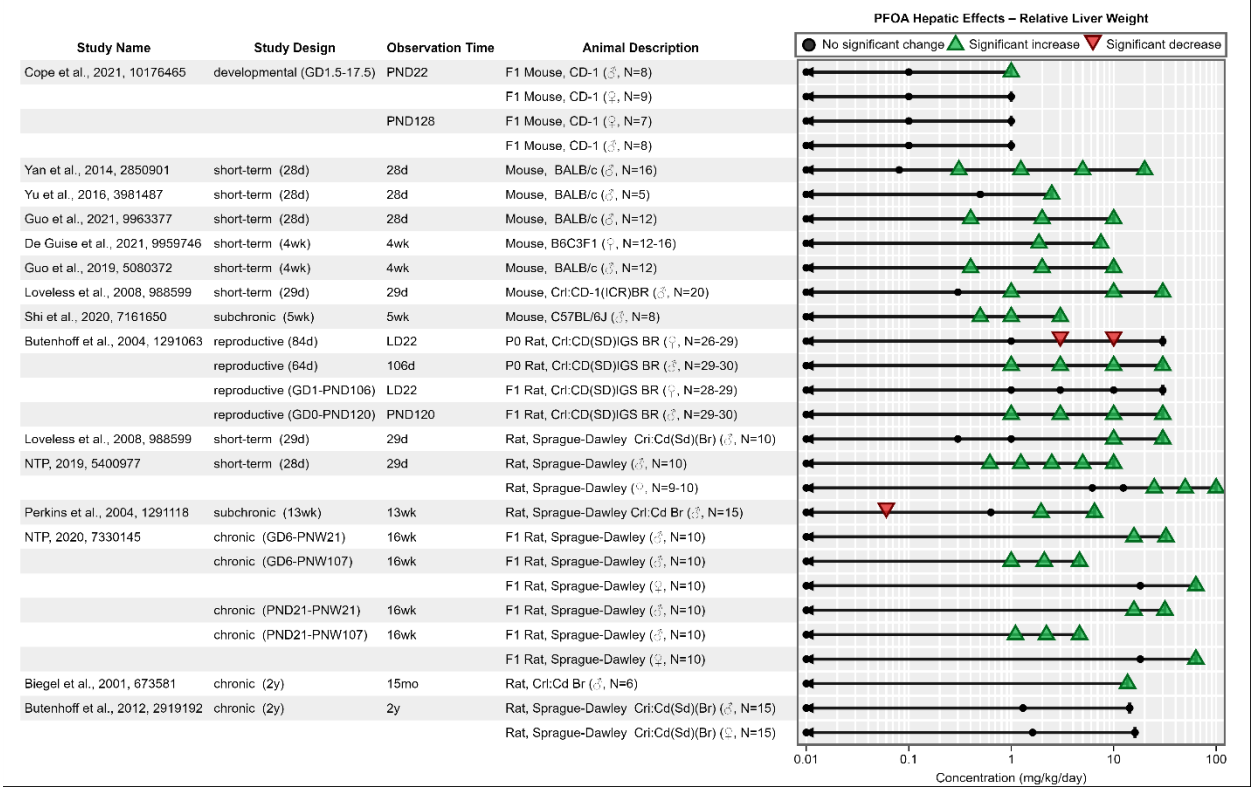


Figure 3-14. Relative Liver Weight in Rodents Following Exposure to PFOA (Continued, logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; PNW = postnatal week; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day; wk = week; y = year.

3.4.1.2.2 Clinical Chemistry Measures

Albumin, a blood protein that plays a major role in PFOA toxicokinetics (Section 3.3), is synthesized by the liver. Increases in serum albumin were reported in several short-term and chronic studies in male rodents, with increases observed at doses as low as 0.4 and 1.3 mg/kg/day in mice and rats, respectively (NTP, 2020; Guo et al., 2019; Yan et al., 2014; Butenhoff et al., 2012). Females appeared to be less sensitive, with increased albumin at doses ≥25 mg/kg/day in rats after short-term or chronic exposures and no significant differences or inconsistent decreases in pregnant mice after gestational exposures (Blake et al., 2020; NTP, 2020, 2019; Butenhoff et al., 2012; Yahia et al., 2010). The albumin/globulin ratio was significantly increased in both adult males and females after PFOA exposure for 28 days or 16 weeks (NTP, 2020; Guo et al., 2019; NTP, 2019).

Similar to albumin, inconsistent results were observed for total protein, with statistically significant decreases observed in some studies in male rats (NTP, 2020, 2019) and pregnant female mice in one study (Blake et al., 2020), and increases or no significant changes observed in several other studies in adult male rats or mice (Guo et al., 2019; Butenhoff et al., 2012) and in female rats (NTP, 2020, 2019; Butenhoff et al., 2012).

Increases in enzymes including ALT, ALP, and AST following PFOA exposures were observed across multiple species, sexes, and exposure paradigms (Figure 3-15 (male mice), Figure 3-16 (male rats), Figure 3-17 (female rodents)). These enzymes are often useful indicators of hepatic enzyme induction, hepatocellular damage, or hepatobiliary damage as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood (U.S. EPA, 2002a). Alterations in serum enzymes are generally considered to reach biological significance and indicate potential adversity at levels ≥twofold compared with controls (i.e., ≥100% change relative to controls) (Hall et al., 2012; U.S. EPA, 2002a).

In adult male mice dosed with PFOA for 4–5 weeks, statistically significant increases in ALT and/or AST were observed at PFOA exposure levels ranging from 2 to 21.6 mg/kg/day (Crebelli et al., 2019; Guo et al., 2019; Yan et al., 2014; Minata et al., 2010). Increases in ALT were ≥100% above control values at doses as low as 1.25 mg/kg/day (Yan et al., 2014). Biologically significant increases in AST were only observed in two of these studies at doses ≥20 mg/kg/day (Yan et al., 2014; Minata et al., 2010). In the only short-term study examining ALP in male mice, ALP was significantly increased at concentrations of 5 and 20 mg/kg/day after 28-day exposure (Yan et al., 2014); serum ALP levels were ≥100% change at doses of 1.25 mg/kg/day and higher.

In male CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day from GD 1.5 to GD 17.5 and then fed either a high- or low-fat diet starting on PND 22, no significant changes were observed in ALT, AST, or ALP on PND 128 (Cope et al., 2021).

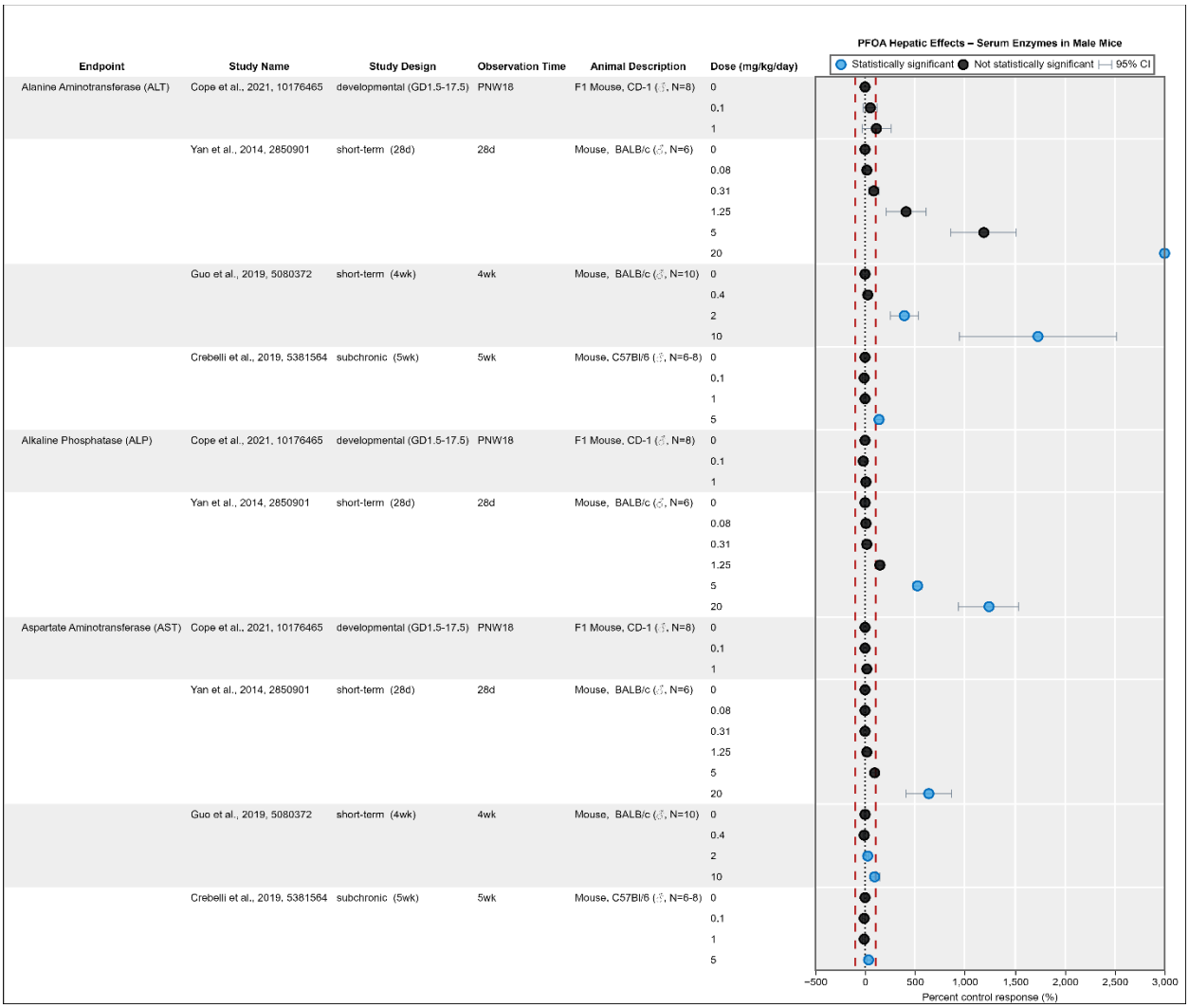


Figure 3-15. Percent Change in Serum Enzyme Levels Relative to Controls in Male Mice Following Exposure to PFOA[a,b]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; d = day; wk = week; CI = confidence interval.
[a] The red dashed lines indicate a 100% increase or 100% decrease from the control response.
[b] Results for Yan et al. (2014) are presented for six doses (0, 0.08, 0.31, 1.25, 5, and 20 mg/kg/day), and a statistically significant response of 7,000% occurred at the highest dose for the ALT endpoint. The axis has been truncated at 3,000% to allow results at lower doses for other studies and endpoints to be legible.

NTP (2020, 2019) reported significantly increased ALT and ALP at all doses tested in the 28-day and 16-week exposures of male Sprague-Dawley rats to PFOA (dose range of 0.625–32.1 mg/kg/day). However, increases in ALT did not exceed 100% change in either study. Similarly, increases in ALP did not exceed 100% change in the 28-day gavage study (NTP, 2019) and only exceeded 100% change with doses ≥15.6 mg/kg/day at the 16-week interim time point of the chronic dietary study (NTP, 2020). In another chronic dietary study, Butenhoff et al. (2012) generally observed increased ALT and ALP in male Sprague-Dawley rats dosed with 1.3 and 14.2 mg/kg/day PFOA at time points ranging from 3 months to 2 years of administration.

Increases in ALT were above or approximately 100% change in both dose groups at 6, 12, and 18 months of exposure. ALP levels were elevated at all time points with 14.2 mg/kg/day PFOA but were only above 100% change at the 18-month time point. AST was also less sensitive than ALT or ALP in male rats. NTP (2019) observed statistically significant but not biologically significant increases in AST at doses of 2.5 mg/kg/day and higher (up to 10 mg/kg/day) after 4 weeks. Butenhoff et al. (2012) did not observe biologically significant increases in AST at any time of assessment during the 2-year feeding study.

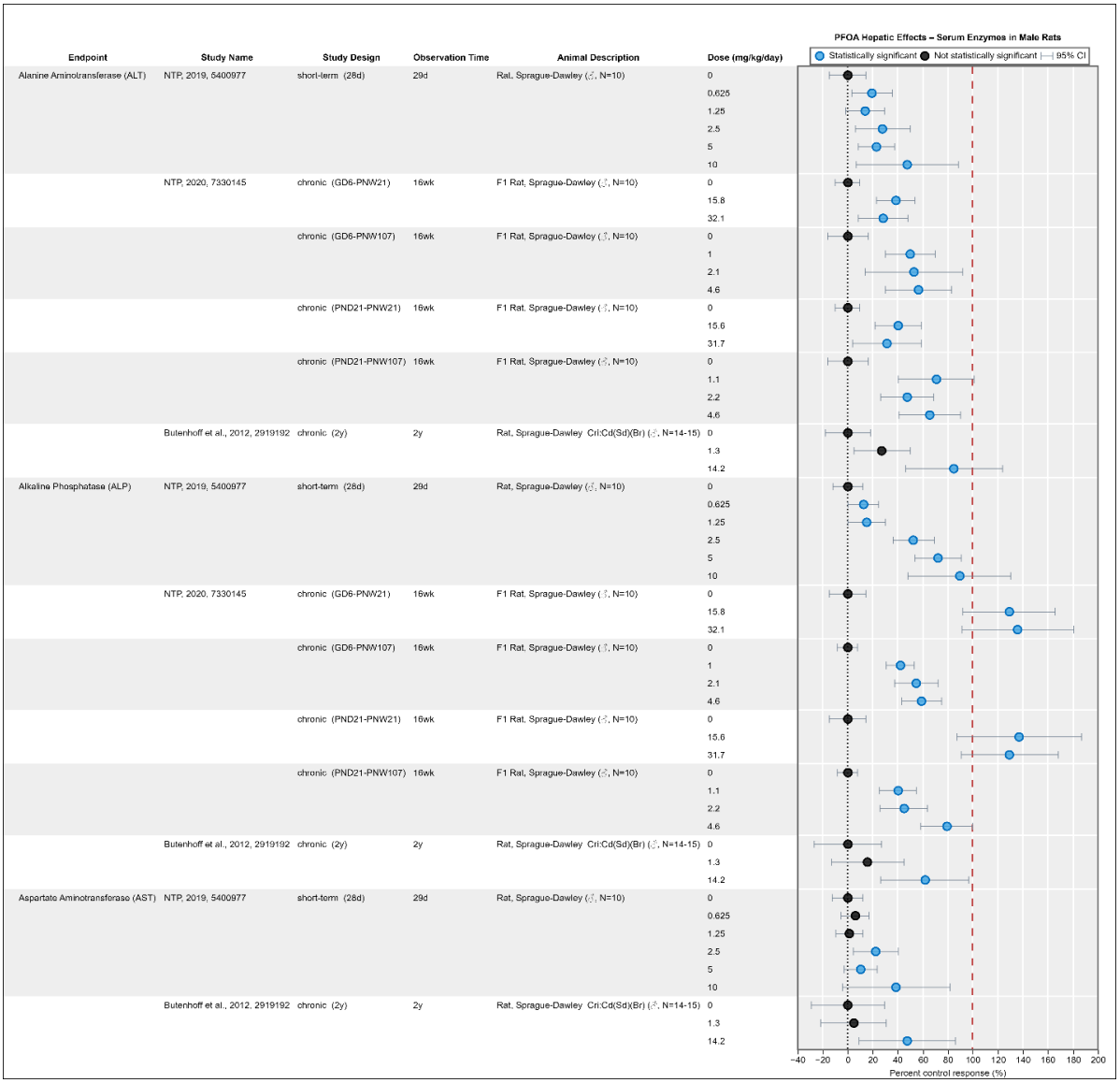


Figure 3-16. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; F_1 = first generation; d = day; wk = week; CI = confidence interval.
[a] The red dashed line indicates a 100% increase from the control response.

In addition to the findings in rodents, no consistent responses of serum enzymes were observed in the one available study in male cynomolgus monkeys dosed with PFOA for 26 weeks (Butenhoff et al., 2002).

The only available studies measuring ALT, AST, or ALP in female mice were after gestational PFOA exposures. Blake et al. (2020) reported no statistically significant effects on ALT or ALP levels in CD-1 dams after gestational PFOA exposure, and significantly increased AST (113% increase over control) only after exposure to the high dose of 5 mg/kg/day from GD 1.5 to GD 17.5. In contrast, Yahia et al. (2010) reported biologically significant increases in ALT and AST in dams after gestational exposure to 5 or 10 mg/kg/day PFOA (150% and 372% increase from control ALT levels, respectively; 312% and 813% increase from control AST levels, respectively). Biologically significant increases in ALT, ALP, and AST were only observed at the highest dose of 10 mg/kg/day. In a study in which female CD-1 mice were gestationally exposed to 0.1 or 1 mg/kg/day from GD 1.5 to GD 17.5 and then given a low-fat diet starting on PND 22, no significant changes were observed in ALT, AST, or ALP on PND 128 (Cope et al., 2021). However, in the group of females exposed to 1 mg/kg/day and then given a high-fat diet, statistically significant increases were observed in ALT (130% control), AST (23% control), and ALP (43% control).

Short-term and chronic studies reported statistically but not biologically significant increases in ALT in female rats after 4- or 16-week PFOA exposures between 50–100 mg/kg/day (NTP, 2020, 2019). The 4- and 16-week studies also reported no biologically significant changes in ALP with any PFOA dose, though PFOA exposures resulted in statistically significant ALP increases at gavage doses as low as 6.25 mg/kg/day after 4 weeks (NTP, 2020, 2019). NTP (2019) and found no statistically or biologically significant differences in AST in adult female Sprague-Dawley rats following 4-week PFOA gavage dosing. Butenhoff et al. (2012) also did not observe statistically significant changes in ALT, AST, or ALP in adult female Sprague-Dawley rats exposed to 1.6 or 16.1 mg/kg/day PFOA for up to 2 years.

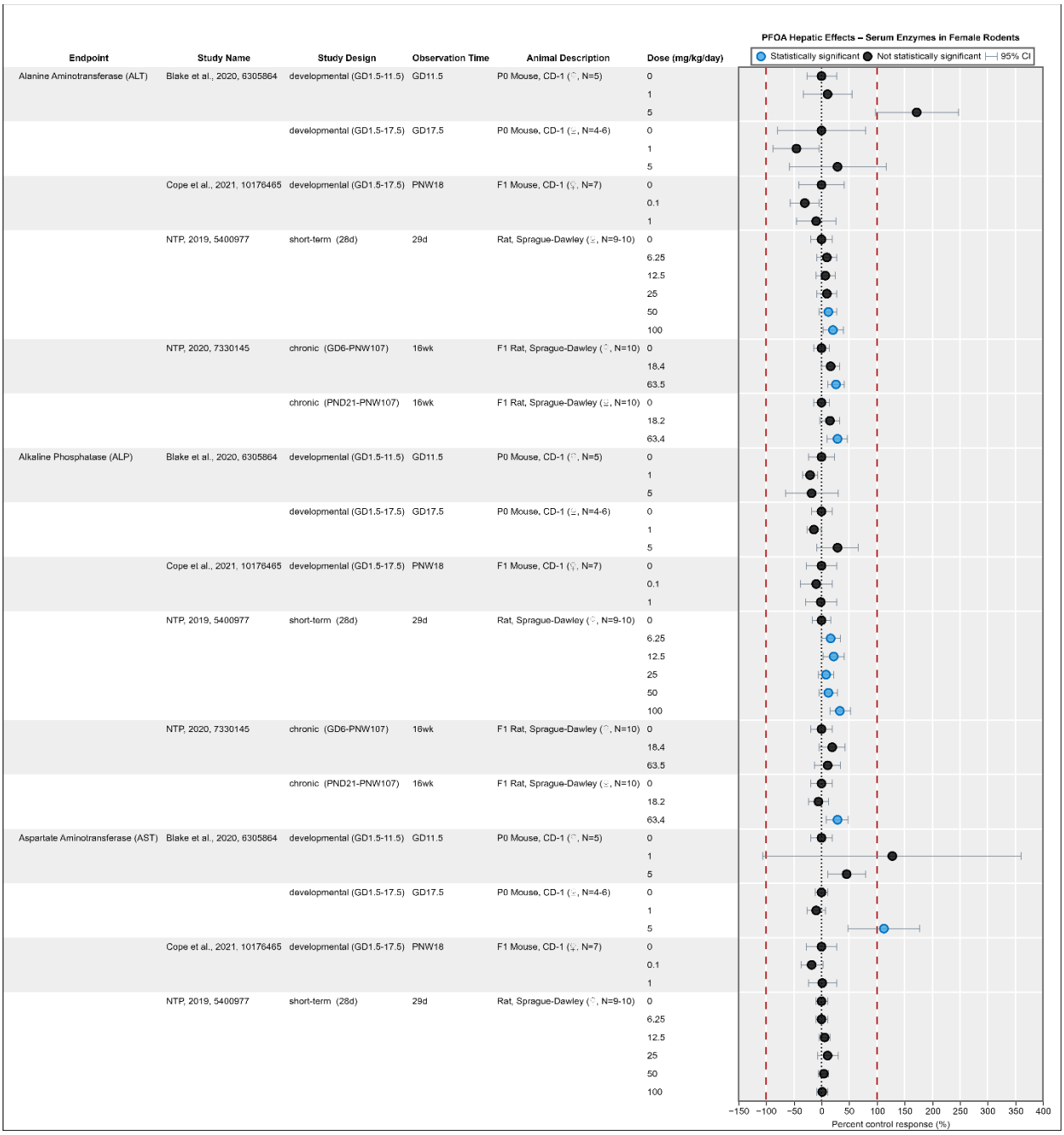


Figure 3-17. Percent Change in Enzyme Levels Relative to Controls in Female Rodents Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; P_0 = parental generation; F_1 = first generation; d = day; wk = week; CI = confidence interval.
[a] The red dashed lines indicate a 100% increase or 100% decrease from the control response.

3.4.1.2.3 Histopathology

The available animal toxicology literature provides evidence of alterations in liver histopathology were observed after PFOA exposure. Increased cell proliferation/division, bile duct hyperplasia, and hepatocellular hypertrophy were common responses across multiple studies. Loveless et al. (2008) reported increased incidence and severity of hepatocellular hypertrophy with increasing doses of PFOA (0.3–30 mg/kg/day) in male CD-1 mice dosed for 29 days (incidences of 0/19, 20/20, 20/20, 20/20, and 19/19 (all severity grades combined) in the 0, 0.3, 1, 10, and 30 mg/kg/day groups, respectively). Several other 28-day studies in adult male mice provided qualitative descriptions and images as evidence of increased hypertrophy, though results were not quantitatively reported (Guo et al., 2019; Li et al., 2017b; Yan et al., 2017; Minata et al., 2010).

Doses as low as 0.3 mg/kg/day PFOA resulted in increased incidence and severity of hypertrophy in male rats dosed for 28 or 29 days (NTP, 2019; Loveless et al., 2008; Perkins et al., 2004); female rats dosed for 28 days showed slight increases at 50 mg/kg/day (20%) and a 100% hypertrophy incidence rate at 100 mg/kg/day compared with 0% incidence at all lower doses (6.25, 12.5, or 25 mg/kg/day) and in controls (n = 10) (NTP, 2019). Butenhoff et al. (2012) reported significant increases in the incidence of hypertrophy in male and female adult Sprague-Dawley rats administered PFOA for 1 or 2 years at the highest dose tested for each sex (14.2 and 16.1 mg/kg/day for males and females, respectively). NTP (2020) also reported increased incidence of hepatocellular hypertrophy in male and female adult rats dosed with PFOA for 16 or 107 weeks (see study design details in Section 3.4.4.2.1.2). At the 16-week interim necropsy, males had significantly increased incidences of hypertrophy at all doses tested (1–32.1 mg/kg/day); significantly increased incidences of hypertrophy were only observed in females at the highest doses tested (63.4/63.5 mg/kg/day) at 16 weeks. At 107-weeks, significantly increased incidences of hypertrophy were observed in males and females at doses ≥1.1 mg/kg/day and ≥18.2 mg/kg/day, respectively.

In a developmental toxicity study, Blake et al. (2020) observed 100% incidence of hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm at both the GD 11.5 and GD 17.5 time points with doses of 1 and 5 mg/kg/day compared with 0% incidence in controls (all n = 5–6); however, control CD-1 mouse dams at the GD 17.5 time point also exhibited what the authors characterized as hepatocellular hypertrophy consistent with pregnancy at that stage of gestation. Quist et al. (2015) similarly reported increased severity of hepatocellular hypertrophy with increasing PFOA doses (0.01–1 mg/kg/day) in PND 91 female CD-1 mouse offspring exposed from GD 1 to GD 17. In a standard 2-generation reproductive toxicity study, significant increases in the incidence of diffuse hepatocellular hypertrophy were reported for male F_1 Sprague-Dawley rat offspring at doses of 3 mg/kg/day and higher (Butenhoff et al., 2004a).

In addition to hepatocellular hypertrophy, significantly increased incidences of mitotic figures and bile duct hyperplasia were observed in adult male CD-1 mice exposed to 10 or 30 mg/kg/day PFOA for 29 days (Loveless et al., 2008). NTP (2020) reported significantly increased incidences of mitoses and bile duct hyperplasia in female Sprague-Dawley rats dosed with 63.5 mg/kg/day PFOA for 2 years, but not in males. In contrast, Filgo et al. (2015) reported the incidence and severity of bile duct hyperplasia in two strains of 18-month-old wild-type female mice exposed to PFOA during gestation and found no alterations in CD-1 mice and a significant

decrease in the severity of bile duct hyperplasia in 129/Sv mice. However, increased mitoses were observed (data not provided) in ICR mouse dams exposed to 1–10 mg/kg/day PFOA during gestation (Yahia et al., 2010).

Several studies reported cytoplasmic alterations including cytoplasmic vacuolization resulting from PFOA exposures. Male mice dosed with PFOA for 28 days were reported to have increased vacuolation at doses between 5.4–21.6 mg/kg/day (incidence data not provided) and significantly decreased numbers of nuclei per unit area with 28-day exposures to ≥0.4 mg/kg/day (Guo et al., 2019; Minata et al., 2010). Male rats were particularly susceptible to cytoplasmic alterations; NTP (2020, 2019) reported incidences of 90%–100% in animals receiving doses ≥1 mg/kg/day for 4 or 16 weeks compared with 0% incidences in controls (all n = 10). In the 2-year study, males receiving ≥2.1 mg/kg/day showed a 58% or greater incidence rate compared with 0% incidence rates in controls (all n = 50) (NTP, 2020).

Female rats receiving doses ≥25 mg/kg/day for 4, 16, or 107 weeks had 98%–100% incidence rates of cytoplasmic alterations compared with 0% incidence rates in controls (NTP, 2020, 2019). In CD-1 mouse dams, 100% incidence rates of cytoplasmic vacuolization were observed only at the highest dose of 5 mg/kg/day but at both gestational time points (GD 11.5 and GD 17.5) compared with 0% incidence rates in controls (n = 5–6) (Blake et al., 2020). In this study, the vacuoles frequently contained remnant membrane material as myelin figures.

Cell and tissue death[8] and degeneration was the final category of hepatic histological effects observed across multiple studies, species, and sexes (Table 3-3). Incidence rates of individual cell necrosis in male CD-1 mice dosed with PFOA for 29 days were above 50% at doses ≥1 mg/kg/day (Loveless et al., 2008). There was similarly a significantly increased percentage of necrotic liver cells, analyzed by flow cytometry, in male C57BL/6 mice administered 5 mg/kg/day PFOA in drinking water for 5 weeks (Crebelli et al., 2019). Significantly increased incidences of single-cell death were observed in male Sprague-Dawley rats after 16 weeks of exposure to doses as low as 1 mg/kg/day but were not increased in females at this time point (NTP, 2020). Incidence rates of single-cell death in male and female rats after 2-year exposures as reported in NTP (2020) are provided in Table 3-3 (see further study design details in Section 3.4.4.2.1.2). Apoptosis and single-cell necrosis were also observed in livers of pregnant CD-1 mice after gestational exposures of 1 and 5 mg/kg/day, with increasing length of exposure resulting in increased incidence rates (Blake et al., 2020). In male and female CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day from GD 1.5 to GD 17.5 and then given a low-fat diet on PND 22, incidences of single-cell necrosis were higher in the exposed groups but not significantly increased at PNW 18 (Table 3-3) (Cope et al., 2021). However, in females exposed to 1 mg/kg/day and then to a high-fat diet, incidences of single-cell necrosis were significantly increased at PNW 18.

In male CD-1 mice exposed to PFOA for 29 days, the incidence of hepatic focal necrosis increased with increasing PFOA doses between 1–30 mg/kg/day (Loveless et al., 2008). In the same study, increased incidences of necrosis were reported in male Sprague-Dawley rats only with the highest dose tested (30 mg/kg/day) (Loveless et al., 2008). Inconsistent incidences of

[8] In this document, EPA used the cell death nomenclature as reported in the individual studies to describe the observed effects. Cell "necrosis" is a type of cell death, the term for which is generally used when a specific method to distinguish necrotic cells from other dying cells (e.g., apoptotic cells) has been employed (Elmore et al., 2016). EPA did not evaluate the methods of individual studies to ensure that the nomenclature used by the authors accurately reflected the type of cell death reported.

hepatic necrosis were observed in male and female Sprague-Dawley rats administered PFOA in feed for 16 weeks, though there were increases reported after 2 years (NTP, 2020). Table 3-3 depicts the 2-year data for males and females. In a separate 2-year study, there were no significant differences in the incidences of hepatic necrosis in male or female Sprague-Dawley rats (Butenhoff et al., 2012). Blake et al. (2020) did not observe consistent increases in the incidence of focal necrosis in mouse CD-1 dams dosed with PFOA during gestation. However, Butenhoff et al. (2004a) reported significant increases in focal and multifocal necrosis in F_1 generation male Sprague-Dawley rats in a 2-generation reproductive toxicity study (data not provided).

Table 3-3. Associations Between PFOA Exposure and Cell Death or Necrosis in Rodents

Reference	Study Design	Endpoint Name	Incidence
		Males	
NTP (2019)	28-d Sprague-Dawley rat oral gavage dosing; 0, 0.625, 1.25, 2.5, 5, 10 mg/kg/d	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 1/10, 0/10
Loveless (2008)	29-d Crl:CD(SD)IGS BR rat oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Focal Necrosis	0/10, 0/10, 0/10, 1/10, 4/10
	29-d Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Individual Cell Necrosis	0/19, 0/20, 11/20, 20/20, 19/19
	29-d Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Focal Necrosis	0/19, 1/20, 3/20, 4/20, 7/19
Perkins (2004)[a]	4-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 0/15, 0/15, 1/15, 2/14
	7-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 0/15, 0/15, 0/15, 1/15
	13-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 1/15, 0/15, 1/15, 0/15
Butenhoff (2012)	2-yr Crl:COBS® CD(SD)BR rat feeding study; 0, 1.3, 14.2 mg/kg/d	Focal Hepatocellular Necrosis	3/50, 5/50, 5/50
Cope (2021)[b]	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (offspring); 0, 0.1, 1 mg/kg/d	Hepatocyte Single-Cell Necrosis	2/8, 5/9, 6/9
NTP (2020)	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/150, 0/300, 150/150, and 300/300 ppm	Hepatocellular Single-Cell Death	0/10, 10/10, 10/10, 9/10, 10/10
		Necrosis	0/10, 6/10, 2/10, 2/10, 4/10
	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40,	Hepatocellular Single-Cell Death	0/10, 7/10, 9/10, 10/10, 0/10, 5/10, 8/10, 10/10
		Necrosis	1/10, 1/10, 6/10, 4/10, 0/10, 2/10, 3/10, 1/10

Reference	Study Design	Endpoint Name	Incidence
	0/80, 300/0, 300/20, 300/40, 300/80 ppm		
	2-yr Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm	Hepatocellular Single-Cell Death	1/50, 1/50, 11/50, 24/50, 1/50, 3/50, 5/50, 29/50
		Necrosis	2/50, 17/50, 23/50, 20/50, 1/50, 11/50, 14/50, 21/50
		Females	
NTP (2019)[c]	28-d Hsd:Sprague-Dawley SD rat oral gavage dosing; 0, 6.25, 12.5, 25, 50, 100 mg/kg/d	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 0/10, 0/10
Butenhoff (2012)	2-yr Crl:COBS® CD(SD)BR rat feeding study; 0, 1.6, 16.1 mg/kg/d	Focal Hepatocellular Necrosis	5/50, 6/50, 2/50
Blake (2020)	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 11.5 (dams); 0, 1, 5 mg/kg/d	Focal Necrosis	1/5, 0/5, 2/5
		Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 1/5, 3/5
	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (dams); 0, 1, 5 mg/kg/d	Focal Necrosis	0/5, 0/5, 0/6
		Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 5/5, 6/6
Cope (2021)[b]	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (offspring); 0, 0.1, 1 mg/kg/d	Hepatocyte Single-Cell Necrosis	1/9, 3/9, 4/10
NTP (2020)	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000, 150/300, and 300/1,000 ppm	Hepatocellular Single-Cell Death	0/10, 0/10, 1/10, 0/10, 0/10
		Necrosis	0/10, 0/10, 2/10, 0/10, 0/10
	2-yr Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000, 150/300, and 300/1,000 ppm	Hepatocellular Single-Cell Death	0/50, 4/50, 29/50, 5/50, 32/50
		Necrosis	0/50, 1/50, 8/50, 4/50, 5/50

Notes: GD = gestation day.
[a] Incidence data as reported by Perkins et al. (2004) were split into severity categories within the original study. For the purposes of this table, all non-grade 0 severities were considered an incidence (results for severity grades 1–3 were combined).
[b] Data are summarized for low-fat diet only from Cope et al. (2021).
[c] Incidence data not explicitly reported by NTP (2019).

Cystic degeneration was also observed across two chronic feeding studies in male rats. Butenhoff et al. (2012) reported incidences of cystic degeneration characterized as areas of multilocular microcysts in the liver parenchyma in 4/50 (8%), 7/50 (14%), and 28/50 (56%) male rats dosed for 2 years with 0, 1.3, or 14.2 mg/kg/day, respectively. NTP (2020) similarly reported increases

in the incidence of cystic degeneration in the liver of male rats administered 4.6 mg/kg/day PFOA for 107 weeks.

3.4.1.2.4 Additional Hepatic Endpoints

A suite of other liver effects was observed but were either not included as endpoints of interest across multiple studies or had inconsistent results between studies, sexes, and/or species. These included serum measures of gamma-glutamyl transpeptidase (only measured in one short-term study of male BALB/C mice that showed increases at 2 and 10 mg/kg/day exposures) (Guo et al., 2021a), bile acids (study results generally showed no response or increases at high doses) (Guo et al., 2021a; Blake et al., 2020; NTP, 2020, 2019; Yan et al., 2014; Butenhoff et al., 2002), bilirubin (study results showed no change or minimal increases at high doses) (Guo et al., 2021b; NTP, 2019; Butenhoff et al., 2012; Yahia et al., 2010; Butenhoff et al., 2002), and histopathological findings such as hepatic inflammation (study results showed increased incidence/severity, decreased incidence, or no response) (NTP, 2020; Filgo et al., 2015; Quist et al., 2015), increased incidence of cellular infiltration (Cope et al., 2021; Butenhoff et al., 2012), and increased incidence of hepatocytomegaly (Zhang et al., 2020b). NTP (2020) also reported a variety of other histopathological outcomes including eosinophilic or mixed-cell foci (significant increases in male Sprague-Dawley rats) and pigmentation (significant increases in males and females). Butenhoff et al. (2004a) similarly reported increased discoloration of the liver in male F_1 Sprague-Dawley rats analyzed during a standard 2-generation reproductive toxicity study.

3.4.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse hepatic outcomes is discussed in Sections 3.2.1, 3.2.2, 3.2.3, 3.2.7, 3.2.8, 3.2.9, 3.3.2, 3.3.3, 3.3.4, 3.4.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 81 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to hepatic effects. A summary of these studies as organized by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-18.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling				
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	9	0	11	19
Cell Growth, Differentiation, Proliferation, Or Viability	17	1	36	50
Cell Signaling Or Signal Transduction	14	1	17	30
Extracellular Matrix Or Molecules	1	0	1	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	21	0	19	37
Hormone Function	6	1	1	8
Inflammation And Immune Response	5	1	3	9
Oxidative Stress	8	0	14	21
Renal Dysfunction				
Xenobiotic Metabolism	8	1	12	20
Other	0	0	3	3
Not Applicable/Not Specified/Review Article				
Grand Total	42	2	47	83

Figure 3-18. Summary of Mechanistic Studies of PFOA and Hepatic Effects

Interactive figure and additional study details available on HAWC.

3.4.1.3.1 Nuclear Receptor Activation

3.4.1.3.1.1 Introduction

The ability of PFOA to mediate hepatotoxicity via nuclear receptor activation has been investigated for several receptor-signaling pathways, including that of the peroxisome proliferator-activated receptors (PPARα, PPARδ, PPARγ), the pregnane X receptor (PXR), and the constitutive androstane receptor (CAR). PPARα is a major target for PFOA. A primary mechanism of hepatic injury associated with PFOA-mediated activation of PPARα relates to impacts on hepatic lipid metabolism caused by altered expression of genes and proteins within the PPARα signaling pathway (Li et al., 2019b; Pouwer et al., 2019; Wen et al., 2019c; Das et al., 2017; Hui et al., 2017; Rebholz et al., 2016; U.S. EPA, 2016c; van Esterik et al., 2015; Yan et al., 2015a; Yang et al., 2014; Wang et al., 2013). Activation of PPARα has been cited as a mechanism of action for PFAS, including PFOA (U.S. EPA, 2016c), because of the association between hepatic lesions and/or increased liver weight and peroxisome proliferation downstream of PPARα activation in rats. However, increased hepatic lipid content in the absence of a strong

PPARα response (i.e., activation of downstream target genes) is a characteristic of exposure to PFOA. Additionally, many of the genes activated by PFOA are regulated by transcription factors other than PPARα, including CAR, PPARγ, PXR, Erα, and HNF4α (U.S. EPA, 2016c). PPARs, CAR, and PXR are nuclear receptors that can form heterodimers with one another to induce transcription of linked genes. Other factors impacting nuclear receptor activation in hepatocytes include dose and duration of PFOA exposure and the genetic background, diet, and sex of exposed animals. Sex-specific hepatic effects varied by strain, and long-term PFOA oral exposure in mice with pre-existing steatosis had protective effects against hepatic injury (Li et al., 2019c; NTP, 2019; Li et al., 2017b). Thus, the underlying mechanism(s) of PFOA-induced hepatotoxicity may involve multiple nuclear receptors. Additionally, hepatic effects observed with PFAS exposure, including inflammation and necrosis, cannot be fully explained by PPARα activation (Section 3.4.1.2.3). This updated assessment includes a summary of studies that have examined PPARs, CAR, PXR, Erα, and HNF4α activation as potential mechanisms underlying the health effects induced by PFOA.

3.4.1.3.1.2 PPARα Receptor Binding and Activation

Receptor binding and activation assays have been performed to examine the association between activation of PPARs, CAR, and/or PXR, and PFOA-mediated hepatotoxicity. PPARs modulate gene expression in response to exogenous or endogenous ligands and play essential roles in lipid metabolism, energy homeostasis, development, and cell differentiation (U.S. EPA, 2016c).

Several studies used luciferase reporter assays to examine the activation of PPARα by PFOA in vitro using human and animal cell lines transfected with mouse and human PPARα (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Buhrke et al., 2013). In African green monkey kidney COS-1 cells transfected with mouse PPARα, PFOA was the most potent activator of PPARα among the 5 PFAS tested, with PPARα activation observed at less than 1 μM after a 24 h exposure (Wolf et al., 2014). A study in human HEK293T cells found that human PPARα was activated at a concentration of 50 μM PFOA after a 24 h exposure (Behr et al., 2020b). Whether PFOA activates other nuclear receptors is less clear from studies conducted in HEK293 cells and may be cell type- and dose-dependent. PFOA had no activity in HEK293 cells transfected with constructs encoding other nuclear receptors, including PPARδ, CAR, PXR, the farnesoid X receptor (FXR), the liver X receptor α (LXRα), the retinoid X receptor α (RXRα) and retinoic acid receptor α (RARα), at concentrations up to 100 μM for 24 hours (Behr et al., 2020b). In a second study using a human PPARα construct in HEK293 cells, PFOA induced PPARα activation at concentrations of 25 μM and higher, whereas PFOA concentrations of at least 100 μM were necessary to activate PPARγ and PPARδ (Buhrke et al., 2013). Results from the single study conducted in a human hepatic cell line (HepG2) were consistent with results in other cell lines (Rosenmai et al., 2018). Of the 14 PFAS substances tested, PFOA was the most potent PPARα activator, showing significant elevation of luciferase activity after a 24-hour exposure to 30 and 100 μM PFOA. While luciferase levels were elevated at 10 μM of PFOA, the increase did not reach significance. These in vitro studies support PPARα activation by PFOA.

Another study measured the expression of hepatic carboxylesterases (*Ces*) that function in the metabolism of drugs, chemical toxicants, and endogenous lipids (Wen et al., 2019c). PFOA upregulated expression of the PPARα target gene, Cyp4a14, in the livers of male C57BL/6 NCrl mice after exposure to 3 mg/kg/day by gavage for 7 days. PFOA exposure also led to alterations to the expression of Ces genes: *Ces1d*, *1e*, *1f*, *1g*, *2c*, and *2e* mRNA levels were increased

between 1.5- and 2.5-fold, while *Ces1c* and *2b* transcripts were decreased. In a second study within Wen et al. (2019c), *Ces* genes were measured in the livers of C57BL/6NTac mice and PPARα-null mice also exposed to 3 mg/kg/day PFOA by gavage for 7 days. *Ces1e* and *1f* mRNA and protein levels were PPARα dependent, whereas *Ces1c, 1d, 1g, 2a, 2b*, and *2e* mRNA and CES2 protein levels were induced by PFOA in PPARα-null mice, implicating a CAR-mediated pathway for differential expression of these genes.

The mechanism by which PFOA activates PPARα is likely dependent on interactions with liver fatty acid binding protein (L-FABP). L-FABP facilitates the nucleo-cytoplasmic shuttling of activator ligands, such as fatty acids, for nuclear receptors, including PPAR activators, PXRs, and LXRs. PFOA is structurally similar to fatty acids, and both exhibit a strong binding affinity with L-FABP (Section 3.3.1.2). Thus, L-FABP is responsible for delivering PFOA to the nuclei of hepatic cells for access to nuclear receptors. Sheng et al. (2018) used circular dichroism (CD) spectroscopy, fluorescence displacement assays, and molecular docking approaches to evaluate the binding mode and capacity of PFOA as well as PFOS and PFAS replacement chemicals to purified human L-FABP (hL-FABP). The purified recombinant hL-FABP was calculated to consist of 15.7% α-helix and 54.4% β-sheet. In the presence of PFOA, α-helix content of the protein increased slightly, whereas the β-sheet content decreased. The dissociation constant (Kd) of PFOA to hL-FABP was 8.03 ± 2.10 μM, which was higher than PFOS and lower than some (but not all) replacement PFAS substances. By molecular docking, PFOA bonded with hL-FABP in a "head-out" mode, such that the carboxyl head of PFOA will interacted with R122 amino acid residue through hydrogen bonding and N111 amino acids residue through hydrophobic interactions. Introduction of oxygen molecules into the backbone could flip the binding prediction to a "head-in" mode characterized by interactions with amino acid residue N61. By comparing PFOA to PFOS and replacement PFAS chemicals, the authors demonstrated that these three parameters correlated both with cytotoxicity in human liver HL-7702 cells and binding affinity for hL-FABP. Notably, expression of select PPARα-regulated genes showed no significant change across the chemicals tested, with one exception, the Cd36 gene. Expression of other genes, including cell cycle genes, did correlate with these binding parameters. These findings suggest that binding of PFAS to hL-FABP can mediate toxicity in a manner that is not exclusively dependent on PPARα-mediated changes in gene expression in liver cells, but possibly through effects on other FABP-related events such as binding to the CD36 protein or effects on cell proliferation.

3.4.1.3.1.3 Receptor Binding and Activation of Other Nuclear Receptors

PFOA can activate PPARα in the liver of rodents and humans. However, the extent by which activation of PPARα mediates hepatoxicity may be species-specific, and activation of other receptors may also contribute to toxicity (U.S. EPA, 2016c). Indeed, studies in mice and rats indicate that PFOA may activate PPARα, CAR, and PXR in the liver (Li et al., 2019c; NTP, 2019; Wen et al., 2019c; Rose et al., 2016).

Several studies observed perturbations in lipid transport, fatty acid metabolism, triglyceride synthesis, and cholesterol synthesis in PFOA-exposed mice (Li et al., 2019b; Das et al., 2017; Rosen et al., 2017). A few of these studies, Das et al. (2017), Rosen et al. (2008b), and Rosen et al. (2017), investigated the effects of PFOA on lipid metabolism and homeostasis in the absence of PPARα by using knockout mouse models. After exposure to 10 mg/kg/day PFOA for 7 days, Das et al. (2017) observed that a smaller subset of genes related to lipid homeostasis was

activated in PPARα null mice compared with wild-type (WT) mice. Increased expression of genes regulating fatty acid and triglyceride synthesis and transport into hepatocytes was attenuated but not entirely abolished in PFOA-exposed PPARα null mice compared with WT mice. Gene expression changes in PPARα null mice implicate a role for PPARβ/δ and/or PPARγ in the absence of PPARα (Rosen et al., 2008b). Mechanistically, these changes correlated with the development of steatosis in PFOA-exposed WT mice consistent with increased triglyceride accumulation. In contrast, elevated triglyceride levels and steatosis develop in PPARα null mice even in the absence of PFOA exposure. The authors propose that PFOA exposure alters lipid metabolism to favor biosynthesis and accumulation over β-oxidation, leading to hepatic steatosis. PFOA increased the expression of genes related to fatty acid β-oxidation, lipid catabolism, lipid synthesis, and lipid transport in both strains; however, gene induction was lower in PPARα null mice (Rosen et al., 2017; Rosen et al., 2008b). In fact, the authors suggest that the transcriptome of the mice resembled that of mice treated with PPARγ agonists, thus indicating a role for other PPAR isoforms in the dysregulation of lipid synthesis (Rosen et al., 2017). Furthermore, Rosen and colleagues (Rosen et al., 2017) demonstrated that PFOA significantly downregulated the Signal Transducer and Activator of Transcription 5B gene (STAT5B), a transcription factor and member of the STAT family, in a PPARα-dependent manner. STAT5B has been demonstrated in regulation of sexually dimorphic gene expression in the liver between males and females, raising the possibility that that PFOA exposure may promote feminization of the liver in male mice (Rosen et al., 2017; Oshida et al., 2016).

Increasing evidence links CAR activation as a mechanism of PFOA-induced liver toxicity (Li et al., 2019c; NTP, 2019; Wen et al., 2019c). The use of genetically modified mice and gene expression analyses has demonstrated that PFOA exposure activates both PPARα and CAR receptors (Li et al., 2019c; NTP, 2019; Wen et al., 2019c; Abe et al., 2017; Rosen et al., 2017; Oshida et al., 2015a; Oshida et al., 2015b).

Five recent studies also examined PFOA activation of CAR-specific genes (NTP, 2019; Wen et al., 2019c; Abe et al., 2017; Rosen et al., 2017; Rose et al., 2016). Additionally, one study used both a cell-based reporter assay and in silico approaches to examine PFOA activation of PXR (Zhang et al., 2020d), and one study examined other PFOA effects on other nuclear receptors in vitro (Buhrke et al., 2015). In support of PFOA as a CAR receptor activator, PFOA induced expression of the CAR target genes CYP2B6 in a human hepatocyte cell line in vitro (HepaRG), and *Cyp2b10* in wild-type mice but not CAR-null mice in vivo (Abe et al., 2017). Evidence of CAR-specific gene expression was also noted in male and female rats administered PFOA. Exposed animals exhibited significant increases in expression of PPARα-stimulated genes (*Acox1*, *Cyp4a1*) and CAR-specific genes (*Cyp2b1, Cyp2b2*) in livers compared with controls, suggesting increases in PPARα and CAR activity (NTP, 2019). Males were exposed to a range of doses between 0 and 10 mg/kg/day and females to between 0 and 100 mg/kg/day PFOA for 28 days. Gene expression in liver tissue was analyzed using qRT-PCR. Female rats displayed the greatest fold increase for the CAR-related genes *Cyp2b1* whereas males exhibited the greatest fold increase for *Cyp4a1* and *Cyp2b1* compared with controls.

Rosen et al. (2008b) postulated that gene expression changes in the liver should overlap between PFOA and phenobarbital, a known CAR activator. To test this, differentially expressed genes in wild-type or CAR-null mice treated with PFOA by gavage (3 mg/kg/day) for 7 days were compared with differentially expressed genes in the livers of mice exposed to 100 mg/kg/day

phenobarbital for three days (Rosen et al., 2017). Similarity in differentially expressed genes between the two studies (i.e., overlap) was analyzed using a Running Fisher Test for pairwise comparisons. As expected, there was significant similarity between the lists of differentially expressed genes for PFOA and phenobarbital in WT mice, but not in CAR-null mice. In fact, close to 15% of genes differentially expressed upon PFOA exposure in liver were considered PPARα-independent. Two gene expression compendium studies further analyzed these data using gene expression biomarker signatures built using microarray profiles from livers of WT mice, CAR-null mice (Oshida et al., 2015a), and PPARα-null mice (Oshida et al., 2015b). These analyses found that both CAR and PPARα were activated by PFOA, and that CAR activation was generally more significant in PPARα-null mice. The authors concluded that CAR likely plays a subordinate role to PPARα in mediating the adverse hepatic effects of PFOA (Oshida et al., 2015a).

Activation of CAR may occur via direct activation or indirect activation. Indirect activation of CAR by phenobarbital involves blockade of the downstream phosphorylation pathway of EGFR protein phosphatase 2A (PP2A), which dephosphorylates CAR to enable nuclear translocation. Using a COS-1 fibroblast cell-based reporter gene assay that is capable of detecting CAR ligands but not indirect activators, Abe et al. (2017) observed that PFOA failed to activate reporter gene expression. In a second study using primary mouse hepatocytes, PFOA exposure led to CAR-mediated expression of *Cyp2b10* even in the presence of okadaic acid, a PP2A drug inhibitor. Together these findings suggest the mechanism of PFOA-mediated CAR activation indirect and distinct from that of phenobarbital. Moreover, an analysis of historical and new data of gene expression in PPARα- and CAR-null mice indicate the pathway of PFOA-mediated CAR activation is PPARα-independent (Rosen et al., 2017). Thus, the precise mechanism of CAR activation by PFOA remains to be determined.

Several studies evaluated PFOA activation of other nuclear receptors. Rosen et al. (Rosen et al., 2017) noted that PFOA activated PPARγ and ERα in trans-activation assays from the ToxCast screening program. Zhang et al. (Zhang et al., 2020d) used a cell-based reporter assay and an in silico approach to estimate PFOA-mediated activation of the PXR receptor. The PFOA log EC50 was 5.04 M in the luciferase-based PXR reporter assay, a higher concentration (i.e., less potent) than observed for PPARα. These authors also developed classical QSAR and 3D-QSAR models that predicted very similar values of log EC50 of 4.92 M and 4.94 M, respectively. Both models suggested that molecular structural factors including molecular polarizability, charge, and atomic mass are key parameters dictating hPXR agonistic activity of PFOA and other perfluoroalkyl chemicals.

In addition to the key role of PPARα and other nuclear receptors discussed above, other transcription factors and epigenetic mechanisms influence PFOA-mediated changes in lipid metabolism and storage. Beggs et al. (2016) observed a decrease in hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator or hepatic differentiation, in the livers of ten-week-old CD-1 mice exposed to 3 mg/kg/day PFOA once daily by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcriptional regulation of liver-specific genes, and regulation of lipid metabolism. In this study, PFOA exposure correlated with downregulation of HNF4α target genes involved in differentiation (*Cyp7a1*) and induced pro-mitogenic genes including CCND1. Other genes altered by PFOA exposure mapped to pathways involved in lipid metabolism, liver cholestasis, and hepatic

steatosis. PFOA also led to diminished accumulation of HNFα protein. This decrease in HNF4α was not accompanied by a change in expression of the gene, suggesting that the decrease in HNF4α occurs post-translationally. The decreased HNFα correlated with upregulation of genes that are negative targets of HNF4α. HNF4α is considered an orphan receptor, with various fatty acids as its endogenous ligands. These fatty acids maintain the structure of the receptor homodimer. PFOA and PFOS are analogous in structure to fatty acids and may also provide stabilization of the homodimer. The authors investigated the role of PFOA and PFOS interaction with this protein via in silico docking models, which showed a displacement of fatty acids by PFOA/PFOS, possibly tagging HNF4α for degradation. The authors hypothesize that steatosis, hepatomegaly, and carcinoma in rodents may be a consequence of the loss of this protein and also presents a mechanism for PFOA-induced hepatic effects in humans.

In primary human hepatocytes exposed to 1, 25, or 100 μM PFOA for 24 hours, the number of differentially regulated genes was 43, 109, and 215, respectively, as measured using a human genome gene chip (Buhrke et al., 2015). Given known activators of the differentially expressed genes, the authors suggest that in addition to PPARα, PPARγ and HNF4α may contribute to changes in expression of genes involved in carnitine metabolism. PFOA-mediated induction of ERα signaling was also predicted based on pathway analysis.

3.4.1.3.1.4 Host Factors Impacting PPARα Signaling

The effects of PFOA on PPARα activation depend on diet and pre-existing conditions (Li et al., 2019c). Mice were subjected to control diet or high-fat diet (HFD) for 16 weeks to induce nonalcoholic fatty liver disease (NAFLD), after which they were exposed to vehicle or 1 mg/kg/day PFOA by oral gavage for 2, 8, or 16 weeks; control diet and HFD were continued throughout this exposure period. Preexisting NAFLD in mice fed a HFD enhanced the induction of PPARα activation by PFOA early in the exposure but reduced the severity of macrovesicular steatosis and sinusoidal fibrosis induced by a HFD, and reversed HFD-induced increase in body weight and serum alanine aminotransferase (ALT). The authors hypothesized that PFOA exposure in animals with a lipid burden in the liver leads to PFOA-mediated inhibition of fatty acid biosynthesis pathways by the metabolic end-product feedback effect. The authors also observed reduced Tgf-β gene expression in PFOA-treated HFD-fed mice compared with vehicle-treated HFD-fed mice, which could account for the diminished level of hepatic stellate cell activation and collagen production associated with fibrosis. Furthermore, the duration of PFOA exposure impacted gene expression and hepatic injury. For example, PFOA induced Srebf1 and Srebf2 genes in the fatty acid biosynthesis pathway following 2 weeks of treatment, but this effect was not seen following 8 or 16 weeks of PFOA treatment. Notably, this increase in Srebf1 expression following 2 weeks of PFOA exposure was only observed with the co-treatment of PFOA and HFD; the Srebf1 effect was not observed in the PFOA-treated mice fed the control diet.

PFOA-driven changes in PPARα-mediated gene expression may also be modified be age, strain, or species. Pregnant Kunming mice were exposed to PFOA at doses of 1, 2.5, 5 and 10 mg/kg/day from gestational days 1–17, and female offspring were analyzed on postnatal day 21 (Li et al., 2019b). Genes involved in fatty acid β-oxidation including acyl-CoA synthetase (Acsl1), carnitine palmitoyl transferase I, Palmitoyl-CoA oxidase (Acox1), acyl-CoA thioesterase 1 (Acot1), and carnitine palmitoyltransferase 1a (Cpt1a) were significantly downregulated at the two highest doses, as was the PPARα gene. In this strain of mouse,

perinatal PFOA disrupts the gene expression of enzymes involved in fatty acid oxidation induced by PPARα, possibly through an epigenetic mechanism. In contrast, several studies have shown PFOA to upregulate expression of PPAR signaling pathway genes, including Acox in rats and mice (Li et al., 2019c; NTP, 2019; Cavallini et al., 2017). One such study proposed that the PFOA-mediated gene expression changes are due to changes in the activity of histone acetyltransferase (HAT) and HDAC (histone deacetylase) (Li et al., 2019b). In female offspring of pregnant Kunming mice treated with PFOA by oral gavage at doses between 0 and 10 mg/kg/day on GD 1–17, the overall levels of histone H3 and H4 acetylation were decreased in a dose-dependent manner in liver tissues in the pups at post-natal day 21. Histone acetylase (HAT) activity was reduced in pups at all doses except for the highest dose (10 mg/kg/day), in which there was no significant difference in HAT activity compared with controls. HDAC activity was increased in all dose groups. The changes in HAT and HDAC activity did not follow a dose-responsive pattern. Notably, gene-specific alterations in histone acetylation activity were not measured; thus, follow-up studies are needed to clarify the relationship between the global histone modifications and the gene expression changes.

Additional support for species specificity derives from studies demonstrating that PFOA-mediated gene expression changes were distinctly different in primary human hepatocytes compared with primary mouse hepatocytes (Rosen et al., 2013). Custom Taqman PCR arrays were generated to include transcripts regulated by PPARα as well as transcripts regulated independently of this nuclear receptor. Mouse and human hepatocytes were exposed to PFOA at doses ranging from 0 to 100 and from 0 to 200 μM, respectively, or the PPARα activator Wy14,643. In mouse cells, many fewer genes were altered by PFOA treatment compared with whole livers from mice exposed in vivo. Also, genes typically regulated by PPARα agonists were not altered by PFOA in mouse cells, including Acox1, Me1, Acaa1a, Hmgcs1, and Slc27a1. The CAR target gene Cyp2b10 was also unchanged in cultured mouse hepatocytes. In contrast, a larger group of genes were differentially expressed in primary human hepatocytes, including PPARα-independent genes (CYP2B6, CYP3A4, and PPARγ). These findings underscore some of the difficulty in extrapolating in vitro results from rodents to humans after PFOA exposure and suggest PPARα may elicit species-specific changes in gene expression.

3.4.1.3.1.5 Conclusions

Although activation of PPARα is a widely cited mechanism of liver toxicity induced by PFAS exposure, PFOA has been shown to activate a number of other nuclear receptors, including PPARγ, CAR/PXR, Erα, and HNF4α. Many of these nuclear receptors, including CAR and PPARγ, are also known to play an important role in liver homeostasis and have been implicated in liver dysfunction, including steatosis (Armstrong and Guo, 2019). Therefore, there is accumulating evidence that PFOA exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans. However, the contribution of gene expression changes induced and associated toxicity by these other receptors is not clear. Also, it is possible that other receptors may play compensatory roles in PPARα null mice. In addition, PFOA-mediated changes in hepatic gene expression and toxicity exhibit strain, sex, and species specificity. Thus, the interplay between nuclear receptor activation and host factors may dictate the nature and severity of liver toxicity in response to PFOA exposure.

3.4.1.3.2 Lipid Metabolism, Transport, and Storage

3.4.1.3.2.1 Introduction

The liver is the prime driver of lipid metabolism, transport, and storage within an organism. It is responsible for the absorption, packaging, and secretion of lipids and lipoproteins. Lipids are absorbed from digestion through biliary synthesis and secretion, where they are converted to fatty acids (Trefts et al., 2017). These fatty acids are then transported into hepatocytes, cells that make up roughly 80% of the liver mass, via a variety of transport proteins such as CD36, FATP2, and FATP5 (Lehner and Quiroga, 2016). Fatty acids can be converted to triglycerides, which can be packaged with high or very-low-density lipoproteins (HDL or VLDL) for secretion. Lipid handling for the liver is important for energy metabolism (e.g., fatty acid β-oxidation) in other organs and for the absorption of lipid-soluble vitamins (Huang et al., 2011). De novo cholesterol synthesis is another vital function of the liver. Cholesterol is important for the assembly and maintenance of plasma membranes. Dysregulation of any of these functions of the liver can have implications for metabolic and homeostatic processes within the liver itself and other organs, and can contribute to the development of diseases such as nonalcoholic fatty liver disease, steatosis, hepatomegaly, and obesity.

PFOA accumulates in liver tissue, and as such, not only influences lipid levels but can also alter gene expression for a variety of pathways involved in biological processes (U.S. EPA, 2016c). PFAS have been shown to induce steatosis and increase hepatic triglyceride levels in rodents via inducing changes in genes directly involved with fatty acid and triglyceride synthesis that may have variable effects on serum triglyceride levels depending on species, sex, and exposure conditions (Li et al., 2019b; Liang et al., 2019; Das et al., 2017; Rosen et al., 2017; Beggs et al., 2016; Rosen et al., 2013). These include genes such as fatty acid binding protein 1 (Fabp1), sterol regulatory element-binding protein 1 (Srebp1), VLDL receptor (Vldlr), and lipoprotein lipase (Lpl1) (Armstrong and Guo, 2019). Various studies have also shown that PFOA alters expression of genes directly involved in cholesterol biosynthesis (Li et al., 2019b; Pouwer et al., 2019; Das et al., 2017; Rosen et al., 2017) and in β-oxidation of fatty acids (e.g., Acox1 and/or carnitine palmitoyltransferase 1A (Cpt1a)) (Lee et al., 2020; Schlezinger et al., 2020; Li et al., 2019b; NTP, 2019; Cavallini et al., 2017; Rosen et al., 2013). Genes involved in lipid metabolism and homeostasis can be altered through PPARα, PPARγ, CAR, and HNF4α induction pathways and are dose-, lifestage-, species-, and sometimes sex-dependent.

3.4.1.3.2.2 In Vivo Models

3.4.1.3.2.2.1 Rats

Two studies conducted in Sprague-Dawley rats reported marked effects on lipid metabolism, including sex-dependent effects, of PFOA on hepatic outcomes (NTP, 2019; Cavallini et al., 2017).

The study conducted by NTP in 2019 (NTP, 2019) used an oral dosing paradigm of 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg (males) or 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day (females) for 28 days. Males exhibited higher plasma levels of PFOA despite receiving a 10-fold lower dose across the dose groups.

Serum cholesterol levels were decreased in PFOA-exposed males and females, whereas serum triglyceride levels were decreased in males but increased in females. In liver, PPARα- and CAR-

induced genes including Acox1, Cyp4a1, Cyp2b1, and Cyp2b2 were upregulated in both males and females compared with controls. In females, the CAR-induced Cyp2b1 and Cyp2b2 exhibited a greater increase than that of Acox1 and Cyp4a1, whereas Cyp4a1 and Cyp2b1 exhibited the greatest fold increase in males. Acox1 was more strongly upregulated in males than females. This gene expression profile indicates a stronger PPARα signal in males relative to females, and stronger CAR activation signal in females. Bile acid concentrations were increased at the two highest dose groups (5 and 10 mg/kg/day) in males, but were not measured in females.

PFOA is known to activate PPAR receptors and proliferation of peroxisomes, and increase expression of acyl-CoA oxidase (ACOX) activity, the first enzyme in the fatty acid beta-oxidation pathway. In one study, a single dose of PFOA (150 mg/kg) in male Sprague-Dawley 2-month-old rats caused increased liver weight associated with an eightfold and a 15-fold increase in ACOX after 2 and 4 days, respectively (Cavallini et al., 2017). PFOA exposure was associated with generation of new, ACOX rich peroxisomes. Autophagy was induced in fasted rats by an injection of an antilipolytic agent (3,5-dimethyl pyrazole (DMP)). In PFOA-treated rats, DMP-induced autophagy delayed the decrease in ACOX activity relative to controls. The authors hypothesized that autophagy may preferentially target older peroxisomes for degradation. However, another possibility not considered by the authors is that PFOA could disrupt drug-induced autophagy, which may represent an interesting area for further research.

3.4.1.3.2.2.2 Mice

Several studies were conducted to investigate the effects of PFOA on lipid accumulation in hepatocytes by histopathological and metabolomic methods using mice of different genetic backgrounds and lifestages, and mice genetically modified to mimic human lipid metabolism (Pouwer et al., 2019; Hui et al., 2017; Rebholz et al., 2016; van Esterik et al., 2015; Wang et al., 2013). Other studies focused on the transcription and translation of genes involved in lipid metabolism and biliary pathways. The focus of these studies was to identify key genes, gene products, and transcriptional regulators affected by PFOA exposure and to examine how PFOA alters metabolism of lipids (Zhang et al., 2020c; Li et al., 2019b; Wu et al., 2018; Das et al., 2017; Rosen et al., 2017; Beggs et al., 2016; Song et al., 2016; Yu et al., 2016; Yan et al., 2015a).

3.4.1.3.2.2.2.1 Changes in Hepatic Lipid Homeostasis

Many biochemical changes occurred with lipids and bile within the liver as well as lipid transport out of the liver (serum/plasma values). In several mouse studies, PFOA increased hepatic lipid levels including triglycerides, total cholesterol, and LDL, which correlated with histopathological changes that are often consistent with steatosis.

In Das et al. (2017), WT male SV129 mice administered 10 mg/kg/day PFOA for 7 days had increased lipid accumulation in liver, as seen by Oil Red O staining, as well as increased liver triglyceride levels. These effects were mainly attributed to activation of PPARα, as they were attenuated in PFOA-exposed PPARα null mice (Section 3.4.1.2). In contrast, in male BALB/c mice administered 0.08, 0.31, 1.25, 5, or 20 mg/kg/day PFOA for 28 days, liver cholesterol was significantly decreased at 0.31 mg/kg/day and above, while triglycerides were significantly decreased at 0.08 and 20 mg/kg/day and significantly increased at 1.25 mg/kg/day (no changes were seen at other concentrations) (Yan et al., 2015a). An increase in the transcriptional activity of PPARα and sterol regulatory element-binding proteins (SREBPs) was also observed. The

authors hypothesize that altered lipid metabolism is induced by PPARα activation, with increased SREBP activity as a mediator in this pathway.

One study evaluated PFOA effects on storage in hepatic lipid droplets (LDs) in BALB/c mice (Wang et al., 2013). LDs are storage structures for neutral lipids that form in the endoplasmic reticulum and release into the cytoplasm. In addition to lipid storage, they influence lipid metabolism, signal transduction, intracellular lipid trafficking, and protein degradation. Four-week-old BALB/c mice fed either regular or HFD were dosed with 5, 10, or 20 mg/kg/day PFOA by gavage for 14 days. Cytoplasmic LDs were apparent in both regular- and HFD-fed mice, though more were observed in HFD-fed mice. However, in PFOA-exposed mice, LDs transferred from the cytoplasm to the nucleus, forming hepatocyte intranuclear inclusions in a dose-dependent manner. The authors suggest that this translocation of LDs to the nucleus is a critical factor in PFOA-mediated liver toxicity. As discussed below (Section 3.4.1.3.2.2.2.2), at least two genes involved in lipid droplet formation, PLIN2 and PLIN4, were increased in PFOA-exposed HepaRG cells in vitro, supporting a role for PFOA in altering lipid droplets in hepatocytes (Louisse et al., 2020).

A targeted metabolomics approach was used to directly identify alterations in 278 metabolites in livers of BALB/c mice exposed to either 0.5 or 2.5 mg/kg/day PFOA for 28 days by gavage (Yu et al., 2016). A total of 274 of these metabolites were identified in liver and were mapped to KEGG metabolic pathways including amino acid, lipid, carbohydrate, and energy metabolism. In liver, nine metabolites mapped to lipid metabolism as evidenced by alterations in the relative concentrations of acylcarnitines, sphingomyelins, phosphatidylcholines, and oxidized polyunsaturated fatty acids. Among the 18 liver metabolites that were significantly different between exposed and control mice were six acylcarnitines, one phosphatidylcholine, and two polyunsaturated fatty acids, which could serve as potential biomarkers of PFOA exposure. The altered lipid profiles are consistent with the finding that PFOA upregulates hepatic nuclear receptors and their target genes directly involved in lipid metabolism and the β-oxidation of fatty acids (Lee et al., 2020). The profile of both phosphatidylcholine and fatty acid metabolites indicated a PFOA-mediated shift to phosphatidylcholines with more carbons and more double bonds. Because a change to fatty acids with more carbon atoms and double bonds is due to biosynthesis reactions of saturated and unsaturated fatty acids, these findings suggest PFOA exposure may stimulate fatty acid biosynthesis, which may account for the altered profile of both phosphatidylcholines and fatty acids in liver. Thus, PFOA may regulate both catabolic and anabolic lipid metabolism in liver.

3.4.1.3.2.2.2.2 Gene Expression and Metabolite Accumulation Impacting Lipid Homeostasis

Several studies probed the genes and pathways by which PFOA alters hepatic lipid homeostasis. Hui et al. (Hui et al., 2017) demonstrated that the expression of genes and proteins associated with lipid storage in was altered in the liver of PFOA-exposed BALB/c mice. Male mice were exposed to 1 or 5 mg/kg/day for 7 days and the expression of lipid metabolism genes was analyzed. Triglyceride and free fatty acid contents in serum were reduced, while hepatic triglyceride levels were increased in the PFOA-exposed mice compared with controls. In liver, transcript levels of hepatic lipoprotein lipase (Lpl) and fatty acid translocase (Cd36) were elevated, while apolipoprotein-B100 (ApoB) expression was diminished. LPL and CD36 regulate lipid intake through lipid hydrolysis and transport of lipids from blood to liver, whereas APOB is required for lipid export from liver. Protein levels aligned with the changes in transcript

levels for these genes. The authors suggest that dysregulation of lipid metabolism and, specifically, fatty acid trafficking, leads to decreased body weights and lipid malnutrition and deposition of lipids in liver. These findings are consistent with observations in male Kunming mice exposed to 5 mg/kg/day PFOA for 21 days (Wu et al., 2018). In these mice, PFOA exposure led to reduced APOB and elevated CD36 protein levels as measured immunohistochemically and correlated to increased liver triglyceride levels. In addition to genes directly involved in regulating lipid metabolism and storage, Eldasher et al. (2013) demonstrated that Bcrp mRNA and protein are increased in the livers, but not the kidneys of male C57BL/6 mice exposed to 1 or 3 mg/kg/day PFOA by gavage for 7 days. BCRP is an ATP-binding cassette efflux transporter protein involved in active transport of various nutrients and drugs and implicated in transport of xenobiotics. In addition, BCRP can function sterol transport and its ATPase activity can be stimulated with cholesterol (Neumann et al., 2017). Further studies are needed to elucidate the role of BCRP or other transport proteins in PFOA-mediated disruption of lipid metabolism.

MicroRNAs (miRNAs or miRs) are also altered after exposure to PFOA in mice in a dose-dependent manner. In serum of male BALB/c mice, 24 and 73 circulating miRNAs were altered in mice exposed to 1.25 and 5 mg/kg/day PFOA, respectively, for 28 days (Yan et al., 2014). Changes in expression of six miRNAs (miR-28-5p, miR-32-5p, miR-34a-5p, miR-200c-3p, miR-122-5p, miR-192-5p) were confirmed in liver, including two (miR-122-5p and miR-192-5p) considered to be biomarkers for drug-induced liver injury. MiRNAs may play a specific role in regulating expression of genes involved in lipid metabolism and storage.

Cui et al. (2019) observed that PFOA exposure (5 mg/kg/day PFOA for 28 day) led to a significant increase of miR-34a, but not miR-34b or miR-34c, in the livers of male BALB/c mice, consistent with the findings of Yan et al. (Yan et al., 2014).

Liver toxicity was evaluated by Cui et al. (2019) by measuring liver weight, elevated liver enzymes, and hepatic cell swelling manifested in both WT mice and in miR-34a-null mice generated on a C57BL/6J background. RNA-Seq analysis of hepatic tissue showed that expression of lipid metabolism genes was significantly altered in both WT mice and in miR-34a-null mice after PFOA exposure; however, fewer genes were altered in livers of miR-34a-null mice. Metabolism genes dominated those changed by miR-34a, including Fabp3, Cyp7a1, and Apoa4. On the basis of the transcriptome analysis, the authors found that miR-34a mainly exerts a metabolic regulation role, rather than the pro-apoptosis and cell cycle arrest role reported previously in vitro.

In addition to perturbed expression of genes as a consequence of activating PPARα and other nuclear receptors, PFOA may directly target enzymes involved in fatty acid metabolism. Shao et al. (2018) postulated that based on the electrophilic properties of PFOA, it may preferentially bind to proteins harboring reactive cysteine residues. To test this hypothesis, proteomic and metabolomic approaches were applied. Two cysteine-targeting probes were used to enrich putative target proteins in mouse liver extracts in the absence or presence of PFOA, resulting in the identification of ACACA and ACACB as novel target proteins of PFOA. Parallel reaction monitoring (PRM)-based targeted proteomics combined with thermal shift assay-based chemical proteomics was used to verify ACACA and ACACB as PFOA binding targets. Next, the authors used a metabolomic approach to analyze liver extracts from female C57BL/6 mice four hours after IP injection with a very high dose (300 mg/kg) of PFOA to confirm abnormal fatty acid

metabolism, including significantly elevated levels of carnitine and acyl-carnitines. ACACA and ACACB are acetyl-CoA carboxylases that can regulate fatty acid biosynthesis. The authors suggest PFOA interactions with these carboxylases leads to a downregulation of malonyl-CoA, required for the rate-limiting step of fatty acid biosynthesis and an inhibitor of carnitine palmitoyl transferase 1 (Cpt1). Despite the correlation to altered fatty acid profiles, additional studies are required to confirm PFOA binding to these lipid enzyme targets and changes in hepatic fatty acid metabolism.

3.4.1.3.2.2.2.3 Host Factors Influencing Lipid Metabolism and Storage

Rebholz et al. (2016) underscored the relevance of genetic background, sex, and diet in PFOA-mediated alterations of hepatic gene expression and highlighted the role of genes involved in sterol metabolism and bile acid production Young, sexually immature male and female C57BL/6 and BALB/c mice were placed on diets to target a dose of approximately 0.56 mg/kg/day of PFOA and supplemented with 0.25% cholesterol and 32% fat. Hypercholesterolemia developed in male and female C57BL/6 mice exposed to PFOA. Hypercholesterolemia was also observed in male BALB/c mice but to a lesser degree than C57BL/6, and did not manifest in female BALB/c mice. The PFOA-induced hypercholesterolemia appeared to be the result of increased liver masses and altered expression of genes associated with hepatic sterol output, specifically bile acid production. These data support genetic background and dietary levels of fat and cholesterol as important variables influencing PFOA-mediated changes in cholesterol. However, an important caveat in this study is that female mice in the control groups for both strains had higher than expected blood PFOA levels.

PFOA-mediated changes in lipid levels may be programmed during early life exposure. C57BL/6JxFVB hybrid mice were exposed during gestation and lactation via maternal feed (van Esterik et al., 2015) to seven doses of PFOA targeting 0.003–3 mg/kg/day. The dose range was chosen to be at or below the NOAEL used for current toxicological assessment. Liver morphology and serum lipids were analyzed at in the pups at 26 weeks (males) and 28 weeks (females) of age. Histopathological changes, including microvesicular steatosis and nuclear dysmorphology, were more frequent in PFOA-exposed mice compared with controls, though the incidence did not reach statistical significance over the dose range. However, perinatal exposure induced a sex-dependent change in lipid levels. In females only, serum cholesterol and triglycerides showed a dose-dependent decrease with a maximum change of −20% for cholesterol and −27% for triglycerides (BMDLs of 0.402 and 0.0062 mg/kg/day, respectively). The authors suggest that perinatal exposure to PFOA in mice alters metabolic programming in adulthood. On the basis of the sexually dimorphic lipid levels, as well as on extrahepatic changes, females appear more sensitive to PFOA-mediated alterations in metabolic programming.

The potential developmental effects of PFOA in liver are also of interest considering recent findings that PFOA regulates expression of homeobox genes involved in both development and carcinogenesis (Zhang et al., 2020c). Adult male C57BL/6 mice, PPARα-null mice, or CAR-null mice were given a single IP administration of 41.4 mg/kg and livers were collected on Day 5. PFOA induced mRNA expression of Hoxa5, b7, c5, d10, Pdx1 and Zeb2 in wild-type mice in a manner dependent on PPARα and CAR. Whether exposure to PFOA alters homeobox genes during perinatal exposure, and the potential for homeobox proteins to alter PFOA susceptibility in different lifestages remains to be determined.

One difference between human and rodent lipid metabolism relates to transfer of cholesterol ester from HDL to the APOB-containing lipoproteins in exchange for triglycerides. Mice lack cholesteryl ester transfer protein (CETP) and rapidly clear APOB-containing lipoproteins. In contrast, a higher proportion of HDL relative to LDL is observed in humans and primates due to the function of CETP. APOE*3-Leiden.CETP transgenic mice, a strain that expresses human CETP, exhibit a more human-like lipoprotein metabolism with transfer of cholesterol ester from HDL to the APOB-containing lipoproteins in exchange for triglycerides resulting in delayed APOB clearance. Pouwer et al. (2019) utilized these transgenic mice to evaluate the effect of PFOA on plasma cholesterol and the mechanism for the hypolipidemic responses observed with PFOA exposures. APOE*3-Leiden.CETP mice were fed a Western-type diet (0.25% cholesterol (wt/wt), 1% corn oil (wt/wt), and 14% bovine fat (wt/wt)) with PFOA (0.01, 0.3, or 30 mg/kg/day) for 4–6 weeks. The doses were chosen to parallel environmental and occupational exposures in humans. PFOA exposure did not alter plasma lipids at lower doses, but did decrease plasma triglycerides, total cholesterol, and non-HDL levels, and increased HDL levels. Overall, these findings mirrored a clinical trial in humans demonstrating PFOA-induced decreases in cholesterol levels. This lipid profile could be attributed to decreased very low-density lipoprotein (VLDL) production and increased VLDL clearance by the liver through increased lipoprotein lipase activity. The concomitant increase in HDL was attributed to decreased CETP activity subsequent to PPARα activation and the downregulation of hepatic genes involved in lipid metabolism, including Apoa1, Scarb1, and Lipc (genes involved in HDL formation, HDL clearance, and HDL remodeling, respectively). On the basis of the lipid profiles, gene expression analysis, and pathway analysis, the authors propose a mechanistic model in which high PFOA exposure increases VLDL clearance by the liver through increased LPL-mediated lipolytic activity. These changes lead to lower VLDL serum levels consistent with reduced VLDL particle formation and secretion from the liver due to reduced ApoB transcript levels and de novo synthesis.

To further explore mechanistic differences in PFOA-induced changes in lipid metabolism between humans and mice, Schlezinger et al. (2020) investigated PFOA-mediated lipid dysregulation in mice expressing human PPARα (hPPARα) and compared results to PPARα-null mice. Male and female mice were fed an American style diet (51.8% carbohydrate, 33.5% fat, and 14.7% protein, based on an analysis of what 2-to-19-year-old children and adolescents eat using NHANES data1) and exposed to PFOA (8 μM) in drinking water for 6 weeks that led to serum PFOA levels of 48 μg/mL. Both hPPARα-null and PPARα-null mice developed hepatosteatosis after PFOA exposure. Changes in gene expression and increased serum cholesterol that was more pronounced in males than females correlated with changes in expression of genes that regulate cholesterol homeostasis. PFOA decreased expression of Hmgcr in a PPARα-dependent manner. Ldlr and Cyp7a1 were also decreased but in a PPARα-independent manner. Apob expression was not changed. While many of the target genes analyzed were similarly regulated in both sexes, some sex-specific changes were observed. PFOA induced PPARα target genes in livers of both sexes including Acox1 (involved in fatty acid β-oxidation), Adrp (involved in coating lipid droplets), and Mogat1 (involved in diacylglyerol biosynthesis). PPARγ target genes were also upregulated in both sexes and included Fabp4 and Cd36 that contribute to lipid storage and transport as was the CAR target gene Cyp2b10. PFOA exposure decreased expression of Cyp7a1 required for conversion of cholesterol to bile acids and efflux, but more so in females than in males.

Sex-specific changes in hepatic gene expression in response to PFOA exposure was also observed in zebrafish (Hagenaars et al., 2013). Adult zebrafish were exposed to 0.1, 0.5, or 1 mg/L PFOA for 28 days. Livers were harvested and subjected to transcriptomic analysis. Similar to observations in mice, expression of genes regulating fatty acid metabolism and cholesterol metabolism and transport were generally upregulated in males and suppressed in females. Thus, sex-specific effects of PFOA on fatty acid and cholesterol metabolism is observed across different vertebrate species, but also exhibits species specificity. For example, genes in the cytochrome P450 family involved in cholesterol metabolism and transport were suppressed in female zebrafish but upregulated in male zebrafish (Hagenaars et al., 2013). However, Cyp2b genes downstream of CAR (e.g., Cyp2b1 and Cyb2b10) were more strongly upregulated in females compared with males in both rats and mice (Schlezinger et al., 2020; NTP, 2019). Differences in expression of Cyp450 genes may in part relate to species-specific activity of nuclear receptors, and the fact that no CAR orthologues have been identified in zebrafish nor any other fish species (Schaaf, 2017).

3.4.1.3.2.2.3 In Vitro Studies

In vitro studies reported genetic profiles and pathway analyses in mouse and human hepatocytes to determine the effect of PFOA treatment on lipid homeostasis and bile synthesis. Six studies investigated the effect of PFOA on lipid homeostasis using primary hepatocytes and human cell lines such as HepG2, HepaRG, and HL-7702 cells. Various endpoints were also investigated in these cell lines such as mRNA expression through microarray and qRT-PCR assays; lipid, triglyceride, cholesterol, and choline content; and protein levels via ELISA or western blot. In addition, two studies evaluated PFOA-mediated changes to lipids using metabolomic approaches.

Franco et al. (2020a) exposed HepaRG cells to PFOA and PFOS and evaluated metabolomics at a dose range of 100 pM to 1 μM. The highest PFOA exposure levels (10–100 μM) were associated with significant increases in total lipid concentrations, especially at the three highest concentrations tested (10, 100, and 1,000 nM). Interestingly, hepatocyte lipids were decreased in response to increasing PFOS exposure in this system. The affected classes of lipids also diverged, with PFOA associated with increased diglycerides, triglycerides, and phosphatidylcholines, whereas PFOS was associated with decreased diglycerides, ceramides, and lysophosphatidylcholines. Staining of neutral lipids was also prominent in PFOA-treated hepatocytes, suggesting an obesogenic role PFOA that may directly impact hepatic steatosis. The authors further hypothesized that the concentration-dependent decrease in lipid accumulation associated with PFOS may be related to differential ability of these compounds to interact with PPARs, including PPARγ.

Peng et al. (2013) evaluated disturbances of lipids in the human liver cell line L-02 using metabolomic and transcriptomic approaches. Specifically, PFOA exposure was associated with altered mitochondrial metabolism of carnitine to acylcarnitines. The effect was dose-dependent and correlated with altered expression levels of key genes involved in this pathway. Downstream of this pathway, cholesterol biosynthesis was upregulated as measured by both increased cholesterol content and elevated expression levels of key genes. The profile of PFOA-associated disturbance in lipid metabolism was consistent with initial changes in fatty acid catabolism in cytosol that altered mitochondrial carnitine metabolism, ultimately impacting cholesterol biosynthesis.

In contrast to the findings of Peng et al. (2013) in L-02 cells, Das et al. (2017) reported that PFOA did not inhibit palmitate-supported respiration (mitochondrial metabolism) in HepaRG cells. There was no effect on oxidation or translocation of palmitoylcarnitine, an ester involved the in metabolism of fatty acids, as part of the tricarboxylic acid (TCA) cycle in the mitochondrial fraction. This may indicate less of a perturbation to fatty acid metabolism in this cell line. This suggests that intermediary steps in fatty acid activation, transport, and/or oxidation are affected. The authors suggest that PFOA effects on mitochondrial synthesis of fatty acid and other lipids are secondary and possibly compensatory to any mitochondrial-induced toxicity, rather than as the result of activation of peroxisomes, which are mediated by PPARs.

Rosen et al. (2013) exposed mouse and human primary hepatocytes to 0–100 or 0–200 μM PFOA, respectively. Gene expression was evaluated using microarrays and qRT-PCR. For PFOA-exposed murine hepatocytes, a much smaller group of genes was found to be altered compared with the whole liver. These genes included those associated with β-oxidation and fatty acid synthesis such as Ehhadh and Fabp1, which are upregulated by PFOA. In contrast to the transcriptome of primary mouse hepatocytes, a large group of genes related to lipid metabolism was differentially expressed in primary human hepatocytes including perilipin 2 (PLIN2) and CYPTA1, which were upregulated at 100 μM PFOA. The authors attribute some of these differences between mouse and human hepatocytes to a less robust activation of PPARα in humans. Further, many of the genes investigated were chosen to explore effects of PFOS exposure that are independent of PPARα activation but may include other nuclear receptors such as CAR, LXR, PXR, and AhR (Section 3.4.1.3.1). Beggs et al. (2016) exposed human primary hepatocytes to 0.01–10 μM PFOA for 48 or 96 hours to determine pathways affected by PFOA exposure. PFOA treatment altered 40 genes (20 upregulated and 20 downregulated). Upregulated genes were primarily associated with lipid metabolism, hepatic steatosis and cholestasis, and liver hyperplasia. Among the top 10 upregulated genes were PLIN2, CYP4A22, and apolipoprotein A4 (APOA4).

Differential regulation of lipid metabolism and storage genes was also observed in HepG2 cells exposed to PFOA (dose range of 20–200 μM) for 48 hours (Wen et al., 2020). Some specific metabolic pathway genes were not altered, including genes encoding the acyl-CoA dehydrogenase enzyme. FABP1, which encodes for a key protein responsible for fatty acid uptake, transport, and metabolism, exhibited decreased expression. Acyl-CoA oxidase 2 (ACOX2), which is involved in the peroxisome-mediated degradation of fatty acids, was also decreased. In contrast, a number of genes involved in fatty acid anabolism were upregulated. The authors linked PFOA-mediated gene expression changes to diminished global methylation, implicating epigenetic factors in PFOA-mediated changes in gene expression.

In human hepatic cell lines such as HepaRG, PFOA treatment led to downregulation of genes involved in cholesterol homeostasis. Louisse et al. (2020) noted a concentration-dependent increase in triglycerides, a decrease of cholesterol at a high dose, and a downregulation of cholesterogenic genes especially after 24 hours of exposure to the high dose of 200 μM PFOA in HepaRG cells. Cellular cholesterol biosynthesis genes are regulated by SREBPs, which were also downregulated with PFOA exposure. In contrast, PPARα-responsive genes were upregulated with PFOA exposure, particularly at higher doses. Behr et al. (2020a) also exposed HepaRG cells to 0–500 μM PFOA for 24 or 48 hours. Similar to the results from Louisse et al. (2020), at 24 hours, genes related to cholesterol synthesis and transport were downregulated at

the highest dose except for several genes that were upregulated, including bile and cholesterol efflux transporters (SLC51B and ABCG1), and genes involved in bile acid and bilirubin detoxification (CYP3A4, UGT1A1). The gene profiles after 48 hours of exposure were similar, except at the high dose, at which there was an attenuation of the response in cholesterol synthesis and transport. Cholesterol content was significantly higher in the supernatant at the highest dose of 500 μM but there was no significant difference after 48 hours between treated cells and controls, which aligns with the attenuation of gene expression changes. Both studies also observed a PFOA-associated decrease in CYP7A1, a key enzyme involved in the initial step of cholesterol catabolism and bile acid synthesis.

3.4.1.3.2.2.4 Conclusions

Despite some inconsistencies in the literature, an emerging picture of PFOA-related dyslipidemia is largely initiated by activation of nuclear receptors targeted by PFOA, primarily PPARα, PPARγ, and CAR. A primary consequence of this interaction is altered expression of genes regulating hepatic lipid homeostasis. Gene expression profiles of lipid metabolism genes were observed both in vivo and in vitro, and in a diverse set of study designs. While changes in gene expression were consistently observed, the magnitude of the changes varied according to dose, dose duration, and model system. PPARα appears to be the primary driver regulating gene expression. However, studies in PPARα-null mice and analysis of nuclear receptor-specific genes implicate PPARγ, CAR, and possibly PPARδ as important contributors to the changes in PFOA-mediated gene expression. It should be noted, however, that a thorough analysis of potential compensatory changes in gene knockout mice was not discussed in the literature reviewed here.

Two of the primary pathways targeted by PFOA-induced changes in gene expression include metabolism of fatty acids leading to triglyceride synthesis and metabolism of cholesterol and bile acids. In both mice and rats, gene expression changes generally correlated with increased triglyceride levels in liver, and decreased levels of circulating serum triglycerides. For cholesterol, in vitro studies were conflicting but suggest hepatic cholesterol content generally increases in PFOA-exposed animals. However, serum cholesterol levels were reduced in rats but were generally elevated in mice. Hepatic changes in lipid-regulating gene expression appear to influence circulating levels of lipids in serum in a manner that varies by sex, species, and lifestage. For example, adult male rats exhibited decreases in serum triglycerides, whereas adult female rats exhibited increases (NTP, 2019). However, in mice exposed perinatally and then examined in adulthood, females, but not males, exhibited decreased serum levels of triglycerides, a treatment effect that was not observed in males (van Esterik et al., 2015). Male Kunming mice also exhibited a dose-dependent decrease in serum triglycerides and an increase in liver triglycerides (Wu et al., 2018). For cholesterol, serum levels were decreased in PFOA-exposed male rats and increased in female rats (NTP, 2019). In contrast, young male and female C57BL/6 mice exhibited hypercholesterolemia after PFOA exposure, though this was less striking male among BALB/c mice and did not manifest in female BALB/c mice (Rebholz et al., 2016). Elevated serum cholesterol was also more pronounced in males than females in mice expressing human PPARα (Schlezinger et al., 2020).

Importantly, changes in gene expression and lipid content in liver ultimately manifest in altered hepatocyte morphology. Most strikingly and consistently, steatosis manifests in PFOA-exposed animals. Other pathogenetic changes associated with PFOA included hepatomegaly, cholestasis,

hyperplasia, and carcinoma. The finding of steatosis is interesting in light of observation that PFOA exposure downregulates expression of HNF4α in liver with concomitant changes in HNF4α target genes because HNF4α-deficient mice develop steatosis in the absence of exposure to toxicants.

While the precise events that lead to steatosis have yet to be elucidated, the current studies conducted in animals and in vitro studies supports the following key molecular and cellular events related to PFOA-mediated hepatoxicity specific to changes in lipid metabolism: (1) PFOA accumulation in liver activates nuclear receptors; (2) nuclear receptors, including PPARα, then alter expression of genes involved in lipid homeostasis and metabolism; (3) the products of the genes altered by activated nuclear receptors modify the lipid content of liver to favor triglyceride accumulation, and possibly also cholesterol accumulation; (4) altered lipid content in liver leads to accumulation of lipid droplets promoting development of steatosis and other changes leading to liver dysfunction; and (5) alterations in lipid metabolism leads to alterations in serum levels of triglycerides and cholesterol. An intriguing possibility that may be concurrent to these events is direct binding of PFOA to ACACA and ACACB enzymes in a manner that interferes with fatty acid biosynthesis. Although this series of events is plausible, significant gaps remain in understanding this process, including how these events interface with other cellular processes such as cell growth and survival, oxidative stress, and others in understanding the mechanisms of PFOA-mediated hepatoxicity.

There are challenges in the extrapolation of results from research related to PFOA-mediated changes to lipid metabolism in animals to humans. As presented in the 2016 PFOA HESD (U.S. EPA, 2016c), serum lipid levels were variably altered in humans exposed to PFOA in their environments. In occupationally exposed humans and humans exposed to high levels of PFOA, there was a general association with increased serum total cholesterol and LDL, but not HDL. At least one obstacle to extrapolating from rodent to humans is that the cholesteryl ester transfer protein encoded by the CETP gene in humans is absent in rodents. Mice lack CETP and rapidly clear apoB-containing lipoproteins. In contrast, a higher proportion of HDL relative to LDL is observed in humans and primates due to the function of CETP. New models designed to develop mice that are “humanized” for lipid metabolism, including APOE*3-Leiden.CETP (Pouwer et al., 2019), and mice expressing human nuclear receptors (Schlezinger et al., 2020), are likely to accelerate the extrapolation of mechanistic information from animals to humans.

3.4.1.3.3 Hormone Function and Response

While much of the literature relevant to hormone function and response is focused on reproductive or endocrine outcomes (see Appendix, (U.S. EPA, 2024a)), recent literature has also shown a relationship between hepatic hormonal effects and PFOA exposure. PFOA has been found to affect thyroid mechanisms in hepatic cells. Huang et al. (2013) studied the effect of 5, 10, 25, or 50 mg/L PFOA in a human nontumor hepatic cell line (L-02 cells) and found that PFOA exposure downregulated thyroid hormone binding protein precursor.

While there are a small number of studies regarding hormone function and response specifically within the liver, there is evidence that PFOA has the potential to perturb hormonal balance in hepatic cells, particularly regarding thyroid function. This could have implications for hormone function and responses in other organ systems and may also be important for MOA considerations for hepatotoxicity.

3.4.1.3.4 Xenobiotic Metabolism

Xenobiotic metabolism is the detoxification and elimination of endogenous and exogenous chemicals via enzymes (i.e., cytochrome P450 (CYP) enzymes) and transporters (i.e., organic anion transporting peptides [OATPs]) (Lee et al., 2011). As described in Section 3.3.1.3, the available evidence demonstrates that PFOA is not metabolized in humans or other species. However, several studies have investigated how PFOA could alter xenobiotic metabolism in the liver by downregulating or upregulating the gene expression of enzymes and transporters.

Li et al. (2017a) summarized the literature on molecular mechanisms of PFOA-induced toxicity in animals and humans. The authors noted how Elcombe et al. (2007) and Guruge et al. (2006) reported PFOA activation of PXR/CAR and subsequent manipulation of the expression of genes responsible for xenobiotic metabolism (Li et al., 2017a). For instance, Cheng and Klaassen (Cheng and Klaassen, 2008b) concluded that PFOA induced the gene expression of CYP2B10 in mice.

Overall, results from both in vivo and in vitro model systems suggest that genes responsible for xenobiotic metabolism are upregulated as a result of PFOA exposure.

3.4.1.3.4.1 In Vivo Models

Three studies investigated xenobiotic metabolism endpoints in in vivo models with two using mice (Li et al., 2019c; Wen et al., 2019c) and one using zebrafish (Jantzen et al., 2016b).

Li et al. (2019c) examined 5–6-week-old male C57BL/6 mice administered PFOA (1 mg/kg/day) via oral gavage for 2, 8, or 16 weeks. CYP2B and CYP3A activity were assessed via PROD and BQ assays as an indicator of CAR/PXR activity in the liver. As discussed in Section 3.4.1.3.1, the authors reported upregulation of Cyp2b and Cyp3a gene expression with downstream effects to CAR/PXR activation and xenobiotic metabolism. Similarly, Wen et al. (2019c) investigated CYP gene expression (including Cyp1a1, Cyp2b10, and Cyp3a11) with a focus on the activation of the nuclear receptor PPARα and downstream alteration of metabolism and excretion of xenobiotics. Adult, male wild-type C57BL/6NTac and PPARα-null mice were administered PFOA (3 mg/kg/day) for 7 days (Wen et al., 2019c). Expression of a targeted list of genes, including Cyp1a1, Cyp2b10, and Cyp3a11, was quantified by qRT-PCR. In PFOA-treated wild-type mice, gene expression of Cyp1a1 and Cyp3a11 were not significantly changed. Conversely, in PFOA-treated PPARα-null mice, gene expression of Cyp2b10 and Cyp3a11 were significantly altered compared with the wild-type mice (11-fold increase for Cyp2b10 and 1.7-fold increase for Cyp3a11). Authors noted the differences between wild-type and PPARα mice were consistent with a previous study (Corton et al., 2014).

One study examined the expression of four genes related to xenobiotic metabolism in zebrafish (Jantzen et al., 2016b). Zebrafish embryos (AB strain) were exposed to 2.0 µM PFOA dissolved in water from 3 to 120 hours post-fertilization (hpf) and evaluated 180 days post-fertilization (dpf) at adult lifestage for gene expression. Females and males both had significant reductions in slco1d1 expression; however, only males had significant reductions in slco2b1 expression (Jantzen et al., 2016b). Jantzen et al. (2016b) noted that in their previous study (Jantzen et al., 2016a), PFOA exposure from 5 to 14 dpf resulted in significantly increased slco2b1 expression. Given the fluctuation in gene expression from short-term to long-term, further studies with additional timepoints are needed to elucidate the effect of PFOA exposure on OATPs expression.

3.4.1.3.4.2 In Vitro Models

CYP2B6 is expressed in the liver and is predominately responsible for xenobiotic metabolism; similar to previous studies, Behr et al. (2020b) investigated activation of nuclear receptors by PFAS. Authors exposed HEK293T cells and HepG2 cells to varying concentrations of PFOA (0, 50, 100, or 250 μM) for 24 hours. As discussed further in Section 3.4.1.3.1, the authors reported the downstream effects of PFOA-mediated PPARα activation. At the highest concentration of 250 μM, Behr et al. (2020b) reported that PFOA significantly induced gene expression of CYP2B6 by 11.2-fold. CYP2B6 gene expression was assessed in an additional study that used primary human and mouse hepatocytes (Rosen et al., 2013). In primary human hepatocytes, PFOA concentrations ranged between 0 and 200 μM; in mouse hepatocytes, concentrations ranged between 0 and 100 μM. Results varied between human and mouse hepatocytes, with CYP2B6 upregulated in human hepatocytes but not in mouse hepatocytes. The authors noted that the differences between gene expression of the human and mouse hepatocytes were unclear; however, cell density, collection methods, and time in culture were possible factors.

Franco et al. (2020b) assessed the expression of genes encoding several phase I and II biotransformation enzymes following exposure to PFOA concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) for 24 or 48 hours. Gene expression of phase I enzymes (CYP1A2, CYP2C19, and CYP3A4) varied across concentrations and between the 24- and 48-hour exposures. For CYP1A2, after 24 hours, expression was significantly upregulated at concentrations $\geq 10^{-9}$ M; however, after 48 hours, expression was significantly downregulated at concentrations $\geq 10^{-8}$ M. CYP2C19 was downregulated across all concentrations after both 24- and 48-hour exposures; downregulation was significant for concentrations after both 24- and 48-hour exposures with the exception of 10^{-8} M after 24-hours. The authors concluded that PFOA exposure can significantly reduce expression of phase I biotransformation enzymes.

Evidence varied across studies for the effect of PFOA on the expression of CYP3A4, a phase I enzyme involved in bile acid metabolism and detoxification by hydroxylation and xenobiotic metabolism, depending on the model and duration of exposure, as well as whether gene expression or enzyme activity was assessed (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; Rosen et al., 2013; Shan et al., 2013). Franco et al. (2020b) reported that after 24-hours, there were not significant changes in CYP3A4 expression. However, after 48 hours, there was a fivefold reduction in the expression. Conversely, Behr et al. (2020a) and Louisse et al. (2020) reported upregulation of CYP3A4 enzyme activity following 24- or 48-hour PFOA exposure in HepaRG cells; specifically, Behr et al. (2020a) reported significant upregulation at 50 and 100 μM after both 24- and 48-hour PFOA exposure.

Rosen et al. (2013) also reported upregulation of CYP3A4 expression following PFOA exposure (0–100 μM) in human hepatocytes; however, significant changes were not reported for mouse hepatocytes. Lastly, Shan et al. (2013) reported no significant changes in CYP3A4 enzyme activity following PFOA exposure (0, 100, 200, 300, or 400 μM) in HepG2 cells.

Franco et al. (2020b) also assessed gene expression of phase II enzymes, glutathione-s-transferase mu1 (GST-M1) and UDP glucuronosyltransferase-1A1 (UGT-1A1), which were not significantly affected by exposure to PFOA after 24 or 48 hours. The authors noted that it was unclear where and how PFOA alters gene expression of phase I enzymes and not phase II enzymes. Further research is needed to determine whether altered gene expression occurs by

interference with cytoplasm receptors, inhibition of nuclear translocation, and/or inhibition of the interaction of nuclear translocator complexes with DNA sequences (Franco et al., 2020b).

Orbach et al. (2018) focused on the gene expression of the CYP2E1 enzyme. PFOA was added to primary human hepatocytes and primary rat hepatocytes at either ½ LC50 or LC50 (500 μM for both humans and rats) for 24 hours. CYP2E1 enzymatic activity was estimated by the conversion of 7-methoxy-4-trifluoromethylcoumarin (MFC) to 7-hydroxytrifluoromethylcoumarin (HFC). However, in both human and rat hepatocytes, there were no significant changes in CYP2E1 activity.

Song et al. (2016) analyzed the expression of over 1,000 genes by expression microarray analysis following exposure of HepG2 cells with increasing concentrations (0–1,000 μM) of PFOA for 48 hours. As a result, 1,973 genes expressed ≥1.5-fold changes in the exposed groups compared with the control group, including 20 genes responsible for metabolism of xenobiotics by cytochrome P450.

3.4.1.3.4.3 Conclusions

Several studies are available that assessed xenobiotic metabolism endpoints as a response to PFOA exposure, including studies in mice (Li et al., 2019c; Wen et al., 2019c), zebrafish (Jantzen et al., 2016b), primary hepatocytes (Orbach et al., 2018; Rosen et al., 2013), or hepatic cell lines (Behr et al., 2020b; Franco et al., 2020b; Louisse et al., 2020; Song et al., 2016; Shan et al., 2013). Jantzen et al. (2016b) reported significant reductions in the expression of OATPs (slco1d1 and slco2b1). While the majority of studies reported altered gene expression of CYP enzymes, the direction and magnitude of change varied across doses and exposure durations. Jantzen et al. (2016b) and Franco et al. (2020b) both noted the need for further research to elucidate any potential relationships between PFOA exposure and xenobiotic metabolism.

3.4.1.3.5 Cell Viability, Growth and Fate

3.4.1.3.5.1 Cytotoxicity

Several in vitro studies have examined the cytotoxic effect of PFOA on cell viability assays in both primary hepatic cell cultures (Xu et al., 2019b; Beggs et al., 2016) and in hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Franco et al., 2020a; Ojo et al., 2020; Wen et al., 2020; Zhang et al., 2020a; Lv et al., 2019; Rosenmai et al., 2018; Sheng et al., 2018; Song et al., 2016; Cui et al., 2015; Wielsøe et al., 2015; Yan et al., 2015a; Hu et al., 2014; Huang et al., 2014; Shan et al., 2013; Florentin et al., 2011), with varying results depending on the exposure concentration and duration, cell line, and culturing methods.

In mouse primary hepatocytes, cell viability as determined by cell counting Kit-8 (CCK-8) assay did not significantly change at concentrations of PFOA in the range of 10–500 μM; however, a 41% decrease in viability was observed after 24 hours of exposure to 1000 μM PFOA (Xu et al., 2019b). In primary rat hepatocytes exposed to PFOA for 24 hours showed no changes in cell viability at concentrations ≤25 μM, but cell viability was increased by approximately 16% in the 100 μM concentration (Liu et al., 2017a).

PFOA exposure duration and concentration affect cytotoxicity. In HepG2 cells, 100 μM PFOA did not affect cell viability after 1–3 hours of exposure (Shan et al., 2013; Florentin et al., 2011). However, after 72 hours, cell viability as determined by neutral red assay was reduced by nearly

80% in the same cell line (Buhrke et al., 2013), suggesting that PFOA cytotoxicity is increased with long-term exposure. Additionally, in human HEPG2 cells treated at different concentrations of PFOA for 24 hours, viability as determined by MTT assay did not change with 100 μM PFOA, but was significantly reduced by 14% at 200 μM, 22% at 400 μM, 47% at 600 μM, and 69% at 800 μM, suggesting a concentration-dependent reduction in cell viability (Florentin et al., 2011). In contrast, cell viability dropped below 80% in HepaRG cells exposed to 100 μM PFOA at 24 hours (Franco et al., 2020b). Another study in HepaRG cells (Louisse et al., 2020) showed no effect on cell viability up to concentrations of 400 μM for 24 hours. Although some results are conflicting, overall, these studies suggest that exposure duration and concentration, type of cell lines, species, and viability assessment methods are determinants of PFOA-induced cytotoxicity.

IC50 values in hepatic cell lines ranged from approximately 42 μM PFOA after 72 hours (Buhrke et al., 2013), 102–145 μM after 24 hours (Franco et al., 2020b; Ojo et al., 2020), to 305 μM after 48 hours of exposure in HepG2 cells (Song et al., 2016). In a fetal liver cell line (HL-7702), IC50 values were 647 μM after 24 hours exposure and 777 μM after 48 hours exposure (Sheng et al., 2018; Hu et al., 2014). One study in zebrafish liver cells reported IC50 values of 84.76 μg/mL after 48 hours exposure (Cui et al., 2015).

3.4.1.3.5.2 Apoptosis

To determine the mechanism underlying PFOA-induced cytotoxicity, several studies have interrogated the apoptosis pathway as a potential mechanism (Li et al., 2017b; Cui et al., 2015; Buhrke et al., 2013). Apoptosis is characterized by biochemical and morphological changes in cells. Flow cytometry has been used to quantify the percentage of apoptotic cells and their phase in cells exposed to PFOA. The percentage of apoptotic cells in the early and late phases of apoptosis nearly doubled in isolated C57BL/6J mice hepatocytes exposed to 500 μM and 1,000 μM PFOA for 24 hours (Xu et al., 2019b). In zebrafish liver cells exposed to the IC50 (84.76 μg/mL) and IC80 (150.97 μg/mL) for 48 hours, the percentage of dead cells in the late phase of apoptosis did not change in cells exposed to the IC50 compared with control, while a significant increase in the percentage of apoptotic cells in the late phase of apoptosis was observed in the cells exposed to the IC80 (Cui et al., 2015).

Activation of cysteine aspartic acid-specific protease (caspase) family is essential for initiation and execution of apoptosis. PFOA-induced apoptosis via caspase activities have been examined in primary mouse hepatocytes, mouse cell lines, and human cell lines after exposure to various PFOA concentrations (Xu et al., 2020b; Sun et al., 2019; Li et al., 2017b; Cui et al., 2015; Buhrke et al., 2013; Huang et al., 2013). In mouse hepatocytes, PFOA induced caspase activity in a dose-dependent manner (Li et al., 2017b). In male C57BL/6J mouse hepatocytes treated with PFOA for 24 hours, caspase 3 activity did not change at doses below 1,000 μM but increased by more than 1,000% at 1,000 μM (Xu et al., 2020b). In a spheroid model of mouse liver cells (AML12), increased activity of caspase 3/7 was detected from 14 to 28 days of ≥100 μM PFOA exposure (Sun et al., 2019). In contrast, 100 μM PFOA did not change caspase 3/7 activity in HepG2 cells exposed for 48 hours (Buhrke et al., 2013).

Another key feature of cells undergoing apoptosis is the release of lactate dehydrogenase (LDH). Many studies have reported intracellular release of LDH in hepatocytes treated with PFOA (Sun et al., 2019; Wielsøe et al., 2015; Yan et al., 2015b; Shan et al., 2013). In male C57BL/6J mouse

primary hepatocytes treated with PFOA for 24 hours, 35% increase in LDH was observed at the 10 mM dose compared with control. However, for all concentrations below 10 mM, the difference was not significant (Xu et al., 2020b).

Changes in mRNA and protein expression of apoptotic genes is a hallmark of apoptosis. Increased expression of p53, Bcl-2, Bcl-2 associated X-protein (Bax), caspase-3, nuclear factor kappa B (NF-κB) mRNA and protein was observed in zebrafish liver (Cui et al., 2015). In human hepatoma SMM-721 cells treated with 10 or 100 μg/mL PFOA for 3 hours, BAX mRNA was significantly increased while B cell lymphoma 2 (Bcl-2) decreased compared with control (Lv et al., 2019). Proteomic analysis of 28 proteins differentially expressed in PFOA-exposed human nontumor hepatic cells (L-02) led the authors to conclude that PFOA induces apoptosis by activating the p53 mitochondria pathway (Huang et al., 2013). This result is consistent with several studies showing that PFOA-induced liver apoptosis is in part mediated through p53 activation (Sun et al., 2019; Li et al., 2017b). In a third study that examined miRNA expression in the mouse liver, an increase in the expression of miR-34a-5p, which has been shown to be involved in p53-mediated apoptosis, was observed (Yan et al., 2014).

PFOA has been shown to induce apoptosis through morphological changes to the mitochondrial membrane (Xu et al., 2020b; Li et al., 2017b). One study in Balb/c male mice gavaged with PFOA (0.08–20 mg/kg/day) for 28 days suggested that hepatocyte apoptosis following exposure to PFOA may be caused by endoplasmic reticulum stress, mediated by the induction of ER stress markers including phosphorylated eukaryotic initiation factor 2α (p-elf2α), spliced X box-binding protein 1 (XBP1), and C/EBP homologous protein (CHOP) (Yan et al., 2015b).

An RNA-sequencing study in primary human hepatocytes found that PFOA exposure was associated with changes in gene expression that aligned with cell death and hepatic system disease, including necrosis, cholestasis, liver failure, and cancer (Beggs et al., 2016). Another RNA-sequencing study showed that PFOA induced intracellular oxidative stress in Sprague-Dawley rats leading to apoptosis (Liu et al., 2017a). Other mechanisms underlying PFOA-induced apoptosis include DNA damage (Wielsøe et al., 2015), autophagosome accumulation (Yan et al., 2017; Yan et al., 2015b), induction of ER stress biomarkers and oxidative stress (Li et al., 2017b; Wielsøe et al., 2015; Huang et al., 2013; Panaretakis et al., 2001), and reduction of mitochondrial ATP (Sun et al., 2019; Mashayekhi et al., 2015). Although many studies have reported oxidative stress as a potential mechanism underlying PFOA-induced apoptosis, Florentin et al. (2011) did not observe an increase in DNA damage or ROS at doses that proved cytotoxic to HEPG2 cells, leading the authors to conclude that PFOA-induced apoptosis is not related to DNA damage nor oxidative stress.

PFOA-induced apoptosis has been shown to differ between males and females. In male and female Balb/c mice gavaged with PFOA at doses ranging from 0.01 to 2.5 mg/kg/day for 28 days, caspase-9 activity and dissipation of the mitochondrial membrane potential were higher in females than males. Specifically, mitochondrial membrane dissipation was 25% in males and 39% in females for mice in the 2.5 mg/kg/day groups. In the 0.05 mg/kg/day group, caspase-9 activity was elevated by 72% in females compared with 40% in males. The sexual dimorphic changes in caspase-9 and mitochondrial membrane dissipation were accompanied by morphological changes in the mitochondria characterized by increased mitochondrial vesicle formation and swelling in female than male hepatocytes, suggesting that female livers are more susceptible to PFOA-induced apoptosis than males (Li et al., 2017b).

3.4.1.3.5.3 Cell Cycle and Proliferation

Alterations in cell proliferation and cell cycle were also seen in many in vivo and in vitro studies (Wen et al., 2020; Zhang et al., 2020a; Lv et al., 2019; Beggs et al., 2016; Song et al., 2016; Zhang et al., 2016a; Buhrke et al., 2015; Buhrke et al., 2013). In mice exposed to 3 mg/kg/day PFOA for 7 days by oral gavage, proliferation in the liver, as seen through proliferation cell nuclear antigen (PCNA) staining, was increased relative to control (Beggs et al., 2016). HL-7702 cells were treated with PFOA at concentrations of 50–400 μM for 48 or 96 hours (Zhang et al., 2016a). All except the highest dose (400 μM) group showed an increase in cell proliferation compared with control at 48 hours. Other studies have reported a similar pattern for which proliferation is significantly increased at low doses and decreased at high doses of PFOA in human primary hepatocytes (Buhrke et al., 2015), HepG2 (Buhrke et al., 2013), and HepaRG cells (Behr et al., 2020a). Together these studies suggest that higher concentration of PFOA may interfere with cell cycle progression by reducing cell proliferation rather than severely inducing apoptosis.

In contrast, a study in primary hepatocytes of Sprague-Dawley rats found increased proliferation at the highest dose and no proliferative effect at low doses. Approximately 16% increase in proliferation was observed with PFOA exposures of 100 μM for 24 hours compared with controls (Liu et al., 2017a). However, no changes in cell number as measured by MTT assay was observed at the PFOA concentration range of 0.4–25 μM at the same duration, adding to the evidence that PFOA-induced proliferation is dose-dependent and may vary by cell type.

PFOA has also been shown to disrupt cell cycle progression. Using flow cytometry, Zhang et al. (2016a) found that in HL-7702 cells, the proportion of cells in the G0/G1 phase (nondividing) significantly decreased while cells in the S-phase increased after 48 hours of exposure to 50 and 100 μM PFOA. However, at the 200 μM and 400 μM exposure for 48 hours, percentage of cells in the G0/G1 phase increased while cells in the G2/M/S phase (interphase growth/mitosis) decreased significantly compared with control. Interestingly, the same trend was observed in cells incubated at the same dose for 96 hours (Zhang et al., 2016a). A second study in immortalized nontumor cells derived from human normal liver tissue (L-02 cells) also used flow cytometry to examine changes in the cell cycle after 72 hours at 25 and 50 mg/L and found that PFOA increased the percentage of cells in G2/M phases but decreased the number of cells in G0/G1 and S phases (Huang et al., 2013). Additionally, the percentage of cells in apoptotic sub-G1 (G1-) phase increased significantly from 19% to 33% compared with 10% of cells in the G1-phase in the control group, leading the authors to conclude that PFOA treatment disrupt cell cycle in L-02 cells by arresting cells in G2/M phase while inducing apoptosis. A third study in a zebrafish liver cell line also used flow cytometry to identify changes in the cell cycle after 85 and 151 μg/mL PFOA exposure for 48 hours. In corroboration with the study in L-02 cells, PFOA concentration of 151 μg/mL showed an increase in the percentage of cells in the G2/M/S stage and a decrease in the percentage of cells in the G1/G0 phase (Cui et al., 2015). Together, these studies suggest that PFOA interferes with the balance between apoptosis and proliferation by disrupting cell cycle progression.

PFOA-induced changes in cell proliferation and cell cycle progression are often accompanied with changes in mRNA and protein expression of genes implicated in cell cycle progression. Pathway analysis of protein expression in human HL-7702 normal liver cells exposed to 50 μM PFOA for 48 and 96 hours identified 68 differentially expressed proteins that are related to cell

proliferation and apoptosis (Zhang et al., 2016a). Western blot analysis from the same study showed differential protein expression of positive cell cycle-regulators, including cyclins and cyclin-dependent kinases (Cyclin/CDKs) that are known to control G1/G2/S/M cell cycle progression, as well as negative regulators (p53, p21, MYTI, and WEE1). Interestingly, expression of cell cycle regulations was dose-dependent. Significant induction of cyclin D1, CDK6, cyclin E2, cyclin A2, CDK2, p-CDK1, p53, p21, p-WEE1 and myelin transcription factor 1 (MYT1) was observed at low dose (50 or 100 μM). However, cyclin A2, cyclin B1 and p21 proteins were significantly inhibited at high dose (400 μM) at the same duration (48 hours) (Zhang et al., 2016a). In primary human hepatocytes treated with 10 μM PFOA, CCND1 and Aldo-keto reductase family 1 member B10 (AKR1B10) mRNA were significantly induced after 96 hours (Beggs et al., 2016). AKR1B10 is a promitogenic gene that has been associated with the progression of hepatocellular carcinoma (Matkowskyj et al., 2014). In addition, two microarray studies in hepatic cell lines found that PFOA exposures ranging from 100 to 305 μM for up to 48 hours were associated with pathways involved in the regulation of cellular proliferation or the cell cycle (Louisse et al., 2020; Song et al., 2016).

PFOA has been shown to decrease the expression of hepatocyte nuclear factor 4-alpha (HNF4α), a regulator of hepatic differentiation and quiescence, in multiple studies and is thought to mediate steatosis following PFOA exposure (Behr et al., 2020a; Beggs et al., 2016). One study suggested that PFOA-induced proliferation may be mediated by the degradation of HNF4α (Beggs et al., 2016). This study, using wild-type CD-1 and HNF4α knockout mice, reported that 11 out of 40 genes altered by PFOA exposure were regulated by HNF4α. PFOA exposure decreased the expression of HNF4α in both male mice and primary human hepatocytes and increased the expression of Nanog, a stem cell marker, suggesting that PFOA may be de-differentiating hepatocytes. Increased relative liver weight in PFOA-exposed mice was observed in this study and the authors concluded that hepatomegaly, along with other liver effects such as steatosis, may be mediated by PFOA-induced dysregulation of HNF4α.

3.4.1.3.5.4 Conclusions

Hepatotoxicity is widely cited as a type of toxicity induced by PFOA exposure. PFOA has been shown to trigger apoptosis at high doses and induce cell proliferation at low doses. PFOA-induced apoptosis is activated through a cascade of mechanisms including activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, and activation of p53 mitochondria pathway. Additionally, PFOA induced hepatocyte proliferation both in vivo and in vitro by disrupting cell cycle progression leading to liver dysfunction, including steatosis and hepatomegaly. Therefore, PFOA exposure may lead to liver cytotoxicity through a myriad of intracellular events.

3.4.1.3.6 Inflammation and Immune Response

The liver is an important buffer between the digestive system and systemic circulation and is thus exposed to compounds that are potentially immunogenic, resulting in protective immune and inflammatory responses. Kupffer cells constitute the majority of the liver-resident macrophages and make up one-third of the non-parenchymal cells in the liver. Kupffer cells phagocytose particles, dead erythrocytes, and other cells from the liver sinusoids and play a key role in preventing immunoreactive substances from portal circulation from entering systemic circulation (Dixon et al., 2013). While Kupffer cells can be protective in drug- and toxin-induced liver toxicity, dysregulation of Kupffer cell-mediated inflammatory responses is associated with

a range of liver diseases, including steatosis. Other liver-resident immune cells include natural killer (NK) cells, invariant NKT cells, mucosal associated invariant T (MAIT) cells, γδT cells, and memory CD8 + T cells (Wang and Zhang, 2019). The non-immune cells of the liver, liver sinusoidal endothelial cells (LSECs), hepatocytes, and stellate cells, also participate in immunity. They can express pattern recognition receptors and present antigens to T cells (Robinson et al., 2016). However, the impact of PFOA on the immune function of these cell types has not been thoroughly investigated.

3.4.1.3.6.1 In Vivo Studies

Investigations into the liver immune response have been conducted in a single human study in the C8 Health Project cohort (Bassler et al., 2019), and in several rodent studies (Li et al., 2019c; Wu et al., 2018; Hui et al., 2017; Liu et al., 2016; Yu et al., 2016; Botelho et al., 2015). Bassler et al. (2019) collected 200 serum samples from participants of the C8 Health Project to analyze mechanistic biomarkers of non-alcoholic fatty liver disease (NAFLD) and test the hypothesis that PFAS exposures are associated with increased hepatocyte apoptosis and decreased proinflammatory cytokines. PFOA levels were significantly correlated with decreases in serum levels of the proinflammatory cytokine tumor necrosis factor α (TNFα). In contrast, both interferon γ (IFNγ) and cleaved complement 3 (C3a) were positively associated with PFOA levels. The authors state that these results are consistent with other findings that PFAS are immunotoxic and downregulate some aspects of the immune responses, but paradoxically result in increased apoptosis, which may subsequently result in progression of liver diseases (including NAFLD).

A study in mice acutely exposed to PFOA also linked hepatic injury to activation of the complement system. In contrast to the human study (Bassler et al., 2019), a decrease in serum C3a was observed in mice (Botelho et al., 2015). C57BL/6 mice exposed to a 10-day dietary treatment with PFOA (0.002–0.02%, w/w) exhibited hepatomegaly, elevated serum triglycerides, elevated alanine aminotransferase (ALAT), hepatocyte hypertrophy, and hepatocellular necrosis at all doses. At the highest dose only, PFOA-induced hepatic injury coincided with deposition of the complement factor C3a fragment in the hepatic parenchyma. The findings support activation of the classical, but not alternative complement cascade in liver, and correlated with diminished C3 levels in serum. In serum, commercial hemolytic assays indicated attenuation of both the classical and alternative complement pathways. These authors proposed that that PFOA-mediated induction of hepatic parenchymal necrosis is the initiation event that leads to activation of the complement cascade and pro-inflammatory responses.

In another study in mice, the effects of PFOA exposure on inflammatory changes in liver varied depending on the presence of pre-existing NAFLD (Li et al., 2019c). Mice were subjected to control diet or HFD for 16 weeks to induce NAFLD, after which they were exposed to vehicle or 1 mg/kg/day PFOA by oral gavage for 2, 8, or 16 weeks; the control diet and HFD were continued throughout the exposure period until necropsy. In mice on the control diet, inflammatory changes were not observed in the first 8 weeks of PFOA treatment. However, after 16 weeks of PFOA treatment, mild hepatic lobular inflammation was observed in 3 of 5 animals, suggesting that chronic exposure to PFOA induces inflammatory changes in liver. In HFD-fed mice, focal inflammation was seen as early as 2 weeks after initiating PFOA treatment and inflammatory foci were observed in 2 of 5 mice after 16 weeks of PFOA exposure. Gene expression of Tnfα measured by qRT-PCR was elevated in the HFD group exposed to PFOA for

all three treatment durations (2, 8, or 16 weeks of PFOA). Similarly, Liu et al. (2016) observed an induction of TNFα in liver homogenates, measured by ELISA, in male Kunming mice fed a regular diet (Liu et al., 2016) and exposed to a higher dose of PFOA (10 mg/kg/day for 2 weeks). This study observed significantly elevated levels of both TNFα and IL-6 in liver homogenates.

Li et al. (2019c) also confirmed increased expression of inflammatory genes using an RNA-Seq transcriptomic approach. Compared to mice on the control diet, the HFD group exposed to PFOA resulted in 537 differentially expressed genes. The inflammatory response was among the top enriched Gene Ontology (GO) terms for the gene set specific to the PFOA-exposed HFD. Analysis using Ingenuity Pathway Analysis showed significant upregulation of chemokines and chemokine-related genes and toll-like receptor (TLR) related genes in the PFOA-exposed HFD group compared with mice fed the control diet. Taken together with the histopathological findings, these gene expression changes suggest that preexisting fatty liver may enhance PFOA-mediated inflammatory changes in liver.

Another potential nexus between changes in hepatic lipid metabolism and inflammation comes from a high-throughput metabolomics study in male BALB/c mice (Yu et al., 2016). After a 28-day exposure to 0, 2.5 or 5 mg/kg/day PFOA, livers were subjected to metabolomic analysis. Metabolite analysis indicated PFOA altered polyunsaturated fatty acid metabolism including the arachidonic acid pathway. Arachidonic acid is a precursor in production of inflammatory mediators including prostaglandins, thrombaxanes, and leukotrienes. Prostaglandins (PGD2, PGE2, and PGF2α) were slightly elevated but increases did not reach statistical significance. However, the ratio of the thromboxane A2 (TXBA2) metabolite thromboxane X2 (TXB2) to prostaglandin I2 (PGI2) was significantly decreased in PFOA-exposed mice. Given the prothrombotic role of TXBA2 and the vasodilatory role of PGI2, the authors suggest these changes are consistent with ischemic liver injury that is characterized by vasodilation of microvasculature, lessened adherent leukocytes, and improved flow velocity in liver. Two leukotrienes, LTD4 and LTB4 were significantly lower in the high dose group. Both leukotrienes can also regulate vascular permeability and the authors suggest these changes are consistent with PFOA-induced inflammation in liver. PFOA also upregulates CD36 gene expression in hepatocytes (Wu et al., 2018; Hui et al., 2017), which is a negative regulator of angiogenesis (Silverstein and Febbraio, 2009). Together with the PFOA-mediated changes in abundance of prostaglandins and thrombaxanes, these findings raise the possibility that PFOA-mediated alterations of the hepatic microvasculature are key events in the development or persistence of liver inflammation.

3.4.1.3.6.2 In Vitro Studies

In a study investigating the hepatic effects of PFOA in vitro, Song et al. (2016) evaluated gene expression changes in human liver hepatocellular carcinoma HepG2 cells using a whole genome expression microarray. After exposing these cells to 306 μM PFOA (the IC20 dose for cell viability inhibition) for 48 hours, gene expression changes were evaluated. PFOA exposure led to differential regulation of 1,973 genes. Through KEGG pathway analyses, the authors reported that genes related to immune response were among the most differentially expressed biological process out of the 189 processes with altered genetic profiles. The authors identified 17 immune-associated genes that were differentially expressed. These genes mapped to the TNF signaling pathway, nucleotide-binding and oligomerization domain (NOD)-like receptor signaling,

cytokine-cytokine receptor interactions, and the complement and coagulation cascade system. These findings support a role for PFOA in dysregulating innate immune mechanisms.

Alterations in cytokines associated with regulation of adaptive immunity were also observed using multicellular hepatic organotypic culture models composed of primary human or rat cells (Orbach et al., 2018). This system involved seeding primary liver sinusoidal epithelial cells and Kupffer cells encapsulated in extracellular matrix proteins above the hepatocytes. This culture system forms a stratified three-dimensional (3D) structure designed to more accurately mimic liver tissue. Organotypic cultures were exposed to 500 μM PFOA for 24 hours (the LC50 in human cultures). PFOA exposure led to a 62% decrease in IL-10 levels. In addition to being a key cytokine in development of T helper lymphocytes, IL-10 has anti-inflammatory properties. Thus, the decrease in IL-10 observed in organotypic culture is consistent with the proinflammatory changes in liver associated with PFOA exposure. Using a proteomic approach, another cytokine, IL-22, has also been shown to be downregulated in PFOA-exposed human hepatic L-02 cells (Huang et al., 2013). IL-22, a member of the IL-10 cytokine family, exerts protective effects in liver during acute inflammation and alcoholic liver injury (Ki et al., 2010; Zenewicz et al., 2007). T helper (Th22) cells are a T cell subset responsive to IL-22. Th22 cells function in maintaining the integrity of the epithelial barriers (Hossein-Khannazer et al., 2021). As such, diminished levels of IL-22 in the liver suggest that PFOA could interfere with the protective effects of IL-22 and Th22 cells.

3.4.1.3.6.3 Conclusions

The limited number of studies reviewed support a role PFOA in inducing hepatic inflammation through dysregulation of innate immune responses. This includes elevated levels of TNFα as well as changes in prostaglandin and thromboxane levels. Gene expression studies also suggest a role for chemokines in elaborating inflammation in liver. Expression of genes coding for products involved in innate immune defense systems were altered, including TLRs, molecules involved in NOD signaling, and C3a, a key indicator of complement cascade activation. Far less is known regarding PFOA effects on adaptive immunity in liver. PFOA exposure caused a reduction in IL-10 levels in organotypic culture of liver. IL-10 has anti-inflammatory properties in addition to promoting differentiation of Th2 CD4+ T cells. Intriguingly, IL-22 levels were diminished in PFOA-exposed hepatic cells. This cytokine may impact the function of Th22 T lymphocytes and impact the epithelial barriers in liver. Moreover, IL-22 reduction may reduce the protective effects of this cytokine during inflammation. Altogether, induction of inflammation appears to be an important mechanism that impacts liver pathogenesis in response to PFOA exposure, though the contribution of specific populations of resident or infiltrating liver immune cells and the series of events that produce inflammation have yet to be elucidated. Adaptive immune responses are disrupted in PFOA-exposed animals (Section 3.4.2.2). However, whether alterations in adaptive immunity impact pathogenetic mechanisms in liver remain unknown.

3.4.1.3.7 Oxidative Stress and Antioxidant Activity

3.4.1.3.7.1 Introduction

Oxidative stress, caused by an imbalance of reactive oxygen species (ROS) production and detoxification processes, is a key part of several pathways, including inflammation, apoptosis, mitochondrial function, and other cellular functions and responses. In the liver, oxidative stress

contributes to the progression and damage associated with chronic diseases, such as alcoholic liver disease, non-alcoholic fatty liver disease, hepatic encephalopathy, and Hepatitis C viral infection (Cichoz-Lach and Michalak, 2014). Indicators of oxidative stress include but are not limited to increased oxidative damage (e.g., malondialdehyde (MDA) formation); increased reactive oxygen species (ROS) production (e.g., hydrogen peroxide and superoxide anion); altered antioxidant enzyme levels or activity (e.g., superoxide dismutase (SOD) and catalase (CAT) activity); changes in total antioxidant capacity (T-AOC); changes in antioxidant levels (e.g., glutathione (GSH) and glutathione disulfide (GSSG) ratios); and changes in gene or protein expression (e.g., nuclear factor-erythroid factor 2-related factor 2 (Nrf2) protein levels). PFOA has been implicated as a chemical that can induce these indicators of oxidative stress, inflammation, and cell damage.

3.4.1.3.7.2 In Vivo Models

3.4.1.3.7.2.1 Mouse

Yan et al. (2015b) examined livers from male Balb/c mouse following PFOA exposure of 0.08, 0.31, 1.25, 5, or 20 mg/kg/day for evidence of oxidative stress, including changes in expression of oxidative stress-related genes. While no change was observed in Cat expression levels, increases in Sesn1, Sod1, and Sod2 were observed in livers from mice exposed to 1.25, 5, and 20 mg/kg/day PFOA, respectively. PFOA exposure led to increased CAT activity and decreased SOD activity in mouse livers. MDA contents were decreased at all dose levels, and levels of the antioxidant GSH increased at 5 and 20 mg/kg/day PFOA. Authors concluded that the changes in SOD, CAT, GSH, and MDA reflect PFOA-induced disruptions to the antioxidant defense system in the livers of exposed mice. However, no significant oxidative damage was observed.

Li et al. (2017b) explored the role of ROS accumulation in apoptosis in male and female Balb/c mice dosed with 0.05, 0.5, or 2.5 mg/kg/day PFOA for 28 days. The authors explored how activation of PPARα and suppression of the electron transport chain (ETC) sub-unit Complex I influenced ROS generation. Excluding the lowest male dose group, PFOA exposure significantly increased 8-OHdG levels in the liver, a key indicator of oxidative DNA damage. 8-OHdG levels were higher among dosed females compared with males, which authors suggest signals stronger genotoxicity in females. Authors explored the connection between the oxidative stress and apoptosis through the p53 signal pathway. Increases in p53 levels occurred in the same dose groups with elevated 8-OHdG, which authors suggest indirectly links oxidative stress to apoptosis. Authors posited that ROS hypergeneration led to increased 8-OHdG levels, and DNA damage then leads to increases in programmed cell death protein 5 (PDCD5), which activates p53 to induce apoptosis. At 0.5 and 2.5 mg/kg/day, PFOA exposure decreased expression of electron transport chain (ETC) proteins, which corresponds to an increase in ROS generation and accumulation. For two ETC subunits, ACP and NDUV2, expression was increased, which also indicates an accumulation of ROS and an increase in antioxidant activity to counter ROS generation. At 0.05 mg/kg/day, female mice showed more oxidative stress than males. In these females, Complex I suppression drove ultimate apoptosis, while PPARα activation drove apoptosis among males.

Two studies examined changes in oxidative stress endpoints in male Kunming mice exposed to PFOA (Liu et al., 2016; Yang et al., 2014), and an additional two studies evaluated oxidative stress endpoints in pregnant female Kunming mice and their pups (Li et al., 2019a; Song et al.,

2019). In the livers of male Kunming mice exposed to 2.5, 5, or 10 mg/kg/day PFOA for 14 days, MDA at all doses and H_2O_2 at 5 and 10 mg/kg/day levels were significantly increased compared with controls (Yang et al., 2014). Liu et al. (2016) explored grape seed proanthocyanidn extract (GSPE) as a protective agent against PFOA damage in the liver. The authors reported significantly increased MDA and H_2O_2, significantly decreased Nrf2 protein levels, and significantly decreased SOD and CAT activity in the liver following PFOA exposure. Additionally, expression of SOD and CAT, measured via qRT-PCR, were significantly decreased in the livers of exposed mice. Li et al. (2019b) found that serum levels of SOD and 8-OHdG were significantly increased in pups of females dosed at 2.5, 5, and 10 mg/kg/day PFOA. Serum levels of CAT were increased at 5 and 10 mg/kg/day PFOA. PFOA-induced changes in SOD, CAT, and 8-OHdG reflect increased antioxidant activity in response to increased oxidative stress and increased DNA damage. In their study examining the protective effects of lycopene against PFOA-induced damage, Song et al. (2019) exposed pregnant mice to 20 mg/kg/day PFOA via oral gavage from gestational days (GD) 1–7. After sacrifice on GD 9, levels of MDA were significantly increased in livers of pregnant mice treated with 20 mg/kg/day PFOA, while SOD and GSH-Ps levels were significantly decreased compared with controls, providing evidence of oxidative damage in the liver following PFOA exposure.

Three studies dosed C57Bl/6 mice with PFOA to study impacts on oxidative stress endpoints (Crebelli et al., 2019; Wen et al., 2019c; Kamendulis et al., 2014). In male C57Bl/6 mice dosed with 28 mg/L PFOA, Crebelli et al. (2019) found slightly decreased T-AOC, but the results were not statistically significant. MDA levels were below detection limits in all collected samples. Additionally, there was no statistically significant change in the levels of liver TBARS that would indicate lipid peroxidation. Kamendulis et al. (2014) exposed male C57Bl/6 mice to 5 mg/kg/day and found that PFOA exposure led to a 1.5-fold increase in 8-iso-PGF2α levels, a measure of lipid peroxidation that indicates oxidative damage. Additionally, PFOA led to a nearly twofold increase in mRNA levels of Sod1 in liver cells extracted from mice dosed at 2.5 and 5 mg/kg/day PFOA. mRNA levels of Sod2 and Cat were increased threefold and 1.3-fold, respectively. The same doses of PFOA also led to a nearly twofold increase in Nqo1 mRNA levels. The induction of genes for detoxifying enzymes following PFOA exposure suggests PFOA causes increased oxidative stress activity. In a different study (Wen et al., 2019c), 1 and 3 mg/kg/day PFOA exposure in wild-type C57BL/6 NCrl male mice increased gene expression of Nrf2 and Nqo1, measured via qRT-PCR assays, by 50%–300%.

One gene expression compendium study aimed to examine the relationship between activation of xenobiotic receptors, Nrf2, and oxidative stress by comparing the microarray profiles in mouse livers (strain and species not specified) (Rooney et al., 2019). The study authors compiled gene expression data from 163 chemical exposures found within Illumina's BaseSpace Correlation Engine. Gene expression data for PFOA exposure was obtained from a previously published paper by Rosen et al. (2008b). In WT (129S1/SvlmJ) and Pparα-null male mice, Nrf2 activation was observed (as seen by increases in gene expression biomarkers) after a 7-day exposure to 3 mg/kg/day PFOA via gavage. Similar to Nrf2, CAR was also activated in both mouse strains after PFOA exposure. The authors proposed that CAR activation by chemical exposure (PFOA or otherwise) leads to Nrf2 activation, and that oxidative stress may be a mediator.

3.4.1.3.7.3 In Vitro Models

Rosen et al. (2013) assessed oxidative stress-related gene expression changes using Taqman low-density arrays (TLDA) in both mouse and human primary hepatocytes exposed to levels of PFOA ranging from 0 to 200 μM. PFOA exposure led to a decrease in the expression of the heme oxygenase 1 (Hmox1) gene in human primary hepatocytes. There were no changes observed in the nitric oxide synthase 2 (Nos2) gene nor in either gene in primary mouse hepatocytes.

Orbach et al. (2018) examined the impacts of 500 μM PFOA exposure in multicellular organotypic culture models (OCM) of primary human and rat hepatocytes and in collagen sandwich (CS) models via high-throughput screening. In exposed rat and human cells, PFOA decreased GSH levels by <10%. The authors suggest that PFOA did not bind to or oxidize GSH. In human OCMs, mitochondrial integrity decreased 37% following PFOA exposure. In human CS models, the decrease was 39%. In rat OCMs, exposure decreased mitochondrial integrity by 47%, and by 45% in rat CS models.

In primary rat hepatocytes incubated with 100 μM PFOA for 24-hours, Liu et al. (2017a) found that intracellular oxidant intensity increased to more than 120% of control levels as measured by mean fluorescence intensity of 2′,7′-dichlorofluorescein (DCF). In addition, cells incubated with 6.25, 25, or 100 μM PFOA displayed significantly increased levels of mitochondrial superoxide, measured by MitoSOX fluorescence. In cells exposed to 100 μM PFOA, mitochondrial superoxide levels were elevated to 130% of those of controls. Authors suggest that these results indicate that mitochondrial superoxide is a more sensitive marker of oxidative stress than intracellular ROS levels.

Two studies examined oxidative stress endpoints following PFOA exposure in mitochondria isolated from Sprague-Dawley rats (Das et al., 2017; Mashayekhi et al., 2015). Mashayekhi et al. (2015) examined oxidative damage in the mitochondria, an important organelle in the oxidative stress pathway, associated with PFOA exposure. In mitochondria isolated from the livers of male Sprague-Dawley rats, significant increases in the percent ROS formation were observed following exposure to 0.75, 1, or 1.5 mM PFOA for up to 20 minutes. At 30 minutes and longer, significant increases were observed at the two highest concentrations only. Mashayekhi et al. (2015) also observed significantly increased levels of ROS formation in complexes I and III of the mitochondrial respiratory chain, key sources of ROS production. Disruption to the chain can lead to accumulation of ROS and, ultimately, oxidative stress. In complex II, activity levels were significantly decreased at 0.75 and 1.5 mM PFOA exposure. There was no significant difference in MDA of GSH content in liver mitochondria following PFOA exposure. PFOA exposure from 0.5–1.5 mM significantly decreased mitochondrial membrane potential and ATP levels and significantly increased mitochondrial swelling, suggesting a decrease in mitochondrial function following exposure to PFOA.

Xu et al. (2019b) exposed mouse hepatic primary cells from C57Bl/6J male mice to 0.01, 0.1, 0.5, or 1 mM PFOA for 24 hours. ROS levels, measured by a CM-H2DCFA fluorescent probe, were significantly increased in cells exposed to 0.5 and 1 mM PFOA. Interestingly, SOD activity was significantly increased in cells exposed to 0.5 and 1 mM PFOA, up to 123% with 1 mM, while CAT activity was reduced to 7.7% in cells at the highest concentration. Increasing PFOA exposure also led to alterations in the structure of SOD, resulting in a significantly decreased

percentage of α-helix structures (20%) and an increased percentage of β-sheet structures (29%), providing evidence of polypeptide chain unfolding and decreased helical stability. These structural changes suggest that PFOA interacts directly with SOD, resulting in polypeptide chain extension and, ultimately, diminished antioxidant capacity. Additionally, GSH content was increased by 177% and 405% in cells exposed to 0.5 mM and 1 mM PFOA, respectively. The authors suggest that increases in GSH may reflect cellular adaptations to oxidative stress and can lead to detoxification of oxidized GSSG to GSH.

Xu et al. (2020b) exposed cultured primary mouse hepatocytes to 0.01, 0.1, 0.5, or 1 mM of PFOA for 24 hours to examine oxidative stress-related apoptosis. The authors examined the impact of PFOA exposure on endogenous levels of lysozyme (LYZ), an enzyme that inhibits oxidative stress-induced damage, and demonstrated that PFOA exposure impacted LYZ molecular structure, subsequently decreasing activity levels, leading to oxidative stress-induced apoptosis. Decreases in peak intensity at 206 nm during ultraviolet-visible (UV-vis) absorption spectrometry represented an unfolding of the LYZ molecule following exposure to PFOA, which inhibited enzyme activity. At concentrations of 100 μM and above, LYZ enzyme activity decreased to 91% of control levels. Such an impact on LYZ activity was deemed to be related to the high affinity of PFOA for key central binding sites on the LYZ molecule.

In human HL-7702 liver cells, 24 hours of PFOA exposure at 1, 2.5, or 7.5 μg/mL led to a dose-dependent increase in 8-OHdG levels in cells exposed to the two highest concentrations (Li et al., 2017b). The authors noted that DNA damage, which frequently accompanies increases in 8-OHdG, was observed in their in vivo models following PFOA exposure, suggesting increased oxidative stress following exposure. In human non-tumor hepatic cells (L-02) exposed to 25 or 50 mg/L PFOA for 72 hours, Huang et al. (2013) observed concentration-dependent increases in ROS levels measured via DCFH-DA fluorescent probe, evidence of the role of PFOA in inducing oxidative stress.

Six additional studies examined oxidative stress endpoints following PFOA exposure in HepG2 cell lines (Wan et al., 2016; Wielsøe et al., 2015; Yan et al., 2015b; Shan et al., 2013; Florentin et al., 2011; Panaretakis et al., 2001). Four studies reported increases in ROS levels following PFOA exposure (Wan et al., 2016; Wielsøe et al., 2015; Yan et al., 2015b; Panaretakis et al., 2001), while two studies did not observe statistical differences in ROS levels following 1- or 24-hour PFOA exposures up to 400 μM (Florentin et al., 2011) or following 3-hour PFOA exposures up to 400 μM (Shan et al., 2013).

Wielsøe et al. (2015) incubated HepG2 cells with up to 2×10^{-4} M PFOA to detect changes in ROS, T-AOC, and DNA damage. PFOA exposure significantly increased ROS production, as measured with the carboxy-H2DCFDA, and significantly decreased T-AOC at all concentrations by 0.70–0.82-fold compared with controls. Additionally, PFOA induced DNA damage, specifically, increased mean percent tail intensity, an indicator of strand breaks, measured via comet assay. In cells exposed up to 400 μM PFOA for up to 24 hours, Panaretakis et al. (2001) observed increased ROS levels, measured via DCFH-DA and dihydroethidium fluorescent probes, following 3 hours PFOA exposure. H_2O_2 levels were detectable in 91% and 98% of the cell population at 200 and 400 μM PFOA, respectively. Additionally, superoxide anion levels were detectable in 43% and 71% of cells exposed to 200 and 400 μM PFOA, respectively. Authors reported evidence of depolarized mitochondrial membranes in cells exposed up to 24 hours. Yan et al. (2015b) observed significantly increased ROS levels in cells incubated with

100 and 200 μM PFOA for 24 hours, but no changes were observed in superoxide anion levels. After 72 hours of exposure, however, ROS levels decreased at those concentrations, with statistically significant results observed at 200 μM PFOA. Activity levels of SOD and CAT were not altered in exposed cells compared with controls, nor were MDA or GSH contents. Similarly, in HepG2 cells treated with PFOA for 24 hours, Yan et al. (2015b) found ROS levels significantly increased, but no significant changes were observed in SOD and CAT activity or MDA and GSH levels. Yarahalli Jayaram et al. (2018) examined the impacts of PFOA exposure on oxidative stress endpoints and small ubiquitin-like modifiers (SUMO), which play a key role in posttranslational protein modifications. SUMOylation of a protein has been identified as a key part of the oxidative stress pathway. In cells incubated with 250 μM PFOA, ROS levels were significantly increased. Cells incubated with PFOA also showed increased levels of nitric oxide (NO). Additionally, expression levels of genes related to SUMOylation were measured. PFOA treatment significantly increased levels of SUMO2 in HepG2 cells, but did not impact SUMO1, SUMO3, or UBC9 mRNA levels.

In cells exposed to 10 and 200 μM PFOA for 24 hours, Florentin et al. (2011) observed significant increases in the percentage of DNA tails, an indicator of DNA damage measured via comet assay. However, no such changes were observed at the 1-hour time point or at other concentrations (5, 50, 100, or 400 μM) after 24 hours. Additionally, no significant changes in ROS generation were observed. Shan et al. (2013) exposed HepG2 cells to 100 μM PFOA for 3 hours and found an increase in ROS generation, though the effect was not statistically significant. Additionally, no changes were observed in the GSH/GSSG ratio.

In two cell lines derived from Hepa1c-1c7 mouse cells, CR17 and HepaV cells, Melnikov et al. (2018) found that Hmox1 gene expression was significantly decreased in cells exposed to PFOA for 24 hours compared with controls. Additionally, exposed HepaV cells showed significantly decreased expression of Gclc and Gclm. There were no significant changes in GSH levels after exposure to 100 μM PFOA for 24 hours. CR17 cells have increased glutamate-cysteine ligase (GCL) activity, leading to increased GSH content. Authors anticipated that the elevated GSH levels in the CR17 cell line would better resist PFOA toxicity. They concluded that the observed changes in gene expression in PFOA-exposed HepaV cell lines, but not in CR17 cell lines, supported this hypothesis.

Sun et al. (2019) examined the impacts of PFOA exposure on both a monolayer and a scaffold-free three-dimensional spheroid model of mouse liver cells (AML12). Monolayer cells were exposed to 6.25–2,000 μM PFOA for 24 and 72 hours. The spheroid cell model was exposed to 50, 100, and 200 μM PFOA for up to 28 days. In monolayer cells exposed to 200 μM PFOA for 72 hours, ROS levels, measured via an ROS-Glo assay kit, increased 1.6-fold compared with controls. In the spheroid cell models, however, ROS levels decreased in cells exposed to 100 and 200 μM PFOA for 24 and 72 hours, which authors report suggests that monolayer cells demonstrate higher PFOA toxicity due to the absence of an endogenous extracellular matrix with the potential to inhibit PFOA diffusion. After 14 days of exposure, ROS levels in spheroid cells significantly increased at all concentrations. Gene expression of glutathione S-transferases alpha 2 (Gsta2), Nqo1, and Ho-1 increased with increasing PFOA concentration and duration of exposure, which provides additional evidence of PFOA's effect on oxidative stress.

3.4.1.3.7.4 Conclusions

Results from new studies published since the 2016 PFOA HESD (U.S. EPA, 2016c) further support the 2016 conclusions that PFOA can cause oxidative stress and related cellular damage. Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in T-AOC, was observed following both in vivo and in vitro exposures to PFOA. PFOA exposure was also associated with increased levels of markers of oxidative damage and decreased activity or levels of protective antioxidants that play a role in the reduction of oxidative damage. There was also evidence that PFOA can disrupt the structure and subsequent function of crucial enzymes that mitigate ROS production and oxidative damage, SOD and LYZ. While further research is needed to understand the underlying mechanisms of PFOA-induced oxidative stress responses, it is clear that PFOA induces oxidative stress in hepatic tissues.

3.4.1.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and hepatic effects in humans based on associations with liver biomarkers, especially ALT, in several *medium* confidence studies. Across the studies in the 2016 PFOA HESD (U.S. EPA, 2016c) and this updated systematic review, there is consistent evidence of a positive association between exposure to PFOA and ALT in adults (Jain, 2019; Jain and Ducatman, 2019c; Nian et al., 2019; Salihovic et al., 2018; Darrow et al., 2016; Gleason et al., 2015; Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010). An exposure-response gradient observed in one *medium* quality study that examined categorical exposure in adults (Darrow et al., 2016) increases certainty in the association. These associations were observed in studies of the general population, in communities with high exposure from water due to contamination events, and in occupational studies. Consistency in the direction of association across these different population sources increases certainty in the results and reduces the likelihood that they can be explained by confounding across PFAS. For example, studies in communities with high exposure from water and occupational participants are less susceptible to potential confounding from other PFAS due to PFOA exposure predominating over other PFAS. In addition, the single general population that performed multipollutant modeling (Lin et al., 2010) found no attenuation of the association, further increasing confidence in the association between PFOA exposure and increased ALT. The positive associations with ALT are also supported by the recent meta-analysis of 25 studies in adolescents and adults (Costello et al., 2022). Associations for other hepatic outcomes were less consistent, including for functional outcomes such as liver disease. This may be due to a relative lack of *high* confidence studies of these outcomes.

The animal evidence for an association between PFOA exposure and hepatic toxicity is *robust* based on 27 *high* or *medium* confidence animal toxicological studies. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those that indicate functional impairment or lesions (Hall et al., 2012; EMEA, 2010; FDA, 2009; U.S. EPA, 2002a). EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant increases in enzymes indicative of liver toxicity (U.S. EPA, 2002a). Many of the studies discussed in this section reported dose-dependent increases in liver weight and hepatocellular hypertrophy in rodents of both sexes. However, a limited number of these studies additionally examined functional or histopathological hepatic impairment to

provide evidence that the enlargement of hepatic tissue was an adverse, and not adaptive, response (Blake et al., 2020; NTP, 2020; Crebelli et al., 2019; Guo et al., 2019; Yan et al., 2014; Minata et al., 2010; Loveless et al., 2008).

EPA identified the following studies as providing the most comprehensive evidence of dose-dependent hepatoxicity resulting from oral PFOA exposure: a chronic dietary study in male and female Sprague-Dawley rats (NTP, 2020) (see study design details in Section 3.4.4.2.1.2); a developmental study in male and female CD-1 mice (Cope et al., 2021); and a 29-day oral gavage study in male rats and mice (Loveless et al., 2008). NTP (2020) conducted histopathological examinations of liver tissue in male and female rats and reported dose-dependent increases in the incidence of hepatocellular hypertrophy and hepatocellular cytoplasmic vacuolation, as well as increases in the incidence of hepatocellular single-cell death and hepatocellular necrosis at the same dose levels. Cope et al. (2021) also provides evidence of hepatic lesions in adult male and female CD-1 mice offspring exposed gestationally from GD 1.5 to GD 17.5. When the offspring were weaned, they were placed on a low- or high-fat diet. At 18 weeks there were increases in the incidence and severity of hepatocellular single-cell death in females on either the low- or high-fat diets and males on the low-fat diet. Loveless et al. (2008) similarly provides concurrent evidence of liver enlargement and hepatic lesions in male mice gavaged with PFOA for 29 days. Increases in the incidence and severity of hepatocellular hypertrophy and individual cell or focal cell necrosis were dose-dependent. Similar to the NTP (2020) study, Loveless et al. (2008) provides a comprehensive report of hepatotoxicity, with a low-dose range resulting in dose-dependent increases in histopathological outcomes indicating adversity.

An important element of understanding the underlying mechanism(s) of toxicity is species specificity and relevance of data collected from laboratory models in relation to observed human effects as well as in consideration of human hazard. There are several studies that have proposed potential underlying mechanisms of the hepatotoxicity observed in rodents exposed to PFOA, such as induction of hepatocytic proliferation leading to hypertrophy or nuclear receptor activation leading to lipid droplet accumulation and steatosis. Generally, mechanistic evidence supports the ability of PFOA to induce hepatotoxicity which may explain elevated serum ALT levels in humans (and animals). However, mechanistic studies did not specifically relate (or, "anchor") mechanistic data with serum ALT levels in animals, and challenges exist in the extrapolation of evidence for PFOA-mediated changes in rodents to humans. For example, there is substantial evidence that PFOA-induced liver toxicity, specifically alterations to lipid metabolism and accumulation, occurs via the activation of multiple nuclear receptors, including PPARα. Activation of PPARα by PFOA has been demonstrated in multiple studies across various model systems, both in vivo and in vitro. Several studies examined the activation of PPARα in vitro in both human and animal cell lines transfected with mouse and human PPARα using luciferase reporter assays, the results of which demonstrate that PFOA can activate human PPARα in vitro. In addition to PPARα, evidence also exists indicating that PFOA can activate CAR, PXR, PPARγ, ERα, and HNFα, as evidenced by receptor activation assays as well as changes in target genes of these receptors. PFOA showed the highest potency for PPARα in comparison to PPARγ and PPARδ, although PFOA did activate these receptors at concentrations of 100 μM (compared with 25 μM for PPARα). Like PPARα, PPARγ and CAR are known to play important roles in liver homeostasis, and dysregulation of these nuclear receptors can lead to steatosis and liver dysfunction, potentially presenting an important mechanism for the liver

effects observed in rodent studies. Beyond receptor activation assays, individual target genes that represent reliable markers of CAR and PPARα activation (e.g., *Cyp2b1* and *Cyp4a1*, respectively) have been clearly demonstrated to be altered by PFOA, and changes to these nuclear receptors have important implications regarding hepatotoxicity, specifically steatosis. PPARα has a vastly different expression in rodents compared with humans, and this species difference is known to play a major role in differences in liver effects between the two species. PPARα is the most demonstrated nuclear receptor to be activated by PFOA, and it should be noted that using PPARα-null mice to study PPARα-independent effects of PFOA may lead to compensatory mechanisms involving other nuclear receptors.

Another example of species specificity for an effect of PFOA is the presence or absence of a transfer protein that is important in cholesterol accumulation, CETP, which is expressed in humans but not in rodents. Transgenic mice that express human CETP exhibit a more human-like lipoprotein metabolism. Laboratory models that are designed to better predict human-relevant mechanisms, such as mice expressing human CETP or PPARα, will continue to aid in accuracy of the extrapolation of mechanistic findings in rodents to humans. Despite these challenges, the evidence that PFOA leads to hepatotoxicity via activation of hepatic nuclear receptors and dysregulation of lipid metabolism and accumulation is clear.

When considering the evidence from both in vivo and in vitro studies, PFOA-mediated hepatoxicity specific to changes in lipid metabolism leading to steatosis, the most commonly reported hepatocytic morphological alteration in PFOA-exposed animals, likely occurs through the following molecular and cellular events: (1) PFOA accumulation in liver activates nuclear receptors, including PPARα; (2) expression of genes involved in lipid homeostasis and metabolism is altered by nuclear receptor activation; (3) gene products (translated proteins) modify the lipid content of liver to favor triglyceride accumulation and potentially cholesterol accumulation; (4) altered lipid content in the liver leads to accumulation of lipid droplets, which can lead to the development of steatosis and liver dysfunction; and (5) alterations in lipid metabolism lead to alterations in serum levels of triglycerides and cholesterol. Although individual studies have not demonstrated every step of this proposed process, each event has been demonstrated for PFOA, including steatosis in PFOA-exposed animals. It has also been suggested that PFOA could interfere with fatty acid biosynthesis by binding to the Acetyl-CoA carboxylase 1 and Acetyl-CoA carboxylase 2 enzymes; however, only a single study has demonstrated such a binding event and further research is needed to understand the plausibility of this binding occurring across species and exposure scenarios.

In addition (and potentially related) to the abundance of evidence related to hepatic nuclear receptors, PFOA also alters apoptosis and cell proliferation in the liver. Specifically, PFOA exposure at high doses causes apoptosis through a cascade of mechanisms including activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, autophagy, and activation of the p53 mitochondria pathway. PFOA has been shown to induce hepatocytic proliferation at low doses by disrupting cell cycle progression, leading to steatosis, hepatomegaly, and liver dysfunction in general.

There are other mechanisms that may be involved in PFOA-induced hepatotoxicity, but the evidence for such is limited and the relevance to liver outcomes is less clear. These include hormone perturbation, inflammatory response, and oxidative stress. There are very limited data demonstrating the potential of PFOA to perturb hormone balance, particularly related to thyroid

function. There are also a limited number of studies that reported inflammation in the liver, including changes in cytokine levels and the expression of genes involved in innate immunity. PFOA can cause oxidative stress in the liver, as demonstrated by standard indicators of oxidative stress including increased ROS levels, changes in GSH and GSSG levels, and decreased total antioxidant capacity in both in vivo and in vitro exposures to PFOA. The direct relevance of oxidative stress to liver pathology induced by PFOA requires further study, but it is clear that PFOA can cause oxidative stress. These other mechanisms that have a limited evidence base may also occur in relation to the more well-characterized mechanisms of PFOA-induced hepatotoxicity. For example, while the role of alterations in adaptive immunity in PFOA-induced liver pathology is not clear, it is plausible that the inflammatory response is related to fatty liver and associated liver dysfunction, such as the liver outcomes observed in humans and rodents, which can occur via nuclear receptor-mediated pathways.

3.4.1.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause hepatotoxicity in humans under relevant exposure circumstances (Table 3-4). This conclusion is based primarily on coherent liver effects in animal models following exposure to doses as low as 0.3 mg/kg/day PFOA. In human studies, there is consistent evidence of a positive association with ALT in adults, at median PFOA levels as low as 1.3 ng/mL. The available mechanistic information provides support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.

Table 3-4. Evidence Profile Table for PFOA Exposure and Hepatic Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.1.1)					⊕⊕⊙ *Evidence Indicates (likely)*
Serum biomarkers of hepatic injury 17 *Medium* confidence studies 5 *Low* confidence studies	Studies in adults consistently reported significant increases in ALT (9/11). Findings in adults were generally positive for AST (5/7) and GGT (7/10). Some studies reported conflicting or nonsignificant associations, however, these were mostly of *low* confidence. Occupational studies generally reported significant increases in ALT (4/7), but there were some nonsignificant associations based on type of analysis, location, or years analyzed. In occupational studies, findings for liver enzymes other than ALT were mixed, varying at times by time, location, or sex. Findings in studies of children were limited, but one study observed	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for ALT • *Coherence* of findings across biomarkers	• *Low* confidence studies	⊕⊕⊙ *Moderate* Evidence for hepatic effects is based on increases in ALT in adults, including increases in ALT in occupational populations. Supporting evidence includes increases in other liver enzymes such as AST and GGT, and increased incidence of liver disease mortality in occupational settings. Minor uncertainties remain regarding mixed liver enzyme findings in children and coherence across biomarkers and limited availability of	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of hepatoxicity as noted by increased serum biomarkers of hepatic injury (primarily ALT) with coherent results for increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury in animal models. Although associations between PFOA exposure and other serum biomarkers of hepatic injury were identified in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies. *Human relevance and other inferences:* The available mechanistic information overall provide support for the biological

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
				high-quality studies on liver disease.	plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.
	significant positive associations for ALT, AST, and GGT in girls (1/3).				
Liver disease or injury 4 *Medium* confidence studies 3 *Low* confidence studies 1 *Mixed* confidence study	A limited number of studies examined liver disease or injury in general population adults and occupational populations. One occupational study reported significantly higher mortality from cirrhosis of the liver compared with a group of similar, non-exposed workers (1/1). Two occupational and one general population study reported no significant	• No factors noted	• Association only observed in *Low* confidence studies • *Lack of coherence* of across measures of liver inflammation		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	association with any form of liver disease (0/3). Other measures of inflammation in the liver were mixed and lacked coherence.				
Serum protein 4 *Medium* confidence studies 2 *Low* confidence studies	Significant increases in albumin were consistently observed in adults (4/5), while findings from the single occupational study were nonsignificant. Findings for total serum protein and fibrinogen were mixed or imprecise.	• *Medium* confidence studies • *Consistent direction of effect* for albumin	• *Low* confidence studies • *Imprecision* of findings for fibrinogen and other serum proteins		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Serum iron 1 *Medium* confidence study	Only one large cross-sectional study examined serum iron concentrations and reported a significant positive association.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.1.2)					
Histopathology 3 *High* confidence studies 11 *Medium* confidence studies	Histopathological alterations in liver were observed in male and female rodents exposed to PFOA for various durations (14/14). Increased hepatocellular hypertrophy (10/14) and necrosis (5/12) were the most common lesions. Other lesions included inflammation or cellular infiltration (5/14), cytoplasmic alteration or vacuolation (3/12), mitosis or mitotic figures (3/12), bile duct hyperplasia (2/13), cystic/cystoid degeneration	• *High* and *medium* confidence studies *Consistent direction of effects* across study design, sex, and species • *Dose-dependent response* • *Coherence* of findings across other endpoints indicating liver damage (i.e., increased serum biomarkers and liver weight) • *Large magnitude of effect,* with some responses reaching 100%	• No factors noted	⊕⊕⊕ *Robust* Evidence is based on 26 *high* or *medium* confidence animal toxicological studies indicating increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	(2/12), fatty change (2/13), and/or pigment (1/12).	incidence in some dose groups (i.e., hypertrophy, vacuolation, single-cell death) or are considered severe		that indicate functional impairment or lesions. EPA considers responses such as increased	
		(i.e., cell or tissue death/necrosis and cystoid degeneration)		relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant (i.e., greater than 100% change) increases in enzymes indicative of hepatobiliary damage. Many of the studies discussed in this section reported dose-dependent increases in liver weight and	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
			hepatocellular hypertrophy in rodents of both sexes. Although a limited number of these studies additionally examined functional or histopathological hepatic impairment, several provide evidence of adverse hepatic responses.		
Liver weight 5 *High* confidence studies 21 *Medium* confidence studies	Absolute (17/21) and relative (18/22) liver weights were increased in male and female rodents exposed to PFOA for various durations. Several studies that included both males and females suggested that males may be more sensitive than females (4/7).	• *High* and *medium* confidence studies • *Consistent direction* of effects across study design, sex, and species • *Dose-dependent response* • *Coherence* of effects with other responses indicating	• *Confounding* variables such as decreases in body weights		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
		increased liver size (e.g., hepatocellular hypertrophy)			
Serum biomarkers of hepatic injury 3 *High* confidence studies 7 *Medium* confidence studies	Increases were observed in ALT (6/9), AST (6/7), ALP in (4/6), and GGT (1/1). Biologically significant changes (≥100%) in an enzyme level were observed in 6/9 studies. Albumin (5/6) and albumin/globulin ratio (3/3) were increased. Bile acids were increased in males (4/4) and	• *High* and *medium* confidence studies • *Consistent direction of effects* across study design, sex, and species • *Dose-dependent response* • *Coherence* of findings with other responses indicating	• *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation						Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**		**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	unchanged in females (3/3). Inconsistent changes in	hepatobiliary damage (i.e., histopatho logical lesions)				
	bilirubin were observed with direct bilirubin increased in males (2/2) or females (0/1), increased indirect bilirubin in males (1/1), and mixed effects on total bilirubin in males (2) and transient effects in females (1). Total protein was decreased in males (3/5) and females (1/4).	• *Large magnitude of effect*, with evidence of biologically significant increases (i.e., ≥100% control responses) in serum liver enzymes indicating adversity				

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Mechanistic Evidence and Supplemental Information (Section 3.4.1.3)					
Biological Events or Pathways	**Summary of Key Findings, Interpretation, and Limitations**		**Evidence Stream Judgment**		
Molecular initiating events – PPARα	**Key findings and interpretation:** • Activation of human PPARα in vitro. • Increased expression of PPARα-target genes in vitro in rat and human hepatocytes, and cells transfected with rat or human PPARα. • Altered expression of genes involved in lipid metabolism and lipid homeostasis. **Limitations:** • Increased hepatic lipid content has also been reported for PFOA in the absence of a strong PPARα response.		Overall, studies in rodent and human in vitro and in vivo models suggest that PFOA induces hepatic effects, at least in part, through PPARα. The evidence also suggests a role for PPARα-independent pathways in the MOA for noncancer liver effects of PFOA.		
Molecular or cellular initiating events – other pathways	**Key findings and interpretation:** • Increased apoptosis is a high dose effect demonstrated in vivo, as well as in vitro, occurring through a cascade of mechanisms: ○ activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, autophagy, and activation of p53 mitochondria pathway. • Inflammation of the liver (e.g., changes in cytokine levels and the expression of genes involved in innate				

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	immunity) has been reported in a limited number of studies. • Induction of oxidative stress in vivo and in vitro, including increased ROS levels, changes in GSH and GSSG levels, and decreased total antioxidant capacity. • Indirect evidence of activation of alternative pathways, including activation of other nuclear receptors, primarily CAR and PPARγ, following observations in knockout or humanized PPARα mice. **Limitations:** • The direct relevance of oxidative stress to liver pathology induced by PFOA requires further study. • Very limited database for other pathways, with the exception of apoptosis and cell cycle changes.				

Notes: ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CAR = constitutive androstane receptor; EPA = Environmental Protection Agency; GGT = gamma-glutamyl transferase; GSH = glutathione; GSSG = glutathione disulfide; LDH = lactate dehydrogenase; MOA = mode of action; PPARγ = peroxisome proliferator-activated receptor gamma; PPARα = peroxisome proliferator-activated receptor alpha; ROS = reactive oxygen species.

3.4.2 Immune

EPA identified 50 epidemiological and 13 animal toxicological studies that investigated the association between PFOA and immune effects. Of the epidemiological studies, 1 was classified as *high* confidence, 29 as *medium* confidence, 12 as *low* confidence, 6 as *mixed* (6 *medium/low*) confidence, and 2 were considered *uninformative* (Section 3.4.2.1). Of the animal toxicological studies, 3 were classified as *high* confidence, 9 as *medium* confidence, and 1 was considered *mixed* (*medium*/*low)* confidence (Section 3.4.2.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.2.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.2.1.1 Immunosuppression

Immune function – specifically immune system suppression – can affect numerous health outcomes, including risk of common infectious diseases (e.g., colds, influenza, otitis media) and some types of cancer. The WHO guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications (WHO, 2012).

There are 13 epidemiological studies (14 publications[9]) from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and immunosuppresive effects. Study quality evaluations for these 14 studies are shown in Figure 3-19. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-5 and below.

[9] Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407

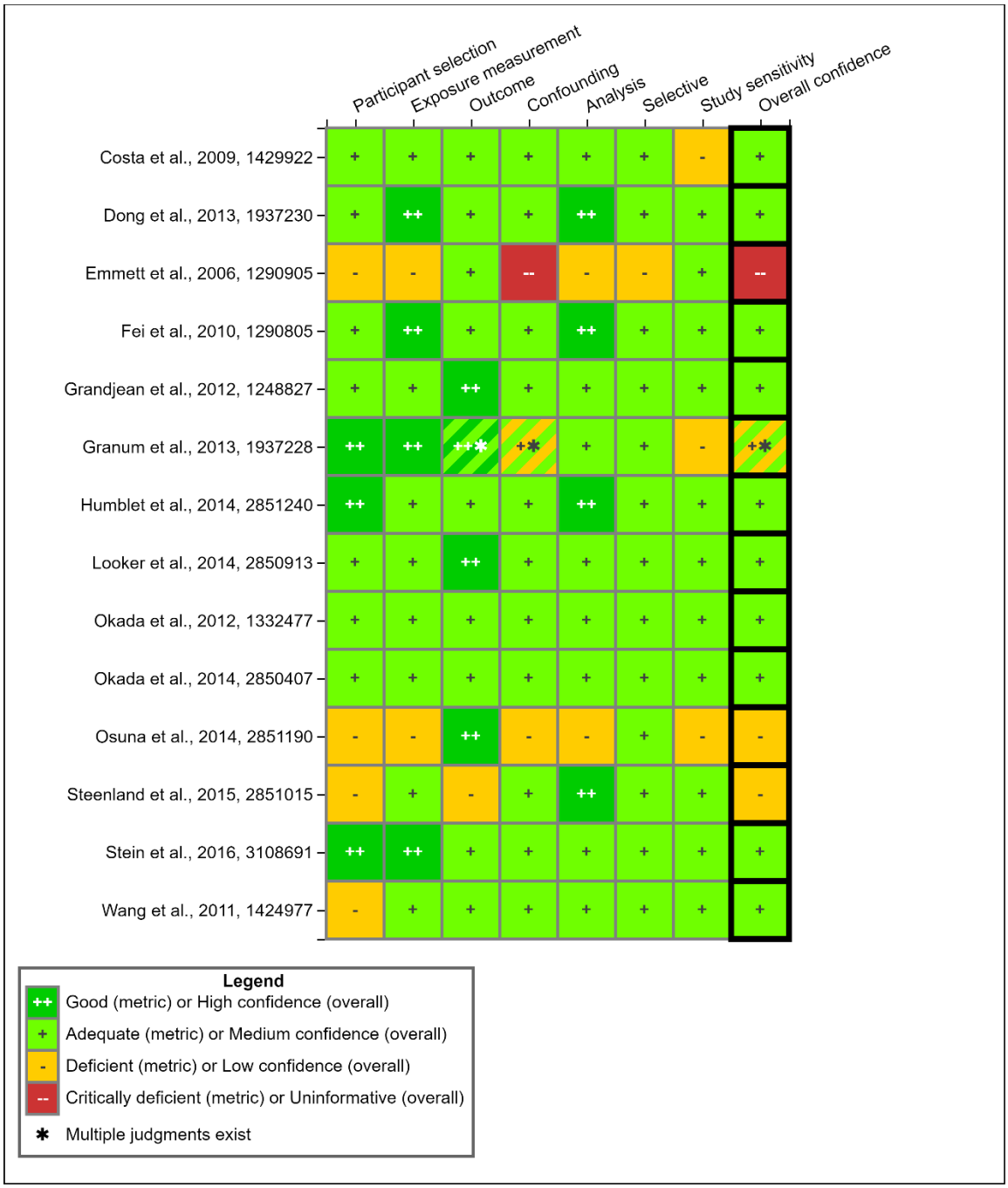


Figure 3-19. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immune Effects Published Before 2016 (References in 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Three studies reported decreases in response to one or more vaccines in relation to higher PFOA exposure in children (Granum et al., 2013; Grandjean et al., 2012) and adults (Looker et al., 2014). Antibody responses for diphtheria and tetanus in children (n = 587) were examined at multiple timepoints in a study on a Faroese birth cohort (Grandjean et al., 2012). Prenatal and

age five serum PFOA concentrations were inversely associated with childhood anti-diphtheria antibody response at all measured timepoints, and the association was significant for anti-diphtheria antibody response at age seven in separate models for prenatal and age five serum PFOA concentrations. The association was less pronounced when examining anti-tetanus antibody responses in relation to prenatal PFOA measurements, but the anti-tetanus antibody response (age seven) was significantly decreased in relation to PFOA measured in child serum at five years of age. Another study on Faroese children conducted a pilot investigation on the association between elevated PFOA exposure and autoantibodies to antigens indicating tissue damage, but the results were unclear (Osuna et al., 2014). Prenatal PFOA exposure was associated with diminished vaccine response in a different birth cohort study (Granum et al., 2013). Decreases in the anti-rubella antibody response were significantly associated with elevated prenatal PFOA concentrations among three-year-old children. Stein et al. (2016b) reported significant inverse associations between PFOA exposure and mumps and rubella antibody concentrations in adolescents (12–19 years old) from multiple NHANES cycles (1999–2000, 2003–2004), but no association was observed for measles. A C8 Health Project study examining influenza vaccine responses in highly exposed adults (Looker et al., 2014) observed that pre-vaccination PFOA concentrations were inversely associated with GM A/H3N2 antibody titer rise, but no association was found with antibody titers for A/H1N1 and influenza type B. In the studies of children, there was concern that the associations were also seen with other correlated PFAS, but this was not considered a limitation in the study in adults, which was conducted in a population with known high PFOA exposure (the C8 Health Project study).

Associations between prenatal PFOA exposure and risk of infectious diseases (as a marker of immune suppression) were not observed in one study, although there was some indication of effect modification by gender (i.e., associations seen in females but not in males). Fei et al. (2010b) examined hospitalizations for infectious diseases in early childhood in a Danish birth cohort with mean maternal PFOA concentration of 0.0056 μg/mL. A slightly higher risk for hospitalizations was observed in females whose mothers had higher PFOA concentrations (incidence rate ratio [IRR] = −1.20, 1.63, 1.74 for quartile 2 [Q2], quartile 3 [Q3], and quartile 4 [Q4], respectively compared with quartile 1 [Q1]; see Appendix D, (U.S. EPA, 2024a)), and the risk for males was below 1.0 for each quartile. Overall, there was no association between hospitalizations due to infectious diseases and maternal PFOA exposure.

Overall, the 2016 PFOA HESD (U.S. EPA, 2016c) found consistent evidence of an association between PFOA exposure and immunosuppression.

Table 3-5. Associations Between Elevated Exposure to PFOA and Immune Outcomes from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Population	Tetanus Ab[a]	Diphtheria Ab[a]	Rubella Ab[a]	Influenza Ab[a]	Infectious Disease[b]	Asthma[b]	Eczema[b]	Autoimmune Disease[b]	White Blood Cell Count[a]
Costa 2009, 1429922 *Medium*	Cohort	Occupational	NA	NA	NA	NA	NA	NA	NA	NA	↑
Dong, 2013, 1937230 *Medium*	Case-control	Children	NA	NA	NA	NA	NA	↑↑	NA	NA	NA
Fei, 2010, 1290805 *Medium*	Cohort	Children	NA	NA	NA	NA	–	NA	NA	NA	NA
Grandjean, 2012, 1248827 *Medium*	Cohort	Children	↓↓	↓↓	NA	NA	NA	NA	NA	NA	NA
Granum, 2013, 1937228 *Mixed*[c]	Cohort	Children	–	NA	↓↓	NA	↑↑	–	–	NA	NA
Humblet, 2014, 2851240 *Medium*	Cross-sectional	Adolescents	NA	NA	NA	NA	NA	↑↑	NA	NA	NA
Looker, 2014, 2850913 *Medium*	Cohort	Adults	NA	NA	NA	↓↓	–	NA	NA	NA	NA
Okada, 2014, 2850407 *Medium*	Cohort	Children	NA	NA	NA	NA	↑	↑	–	NA	NA
Steenland, 2015, 2851015 *Low*	Cohort	Adults	NA	NA	NA	NA	NA	NA	NA	↑↑	NA
Stein, 2016, 3108691 *Medium*	Cross-sectional	Adolescents	NA	NA	↓↓	NA	NA	↑	NA	NA	NA
Wang, 2011, 1424977 *Medium*	Cohort	Children	NA	NA	NA	NA	NA	NA	↓	NA	NA

Notes: Ab = antibody; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Emmett et al., 2006, 1290905 was not included in the table due to their *uninformative* overall study confidence ratings.

Osuna, 2014, 2851190 analyzed autoantibody response to indicators of tissue damage and was not included in the table.

Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407, which was considered the most updated data.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Granum, 2013, 1937228 was rated *medium* confidence for antibody response, common cold, and gastroenteritis, and *low* confidence for all other outcomes.

3.4.2.1.2 Immunosuppression Study Quality Evaluation and Synthesis from the Updated Literature Review

There are 27 epidemiological studies identified from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated associations between prenatal, childhood, or adult PFOA exposure and immunosuppression since publication of the 2016 PFOA HESD. Study quality evaluations for these 27 studies are shown in Figure 3-20 and Figure 3-21.

One study from the 2016 assessment (Grandjean et al., 2012) was updated during this period, and the update was included in the systematic review (Grandjean et al., 2017a).

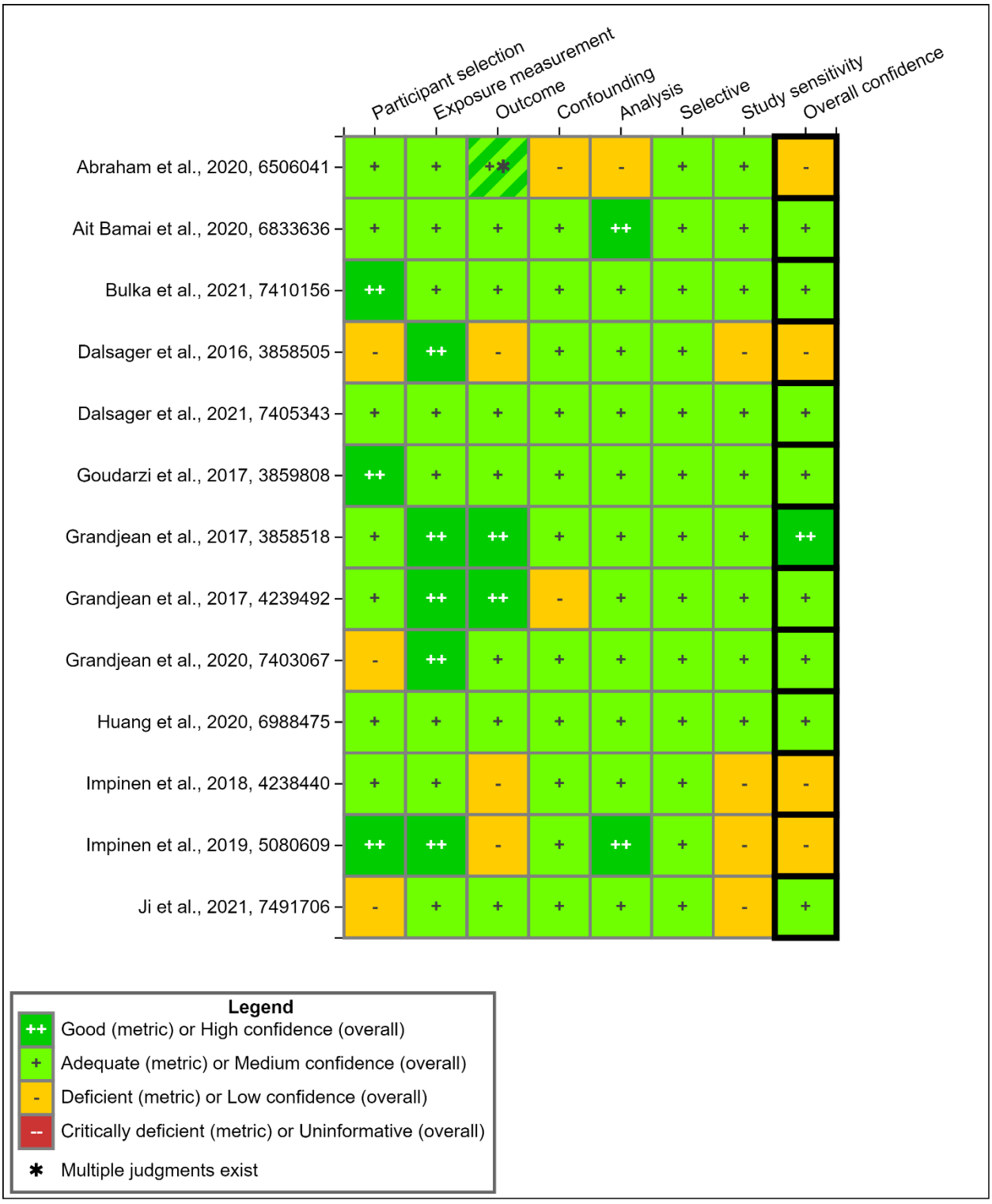


Figure 3-20. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immunosuppression Effects

Interactive figure and additional study details available on HAWC.

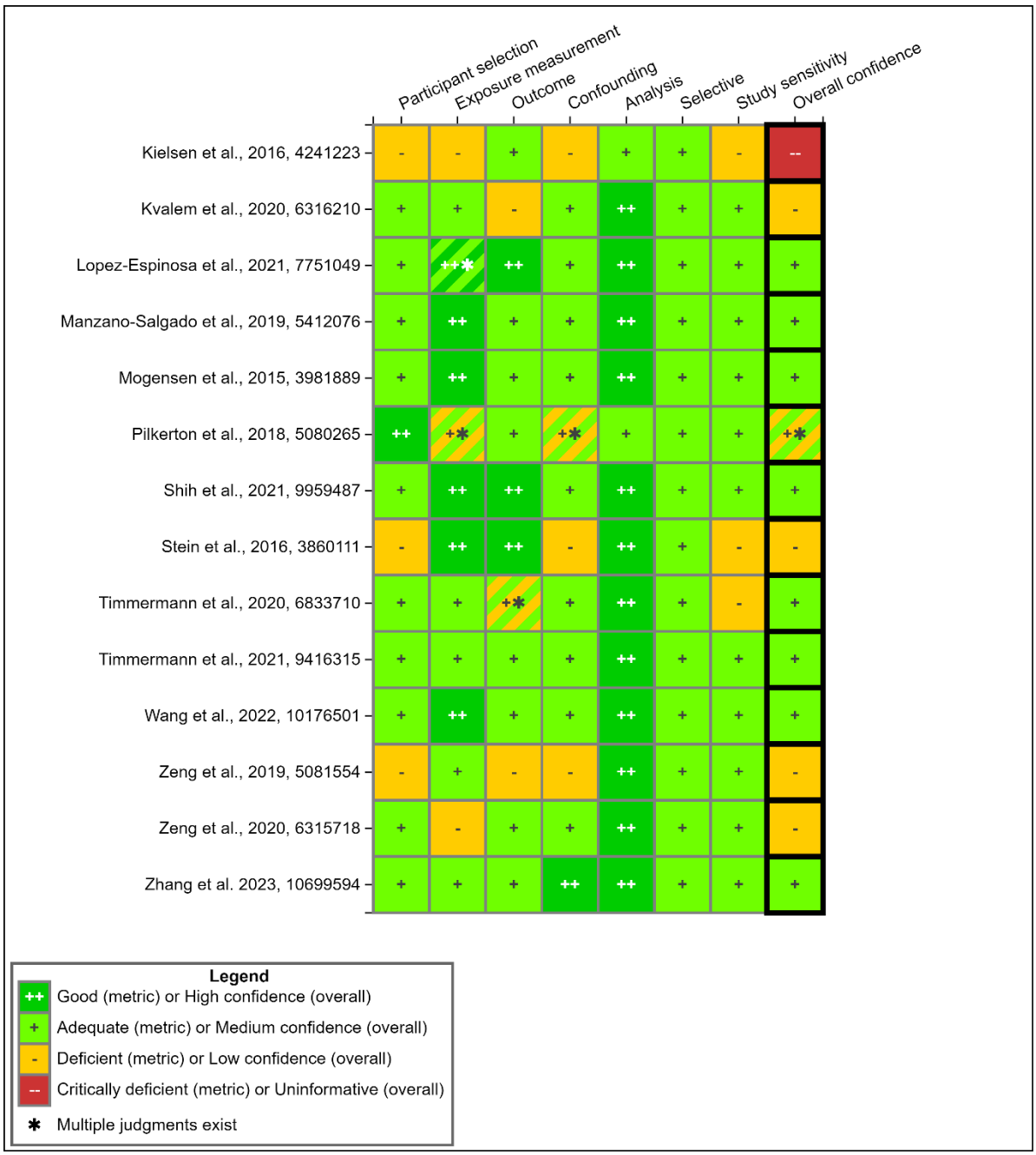


Figure 3-21. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immunosuppression Effects (Continued)

Interactive figure and additional study details available on HAWC.

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

3.4.2.1.2.1 Vaccine Response

Ten studies (11 publications[10][11]) studied the relationship between antibody response to vaccination and PFOA exposure. Five of these studies (six publications) investigated antibody response to vaccination in children (Timmermann et al., 2021; Abraham et al., 2020; Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a). In adults, two studies investigated antibody response to diphtheria and tetanus (Shih et al., 2021; Kielsen et al., 2016), one study investigated hepatitis vaccine response (Shih et al., 2021), one study investigated adult flu vaccine response (Stein et al., 2016a), one study measured rubella antibodies in both adolescents (aged 12 and older) and adults (Pilkerton et al., 2018), and one study measured rubella, measles, and mumps antibodies in adolescents (Zhang et al., 2023). In addition to these studies on vaccine response, one study (Zeng et al., 2019b) measured natural antibody response to hand, foot, and mouth disease (HFMD), and one study (Zeng et al., 2020) measured antibody response to hepatitis B infection in adults. Overall, eight studies were *medium* confidence (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Timmermann et al., 2020; Pilkerton et al., 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a), four were *low* confidence (Abraham et al., 2020; Zeng et al., 2020; Stein et al., 2016a Zeng, 2019, 5081554), and one study (Kielsen et al., 2016) was *uninformative*.

Of the studies that measured antibody response to vaccination in children and adolescents, four studies were cohorts (Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a), and four were cross-sectional (Zhang et al., 2023; Timmermann et al., 2021; Abraham et al., 2020; Pilkerton et al., 2018) (maternal serum was also available for a subset of participants in Timmermann et al. (2021)). These included multiple prospective birth cohorts in the Faroe Islands, one with enrollment in 1997–2000 and subsequent follow-up to age 13 (Grandjean et al., 2017a) and one with enrollment in 2007–2009 and follow-up to age five (Grandjean et al., 2017b). One additional cohort in the Faroe Islands examined outcomes in adults with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021). Five of these studies measured antibody response to tetanus vaccination (Timmermann et al., 2021; Abraham et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a); the same studies also measured antibody response to diphtheria vaccination; two studies measured antibody response to measles vaccination (Zhang et al., 2023; Timmermann et al., 2020), two studies measured antibody response to rubella vaccination (Zhang et al., 2023; Pilkerton et al., 2018) one study measured antibody response to mumps vaccination (Zhang et al., 2023), and one study to *Haemophilus influenza* type b (Hib) antibodies (Abraham et al., 2020).

The results for this set of studies in children are shown in Table 3-6 and Appendix D (U.S. EPA, 2024a). The Faroe Islands studies (Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a) observed associations between higher levels of PFOA and lower antibody levels against tetanus and diphtheria in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. These studies measured PFOA exposure levels in maternal blood during the perinatal period and at later time periods from children (at ages 5, 7, and 13 years). There are a few results in the opposite direction for sub-

[10] Multiple publications of the same study: the study populations are the same in Grandjean et al. (2017a) and Mogensen et al. (2015a).
[11] Zhang (2023) analyzes NHANES cycles 2003–2004 and 2009–2010 partially overlapping with Pilkerton (2018) and Stein (2016b) which both analyze cycles 1999–2000 and 2003–2004.

analyses of the Faroe Island cohorts (Grandjean et al., 2017b; Grandjean et al., 2017a), such as maternal PFOA exposure and anti-tetanus antibodies at 7 years (Table 3-6). No biological rationale has been identified as to whether one particular time period or duration of exposure or outcome measurement is more sensitive to an overall immune response to PFOA exposure. Changes in tetanus and diphtheria antibody concentrations in children from all *high* and *medium* confidence studies are provided in Figure 3-22 and Figure 3-23.

It is plausible that the observed associations between decreased antibody concentration and PFOA exposure observed in the Faroe Islands cohort could be partially explained by confounding across the PFAS (e.g., exposure levels to PFOS were higher than PFOA (PFOS 17 ng/mL, PFOA 4 ng/mL); there was a moderately high correlation between PFOA and PFOS, PFHxS, and PFNA (0.50, 0.53, 0.54, respectively) (Grandjean et al., 2017b; Grandjean et al., 2017a). To investigate this, the authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018). In these analyses, estimates were adjusted for PFOS and there was no notable attenuation of the observed effects. The other available studies did not perform multipollutant modeling, so it is difficult to determine the potential for highly correlated PFAS to confound the effect estimates. However, as described above, one study (Looker et al., 2014) observed an association with PFOA in a population where PFOA exposure predominated (the C8 Health Project population), and this is not likely to be confounded by other PFAS. Overall, the available evidence suggests that confounding is unlikely to explain the observed effects.

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOA at 7 years median (25th-75th percentile)= 4.4 ng/mL (3.5-5.7 ng/mL)	percent change (per doubling of PFOA)	--	Age 7	Age 13	9.4
	PFOA at 13 years median (25th-75th percentile)=2.0 ng/mL (1.6-2.5 ng/mL)	percent change (per doubling of PFOA)	--	Age 13	Age 13	3.3
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOA: Geometric mean=4.06 ng/mL (25th-75th percentile=3.33-4.96 ng/mL)	Percent difference (per doubling in age 5 PFOA)	--	Age 5	Age 7	-35.8
			Adj for Age 5 Ab	Age 5	Age 7	-28.2
			Pre-booster	Age 5	Age 5	-13.3
			Post-booster	Age 5	Age 5	-9.7
	Maternal PFOA: Geometric mean=3.20 ng/mL (25th-75th percentile=2.56-4.01 ng/mL)	Percent difference (per doubling in maternal PFOA)	--	Prenatal	Age 7	7.4
			Adj for Age 5 Ab	Prenatal	Age 7	12.3
			Pre-booster	Prenatal	Age 5	-10.5
			Post-booster	Prenatal	Age 5	14.5
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOA)	Cohort 3	Age 1.5	Age 5	-19.24
				Age 5	Age 5	-13.28
				Cord blood	Age 5	-10.46
			Cohort 3 and 5	Age 1.5	Age 5	-16.47
				Age 5	Age 5	-18.75
				Cord blood	Age 5	-17.59
			Cohort 5	Cord blood	Age 5	-22.25
	Median = 2.2 ng/mL (25th - 75th percentile: 1.8 - 2.8 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 5	Age 5	-25.26
	Median = 2.8 ng/mL (25th - 75th percentile: 2.0 - 4.5 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 1.5	Age 5	-16.31
Mogensen et al. (2015, 3981889), Medium	median=4.4 ng/ml (25th-75th percentile=3.5-5.7 ng/ml)	Percent change per doubling of PFOA	Age 7	Age 7	Age 7	-20.5
Timmermann et al. (2022, 9416315), Medium	median=2.13 ng/mL (25th - 75th percentiles: 1.68 - 2.54 ng/mL)	percent difference (per unit increase in maternal PFOA concentration)	Ages 7-12	Prenatal	Age 7-12	-7
	median=2.28 ng/mL (25th - 75th percentiles: 1.89 - 2.88 ng/mL)	percent difference (per unit increase in child PFOA concentration)	Ages 7-12	Age 7-12	Age 7-12	-8

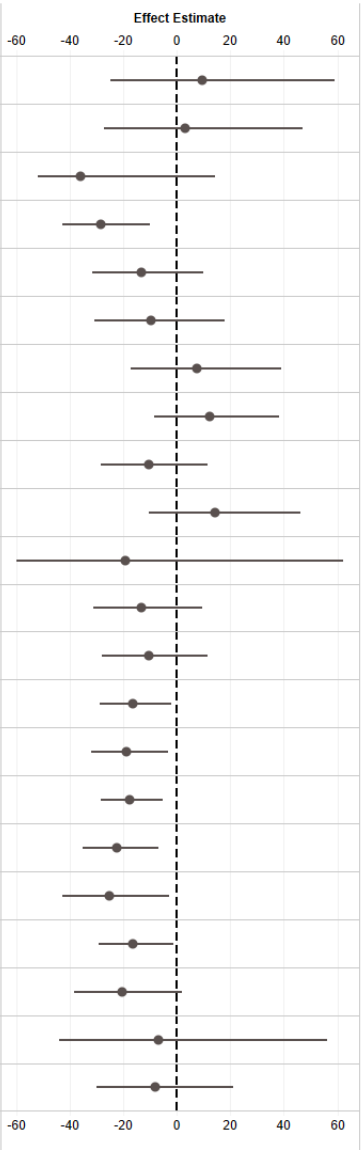
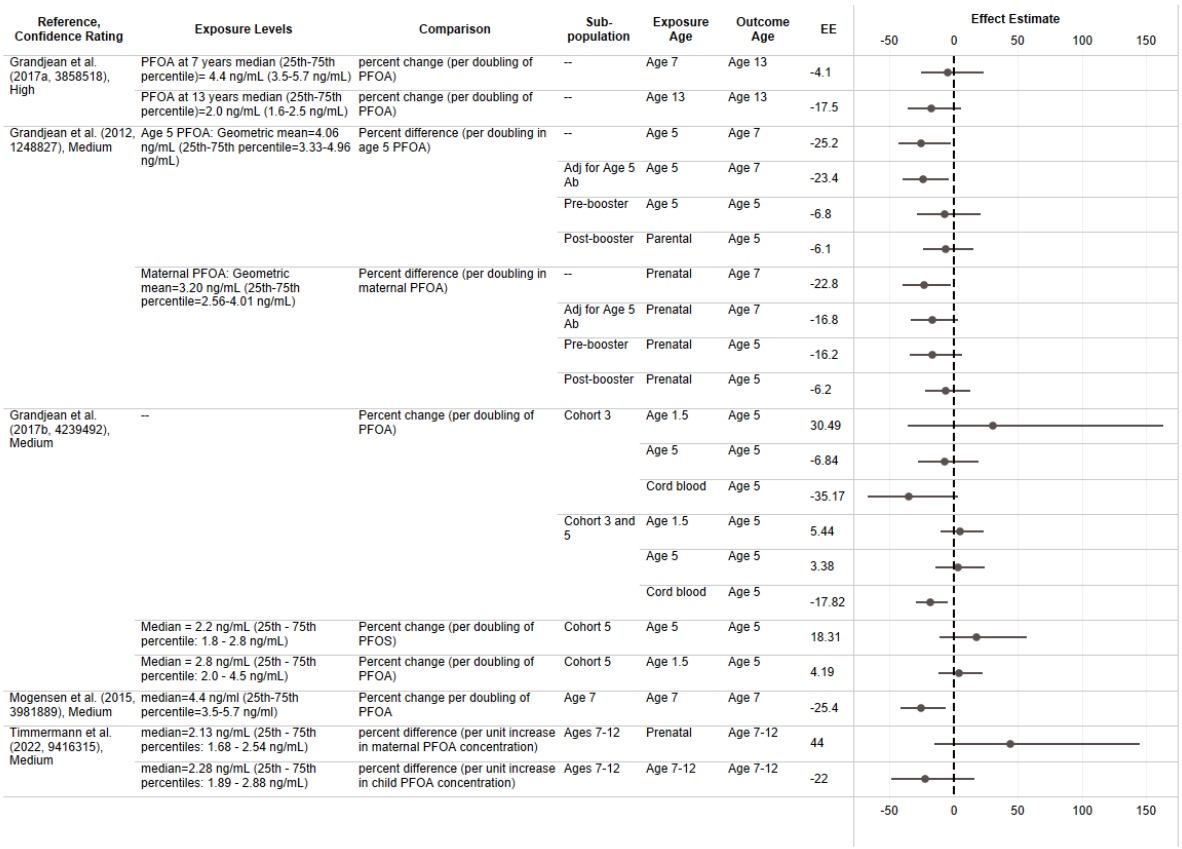


Figure 3-22. Overall Tetanus Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOA HESD.



Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOA at 7 years median (25th-75th percentile)= 4.4 ng/mL (3.5-5.7 ng/mL)	percent change (per doubling of PFOA)	--	Age 7	Age 13	-4.1
	PFOA at 13 years median (25th-75th percentile)=2.0 ng/mL (1.6-2.5 ng/mL)	percent change (per doubling of PFOA)	--	Age 13	Age 13	-17.5
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOA: Geometric mean=4.06 ng/mL (25th-75th percentile=3.33-4.96 ng/mL)	Percent difference (per doubling in age 5 PFOA)	--	Age 5	Age 7	-25.2
			Adj for Age 5 Ab	Age 5	Age 7	-23.4
			Pre-booster	Age 5	Age 5	-6.8
			Post-booster	Parental	Age 5	-6.1
	Maternal PFOA: Geometric mean=3.20 ng/mL (25th-75th percentile=2.56-4.01 ng/mL)	Percent difference (per doubling in maternal PFOA)	--	Prenatal	Age 7	-22.8
			Adj for Age 5 Ab	Prenatal	Age 7	-16.8
			Pre-booster	Prenatal	Age 5	-16.2
			Post-booster	Prenatal	Age 5	-6.2
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOA)	Cohort 3	Age 1.5	Age 5	30.49
				Age 5	Age 5	-6.84
				Cord blood	Age 5	-35.17
			Cohort 3 and 5	Age 1.5	Age 5	5.44
				Age 5	Age 5	3.38
				Cord blood	Age 5	-17.82
	Median = 2.2 ng/mL (25th - 75th percentile: 1.8 - 2.8 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 5	Age 5	18.31
	Median = 2.8 ng/mL (25th - 75th percentile: 2.0 - 4.5 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 1.5	Age 5	4.19
Mogensen et al. (2015, 3981889), Medium	median=4.4 ng/ml (25th-75th percentile=3.5-5.7 ng/ml)	Percent change per doubling of PFOA	Age 7	Age 7	Age 7	-25.4
Timmermann et al. (2022, 9416315), Medium	median=2.13 ng/mL (25th - 75th percentiles: 1.68 - 2.54 ng/mL)	percent difference (per unit increase in maternal PFOA concentration)	Ages 7-12	Prenatal	Age 7-12	44
	median=2.28 ng/mL (25th - 75th percentiles: 1.89 - 2.88 ng/mL)	percent difference (per unit increase in child PFOA concentration)	Ages 7-12	Age 7-12	Age 7-12	-22

Figure 3-23. Overall Diphtheria Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOA HESD.

Table 3-6. Associations between PFOA Exposure and Vaccine Response in Faroe Islands Studies

Exposure measurement timing, PFOA levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOA by Age at Assessment			Tetanus Antibody Associations with PFOA by Age at Assessment		
	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)
Maternal C3: GM: 3.20 (2.56–4.01)	↓ (C3; age, sex)[b] BMD/BMDL (C3 and 5; sex, birth cohort, log-PFOA)[c]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b]	–	↓ (C3; age, sex)[b] BMD/BMDL (C3 and 5; sex, birth cohort, log-PFOA)[c]	↑ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b]	–
Birth (modeled)	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	–	–
18 mo C3: NR C5: 2.8 (2.0–4.5)	↑ (C3; age, sex)[d] ↑ (C3 and 5; age, sex)[d] ↑ (C5; age, sex)[d]		–	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]		–
5 yr C3: GM: 4.06 (3.33–4.96) C5: 2.2 (1.8–2.8)	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↑ (C3 and 5; age, sex)[d] ↑ (C5; age, sex)[d]	↓↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b] BMD/BMDL (C3; sex, age, and booster type at age 5 yr)[e] BMD/BMDL (C3; sex, booster type at age 5 yr, log-PFOA)[c]	–	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	↓↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b] BMD/BMDL (C3; sex, age, and booster type at age 5 yr)[e] BMD/BMDL (C3; sex, booster type at age 5 yr, log-PFOA)[c]	–

Exposure measurement timing, PFOA levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOA by Age at Assessment			Tetanus Antibody Associations with PFOA by Age at Assessment		
	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)
7 yr C3: 4.4 (3.5–5.7)	–	↓↓ (C3; age, sex, booster type)[f] ↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	–	↓ (C3; age, sex, booster type)[f] ↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]
13 yr C3: 2.0 (1.6–2.5)	–	–	↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	–	–	↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]

Notes: C3 = cohort 3, born 1997–2000; C5 = cohort 5, born 2007–2009; GM = geometric mean; NR = not reported.
Arrows indicate direction of association with PFOA levels; double arrows indicate statistical significance ($p < 0.05$) where reported. Arrows are followed by parenthetical information denoting the cohort(s) studied and confounders (factors the models presented adjusted for).
[a] Exposure levels reported from serum as median (25th–75th percentile) unless otherwise noted.
[b] Grandjean et al. (2012); *medium* confidence
[c] Budtz-Jørgensen and Grandjean (2018); *medium* confidence
[d] Grandjean et al. (2017b); *medium* confidence
[e] Grandjean and Budtz-Jørgensen (2013); *medium* confidence
[f] Mogensen et al. (2015a); *medium* confidence
[g] Grandjean et al. (2017a); *high* confidence

A cross-sectional study of these antibody levels in Greenlandic children (Timmermann et al., 2021) reported results that differed in direction of association based on the covariate set selected. The exposure measurement in these analyses may not have represented an etiologically relevant window; cross-sectional analyses in the Faroe Islands studies at similar ages also found weaker associations than analyses for some other exposure windows. A subset of the study population did have maternal samples available, and those results were also inconsistent by vaccine. However, this study was the only one to examine the OR for not being protected against diphtheria (antibody concentrations, which has clear clinical significance, and they reported elevated odds of not being protected (based on antibody concentrations <0.1 IU/mL, OR (95% CI) per unit increase in exposure: 1.41 (0.91, 2.19)).

In children from Guinea-Bissau, West Africa, Timmermann et al. (2020) observed nonsignificant associations between elevated levels of PFOA and decreased adjusted anti-measles antibody levels across time in the group with no measles vaccination at age 9 months. This association was not seen in the group with one measles vaccination. The same pattern was observed at the 2-year follow-up.

Two *medium* cross-sectional studies of adolescents examined associations between elevated levels of PFOA and vaccine response (Zhang et al., 2023; Pilkerton et al., 2018). Inverse associations were observed in cross-sectional analyses in adolescents from NHANES (2003–2004; 2009–2010) for rubella, mumps, and measles (Zhang et al., 2023), including a significant reduction in the antibody response to mumps per 2.7-fold increase in serum (Figure 3-24). No association was observed for rubella vaccine response in the other cross-sectional study of adolescents (Pilkerton et al., 2018), however, an overlapping study (Stein et al., 2016b) reporting on adolescents from the same NHANES cycles (i.e., 1999–2000 and 2003–2004) observed a significant inverse association in adolescents seropositive for rubella.

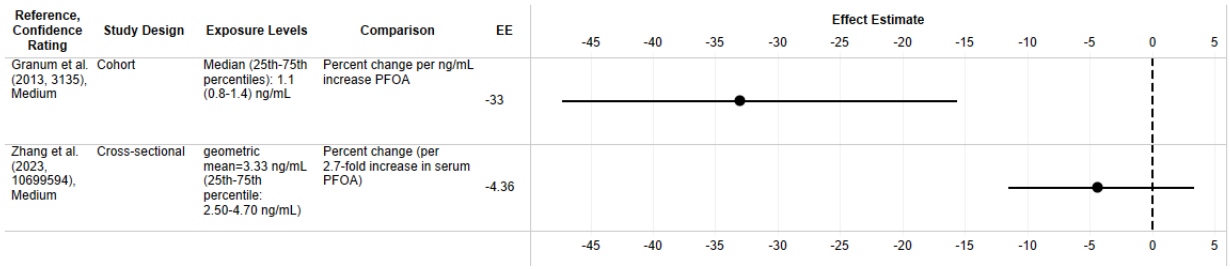


Figure 3-24. Overall Rubella Antibody Levels in Children and Adolescents from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Adolescent regression coefficients from Pilkerton, 2018, 5080265 were not reported quantitatively.
Regression coefficients from Granum, 2013 were re-expressed as percent change.

Lastly, the *low* confidence cross-sectional study of one-year-old children in Germany, Abraham et al. (2020), reported statistically significant correlations between PFOA concentrations and adjusted levels of antibodies against tetanus, Hib, and diphtheria.

Of the three studies that measured vaccine response in adults, two were cohorts (Shih et al., 2021; Stein et al., 2016a) and one was a cross-sectional analysis (Pilkerton et al., 2018). The

medium confidence study by Shih et al. (2021) measured PFOA in cord blood and at multiple points through childhood to early adulthood in people in the Faroe Islands, with outcome measurement at age 28 years. The study by Stein et al. (2016a) was rated *low* confidence because it utilized convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding. The study of the adult population in Pilkerton et al. (2018) was considered *low* confidence as the analysis suffered from potential exposure misclassification due to concurrent exposure and outcome measurements, considering the amount of time since rubella vaccination in childhood. This was less of a concern for the study of adolescent participants, which was rated as *medium* confidence.

In adults and adolescents, results were less consistent than in children. Shih et al. (2021) reported inverse associations for all exposure windows in the total cohort (not statistically significant) for hepatitis B antibodies but for other vaccines (diphtheria, tetanus, and hepatitis A), the direction of association was inconsistent across exposure windows. Results also differed by sex for all vaccines, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes in men). Similar to the results in 13-year-old children in the other Faroe Islands cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Pilkerton et al. (2018) observed statistically significant associations between high-quartile PFOA levels and decreased rubella IgA levels compared with low-quartile PFOA levels in adult men. Stein et al. (2016a) reported no immunosuppression based on seroconversion following FluMist vaccination.

Despite the imprecision (i.e., wide CIs) of some of the exposure-outcome analysis pairs, the findings are generally consistent with respect to an association between PFOA exposure and immunosuppression in children. Changes in antibody levels of 10%–20% per doubling of exposure were observed in the Faroe Islands cohorts (Grandjean et al., 2017b; Grandjean et al., 2017a). The variability in some of the results could be related to differences in etiological relevance of exposure measurement timing, vaccine type, and timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available evidence. Overall, the evidence indicates an association between increased serum PFOA levels and decreased antibody production following routine vaccinations, particularly in children.

In addition to these studies of antibody response to vaccination, there are two studies that examined antibody response to HFMD (Zeng et al., 2019b) and hepatitis B infection (Zeng et al., 2020). This birth cohort study in China (Zeng et al., 2019b) measured antibody levels in infants at birth and age 3 months, which represent passive immunity from maternal antibodies. This study (Zeng et al., 2019b) was rated *low* confidence because the clinical significance of the outcome is difficult to interpret in infants and there are concerns for confounding by timing of HFMD infection as well as other limitations. Statistically significant increased odds of HFMD antibody concentration below clinically protective levels per doubling of PFOA were observed. This is coherent with the vaccine antibody results, but there is uncertainty due to study deficiencies. Zeng et al. (2020) observed negative associations ($p > 0.05$) between serum PFOA concentration and hepatitis B surface antibody; however, there are study limitations due to concurrent measurement of exposure and outcome and potential for reverse causality, and this study was rated *low* confidence.

In a C8 Health Project study, Lopez-Espinoza et al. (2021) measured serum PFAS and white blood cell types in 42,782 adults in 2005–2006 and 526 adults in 2010 from an area with PFOA drinking water contamination in the Mid-Ohio Valley (USA). Generally positive monotonic associations between total lymphocytes and PFOA were found in both surveys (difference range: 1.12%–5.50% for count and 0.36–1.24 for percentage, per PFOA IQR increment). Findings were inconsistent for lymphocyte subtypes. However, the magnitude of the differences was small.

3.4.2.1.2.2 Infectious Disease

Overall, 10 studies (11 publications)[12] measured associations between PFOA exposure and infectious diseases (or disease symptoms) in children with follow-up ranging between 1 and 16 years. Infectious diseases measured included common cold, respiratory tract infections, respiratory syncytial virus, otitis media, pneumonia, chickenpox (varicella), bronchitis, bronchiolitis, ear infections, gastric flu, urinary tract infections, and streptococcus. Of the studies measuring associations between infectious disease and PFOA exposure, eight (nine publications) were cohorts (Wang et al., 2022; Dalsager et al., 2021; Ait Bamai et al., 2020; Huang et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016), one was a case-control study nested in a cohort (Impinen et al., 2018), and one was a cross-sectional study (Abraham et al., 2020). Six studies measured PFOA concentrations from mothers during pregnancy (Wang et al., 2022; Ait Bamai et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016). Two studies (Huang et al., 2020; Impinen et al., 2018) measured PFOA concentrations from cord blood at delivery. Two studies measured PFOA concentrations in children's serum at age one year (Abraham et al., 2020) and at age 10 years (Kvalem et al., 2020).

Several of the studies measured infectious disease incidences as parental self-report, which may have led to outcome misclassification (Abraham et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Impinen et al., 2018). Four studies measured infections as the doctor-diagnosed incidence of disease over a particular period (Ait Bamai et al., 2020; Huang et al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017), and Wang et al. (2022) used a combination of parental report and medical records. One study used hospitalizations as an outcome, with events identified based on medical records (Dalsager et al., 2021). Overall, six studies were *medium* confidence (Wang et al., 2022; Dalsager et al., 2021; Ait Bamai et al., 2020; Huang et al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017) and five were *low* confidence (Abraham et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Impinen et al., 2018; Dalsager et al., 2016).

Increased incidence of some infectious diseases in relation to PFOA exposure was observed, although results were not consistent across studies (see Appendix D, (U.S. EPA, 2024a)). The most commonly examined types of infections were respiratory, including pneumonia/bronchitis, upper and lower respiratory tract, throat infections, and common colds. Dalsager et al. (2021) reported higher rates of hospitalization for upper and lower respiratory tract infections with higher PFOA exposure (statistically significant only for lower respiratory tract). Among studies that examined incidence, two studies (one *medium* and one *low* confidence) examining pneumonia/bronchitis observed statistically significant associations between elevated PFOA concentrations and increased risk of developing pneumonia in 0- to 3-year-old children (Impinen

[12] Multiple publications of the same study: both Dalsager et al. (2016) and Dalsager et al. (2021) use data from the Odense cohort in Denmark and thus have overlapping, though not identical populations. They received different ratings due to outcome ascertainment methods.

et al., 2019) and 7-year-old children (Ait Bamai et al., 2020); one other *low* and one other *medium* confidence study did not report an increase in infections (Wang et al., 2022; Abraham et al., 2020). Huang et al. (2020), a *medium* confidence study, examined recurrent respiratory infections and found no association. Two *low* confidence studies and one *medium* confidence study found positive associations with lower respiratory tract infection (Dalsager et al., 2021; Kvalem et al., 2020; Impinen et al., 2018), while another *medium* confidence study reported no association (Manzano-Salgado et al., 2019). In addition, non-statistically significant positive associations were reported with upper respiratory tract infection (Dalsager et al., 2021) and throat infection (Impinen et al., 2019). There were also statistically significant associations seen for PFOA in relation to respiratory syncytial virus, rhinitis, throat infection, and pseudocroup (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019), but findings were inconsistent across studies. No positive associations were reported with common cold (Kvalem et al., 2020; Impinen et al., 2019). Outside of respiratory tract infections, two *medium* confidence studies examined total infectious diseases. Dalsager et al. (2021) reported higher rates of hospitalization for any infections with higher PFOA exposure (not statistically significant), while Goudarzi et al. (2017) reported higher odds of total infectious disease incidence in girls ($p > 0.05$) but not boys. Results for other infection types, including gastrointestinal, generally did not indicate a positive association. Lastly, one study (Dalsager et al., 2016) measured common infectious disease symptoms in children aged 1-to-4 years and found a positive association with fever and nasal discharge, but not cough, diarrhea, or vomiting. Overall, the observed associations provide some coherence with the associations observed with vaccine response, but inconsistency across studies reduces confidence in the evidence.

In addition to the studies in children, three studies examined infectious disease in adults, (Bulka et al., 2021; Ji et al., 2021; Grandjean et al., 2020) (see Appendix D, (U.S. EPA, 2024a)). All three studies were *medium* confidence. Ji et al. (2021) was a case-control study of COVID-19 infection. They reported higher odds of infection with higher PFOA exposure (OR (95% CI) per log-2 SD increase in PFOA: 2.73 (1.71, 4.55)). In contrast, a cross-sectional study examining severity of COVID-19 illness in Denmark using biobank samples and national registry data (Grandjean et al., 2020) reported no association between PFOA exposure and increased COVID-19 severity. Bulka et al. (2021) used NHANES data from 1999–2016 in adolescents and adults and examined immunoglobulin G (IgG) antibody levels to several persistent infections, including cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, HIV, *Toxoplasma gondii* and *Toxocara* species. High levels of these antibodies were interpreted as presence of a persistent infection. They found higher prevalence of herpes simplex viruses 1 and 2 and total pathogen burden with higher PFOA exposure in adults but no association with other individual pathogens.

3.4.2.1.3 Immune Hypersensitivity Study Quality Evaluation and Synthesis from the Updated Literature Review

Another major category of immune response is the evaluation of sensitization-related or allergic responses resulting from exaggerated immune reactions (e.g., allergies or allergic asthma) to foreign agents (IPCS, 2012). A chemical may be either a direct sensitizer (i.e., promote a specific immunoglobulin E (IgE)-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. For example, chemical exposure could promote a physiological response resulting in a propensity for

sensitization to other allergens (e.g., pet fur, dust, pollen). Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same or in some cases, a similar agent leads to the second phase, elicitation, and symptoms of allergic disease. While these responses are mediated by circulating factors such as T cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of health effects such as allergies or asthma and skin prick tests.

In the 2016 PFOA HESD, two *medium* confidence epidemiological studies reported higher odds of asthma with higher PFOA exposure in children (Humblet et al., 2014; Dong et al., 2013). A case-control study (Dong et al., 2013) of children in Taiwan reported increased odds of asthma with increasing childhood PFOA exposure. The magnitude of association was particularly large comparing each of the highest quartiles of exposure to the lowest. In cross-sectional analyses of asthmatic children, the study authors reported monotonic increases for IgE in serum, absolute eosinophil counts, eosinophilic cationic protein, and asthma severity score. A study on NHANES (1999–2000, 2003–2008) adolescents also reported significantly increased odds of 'ever asthma' per doubling of concurrent PFOA measurements, where 'ever asthma' was defined as ever having received an asthma diagnosis from a healthcare professional (Humblet et al., 2014). Results were less consistent for measures of hypersensitivity (e.g., food allergy, eczema); however, among female infants, decreased cord blood IgE (Okada et al., 2012) was significantly associated with prenatal PFOA exposure.

There are 23 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and hypersensitivity (i.e., asthma, allergy, and eczema) effects. Study quality evaluations for these 23 studies are shown in Figure 3-25. *High* and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

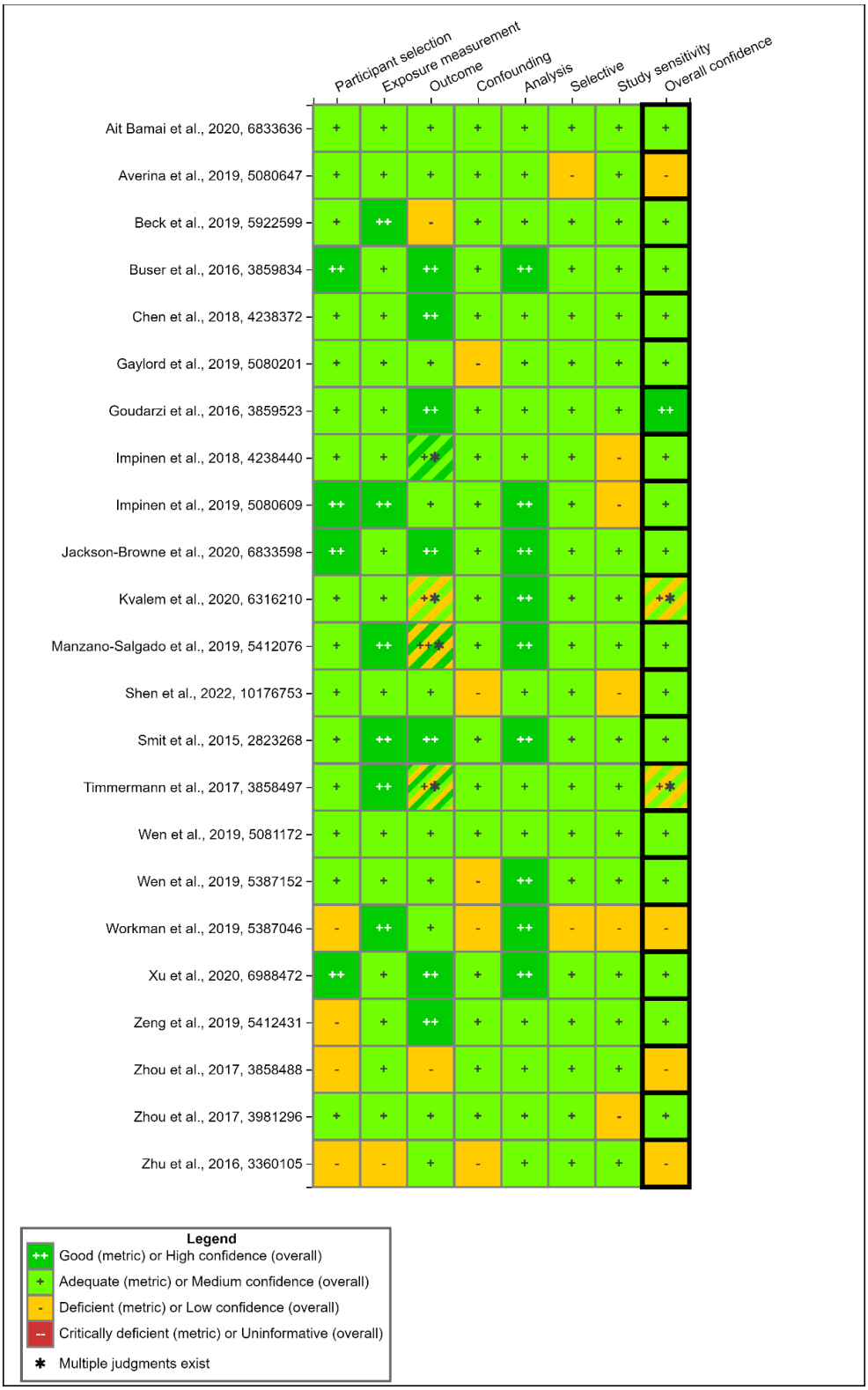


Figure 3-25. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immune Hypersensitivity Effects

Interactive figure and additional study details available on HAWC.

Thirteen studies (15 publications)[13] examined asthma (or asthma symptoms) and PFOA exposure. Nine of these studies were cohorts (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Workman et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017a; Smit et al., 2015); three studies (five publications) were case-control investigations (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016), including one nested case-control, (Gaylord et al., 2019; Impinen et al., 2018); and one was a cross-sectional analysis (Jackson-Browne et al., 2020). Seven studies measured the prevalence of "current" asthma for at least one time point (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Zeng et al., 2019a; Impinen et al., 2018). Nine studies measured 'ever asthma' for at least one time point (Jackson-Browne et al., 2020; Kvalem et al., 2020; Averina et al., 2019; Gaylord et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018; Timmermann et al., 2017a; Smit et al., 2015). Incident or recurrent wheeze was examined in one study (Workman et al., 2019). For asthma, 10 publications were rated *medium* confidence and five publications were rated *low* confidence (Figure 3-25). Timmermann et al. (2017a) was *low* confidence for asthma because the questionnaire used to ascertain status was not validated. Averina et al. (2019) was considered *low* confidence because results were not provided quantitatively. Two studies from the Genetic and Biomarker Study for Childhood Asthma (GBCA) (Zhou et al., 2017c; Zhu et al., 2016) were considered *low* confidence based on participant selection. Cases and controls were recruited from different catchment areas, and the resulting differences between cases and controls indicated potential for residual confounding by age. Additionally, the timing of exposure assessment in relation to outcome assessment was unclear, and it was not reported whether outcome status was confirmed in controls.

Results across these studies were inconsistent (see Appendix D, (U.S. EPA, 2024a)), and few statistically significant results were observed. Several studies observed positive associations with ORs greater than 1.2 between PFOA concentration levels and increased "current" or "ever" asthma (Jackson-Browne et al., 2020; Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017a), but often only within population subgroups. Averina et al. (2019) observed statistically significant increased odds of self-reported doctor-diagnosed asthma among adolescents in their first year of high school. Beck et al. (2019) observed statistically significant increased odds of self-reported asthma per PFOA increase in boys, but this was not observed in girls. For doctor-diagnosed asthma in the same study, an inverse association ($p > 0.05$) was observed in boys and a positive association ($p > 0.05$) was observed in girls. Kvalem et al. (2020) reported increased odds of asthma in girls at age 10 ($p < 0.05$) and between 10 and 16 years of age, but null associations at 16 years, while the opposite was true for boys. Zeng et al. (2019a) observed a positive association in girls and an inverse association in boys (both $p > 0.05$). Jackson-Browne et al. (2020) also observed statistically significant increased odds of "ever" asthma from increased PFOA concentrations in children aged 3–5. However, these associations were null in other age groups and in sex and race categories. Gaylord et al. (2019) reported nonsignificant positive associations in youths of 13–22 years in age. The *low* confidence study by Timmermann et al. (2017a) observed positive associations ($p < 0.05$) between increased asthma odds and elevated PFOA concentrations in a small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella

[13] Three publications (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) reported on the same cohort (Genetic and Biomarker study for Childhood Asthma) and outcome and are considered one study.

(MMR) vaccination before age 5. However, in children of the same ages who had received their MMR vaccination before age 5, an inverse association was observed ($p > 0.05$). *Low* confidence studies from the GBCA study (Zhou et al., 2017c; Zhu et al., 2016) observed elevated PFOA levels ($p < 0.001$) in children with asthma compared with those without (Zhou et al., 2017b), and the odds of current asthma were also found to be elevated among boys and girls with increasing PFOA exposure (Zhu et al., 2016). Two other studies (Impinen et al., 2019; Impinen et al., 2018) observed small positive associations (OR: 1.1); in Impinen et al. (2019), this was only observed for current asthma in boys. Two studies reported nonsignificant inverse associations with asthma (Manzano-Salgado et al., 2019; Smit et al., 2015), and one *low* confidence study did not observe a significant effect for recurrent wheeze (Workman et al., 2019).

In addition to the studies of asthma in children, one *medium* confidence study using data from NHANES examined fractional exhaled nitric oxide (FeNO), a measure of airway inflammation, in adults ((Xu et al., 2020a); see Appendix D, (U.S. EPA, 2024a)). Among participants without current asthma, this study found higher FeNO levels with higher PFOA exposure, indicating greater inflammation (percent change (95% CI) for tertiles vs. T1, T2: 5.29 (1.88, 8.81); T3: 6.34 (2.81, 10.01)).

Overall, there is some evidence of an association between PFOA exposure and asthma, but there is considerable uncertainty due to inconsistency across studies and sub-populations. Sex-specific differences were reported in multiple studies, but there was inconsistency in the direction of association within each sex. There is not an obvious pattern of results by analysis of "ever" versus "current" asthma, and no studies beyond the Dong et al. (2013) study described in the 2016 PFOA HESD examined asthma incidence.

Seven studies observed associations between PFOA exposure and allergies, specifically allergic rhinitis or rhinoconjunctivitis, skin prick test, and food or inhaled allergies. Five of these studies were cohorts (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Timmermann et al., 2017a; Goudarzi et al., 2016), one study was a case-control analysis (Impinen et al., 2018), and one study was a cross-sectional study using data from NHANES 2005–2010 (Buser and Scinicariello, 2016). One study was considered *high* confidence (Goudarzi et al., 2016) and the rest were considered *medium* confidence for allergy outcomes. PFOA concentrations were measured at a variety of time points: three studies measured PFOA during pregnancy (Ait Bamai et al., 2020; Impinen et al., 2019; Goudarzi et al., 2016); three studies measured PFOA concentrations in children at age 5 years (Timmermann et al., 2017a), age 10 years (Kvalem et al., 2020), age 13 years (Timmermann et al., 2017a) and ages 12–19 years (Buser and Scinicariello, 2016); and one study measured PFOA in cord blood at delivery (Impinen et al., 2018) (see Appendix, (U.S. EPA, 2024a)).

Results were generally inconsistent across studies. Three studies conducted skin prick tests on participants to determine allergy sensitization at age 10 years (Kvalem et al., 2020; Impinen et al., 2018), at age 13 years (Timmermann et al., 2017a), and at age 16 years (Kvalem et al., 2020). Skin prick tests were conducted to test sensitization to dust mites, pets, grass, trees and mugwort pollens and molds, cow's milk, wheat, peanuts, and cod. Kvalem et al. (2020) reported a statistically significant but small association (OR: 1.1) with a positive skin prick test at ages 10 and 16 years. Timmermann et al. (2017a) also reported a positive association ($p > 0.05$) in children who had received an MMR before age 5 years (but an inverse association in those who had not received an MMR) and results in Impinen et al. (2018) were null. Five studies measured

symptoms of "current" or "ever" allergic rhinitis or rhinoconjunctivitis (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2018; Timmermann et al., 2017a; Goudarzi et al., 2016). Rhinitis was defined as at least one symptom of runny or blocked nose or sneezing. Rhinoconjunctivitis was defined as having symptoms of rhinitis, in addition to itchy and watery eyes. Rhinitis was increased with exposure at age 16 years ($p < 0.05$) but decreased at age 10 years in Kvalem et al. (2020). Nonsignificant increases in rhinitis were also reported in Impinen et al. (2018) and Timmermann et al. (2017a), but results were null in Ait Bamai et al. (2020) and Goudarzi et al. (2016) for rhinoconjunctivitis. Impinen et al. (2019) measured parent-reported, doctor-diagnosed "current" or "ever" allergy symptoms at age 7 years in addition to known food and inhaled allergies and reported higher odds of current food allergies and ever inhaled allergies (both $p > 0.05$), but not ever food allergies or current inhaled allergies. Buser et al. (2016) measured food sensitization (defined as having at least one food-specific serum IgE ≥0.35 kU/L) and self-reported food allergies and reported statistically significant positive associations with self-reported food allergies in NHANES 2007–2010 but not in in NHANES 2005–2006.

Seven studies measured the association between PFOA concentration and eczema (described by some authors as atopic dermatitis). Six of these studies were cohorts (Manzano-Salgado et al., 2019; Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Timmermann et al., 2017a; Goudarzi et al., 2016), and one was a case-control analysis (Impinen et al., 2018). Four studies measured PFOA concentrations in cord blood at delivery (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Impinen et al., 2018), three studies measured maternal PFOA concentrations during pregnancy (Manzano-Salgado et al., 2019; Timmermann et al., 2017a; Goudarzi et al., 2016), and one study measured PFOA concentrations in children at age 5 and 13 years (Timmermann et al., 2017a). All of the studies were considered *medium* confidence for eczema (see Appendix D, (U.S. EPA, 2024a)).

Two studies (three publications) observed statistically significant associations between increased odds of eczema within the highest quantiles of PFOA exposure (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018); however, the associations were nonmonotonic across categories of exposure. Impinen et al. (2018) also observed a nonsignificant association between higher PFOA concentrations and "ever" eczema at age 2 years; however, results were null for "current" eczema at age 10 years. Results from Goudarzi et al. (2016), Manzano-Salgado et al. (2019) and Timmermann et al. (2017a) were null.

One *medium* confidence nested case-control study examined chronic spontaneous urticaria (Shen et al., 2022). They found no association between PFOA exposure and case status.

3.4.2.1.4 Autoimmune Disease Study Quality Evaluation and Synthesis from the Updated Literature Review

Autoimmunity and autoimmune disease arise from immune responses against endogenously produced molecules. The mechanisms of autoimmune response rely on the same innate and adaptive immune functions that respond to foreign antigens: inflammatory mediators, activation of T lymphocytes, or the production of antibodies for self-antigens (IPCS, 2012). Chemical exposures that induce immune response or immunosuppression may initiate or exacerbate autoimmune conditions through the same functions. Autoimmune conditions can affect specific

systems in the body, such as the nervous system (e.g., multiple sclerosis (MS)), or the effects can be diffuse, resulting in inflammatory responses throughout the body (e.g., lupus).

The 2016 PFOA HESD (U.S. EPA, 2016c) identified one *low* confidence occupational study in workers highly exposed to PFOA (part of the C8 Health Project) (Steenland et al., 2015) that reported significant positive trends for rheumatoid arthritis and ulcerative colitis with increasing cumulative PFOA exposure. The C8 Science Panel concluded there was a probable link between PFOA and ulcerative colitis (C8 Science Panel, 2012b).

There are six epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and autoimmune disease. Study quality evaluations for these 6 studies are shown in Figure 3-26. *High* and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

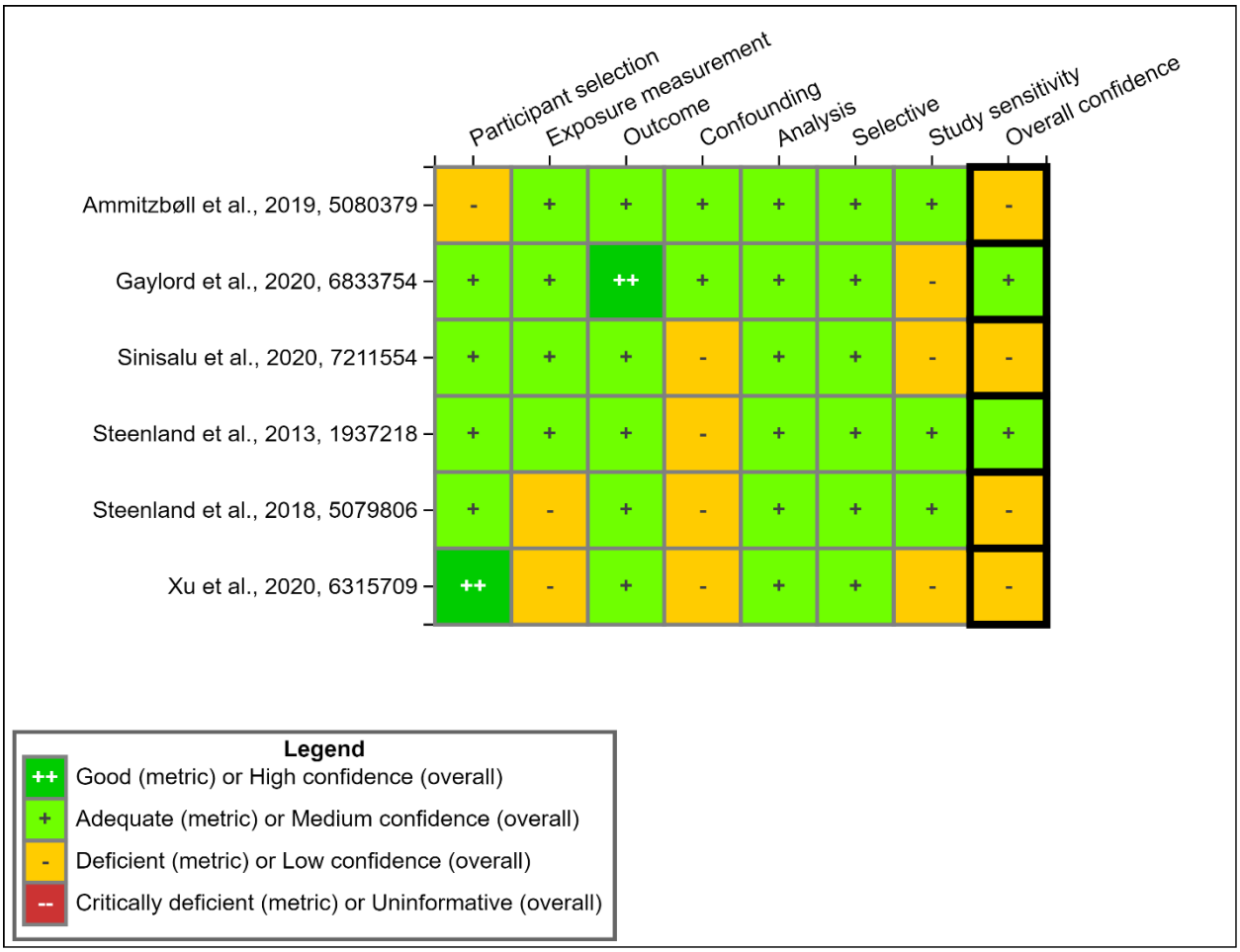


Figure 3-26. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Autoimmune Effects

Interactive figure and additional study details available on HAWC.

One study examined the association between PFOA exposure and multiple autoimmune conditions (rheumatoid arthritis, lupus, MS, ulcerative colitis, and Crohn's disease) in the combined C8 Health Project occupational and community cohort (Steenland et al., 2013). Two case-control studies examined MS (Ammitzbøll et al., 2019) and ulcerative colitis (Steenland et al., 2018b) in adults, and two case-control studies examined celiac disease in children and young adults (Gaylord et al., 2020; Sinisalu et al., 2020). One study was a cohort study that examined ulcerative colitis in children and adults from a high-exposure community in Sweden (Ronneby cohort) (Xu et al., 2020d). The combined occupational and community study used modeled PFOA exposure based on serum concentrations and historical data on residences and drinking water quality (Steenland et al., 2013), and the case-control studies measured PFOA in serum or plasma only (Gaylord et al., 2020; Sinisalu et al., 2020; Ammitzbøll et al., 2019; Steenland et al., 2018b). Two studies were without notable deficiencies and considered *medium* confidence (Gaylord et al., 2020; Steenland et al., 2013). Four studies were considered *low* confidence (Sinisalu et al., 2020; Xu et al., 2020d; Ammitzbøll et al., 2019; Steenland et al., 2018b). Steenland et al. (2018b) examined exposure concentrations 1 to 2 years after diagnosis of celiac disease, resulting in some concern for reverse causation. Additionally, there was potential for residual confounding by SES which was not considered in the analysis. These factors together contributed to a *low* confidence rating. Information on participant selection, particularly control selection, was not reported in Ammitzbøll (2019). Additionally, PFOA was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOA exposure and risk of MS.

In a C8 Health Project study (Steenland et al., 2013), associations for rheumatoid arthritis were generally consistent and positive across unlagged and 10-year lagged PFOA quartiles. The risk of rheumatoid arthritis was significantly elevated compared with those in the third quartile of 10-year lagged exposure to participants in the first quartile, but this was the only significant association. The risk of MS was nonsignificantly elevated in unlagged and 10-year lagged models (Steenland et al., 2013). Significantly increased risk of ulcerative colitis among adults across increasing quartiles of PFOA exposure was also observed (p-trend < 0.0001). Associations with lupus and Crohn's disease were nonsignificant and inconsistent in the direction of effect (Steenland et al., 2013).

Evidence from a case-control study suggested lower PFOA concentrations among healthy controls compared with those with MS (Ammitzbøll et al., 2019). Serum PFOA concentrations were 12% lower (95% CI: −24%, 2%; p = 0.099) in healthy controls compared with cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOA levels were 28% lower (95% CI: −42%, −9%; p = 0.006) in healthy controls compared with cases, but this effect was not seen in women. Steenland et al. (2018b) detected significantly increased levels of PFOA in ulcerative colitis cases versus those with Crohn's disease or controls and observed statistically significantly increased odds of ulcerative colitis with increased PFOA exposure among combined children and adults; however, the trend was not consistent across increasing quintiles of PFOA exposure, with a peak in the third quintile. Xu et al. (2020d) observed significant decreases in risk of Crohn's disease in an early exposure period, but not in later exposure periods, or for UC in children and adults from a high-exposure community in Sweden (Ronneby cohort).

The risk of celiac disease was elevated among children and young adults (≤21 years old) in a case-control study (Gaylord et al., 2020), particularly in females ($p < 0.05$), but the association did not reach significance among the whole population.

In the prospective observational Finnish Diabetes Prediction and Prevention (DIPP) study in which children genetically at risk to develop type 1 diabetes (T1D) and celiac disease were followed from birth, with blood samples taken at birth and 3 months of age (Sinisalu et al., 2020), there was no significant difference in the levels of PFOA exposure in those children that later developed celiac disease, which may be due to the small sample size, but age at diagnosis of celiac disease was strongly associated with the PFOA exposure.

3.4.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are four studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and nine studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and immune effects in animal models. Study quality evaluations for these 13 studies are shown in Figure 3-27.

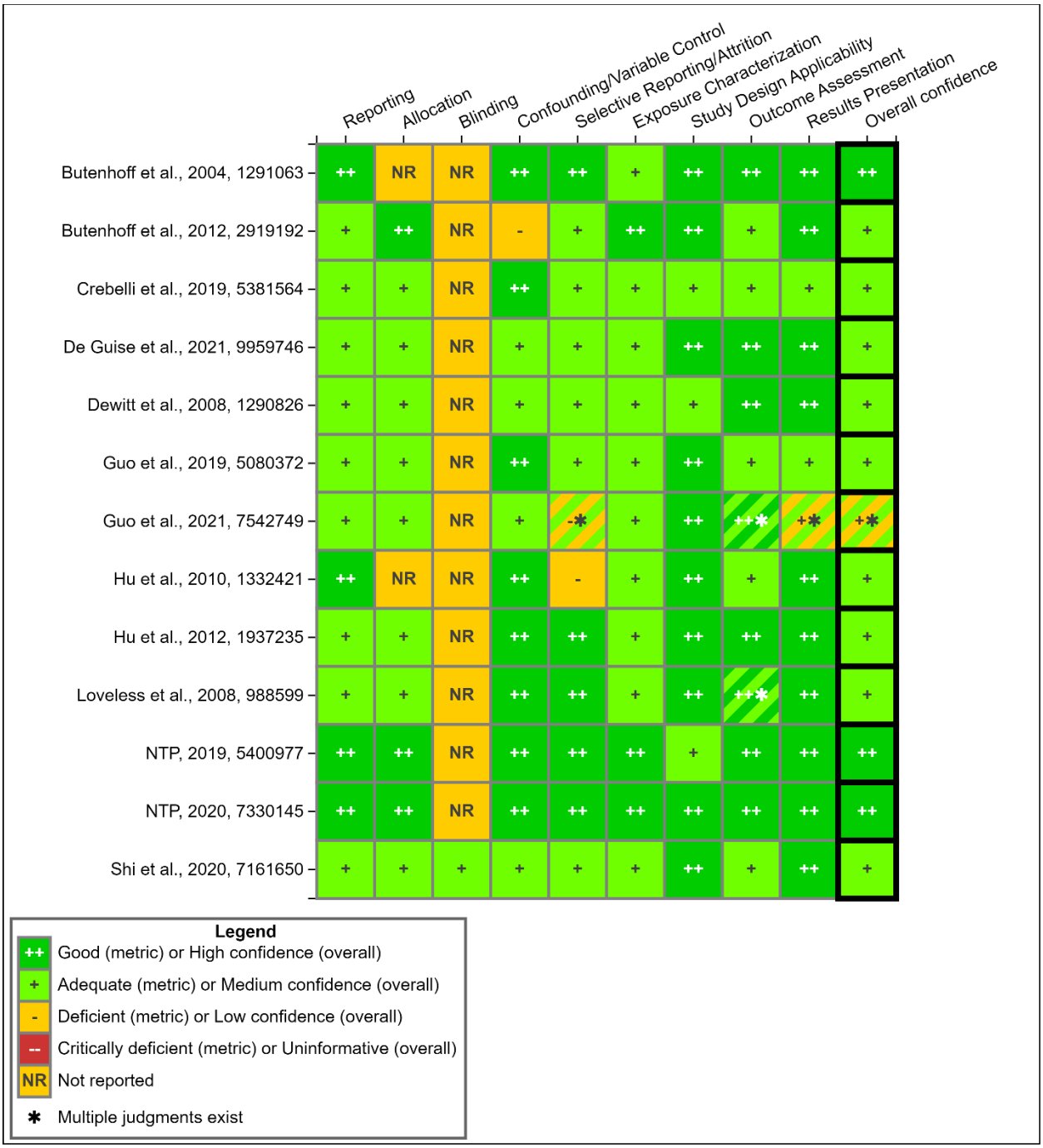


Figure 3-27. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Immune Effects

Interactive figure and additional study details available on HAWC.

The data available on immunological responses of animals following oral exposure to PFOA are extensive, especially as they apply to mice. A number of studies reported effects on spleen and thymus weights, immune system cellular composition, and the ability to generate an immune response following PFOA doses ranging from approximately 1 to 40 mg/kg/day.

3.4.2.2.1 Organ Weight/Histopathology

Short-term exposure studies by Yang et al. (2000), Yang et al. (2001), Qazi et al. (2009), and Yang et al. (2002b) using male C57BL/6 mice, by DeWitt et al. (2008) using female C57BL/6 mice, and by DeWitt et al. (2016b) using female C57BL/6Tac mice were conducted using relatively high PFOA doses (up to approximately 40 mg/kg/day). In each study, the PFOA-treated C57BL/6 mice exhibited significant reductions in spleen and thymus weights after 5–16 days of exposure. Yang et al. (2000) and DeWitt et al. (2008) observed up to an approximately 80% reduction in absolute and relative thymus weight and up to a 30%–48% reduction in absolute and relative spleen weight. Similar reductions in absolute thymus and spleen weights were observed in Yang et al. (2002b); relative weights were not reported. In DeWitt et al. (2016b), relative spleen weights were significantly reduced by 30% after exposure to 30 mg/kg/day, and relative thymus weights were significantly reduced by 55.4% after exposure to 7.5 mg/kg/day (but not after exposure to 30 mg/kg/day). Absolute weights were not reported in this study. In male CD-1 mice exposed for 29 days via gavage to 1, 10, or 30 mg/kg/day PFOA, absolute and relative spleen weights were reduced to approximately 90%, 60%, and 50% of controls, respectively (Loveless et al., 2008). Absolute and relative thymus weights were decreased to approximately 50% of controls in the 10 and 30 mg/kg/day groups. Spleen and thymus weights were only reduced by up to 9% (not statistically significant) in male ICR mice administered 47.21 mg/kg/day PFOA in drinking water for 21 days (Son et al., 2009). In male BALB/c mice dosed with 0.4, 2, or 10 mg/kg/day PFOA via gavage for 28 days, absolute spleen weights were significantly reduced to 88% and 50% of the control in the 2 and 10 mg/kg/day groups, respectively (Guo et al., 2021b). Relative spleen weights in these groups were similarly reduced to 84% and 56% of the control. In the same study, however, no significant changes in spleen or thymus weights were observed in male Sprague-Dawley rats. In a separate 28-day study, male Sprague-Dawley rats administered 2.5–10 mg/kg/day displayed significantly lower absolute spleen weights that reached 76% of control at the highest dose (NTP, 2019). Absolute thymus weight was decreased to 74% of control in males administered 10 mg/kg/day compared with those of the vehicle group. Female spleen and thymus weights were not altered.

In one developmental study, pregnant C57BL/6N mice were exposed to 0.5 or 1 mg/kg/day PFOA from GD 6 to GD 17; the relative spleen and thymus weights of the female offspring were unchanged at PND 48 (Hu et al., 2010). The male offspring were not assessed in this study. However, a reduction in spleen and thymus weights has been reported in male rats following developmental PFOA exposure. NTP (2020) exposed pregnant rats to PFOA beginning on GD 6, and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as "[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]" (see further study design details in Section 3.4.4.2.1.2). Following perinatal and postweaning PFOA exposure (150/150 and 300/300 ppm), significant reductions in absolute and relative spleen weight and absolute thymus weight were observed at 16 weeks in male rats. Reduced absolute and relative spleen weights were also observed in rats following 300/20, 300/40, and 300/80 ppm PFOA exposure. Postweaning exposure alone (0/20, 0/40, 0/150, and 0/300 ppm) significantly reduced absolute and relative spleen weights. Absolute thymus weight was reduced following 0/150 and 0/300 ppm (NTP, 2020). No changes in spleen or thymus weights were reported in females.

Two studies describing effects of subchronic PFOA exposure in adult male mice (Shi et al., 2020; Crebelli et al., 2019) and one chronic study in adult male rats (Butenhoff et al., 2012) did not report reduced spleen weight, and thymus weights were not examined. No changes to spleen weights were observed in C57BL/6 male mice administered ≤5 mg/kg/day for 5 weeks (Shi et al., 2020; Crebelli et al., 2019). Although the changes were not statistically significant, Shi et al. (2020) observed 21%, 32%, and 32% reductions in relative spleen weight (compared with controls) in mice exposed to 0.5, 1, or 3 mg/kg/day, respectively. Body weight gain was also significantly reduced in these groups, and absolute spleen weight was not reported. Similarly, spleen weight was not affected in male Sprague-Dawley rats chronically exposed to 30 or 300 ppm (1.3 or 14.2 mg/kg/day) for 1 or 2 years (Butenhoff et al., 2012). An increase in absolute and relative spleen weight (40% and 30% increase, respectively) was observed only in female rats exposed to 30 ppm (1.6 mg/kg/day) for 2 years.

3.4.2.2.2 Histopathology

Several studies reported on histological evaluations of the spleen and thymus from rodents orally administered PFOA at varying doses and durations. In male Crl:CD-1 (ICR)BR mice administered PFOA for 29 days, decreased spleen weights at 10 and 30 mg/kg/day correlated with the gross observation of small spleens (Loveless et al., 2008). An increased incidence of spleen atrophy was also observed in the 30 mg/kg/day group. The decreased thymus weights at these doses correlated with the microscopic finding of lymphoid depletion and with the gross observation of small thymuses (Loveless et al., 2008). Loveless et al. (2008) also reported increased incidences of granulocytic hyperplasia of the bone marrow in mice in the 10 and 30 mg/kg/day groups.

Other microscopic findings were reported in Son et al. (2009) in the histological evaluation of male ICR mice administered PFOA (0.49–47.21 mg/kg/day) for 21 days. The thymus of mice exposed to 47.21 mg/kg/day PFOA revealed atrophy with decreased thickness of the cortex and medulla compared with control, but increased cellular density of lymphoid cells in the cortex was observed (Son et al., 2009). The authors also reported an enlargement of the spleen with marked hyperplasia of the white pulp in the 47.21 mg/kg/day PFOA-treated group, and an increased area of the lymphoid follicles in the spleen with increased cellular density (Son et al., 2009). In contrast, in a study in male BALB/c mice administered 0.4–10 mg/kg/day PFOA via gavage, the authors noted decreased white pulp content, with the white pulp content in the highest dose group being reduced to nearly in half of that of the control group (quantitative results were not provided) (Guo et al., 2021b).

After 5–6 days of recovery, Loveless et al. (2008) observed increased extramedullary hematopoiesis in the spleens of male Crl:CD(SD)IGS BR rats and Crl:CD-1 (ICR)BR mice exposed to 30 mg/kg/day PFOA for 23–24 days. However, these changes were not observed in rats and mice after a continuous 29-day exposure (Loveless et al., 2008). Likewise, splenic hematopoiesis was not affected in male or female Sprague-Dawley rats administered 0.625–10 or 6.25–50 mg/kg/day PFOA, respectively (NTP, 2019).

Two studies in male Sprague-Dawley rats exposed to up to 30 mg/kg/day PFOA for 28–29 days reported no histopathological changes in the spleen, thymus, and/or lymph nodes (NTP, 2019; Loveless et al., 2008). However, a significant increase in bone marrow hypocellularity of

minimal severity was reported in male rats exposed to 10 mg/kg/day (6/10 compared with 1/10 in controls) but not in female rats (NTP, 2019).

Histological evaluation of the spleen following chronic PFOA exposure was only reported in one study, which administered 30 or 300 ppm PFOA to male and female Sprague-Dawley rats for 2 years. Hemosiderin, an iron-rich pigment, was found in greater amounts in the spleens of males dosed with 300 ppm (approximately 15 mg/kg/day), though this change was not significant, but was significantly reduced in the 30 ppm groups (approximately 1.5 mg/kg/day) and in the 300 ppm females (Butenhoff et al., 2012). However, no histopathological changes in the thymus, spleen, bone marrow, or lymph nodes were reported in a study that exposed Sprague-Dawley rats to up to 300 ppm PFOA for 16 weeks (males and females) or up to 80 ppm PFOA (males) or 300 ppm (females) for 2 years (NTP, 2020).

Histological evaluation of the spleen and thymus following reproductive PFOA exposure was only reported in one study (Butenhoff et al., 2004a). P_0 males and females were administered 1–30 mg/kg/day PFOA from premating until the end of lactation and the F_1 generation was exposed throughout their life. The authors note that no histopathological changes were reported, though quantitative results were not provided.

3.4.2.2.3 Immune Cellularity

3.4.2.2.3.1 White Blood Cells and Differentials

Evidence supporting an effect of PFOA exposure on immune system-associated cellularity has been reported. A decrease in total serum white blood cells to 28% of control was observed in male C57BL/6 (H-2^b) mice fed 40 mg/kg/day for 10 days (Qazi et al., 2009). Total number of circulating neutrophils and lymphocytes (T and B cells) were decreased to 50% and 27% of control, respectively. The numbers of circulating monocytes, eosinophils, and basophils were too small to be determined reliably, according to the study (Qazi et al., 2009).

In a similar study, male Crl:CD-1(ICR)BR mice were exposed to PFOA (10 or 30 mg/kg/day) by oral gavage for 29 days. At both doses tested, increases in total serum neutrophils and monocytes (reaching 296% and 254% of control, respectively, at the highest dose), and a decrease in total number of eosinophils (approximately 60% of control, data not statistically significant) were observed (Loveless et al., 2008). Loveless et al. (2008) also reported a decrease in lymphocytes in male mice dosed with 30 mg/kg/day, but these data were not provided in the study. In a second short-term study, white blood cell count was significantly decreased to 71% and 36% of the control in male BALB/c mice exposed to 2 and 10 mg/kg/day PFOA, respectively, for 28 days (Guo et al., 2021b). White blood cell differentials were not measured in this study.

In a short-term study in male and female Sprague-Dawley exposed to 0.625–10 or 6.25–100 mg/kg/day PFOA, respectively, no changes in white blood cell counts or differentials were reported (NTP, 2019).

In male and female Sprague-Dawley rats chronically exposed to 30 or 300 ppm PFOA (approximately 1.5 or 15 mg/kg/day) for 2 years, PFOA did not affect total white blood cell count, blood lymphocytes, or neutrophils (Butenhoff et al., 2012). However, white blood cell counts were increased in males through the first year of the study. The authors suggest that these

changes were due to increases in absolute counts of lymphocytes at 3 and 6 months and in neutrophils at 12 months (Butenhoff et al., 2012).

3.4.2.2.3.2 Spleen, Thymus, Lymph Nodes, and Bone Marrow Cellularity

Short-term PFOA exposure (10–40 mg/kg/day) significantly decreased splenocyte and thymocyte cell populations by up to approximately 30% and 15% of control, respectively, in male Crl:CD-1 (ICR)BR mice (Loveless et al., 2008) and male C57BL/6 mice (Yang et al., 2001). Similarly, in male C57BL/6 mice administered 40 mg/kg/day PFOA for 7 days, the number of thymocytes was decreased to 14% of control; immature thymocyte populations (CD4 + $CD8^+$) were the most affected (Yang et al., 2000). In the spleen, both B and T cells were significantly reduced in these mice, and the number of total splenocytes was decreased to 20% of control (Yang et al., 2000). Reduced splenocyte and thymocyte CD4 + $CD8^+$ cells were also observed in male ICR mice administered PFOA (0, 0.49, 2.64, 17.63, and 47.21 mg/kg/day) in drinking water for 21 days, reflecting an impairment in cell maturation (Son et al., 2009).

No changes in splenocyte and thymocyte cell populations were observed in one study of male Sprague-Dawley rats exposed to 0.3–30 mg/kg/day PFOA for 29 days (Loveless et al., 2008).

Developmental PFOA exposure may also impact cellularity of the spleen. In one study by Hu et al. (2012), an approximate 22% reduction in splenic regulatory T cells (CD4 + CD25 + $Foxp3^+$) was observed in PND 42 male and female offspring from C57BL/6N dams exposed to 2 mg/kg/day PFOA from gestation through lactation. Thymic cellularity was not examined in this study (Hu et al., 2012).

3.4.2.2.4 Ability to Generate an Immune Response

The ability to generate an immune response following PFOA has been investigated in rodent models. Male Crl:CD-1 (ICR)BR mice were exposed to PFOA (0, 0.3, 1, 10, or 30 mg/kg/day) by oral gavage for 29 days and received an injection of serum sheep red blood cells (SRBC) on day 24 (Loveless et al., 2008). The induced immunoglobulin M (IgM) response was significantly reduced to 80% and 72% of controls in mice exposed to 10 and 30 mg/kg/day, respectively. The same study found no changes in IgM in rats. After an injection with keyhole limpet hemocyanin (KLH), a similar reduction in anti-KLH IgM response was observed in female B6C3F1 mice administered 1.88 and 7.5 mg/kg/day PFOA in drinking water for 28 days (De Guise and Levin, 2021). The IgM response in the mice exposed to 1.88 or 7.5 mg/kg/day was significantly reduced to 29% and 8% of the control's response, respectively. The ability to respond to an immunological challenge was also reduced in female C57BL/6N mice exposed to 3.75 to 30 mg/kg/day PFOA in drinking water for 15 days (Dewitt et al., 2008). The mice showed a dose-dependent reduction in IgM levels (between 11% and 30% decrease) after injection with SRBC to induce an immune response. The IgG response to SRBC significantly increased by approximately 15% following 3.75 and 7.5 mg/kg/day PFOA exposure, but no change was observed at higher doses (Dewitt et al., 2008). In a separate study, female C57BL/6Tac mice were exposed to 0, 7.5, or 30 mg/kg/day PFOA in drinking water for 15 days and injected with SRBC on day 11 (Dewitt et al., 2016b). Exposure to 30 mg/kg/day PFOA reduced SRBC-specific IgM antibody responses by 16%. Similarly, male C57BL/6 mice were fed approximately 40 mg/kg/day PFOA for 10 days and then evaluated for their immune response to horse red blood cells (Yang et al., 2002a). PFOA-exposed mice had no increase in plaque-forming cells in

response to the immune challenge, compared with unimmunized control mice, suggesting a suppression of the humoral immune response.

One developmental study assessed the ability to generate an immune response following gestational exposure to PFOA (Hu et al., 2010). In this study, pregnant C57BL/6N mice were exposed to 0.5 or 1 mg/kg/day PFOA from GD 6 to GD 17. The adult female offspring were immunized with SRBC on PND 44. No change in the immune response was observed, as measured through IgM titers (PND 48) and IgG titers 2 weeks later (PND 63) following an SRBC booster.

Alterations in the serum levels of globulin can be associated with decreases in antibody production (FDA, 2002). PFOA exposure at 12.5 mg/kg/day and up to 100 mg/kg/day for 28 days decreased globulin concentrations in female Sprague-Dawley rats by up to 79% of control. In males, a decrease in globulin concentrations was observed at 0.625 mg/kg/day (74% of control) and up to 10 mg/kg/day (61% of control), highlighting greater PFOA tolerance in females compared with males (Figure 3-28) (NTP, 2019). In contrast, an increase in globulin concentrations, by approximately 7%, was observed in male BALB/c mice exposed to 0.4 or 2 mg/kg/day PFOA (but not 10 mg/kg/day) for 4 weeks (Figure 3-28) (Guo et al., 2019). In a similar study by the same group, immunoglobulins were measured, and IgA concentrations were found to be significantly increased by 12%, 16%, and 33% in male BALB/c mice exposed to 0.4, 2, or 10 mg/kg/day, respectively, PFOA for 4 weeks (Guo et al., 2021b). IgM was increased by 3% and 6% in mice exposed to 2 or 10 mg/kg/day, respectively, and IgG was increased by 6% in mice exposed to 10 mg/kg/day.

Globulin levels were also decreased in pregnant ICR dams on GD 18 following 5 or 10 mg/kg/day PFOA from GD 0 to GD 18 (Yahia et al., 2010). Globulin levels were decreased to 78 and 68% of control, respectively. Globulin levels in offspring were not measured. In a developmental study conducted by NTP (2020), Sprague-Dawley rats were exposed perinatally and/or postweaning for a total of 107 weeks to varying doses of PFOA ((perinatal exposure level (ppm))/(postweaning exposure level (ppm)); see further study design details in Section 3.4.4.2.1.2). In male Sprague-Dawley rats at the 16-week interim timepoint, perinatal exposure to 300 ppm (300/0) and/or postweaning exposure to doses ranging from 20 to 300 ppm (0/150, 0/300, 150/150, 300/300, 0/20, 0/40, 0/80, 300/20, 300/40, or 300/80 ppm) significantly decreased globulin levels. Female rats displayed decreased globulin levels following exposure to 0/300, 0/1,000, 150/300, or 300/1,000 ppm PFOA (NTP, 2020) (Figure 3-28).

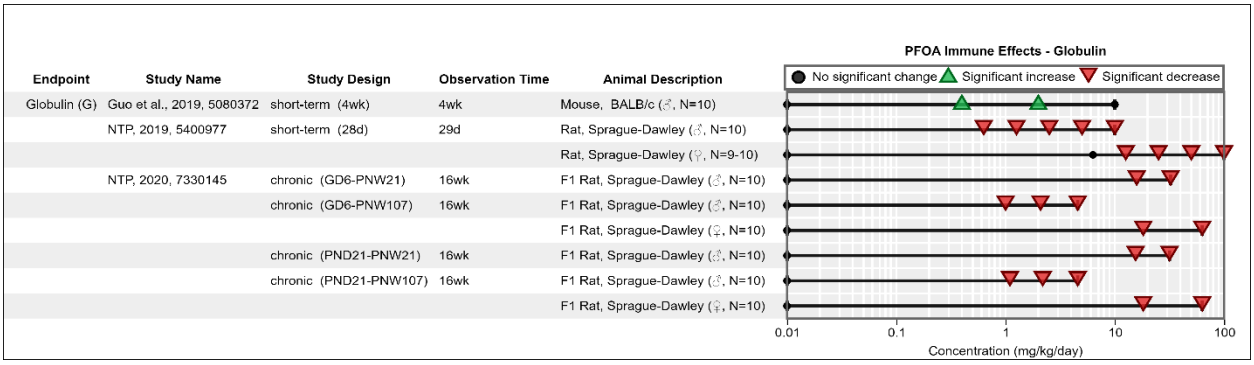


Figure 3-28. Globulin Levels in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; PNW = postnatal week; F_1 = first generation; d = day; wk = week.

3.4.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse immune outcomes is discussed in Sections 3.3.2 and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 22 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to immune effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-29.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	3	0	3	6
Cell Signaling Or Signal Transduction	3	0	1	4
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Inflammation And Immune Response	11	6	5	20
Oxidative Stress	1	0	2	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	12	6	7	22

Figure 3-29. Summary of Mechanistic Studies of PFOA and Immune Effects

Interactive figure and additional study details available on HAWC.

A consistent pattern of findings from human (Section 3.4.2.1) and animal (Section 3.4.2.2) studies support that higher serum concentrations of PFOA are associated with immunosuppression. Additional findings included reduced spleen and thymus weights, reduced cellularity of white blood cells and differentials in circulation, reduced immune cellularity in primary and secondary lymphoid organs, and altered globulin levels. Mechanistic data available from in vitro, in vivo, and epidemiological studies were used to evaluate the mode of action of PFOA-associated immunosuppression and other effects on the immune system.

3.4.2.3.1 Mechanistic Evidence for PFOA-Mediated Effects on Immune System Development and Physiology

Reductions in lymphocyte numbers have been consistently reported in animal toxicological studies (Section 3.4.2.2), with parallel observations of reduced antibody responses in human studies (Section 3.4.2.1). PFOA can alter the number of various B and T cell subsets in primary and secondary lymphoid organs, which may reflect effects on immune system development including effects on proliferation, differentiation, and/or apoptosis of immune cells.

Two in vivo studies were identified that evaluated PFOA-mediated effects on immune system development, reflected in numbers of B and T cell populations. In female BALB/c mice dermally exposed to PFOA for 14 days, the total numbers of splenic CD4+ T cells were reduced, as were the total numbers and percent of CD4+ T cells in the lymph nodes. The percent of splenic CD4+ T cells was increased (Shane et al., 2020). The authors also observed that the absolute number

and percent of splenic B cells were reduced, an observation which could be explained by increased apoptosis of B cells in the spleen or diminished proliferation in the bone marrow, where B cells develop. Effects on B cell differentiation may also reflect reduced cellularity of bone marrow, thymus, and spleen. Qazi et al. (2012) reported reduced percentages of the relatively undifferentiated pro/pre-B cells (CD19+/CD138+/IgM-) in the bone marrow of male C57BL/6 mice fed diets containing 0.02% PFOA for 10 days. Morphological assessment of the bone marrow was consistent with the reduced cell populations; mice treated with 0.02% PFOA displayed hypocellularity in the bone marrow. The authors note that food consumption by the mice exposed to 0.02% PFOA can be reduced up to 35%. Moreover, although experimentally restricting food consumption by 35% in the absence of PFOA exposure affects pro/pre-B cell populations in a similar manner to PFOA, the effect is not identical, which may support that PFOA exposure is associated with decreased pro/pre-B cells in the bone marrow independent of reduced food consumption. The study also demonstrated that the number of myeloid cells (Gr1+/CD11b+) is reduced by 0.02% PFOA but to a lesser magnitude than that of B-lymphoid cells (CD19+), suggesting that the B-lymphoid cell lineage is more sensitive than the myeloid cell lineage.

Several in vitro studies have reported reductions in immune cell viability or increases in cytotoxicity following exposure to PFOA (Sørli et al., 2020; Rainieri et al., 2017), which could also contribute to reduced lymphocyte cellularity or reduced immune organ weight observed in the animal literature (Section 3.4.2.2).

Reductions in immune cellularity of B and T cell populations in the thymus and spleen (Section 3.4.2.2) as well as the bone marrow may reflect perturbations in cellular and/or molecular events including cell proliferation, apoptosis, and oxidative stress. An in vitro study by Rainieri et al. (2017) evaluated the effects of PFOA on cell proliferation by quantifying the distribution of cells in different stages of the cell cycle in a human macrophage cell line (TLT cells). Significantly more cells were in G2/M phase of mitosis following exposure to PFOA in parallel with a lower proportion of cells in the G0/G1 phase, suggesting increased cell proliferation. However, increased cell proliferation is inconsistent with the immune organ atrophy reported in animal toxicological studies (Section 3.4.2.2) and findings of other mechanistic studies in immune organs. Yang et al. (2002b) reported significant reductions in the proportion of thymocytes in the S and G2/M phases and significant increases in the G0/G1 phases of mice treated with PFOA, which were attenuated in PPARα-null mice. These results imply that reductions in cell numbers in the S and G2/M phases of the cell cycle are partially mediated by PPARα.

Two studies (Rainieri et al., 2017; Wang et al., 2014) have reported increased apoptosis in immune cells following PFOA exposure in vivo and in vitro. Increased apoptosis may contribute to the reductions in immune organ weight observed in the animal literature and/or reduced populations of immune cells (Section 3.4.2.2). Wang et al. (2014) exposed BALB/c mice to 0, 5, 10, or 20 mg/kg/day PFOA via gavage for 14 days and reported that the percent of apoptotic cells increased in the spleen at 10 and 20 mg/kg/day and increased in the thymus at 20 mg/kg/day. Increased apoptosis was associated with atrophy of these immune system organs, suggesting that PFOA-induced apoptosis may contribute to organ atrophy. In parallel, the authors explored the association between lipid metabolism and immunotoxicity of PFOA by including a high-fat diet (HFD) group in addition to the regular diet (RD) group; there was a higher percentage of apoptosis in the HFD vehicle control group than the RD vehicle control group,

indicating that HFD could cause or exacerbate apoptosis. Given these diet-related results along with gene expression data showing that PPARα and PPARγ were also upregulated in the thymus and the spleen, the authors concluded that immunomodulation by PFOA occurs via the PPAR pathway and the induction of mitochondrial damage and lymphocyte apoptosis pathway. Rainieri et al. (2017) evaluated apoptosis in TLT cells exposed to 0, 50, 250, or 500 mg/L PFOA for 12 hours. The percentage of apoptotic cells was significantly elevated only at the highest concentration.

Generation of oxidative stress is a potential underlying mechanism linking PFOA to the aforementioned effects on proliferation, differentiation, and/or apoptosis of immune cells. Oxidative stress has been implicated in PFOA immunotoxicity by one in vivo study and several in vitro studies (Rainieri et al., 2017; Yahia et al., 2016; Wang et al., 2014). Wang et al. (2014) observed that the spleens of mice treated with PFOA had mitochondrial swelling and cavitation as well as swollen and ruptured cristae, which suggests impaired oxidative processes. However, there were no significant changes in H_2O_2 concentrations or superoxide dismutase (SOD) activity in spleens of mice exposed to PFOA versus controls. There were no differences in mitochondrial ultrastructure between the HFD group and the RD group, implying that although PFOA-related mitochondrial damage may contribute to apoptosis in lymphocytes, the mechanism may not involve perturbed lipid metabolism. Rainieri et al. (2017) reported increased lipid peroxidation in zebrafish embryos that coincided with a dose-dependent increase in gene expression of glutathione S-transferase pi 1.2 (gstp1) and heat shock cognate 70-kd protein, like (hsp70l), which is typically observed in response to oxidative stress. However, it is important to note that lipid peroxidation and gene expression analyses were evaluated in whole zebrafish embryos and therefore may not necessarily be specific to effects in immune organs. Oxidative DNA damage was reported by Yahia et al. (2016) in a human lymphoblast cell line (TK6 cells) exposed to PFOA at concentrations of 0, 125, 250, and 500 ppm, including a dose-dependent increase in 8-OHdG levels that coincided with increases in tail moment, Olive Tail moment, and tail length in the comet assay at 250 and 500 ppm, which is indicative of DNA damage. Altogether, the evidence suggests that PFOA can induce oxidative stress in immune cells, including oxidation of lipids and DNA, potentially leading to DNA damage.

3.4.2.3.2 Mechanistic Evidence for PFOA-Mediated Effects on Adaptive Immune Responses

3.4.2.3.2.1 Mechanistic Data Informing Suppression of Immune Responses to Vaccines and Infectious Diseases

PFOA-associated immunosuppressive effects are described in Section 3.4.2.2.1. Adaptive immune responses include B and T cell-mediated responses to infection and vaccination, as well as allergic responses related to allergens or autoimmune responses. Mechanistic studies suggest that chemicals, such as PFOA, can perturb the function of mature B or T lymphocytes by acting at several stages of leukocyte function, including antigen recognition, antigen signaling through the antigen receptor, activation, proliferation, and differentiation (Klaassen, 2013). In mice, PFOA has been shown to diminish the immune response to sheep red blood cells (SRBC), a T cell-dependent antibody response (Section 3.4.2.2), indicating that B and/or T cells can be impacted by PFOA. A review of antigen-specific IgM antibody responses by NTP (2016) indicated that both T cell-independent responses (e.g., immunized with dinitrophenyl (DNP) or trinitrophenyl (TNP)) and T cell-dependent responses were reduced by PFOA.

One study provided evidence that antibody glycosylation patterns could be perturbed by PFOA: Liu et al. (2020b) reported that children with higher levels of serum PFOA had altered levels of N-glycosylation of IgG antibodies, which could perturb normal cell-cell interactions through protein receptors involved in antigen recognition and presentation.

Activation of T cells can be demonstrated by transcriptional changes in the genes that encode cytokines (e.g., IL-2) and cell surface proteins (e.g., IL-2 receptor); however, none of the transcriptomic studies reported significant associations with IL-2 levels and PFOA. Although not significant, one study by Zhu et al. (2016) reported trending reductions in the levels of IL-2 and increased serum PFOA concentrations in male and female asthmatic children.

The effect of PFOA on immunoglobulin classes was evaluated in a study by Zhang et al. (2014a), in which zebrafish were exposed to 0, 0.05, 0.1, 0.5, or 1 mg/L PFOA and immunoglobulin gene expression was quantified in spleens. In contrast to mammals, which have five different classes of immunoglobulin (i.e., IgM, IgA, IgD, IgE, and IgG), zebrafish have three (IgM, IgD, and IgZ). The authors reported a dose-dependent reduction in IgM and nonmonotonic dose responses in IgD and IgZ, where the greatest increases in expression were observed at the middle doses. Another zebrafish study by Zhong et al. (2020) reported a similar inverse U-shaped dose-response curve for IgD after 7 or 14 days of exposure to 0, 0.05, 0.1, 0.5, or 1 mg/L PFOA, but reported that IgZ and IgM were elevated in groups exposed to 0.1 or 0.5 mg/L PFOA. Additionally, the effect of PFOA on gene expression of B cell activating factor (baff) paralleled that of IgD, suggesting that PFOA disrupts immunoglobulin levels by interfering with baff mRNA expression.

Differentiation of B and T cells into mature effector cells can also be affected by PFOA exposure. The cytokine milieu surrounding the T cell and antigen presenting cell (APC) influences the fate of the T cell. In addition to the cytokines mentioned above, fluctuations have been reported in IL-10, IL-5, and IL-4 levels. Associations between PFOA exposure and IL-4 or IL-5 are discussed in relation to allergic and asthmatic responses below. The data on IL-10 is limited to a single developmental study by Hu et al. (2012), which exposed pregnant C57BL/6N mice to 0, 0.02, 0.2, or 2 mg/kg PFOA via gavage and examined cytokine levels in the spleens of male and female PND 21 offspring. In males, IL-10 was reduced by approximately 70% relative to IL-10 released from control animals at every exposure level. In contrast, IL-10 was unaffected in females at every exposure level except for an elevation at 0.02%. IL-10 is released by regulatory T (TReg) cells and function to inhibit macrophage responses, therefore the aforementioned impacts of PFOA on macrophages may be downstream of an effect on TRegs.

The impacts of PFOA on the adaptive immune system may reflect dysregulation of cell-signaling pathways involved in adaptive immune responses. The predominant cell-signaling pathways implicated in PFOA-mediated immunotoxicity include the PPAR and NF-κB signaling pathways, which are both involved in the generation of adaptive immune responses. PPARγ activation is involved in the differentiation and development of TH1, TH2, and NK cells, and inhibits the production of inflammatory cytokines in monocytes (Liang et al., 2021).

Multiple in vitro and in vivo studies have investigated the involvement of the PPAR pathway in PFOA immunotoxicity (Dewitt et al., 2016b; Wang et al., 2014; Yang et al., 2002b). Wang et al. evaluated the effects of PFOA in thymocytes of mice exposed to PFOA (0, 5, 10, or 20 mg/kg/day) via gavage and fed RD or HFD. PFOA upregulated gene expression of PPARα

and PPARγ in the thymus of RD animals at the highest dose and elicited a dose-dependent elevation in PPARγ in the thymus for HFD animals that reached significance at 10 mg/kg group. An additional study using PPARα knockout mice suggested the immunosuppressive effects of PFOA are independent of PPARα (Dewitt et al., 2016b). In this study, female C57BL/6Tac PPARα knockout mice and C57BL/6Tac wild-type mice were exposed to 0, 7.5, or 30 mg/kg/day PFOA in drinking water for 14 days and then injected with SRBC on day 11 (Dewitt et al., 2016b). Exposure to 30 mg/kg/day PFOA for 15 days reduced SRBC-specific IgM antibody responses in both wild-type and PPARα knockout mice by 16% and 14%, respectively. There was no significant difference between genotypes, suggesting that PPARα may not be responsible for the suppression of the immune system induced by PFOA exposure. Interestingly, this study also reported reductions in relative spleen weights (30% reduction after exposure to 30 mg/kg/day PFOA) and thymus weights (55.4% after exposure to 7.5 mg/kg/day PFOA) in the wild-type mice, but not in the knockout mice. Similarly, absolute spleen weights of male Sv/129 PPARα-null mice fed approximately 40 mg/kg/day for 7 days were unaffected by PFOA exposure, whereas in male C57BL/6 wild-type mice, absolute spleen weights were significantly reduced by 39% (Yang et al., 2002b). A significant decrease in absolute thymus weight was observed in PFOA-exposed PPARα-null mice, to a lesser degree compared with the reduction observed in PFOA-exposed wild-type mice (39% reduction in PPARα-null mice and 79% reduction in wild-type mice).

One transcriptomics study in humans reported significant associations between maternal blood levels of PFAS (including PFOA), enrichment of genes in neonatal cord blood samples, and episodes of the common cold and antibody titers against the rubella vaccine in children (Pennings et al., 2016). Enrichment of PPARD in neonatal cord blood samples was correlated with maternal PFAS exposure and later common cold episodes in the children. The NF-κB pathway was proposed to be involved in this phenomenon; a comparison of the transcriptomics to the number of common cold episodes revealed that several genes in the NF-κB pathway were altered.

The NF-κB signaling pathway is essential for many parts and functions of the immune system, including a pro-survival role during lymphopoiesis and regulation of T cell differentiation. Wang et al. (2014) provided indirect evidence that NF-κB pathway stimulation may be involved in PFOA immunotoxicity. Gene expression of the glucocorticoid receptor (GR), which stimulates the NF-κB pathway, was increased in the thymus of PFOA-treated animals at the highest exposure level (20 mg/kg), suggesting mechanisms involving NF-κB pathway stimulation may be involved in PFOA immunotoxicity. Additionally, the authors observed that IL-1B gene expression was elevated in the thymus, suggesting that the NF-κB pathway is not suppressed.

3.4.2.3.2.2 Mechanistic Data Informing Allergic or Asthmatic Responses

Several studies evaluated potential associations between PFOA exposure and allergic responses or asthma. An epidemiological study by Zhu et al. (2016) explored the associations between PFOA exposure and TH1/ TH2 polarization in asthmatic children. Male asthmatic children with higher serum levels of PFOA tended to have higher serum IL-4 and IL-5, evident of a TH2 skew. This association was not observed in females, suggesting that the exacerbation of asthma by PFOA involving TH2 cytokines may be male-specific (Table 3-7).

More detailed mechanistic evidence on the relationship between PFOA and allergic responses is available from animal toxicological studies. A dermal exposure study by Shane et al. (2020) applied 0.5–2 % (w/v; equivalent to 12.5–50 mg/kg) PFOA to the skin of BALB/C mice and evaluated allergic sensitization and IgM response. PFOA did not elicit an irritancy response, suggesting that PFOA is not an allergic sensitizer or dermal irritant. However, the splenic IgM response to SRBC was suppressed after 4 days of exposure to 2% PFOA, implying that T cell-dependent immune responses to dermal allergens may be affected by PFOA. Moreover, mice exposed to PFOA had increased expression of Tslp, which is associated with a polarization toward a TH2 response (Shane et al., 2020). In adult zebrafish, the effect of PFOA exposure on mRNA expression of IL-4 was mixed: it was elevated at most doses tested, but reduced at the highest dose (Zhang et al., 2014a). More data from mammalian models on the associations between IL-4 or IL-10 and PFOA are needed to better understand the potential impacts of PFOA on adaptive immune responses involving T cell subsets.

An in vitro study conducted by Lee et al. (2017a) demonstrated that PFOA increased IL-1β gene and protein expression in a dose-related manner in IgE-stimulated RBL-2H3 cells (a rat basophil cell line). Elevated IL-1β was also observed in a study of human bronchial epithelial cells (HBEC3-KT cells) stimulated with a pro-inflammatory agent, Poly I:C, and then treated with 0.13, 0.4, 1.1, 3.3, or 10 μM PFOA (Sørli et al., 2020).

Several studies have evaluated molecular signaling pathways to better understand the mechanistic underpinnings of allergic or asthmatic responses related to exposure to PFOA. At least four mechanistic studies have evaluated the involvement of the NF-κB signaling pathway, which plays an important role in the regulation of inflammation and immune responses, including expression of pro-inflammatory cytokines (Shane et al., 2020; Zhong et al., 2020; Lee et al., 2017a; Zhang et al., 2014a). Histamine release and mast cell degradation were increased in parallel with increased nuclear localization of NF-κB and concomitant reduction in IκB in IgE-stimulated mast cells, suggesting that allergic immune responses and inflammation are exacerbated by PFOA through a mechanism involving the NF-κB pathway (Lee et al., 2017a). Zhang et al. (2014a) reported that PFOA exposure for 21 days can disrupt the NF-κB pathway to mediate inflammatory cytokines in zebrafish. The authors reported a nonmonotonic dose response in gene expression of the p65 transcription factor in RNA isolated from zebrafish splenocytes. In a more recent study, zebrafish were exposed to PFOA for a shorter period (7 or 14 days) and the authors reported that splenic p65 gene expression was increased in all exposed groups (Zhong et al., 2020). Shane et al. (2020) showed that gene expression of NF-κB (Nfkb1) was reduced in the skin of female BALB/c mice dermally exposed to 1 or 2% PFOA after 14 days. However, the study design did not quantify nuclear NF-κB, so it is difficult to discern whether the NF-κB pathway was activated. The authors also reported that gene expression of PPARα was reduced by more than 50% in female mice dermally exposed to 1% or 2% PFOA for 14 days. Mechanistically, PPARα is known to block the NF-κB pathway and thereby modulate immune responses. These data suggest that the NF-κB pathway activity can be reduced independent of action by PPARα in PFOA-mediated immunotoxicity with respect to allergic responses in the skin.

Table 3-7. Effects of PFOA Exposure on Cytokines Impacting Adaptive Immune Responses

Study	Species or Cell Type	Study Type	Cytokine	Measurement	Significant Change in Cytokine	Relevant Immune response
(Zhu et al., 2016)	Human males and females, GBCA study	Epi	IL-2	serum protein (ELISA)	None	Allergy
			IL-4	serum protein (ELISA)	↑[a]	Allergy
			IL-5	serum protein (ELISA)	↑[a]	Allergy
(Hu et al., 2012)	C57BL/6N mice	Ex vivo	IL-10	IL-10 production assay in CD4 + CD25+ T cells[b]		T_{Reg} responses

Notes: ELISA = enzyme-linked immunosorbent assay; GBCA = Genetic and Biomarkers study for Childhood Asthma; IL-2 = Interleukin 2; IL-4 = Interleukin 4; IL-5 = Interleukin 5; IL-10 = Interleukin 10; T_{Reg} = regulatory T cells.
[a] Males only
[b] Purity of CD4 + CD25+ T cells derived by cell estimate to be 84%–95% based on manufacturer specification for the cell isolation kit.

3.4.2.3.2.3 Mechanistic Data Informing Autoimmune Diseases

Select data on PFOA and autoimmune diseases in humans have been summarized by NTP (2016). NTP's conclusion that PFOA was presumed to be an immune hazard to humans was partially based on the positive associations that exist between PFOA exposure and rheumatoid arthritis, ulcerative colitis, and auto-antibodies specific to neural and non-neural antigens. However, the association was considered *low* confidence by the NTP. No animal or in vitro studies have been identified to inform the potential associations between PFOA and autoimmunity.

3.4.2.3.3 Mechanistic Evidence for PFOA-Mediated Effects on Innate Immune Responses

Neutrophils are important cells of the innate immune system that contribute to inflammation and are the first cells to arrive at the site of injury or infection. Reductions in neutrophil migration to the site of injury have been noted in zebrafish exposed to PFOA (Pecquet et al., 2020), suggesting diminished innate immune responses.

Neutrophil migration occurs in response to inflammation and in response to effector cytokines such as IL-8 released from macrophages, which may also be sensitive to PFOA. Qazi et al. (2010) evaluated liver homogenates from male C57BL/6 mice and found that ex vivo production of TNF-α was significantly decreased in animals treated with 0.002% or 0.005% PFOA. Because macrophages are the major producers of TNF-α, the authors propose that PFOA may directly or indirectly affect specialized hepatic macrophages (e.g., Kupffer cells). The decrease in TNF-α release from macrophages could also be related to PFOA effects on the adaptive immune system, given that macrophage responses are inhibited by IL-10 released by TReg cells. Indeed, Hu et al. (2012) demonstrated that ex vivo release of IL-10 from splenocytes was reduced in male mice. Furthermore, cells of the monocyte/macrophage lineage express PPARα and PPARγ (Zhu et al.,

2016; Braissant and Wahli, 1998), which supports a mechanism for immunosuppression involving macrophages and PPAR pathways.

Rainieri et al. (2017) also conducted an in vitro assessment using TLT cells and found that PFOA led to an increase in relative reactive oxygen species (ROS) production measured via the dichlorodihydrofluorescein diacetate (DCF-DA) assay, indicating that PFOA can induce ROS in macrophages.

Although the innate immune system also includes natural killer (NK) cells, no mechanistic studies were identified that evaluated associations with PFOA. One study by Qazi et al. (2010) reported that there were no significant differences in number or percent of NK cells in isolated hepatic immune cells (IHICs) of mice exposed to 0.002% (w/w) PFOA in the diet for 10 days.

3.4.2.3.4 Mechanistic Evidence for PFOA-Mediated Effects on Intrinsic Cellular Defense Pathways

Zhang et al. (2014a) exposed zebrafish to PFOA (0.05, 0.1, 0.5, and 1 mg/L) for 21 days. After exposure, spleens were analyzed for expression patterns of myeloid differentiation 88 (MyD88) and toll-like receptor 2 (TLR2) as well as several cytokines. In addition to the above-mentioned effects on gene expression of *IL-4*, PFOA exerted dose-dependent effects on IL-1β and IL-21 that were stimulated at a low exposure concentration (0.05 mg/L) and inhibited at higher exposure concentrations (≥0.1 mg/L). The Myd88/NF-κB pathway was found to mediate inflammatory cytokine (IL-1 and IL-21) gene expression in zebrafish spleen. Interestingly, exposure of zebrafish to 1 mg/L PFOA reduced TLR2 mRNA expression in spleen by 56% compared with controls. These findings suggest that exposure to PFOA in zebrafish can activate the NF-κB pathway and interfere with TLR2 expression in a dose-dependent manner to enhance pro-inflammatory cytokine gene expression.

3.4.2.3.4.1 Mechanistic Evidence for PFOA-Mediated Effects on Inflammation

The observed increases in circulating leukocytes (neutrophils and monocytes) of experimental animals (Section 3.4.2.2) are consistent with an inflammatory response. Inflammation is a physiological response to tissue damage or infection that can induce components of the innate and adaptive immune system (Klaassen, 2013). Processes that contribute to inflammation and are affected by PFOA include the complement cascade, release and/or upregulation of pro-inflammatory cytokines, and neutrophil migration.

3.4.2.3.4.1.1 Pro-Inflammatory Responses Including Cytokines

The available mechanistic data support that pro-inflammatory cytokines such as IL-1β, TNF-α, and possibly IL-6 are elevated by PFOA exposure (Table 3-8). However, the effect of PFOA (or lack thereof) for some cytokines varies between model organisms and exposure levels. Altered production and/or release of these cytokines may represent an underlying mechanism of the reductions in innate and/or adaptive immune function that has been reported in the human (Section 3.4.2.1) and animal (Section 3.4.2.2) literature.

Elevation of IL-1β is consistent across study designs in mammalian models in vivo and in vitro. Wang et al. (2014) exposed 4–5-week-old male BALB/C mice to 0, 5, 10, or 20 mg/kg/day PFOA via gavage for 14 days in combination with HFD or RD and measured gene expression of cytokines in the thymus and spleen. In the thymus, IL-1β was elevated in mice exposed to

20 mg/kg/day and fed RD. There were no significant effects in the spleen for mice fed RD at any PFOA concentration. In HFD-fed mice, there was an increase in IL-1β in the spleen for the 10 mg/kg/day PFOA group, but no significant changes at any exposure level in the thymus. Likewise, Lee et al. (2017a) and Sørli et al. (2020) have demonstrated that PFOA elevates IL-1β gene and/or protein expression in various cell lines. In contrast to the consistent increases in IL-1β reported in mammalian models, one study in adult zebrafish reported decreased IL-1β mRNA in the spleen following exposure to 0.1, 0.5, or 1 mg/L PFOA for 21 days (Zhang et al., 2014a). More research is needed to determine whether interspecies differences exist in immunomodulation by PFOA. Elevated production of IL-1β is triggered by activation of the inflammasome, which is an innate immune response known to be activated by xenobiotics, and this mechanism may deserve further investigation (Mills et al., 2013).

Several studies have reported elevated levels of TNF-α during immune responses following exposure to PFOA. Qazi et al. (2010) reported decreased levels of TNF-α in liver homogenates of male C57BL/6 mice orally exposed to 0.002% PFOA for 10 days. Lee et al. (2017a) quantified TNF-α levels in blood from male ICR mice following an active systemic anaphylaxis experiment. Mice were sensitized to ovalbumin on day 0 and day 7 via intraperitoneal (i.p.) injection, and PFOA was orally administered on day 9, 11, and 13. Following ovalbumin challenge (i.p.) on day 14, a dose-dependent increase in TNF-α levels in blood was observed, suggesting PFOA aggravates allergic inflammation. In the same study, in vitro experiments using three independent methods (Western blot, RT-PCR, and ELISA) demonstrated a dose-dependent elevation in TNF-α in RBL-2H3 cells sensitized with anti-DNP IgE, then treated with PFOA for 24 hours. Likewise, an in vitro study by Brieger et al. (2011) observed a slight increase in TNF-α released from peripheral blood mononuclear cells (PBMCs) obtained from the blood of 11 human donors. Not all studies reported positive associations of PFOA and TNF-α. Although Bassler et al. (2019) reported positive associations between serum PFOA levels and IFN-γ, the authors found inverse associations with TNF-α.

A few of the studies that observed increases in IL-1β and TNF-α also evaluated other pro-inflammatory cytokines such as IL-8 and IL-6. The in vitro studies by Lee et al. (2017a) did not find significant effects of PFOA on IL-8 expression. This finding was consistent with those of Sørli et al. (2020) and Bassler et al. (2019). IL6 gene and protein expression were elevated in the study by Lee et al. (2017a), which was consistent with results of Brieger et al. (2011) in human PBMCs stimulated with LPS. Most other studies reported either no effect or inverse associations with IL-6 (Mitro et al., 2020; Shane et al., 2020). Giménez-Bastida et al. (2015) reported that PFOA attenuated the elevation in IL-6 levels that normally follows IL-1β-induction in a human colon cell line (CCD-18Co).

IFN-γ is released from activated T cells and NK cells and induces macrophages to produce a variety of inflammatory mediators and reactive oxygen and nitrogen intermediates that contribute to inflammation (Klaassen, 2013). In general, studies did not find associations between PFOA and changes in IFN-γ. The sole exception by Zhong et al. (2020) reported elevations in IFN gene expression in splenocytes of adult zebrafish exposed to 0.05, 0.1, 0.5, or 1 mg/L PFOA for 7 days. Zhu et al. (2016) reported that children with asthma generally had higher serum PFOA concentrations and lower levels of IFN-γ than non-asthmatic children, but there was not a significant association between IFN-γ and PFOA. Qazi et al. (2010) measured IFN-γ levels secreted from IHICs of 6–8-week-old male C57BL/6 (H-2b) mice that were

exposed to 0 or 0.002% (w/w) PFOA in feed for 10 days. A subgroup of IHIC were stimulated with Concanavalin A, which activates T cells to produce IFN-γ. No PFOA-related differences in IFN-γ production were observed in any group in IHICs. The authors also reported a 37% reduction in hepatic levels of IFN-γ, in parallel with reductions in hepatic levels of IL-4 and TNF-α.

Inflammatory responses can be accompanied by increased levels of the activated pro-inflammatory transcription factor, NF-κB. Sirtuins (SIRTs) have been shown to deacetylate NF-κB, which suppresses its transcriptional activation, thereby inhibiting the production of pro-inflammatory cytokines. Park et al. (2019b) exposed a macrophage cell line (RAW 264.7 cells) to 0, 0.5, 5 or 50 μM PFOA and observed significant increases in expression for SIRT3 and SIRT6 at 5 μM exposure, which is inconsistent with a model where PFOA induces inflammation. Interestingly, SIRT4 and SIRT7 expression was more sensitive to PFOA and exhibited non-linear dose-response curves; SIRT4 was significantly reduced at 0.5 μM and significantly elevated at 5 μM, whereas SIRT7 was significantly elevated at 0.5 μM and significantly reduced at 5 and 50 μM. Altogether, the results support that a pro-inflammatory response of PFOA may not follow a linear dose response.

3.4.2.3.4.1.2 Complement Pathways

PFOA can affect both the innate and adaptive immune system to perturb activation of one of the three main pathways of the complement cascade. A study conducted in the C8 Health Project cohort found that serum biomarkers of PFOA were positively associated with serum C3a levels in men, but negatively associated in women, supporting sex-specific perturbations in immune function (Bassler et al., 2019). Also using data from the C8 Health Project, another group of researchers, Genser et al. (2015) found evidence that PFOA blood levels were negatively associated with blood levels of C-reactive Protein (CRP), which is essential for the classical pathway of complement activation (Klaassen, 2013). However, another human study, that measured CRP as one among several blood biomarkers of cardiometabolic disruption reported that serum PFOA was "generally weakly" (i.e., not significantly) associated with CRP and other biomarkers in women 3 years postpartum (Mitro et al., 2020). In contrast to the human evidence, serum C3 levels were reduced in male C57BL/6 (H-2b) mice exposed to 0.02% w/w PFOA in feed for 10 consecutive days (Botelho et al., 2015). Female mice were not studied. Reduced activities of the classical and alternative complement pathways (reflected by CH50 and AH50 response, respectively) were also reported, supporting that PFOA can disrupt the classical (IgM/IgG dependent) and alternative pathways of complement activation, which both require C3.

Table 3-8. Effects of PFOA Exposure on Pro-Inflammatory Cytokines and Markers of Inflammation

Study	Species or Cell Type	Study Type	Cytokine or Inflammatory Marker	Measurement	Direction of Change Following PFOA Exposure
Mitro et al. (2020)		In vivo	IL-6	blood protein (ELISA)	↑

Study	Species or Cell Type	Study Type	Cytokine or Inflammatory Marker	Measurement	Direction of Change Following PFOA Exposure
	Human females, 3 years post-partum		CRP	blood protein (immunoturbidimetric high-sensitivity assay)	↓
Bassler et al. (2019)	Human males and females, C8 Health Project	In vivo	IL-6	serum protein (Multispot Immunoassay)	None
			TNF-α	serum protein (Multispot Immunoassay)	↓
			IL-8	serum protein (Multispot Immunoassay)	None
			IFNγ	serum protein (Multispot Immunoassay)	↑
			C3a	serum protein (ELISA)	None
Sørli et al. (2020)	Human bronchial epithelial cell line	In vitro	Il-6	culture supernatant protein (ELISA)	None
			IL-1α	culture supernatant protein (ELISA)	None
			IL-1β	culture supernatant protein (ELISA)	↑
			CXCL8	culture supernatant protein (ELISA)	None
Wang et al. (2014)	BALB/c mice	In vivo	IL-1β	Gene expression	↑
Shane et al. (2020)	BALB/c mice	In vivo	IL-1β	Gene expression	↑
			IL-6	Gene expression	None
Qazi et al. (2010)	C57BL/6 mice	Ex vivo	IFN-γ	culture supernatant protein (ELISA)	None

Notes: IL-6 = Interleukin 6; CRP = C-Reactive Protein; TNF-α = Tumor Necrosis Factor α; IL-8 = Interleukin 8; IFNγ = Interferon γ; C3a = cleavage product of Complement 3

3.4.2.3.5 Conclusions

Overall, the available evidence supports that PFOA affects the innate and adaptive immune system as well as immune organ physiology at multiple levels including immune system development, survival, proliferation, and differentiation of B and T cells, inflammatory responses, neutrophil migration, and complement activation. One study provided evidence that antibody glycosylation patterns could be perturbed. Mechanistic data available from in vitro, in vivo, and epidemiological studies were used to evaluate the etiology and mode of action of PFOA-associated immunosuppression and other effects on the immune system. The pleotropic immunomodulatory effects of PFOA, including impaired vaccine responses, may reflect perturbed function of B and/or T cells. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating apoptosis, participates in the regulation of CD4+ T cell differentiation, and is involved in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the mechanism underlying

PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro-B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation.

NF-κB activation also facilitates the induction of apoptosis during negative selection of T cells in the thymus, which is essential for the deletion of T cells that recognize self. In contrast, NF-κB acts as a pro-survival factor during the negative selection of B cells. In human studies, PFOA exposure has been associated with autoimmune diseases including ulcerative colitis. Further mechanistic evidence is needed to determine the directionality of the effect of PFOA on NF-κB, which will inform the cell types that predominantly contribute to the etiology of autoimmune diseases associated with PFOA exposure.

3.4.2.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and immunosuppressive effects in human studies based on largely consistent decreases in antibody response following vaccinations (against two different infectious agents: tetanus and diphtheria) in multiple *medium* confidence studies in children (Timmermann et al., 2021; Abraham et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012). Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease. The antibody response results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted (though overlapping) birth cohorts in the Faroe Islands, supported by a *low* confidence study in adults.

The results in human epidemiological studies measuring PFOA concentrations and hypersensitivity were mixed. Significant associations between PFOA exposure and "ever" or "current" asthma were seen primarily in sex- or age-specific subgroups but were null or insignificant in whole study analyses. For allergy and eczema outcomes, results were inconsistent across studies.

The associations between PFOA exposure and human autoimmune disease were also mixed. Two studies (Steenland et al., 2018b; Steenland et al., 2013) found significant associations indicating increased risk of autoimmune disease. Also, PFOA levels were found to be lower in healthy controls compared with cases with MS (Ammitzbøll et al., 2019). Results were most consistent for ulcerative colitis, with significant associations indicating increased risk with increasing PFOA exposure in one *medium* confidence study (Steenland et al., 2013) and one *low* confidence study (Steenland et al., 2018b).

The animal evidence for an association between PFOA exposure and immunosuppressive responses is *moderate* based on 13 *high* or *medium* confidence animal toxicological studies. Short-term and developmental PFOA exposure in rodents resulted in reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. In functional assessment of the immune response, PFOA exposure was associated with reduced globulin and immunoglobulin levels (Dewitt et al., 2008; Loveless et al., 2008). Suppression of the immunoglobulin response in these animals is consistent with decreased antibody response seen in human subpopulations.

Mechanistic data related to the human immunomodulatory effects were similarly inconsistent compared with the human epidemiological data. The available mechanistic data indicate that pro-inflammatory cytokines such as IL-1β, TNF-α, and possibly IL-6 are elevated by PFOA exposure. However, the specific effects vary across model organisms and exposure levels. Altered production and/or release of these cytokines may reflect reductions in innate and/or adaptive immune function that has been reported in the human and animal literature.

While evidence exists for reduced antibody response, such as diminished immune response to sheep red blood cells in mice treated with PFOA (a T cell-dependent antibody response), data are limited. Both T cell-dependent and T cell-independent responses are reduced by PFOA, according to a systematic review conducted by the NTP (NTP, 2016). Alterations to these responses could explain the decreased antibody response in humans. Although the evidence is not consistent across studies or between sexes and/or model systems, several studies have reported that PFOA appears to exacerbate allergic immune and inflammatory response, likely through disruption to the NF-κB pathway, increased TNFα, and/or TH2 response.

One proposed mechanism of immunotoxicity involves apoptosis of immune cells, which appears to be a high-dose phenomenon, as evidenced by in vivo and in vitro studies in which the effects were only seen at ≥10 mg/kg/day in mice or 500 mg/L in the human macrophage TLT cell line. Relatedly, NF-κB activation also facilitates the induction of apoptosis during negative selection of T cells in the thymus, which is essential for the deletion of T cells that recognize host cells (i.e., "self"). In contrast, NF-κB acts as a pro-survival factor during the negative selection of B cells. PFOA has been shown to disrupt the NF-κB pathway. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating apoptosis, participating in the regulation of CD4+ T cell differentiation, and participating in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the mechanism underlying PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation.

There is conflicting evidence regarding the involvement of PPAR signaling in immunotoxic effects of PFOA: there is evidence of PPAR-independent alterations to adaptive immunity, while suppressive effects of innate immunity appear to involve macrophages and PPAR signaling.

3.4.2.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans under relevant exposure circumstances (Table 3-9). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from epidemiological studies at median levels as low as 1.1 ng/mL PFOA. The evidence in animals showed coherent immunomodulatory responses at doses as low as 1 mg/kg/day PFOA that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOA exposure might also have the potential

to affect sensitization and allergic responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and with limited support from animal or mechanistic studies. Given the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOA. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental lifestages represents a source of uncertainty in the immunotoxicity database of PFOA.

Table 3-9. Evidence Profile Table for PFOA Exposure and Immune Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.2.1)					⊕⊕⊙ *Evidence Indicates (likely)*
Immunosuppression 1 *High* confidence study 19 *Medium* confidence studies 8 *Low* confidence studies 3 *Mixed*[a] confidence study	Studies conducted in the Faroe Islands examined antibody levels among children at various timepoints compared with exposure measured prenatally and throughout childhood. Lower antibody levels against tetanus and diphtheria were observed in children at birth, 18 mo, age 5 yr (pre-and post-booster), and at age 7 yr. Similarly, antibody levels against rubella (2/2) were significantly decreased in *medium* confidence studies of children. Findings in the five studies examining adults and adolescents were less consistent than in children. Three studies reported inverse associations, one for rubella, one for hepatitis B antibodies and one for influenza A/H3N2, but other antibody responses were inconsistent across	• *High* and *medium* confidence studies the reported effects • *Consistent direction* of effect • *Coherence* of findings across antibody response and increased infectious disease	• *Low* confidence studies • *Imprecision* of findings	⊕⊕⊙ *Moderate* Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus. Reductions in antibody response were observed at multiple timepoints in childhood, using both prenatal and childhood exposure levels. Similar decreases in antibody response to other pathogens, such as rubella, were observed, although the number of studies analyzing these antibody responses to these pathogens was limited. An increased risk of upper and lower respiratory tract infections was observed among children, coherent with findings of reduced antibody response. There was also supporting	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of reduced antibody response. Evidence in animals showed coherent immunomodulatory responses that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOA exposure might also have the potential to affect sensitization and allergic responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and has only limited support from animal or mechanistic studies.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	all exposure windows. Infectious disease was examined in 14 studies of children. Studies examining infections of the respiratory system observed some positive associations (5/14), although many findings from other studies were not precise. Findings for infectious disease in adults were mixed, with two studies reporting inconsistent results for COVID-19 infections.			evidence of increased risk of asthma, and autoimmune disease, however, the number of studies examining the same type of autoimmune disease was limited.	*Human relevance and other inferences:* Given the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOA. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental life stages represents a source of uncertainty in the immunotoxicity database of PFOA.
Immune hypersensitivity 1 *High* confidence study 20 *Medium* confidence studies 6 *Low* confidence studies 2 *Mixed*[a] confidence studies	Examination of immune hypersensitivity includes outcomes such as asthma, allergies, and eczema. Increased odds of asthma were reported in most *medium* confidence studies (8/12), although associations were often inconsistent by subgroups. *Low* confidence studies supported the findings of increased odds of asthma or higher exposure levels among asthmatics, although results were not always consistent or precise. Eight studies	• *High* and *medium* confidence studies • *Consistent direction* of effect for asthma across *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effect between subpopulations		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	examined allergies, rhinitis, or rhinoconjunctivitis. Some positive associations (3/8) were observed, although this varied by outcome timing and were at times inconsistent. Significantly increased odds of eczema or atopic dermatitis were observed in several studies (4/13), although these associations were sometimes limited to subgroups (2/4).				
Autoimmune disease 2 *Medium* confidence studies 4 *Low* confidence studies	Increased risk of autoimmune disease was reported in several studies (4/6). One study reported a significantly increased risk of rheumatoid arthritis, and two studies reported a significantly increased risk of ulcerative colitis. Two studies reported positive associations for multiple sclerosis, with one reaching significance in men. One study (1/2) observed increased risk of celiac disease among female children and young adults. Findings	• *Medium* confidence studies	• *Low* confidence studies • *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	for Crohn's disease were less consistent.				
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.2.2)					
Organ weights 3 *High* confidence studies 7 *Medium* confidence studies	Decreases in absolute (6/8) and relative (4/8) spleen weights and in absolute (5/5) and relative (3/5) thymus weights were observed across studies regardless of study design. Overall, decreases in spleen and thymus weights were more frequently observed in males than females and tended to coincide with reductions in body weight.	• *High* and *medium* confidence studies • *Dose-response* relationship seen within multiple studies • *Coherence* of findings of other immunological endpoints	• *Inconsistent direction* of effects across sex • *Confounding variables* such as decreases in body weights	⊕⊕⊙ *Moderate* Evidence is based on 13 *high* or *moderate* confidence animal toxicological studies. Short-term and developmental PFOA exposure in rodents resulted in reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. In functional assessments of the immune response, PFOA exposure was associated with reduced globulin and immunoglobulin levels. Suppression of the immunoglobulin response in these animals is consistent with decreased antibody response seen in human subpopulations.	
Immune cellularity 1 *High* confidence study 4 *Medium* confidence studies	Of the studies that measured circulating WBCs and differentials, one short-term study in male mice found decreases in WBC counts, while a chronic rat study observed transient increases in males that were attributed to increased counts of lymphocytes and neutrophils. One short-term study in male rats and mice reported increased neutrophils and monocytes, decreased	• *High* and *medium* confidence studies • *Dose-response* relationship seen within multiple studies • *Coherence* of findings	• *Inconsistent direction* of effects across species, sex, and study design • *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	eosinophils, as well as reduced splenocytes and thymocytes in mice but no changes in rats. One developmental study in mice observed decreases in splenic regulatory T cells in males and females.				
Globulins and immunoglobulins 2 *High* confidence studies 2 *Medium* confidence studies	Mixed results were reported for concentrations of globulins and immunoglobulins. Decreased globulin levels (2/3) were observed in male and female rats, in a dose-dependent manner (1/3), following short-term and chronic exposure to PFOA. One short-term study reported increased globulins (1/3) in male mice. Additional findings, including increases in IgA, IgG, and IgM, were found in male mice.	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Inconsistent direction* of effects between species • *Limited number* of studies examining specific outcomes		
Immune response 4 *Medium* confidence studies	Dose-dependent decreases in IgM following a SRBC or KLH challenge was seen in three short-term studies in mice (3/4).	• *Medium* confidence studies • *Dose-response* relationship seen within multiple studies	• *Inconsistent direction* of effects across study design and species • *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	No changes in IgM were observed in chronically exposed male rats nor developmentally exposed female mice (2/4). In a short-term study that assessed female mice, increased IgG levels were observed after a SRBC challenge (1/2), but a developmental study in female mice found no changes in IgG levels (1/2).				
Histopathology 3 *High* confidence studies 2 *Medium* confidence studies	A short-term study in male mice and rats reported increased incidence of granulocytic hyperplasia of the bone marrow and increased incidence of splenic and thymic atrophy in mice but not rats. One *high* confidence short-term study in male and female rats observed no changes in the spleen, thymus, or lymph nodes but found increased bone marrow hypocellularity in male rats. One chronic study found decreased incidence of splenic hemosiderosis in male and female rats. One	• *High* and *medium* confidence studies • *Coherence* of findings	• *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	chronic and one developmental study observed histopathological changes in the spleen, thymus, bone marrow, and/or lymph nodes of male and female rats.				

Mechanistic Evidence and Supplemental Information (Section 3.4.2.3.4)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
Key findings and interpretation: • Apoptosis of immune cells is a high dose immunotoxic phenomenon that has been observed in both in vivo and in vitro studies of PFOA. • Disruption of the NF-κB signaling pathway, which is involved in T cell responses, regulation of apoptosis, and inflammatory response, has been demonstrated both directly and indirectly in in vivo human and animal data, as well as in vitro. • Inconsistent evidence of exacerbation of allergic immune and inflammatory responses via NF-κB pathway, increased TNFα, and/or T_H2 response. **Limitations:** • Inconsistent findings between sexes, model systems, and studies regarding allergic immune response. • Limited database for immune response data. • While PPARα is mechanistically linked to immune signaling (blocking the NF-κB pathway), it is not clear if PFOA-induced alterations to PPARα are involved in immunomodulatory effects: some PPARα-knockout mouse studies have suggested that immunomodulation occurs independent of PPARα.	Findings support plausibility that PFOA exposure can lead to dysregulation of signaling pathways related to immune response; however, data have inconsistencies.

Notes: HFMD = hand, foot, and mouth disease; A/H3N2 = influenza A virus subtype H3N2; COVID-19 = coronavirus disease 2019; WBC = white blood cells; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; SRBC = sheep red blood cells; KLH = keyhole limpet hemocyanin; NF-κB = nuclear factor kappa B; TNFα = tumor necrosis factor alpha; T_H2 = T helper 2; PPARα = peroxisome proliferator-activated receptor alpha.

[a] Studies may be of *mixed* confidence due to differences in how individual outcomes within the same study were assessed (e.g., clinical test versus self-reported data).

3.4.3 Cardiovascular

EPA identified 112 epidemiological and 10 animal toxicological studies that investigated the association between PFOA and cardiovascular effects. Of the 54 epidemiological studies addressing cardiovascular endpoints, 3 were classified as *high* confidence, 28 as *medium* confidence, 14 as *low* confidence, 5 as *mixed* (1 *high/medium* and 4 *medium/low*) confidence, and 4 were considered *uninformative* (Section 3.4.3.1). Of the 89 epidemiological studies addressing serum lipid endpoints, 1 was classified as *high* confidence, 29 as *medium* confidence, 32 as *low* confidence, 19 as *mixed* (1 *high/medium* and 18 *medium/low*) confidence, and 8 were considered *uninformative* (Section 3.4.3.1). Of the animal toxicological studies, three were classified as *high* confidence, five as *medium* confidence, and two were considered *low* confidence (Section 3.4.3.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.3.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.3.1.1 Cardiovascular Endpoints

3.4.3.1.1.1 Introduction

Cardiovascular disease (CVD) is the primary cause of death in the United States with approximately 12% of adults reporting a diagnosis of heart disease (Schiller et al., 2012). Studied health effects include ischemic heart diseases (IHD), coronary artery disease (CAD), coronary heart disease (CHD), hypertension, cerebrovascular disease, atherosclerosis (plaque build-up inside arteries and hardening and narrowing of their walls), microvascular disease, markers of inflammation (e.g., C-reactive protein), and mortality. These health outcomes are interrelated – IHD is caused by decreased blood flow through coronary arteries due to atherosclerosis resulting in myocardial ischemia. Cardiovascular outcomes were synthesized separately by population (i.e., adults, children, occupational populations), and definitions of certain conditions may vary by age. For example, high blood pressure and/or hypertension is generally defined as SBP ≥140 mmHg and DBP ≥90 mmHg in adults and SBP ≥130 mmHg and DBP ≥80 mmHg in children and adolescents, although consistent blood pressure measurements in youth can be challenging (Falkner et al., 2023).

There are seven epidemiological studies from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cardiovascular effects. Study quality evaluations for these seven studies are shown in Figure 3-30. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-10 and below.

The 2016 PFOA HESD (U.S. EPA, 2016c) did not identify strong evidence for an association between CVD and PFOA, based on five occupational studies. Several occupational studies examined cardiovascular-related cause of death among PFOA-exposed workers at the West Virginia Washington Works plant (Steenland and Woskie, 2012; Sakr et al., 2009; Leonard et al., 2008) and the 3M Cottage Grove plant in Minnesota (Raleigh et al., 2014; Lundin et al., 2009; Gilliland and Mandel, 1993). This type of mortality is of interest because of the relation between lipid profiles (e.g., LDL) and the risk of CVD. A study in West Virginia did not find an association between cumulative PFOA levels and IHD mortality across four quartiles of

cumulative exposure (Steenland and Woskie, 2012). On the basis of these data from the worker cohorts (part of the C8 Health Project), the C8 Science Panel (2012b) concluded that there is no probable link between PFOA and stroke and CAD. A later study of community residents from the C8 Health Project reported an elevated risk of stroke in quintiles 2 through 4 of PFOA concentrations compared with quintile 1 (HRs ranging 1.36 to 1.45); however, the association was null in continuous analyses (HR, linear = 1.00, 95% CI: 0.99, 1.01) (Simpson et al., 2013). Study authors reported a significant increased risk (HR, linear = 1.10, 95% CI: 1.02, 1.18) after excluding the highest quintile of exposure. The analysis of the workers at the Minnesota plant also did not observe an association between cumulative PFOA exposure and IHD risk, but an increased risk of cerebrovascular disease mortality was seen in the highest exposure category (Lundin et al., 2009). These studies are limited by the reliance on mortality (rather than incidence) data, which can result in a substantial degree of under ascertainment and misclassification. Evidence was limited in studies on the general population, with only one high-exposure community study and two NHANES studies examining the association between PFOA and hypertension risk. Increased risk of hypertension was observed in a C8 community study (Winquist and Steenland, 2014); however, the association was imprecise for estimates comparing the highest two quintiles to the lowest quintile of exposure. One NHANES study identified in the 2021 ATSDR *Toxicological Profile for Perfluoroalkyls* (ATSDR, 2021) observed a large increased risk of hypertension for adults not using hypertensive medication in the highest exposure quartile (Min et al., 2012). The other NHANES study reported a decreased risk of hypertension in children (Geiger et al., 2014b).

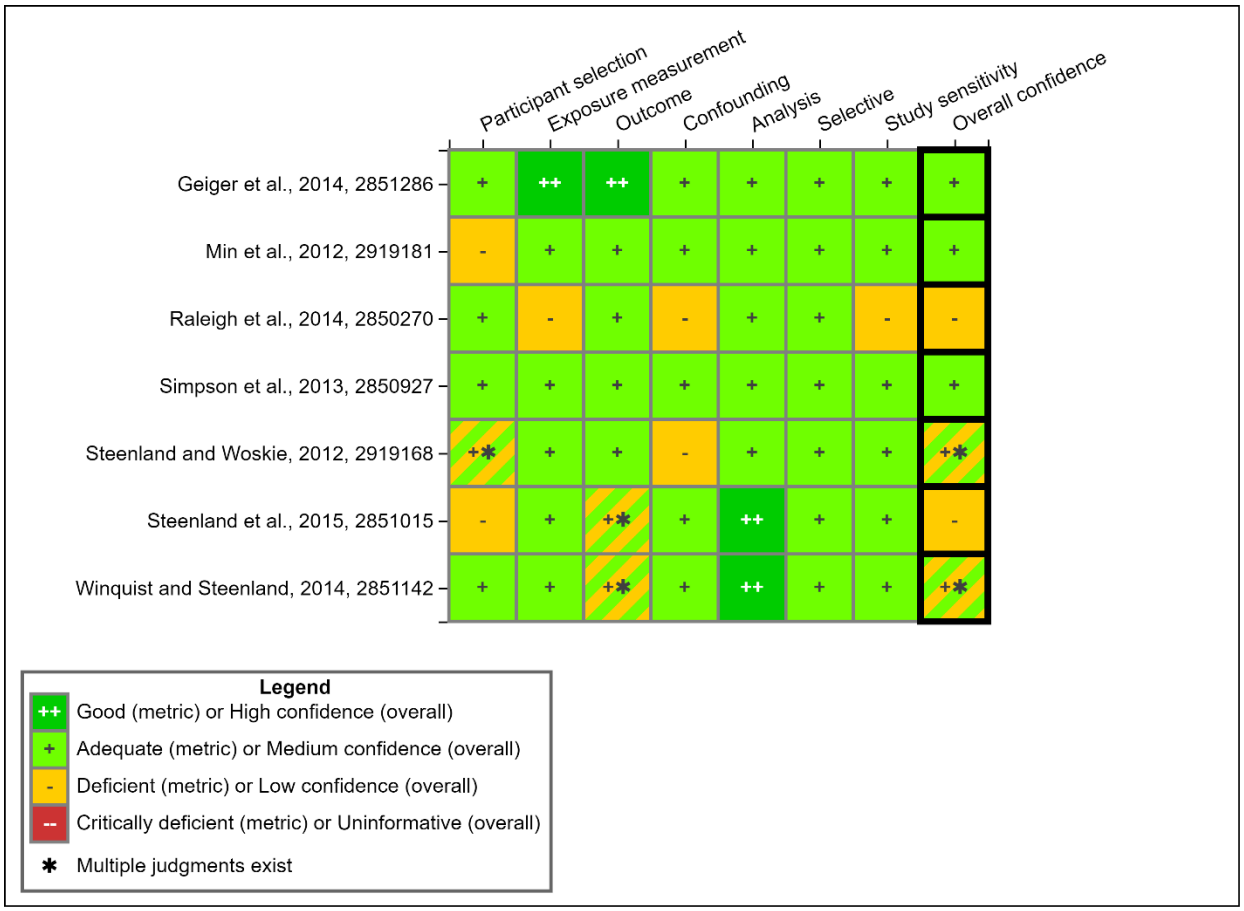


Figure 3-30. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Table 3-10. Associations Between Elevated Exposure to PFOA and Cardiovascular Outcomes from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Population	SBP[a]	DBP[a]	Hypertension[b]	Stroke[b]	CHD, IHD, CAD[b]
Geiger et al., 2014, 2851286 *Medium*	Cross-sectional	Children	↓	–	–	NA	NA
Min, 2012, 2919181	Cross-sectional	Adults	NA	NA	↑↑	NA	NA
Raleigh et al., 2014, 2850270[c]	Cohort	Occupational	NA	NA	NA	NA	–
Steenland and Woskie, 2012, 2919168[d] *Mixed*[e]	Cohort	Occupational	NA	NA	NA	–	–
Simpson, 2013, 2850927 *Medium*	Cohort	Adults and Occupational	NA	NA	NA	↑	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	–	↑	–
Winquist and Steenland, 2014, 2851142 *Mixed*[f]	Cohort	Occupational	NA	NA	↑	NA	–

Notes: SBP = systolic blood pressure; DBP = diastolic blood pressure; CHD = coronary heart disease; IHD = ischemic heart disease; CAD = coronary heart disease; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association; NA = no analysis was for this outcome was performed.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Gilliland, 1993, 1290858 and Lundin, 2009, 1291108 report overlapping data with Raleigh, 2014, 2850270, which was considered the most updated data.

[d] Leonard, 2008, 1291100 and Sakr, 2009, 2593135 report overlapping data with Steenland and Woskie, 2012, 2919168, which was considered the most updated data.

[e] Steenland and Woskie, 2012, 2919168 was rated *medium* confidence for comparisons with the DuPont referent population and *low* confidence for comparisons with the U.S. population.

[f] Winquist and Steenland, 2014, 2851142 was rated *medium* confidence for hypertension and *low* confidence for coronary heart disease.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 48 new epidemiological studies report on the association between PFOA and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 21 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed in the Appendix (U.S. EPA, 2024a). Two of the publications (Girardi and Merler, 2019; Steenland et al., 2015) were occupational studies and the remainder were conducted on the general population. Six general population studies (Ye et al., 2021; Yu et al., 2021; Hutcheson et al., 2020; Mi et al., 2020; Honda-Kohmo et al., 2019; Bao et al., 2017) were conducted in a high-exposure community in China (i.e., C8 Health Project and "Isomers of C8 Health Project" populations), and three studies (Canova et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020) were conducted in a high-exposure community in Italy (i.e., Vento Region). Different study designs were also used including three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b), 11 cohort studies (Li et al., 2021; Papadopoulou et al., 2021; Lin et al., 2020c; Mitro et al., 2020; Donat-Vargas et al., 2019; Girardi and Merler, 2019; Warembourg et al., 2019; Fry and Power, 2017; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Steenland et al., 2015), one case-control study (Mattsson et al., 2015), and 35 cross-sectional studies (Koskela et al., 2022; Averina et al., 2021; Canova et al., 2021; Ye et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020e; Mi et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; He et al., 2018; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Mobacke et al., 2018; Yang et al., 2018; Bao et al., 2017; Koshy et al., 2017; Lind et al., 2017b; Christensen et al., 2016; Lin et al., 2016; Lin et al., 2013; Shankar et al., 2012). The three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b) were not controlled trials of PFAS exposures, but rather health interventions: prevention of type 2 diabetes in the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) (Osorio-Yáñez et al., 2021; Cardenas et al., 2019) and weight loss in Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study (Liu et al., 2018b). Thus, these studies can be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The studies were conducted in different study populations with the majority of studies conducted in the United States (Koskela et al., 2022; Li et al., 2021; Osorio-Yáñez et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Cardenas et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; He et al., 2018; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Fry and Power, 2017; Koshy et al., 2017; Christensen et al., 2016; Steenland et al., 2015; Shankar et al., 2012). The remaining studies were conducted in China (Ye et al., 2021; Yu et al., 2021; Yang et al., 2018; Bao et al., 2017), Taiwan (Lin et al., 2020e; Lin et al., 2016; Lin et al., 2013), Spain (Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017), Croatia (Chen et al., 2019), Sweden (Donat-Vargas et al., 2019; Mobacke et al., 2018; Lind et al., 2017b; Mattsson et al., 2015), Italy (Canova et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020; Girardi and Merler, 2019), Norway (Averina et al., 2021), and two studies conducted in several European countries (Papadopoulou et al., 2021; Warembourg et al., 2019). All the studies measured PFOA in blood components (i.e., serum or plasma) with three studies measuring levels in maternal serum (Li et al., 2021; Papadopoulou et al., 2021; Warembourg et al., 2019), and four studies measuring

levels in maternal plasma (Papadopoulou et al., 2021; Mitro et al., 2020; Warembourg et al., 2019; Manzano-Salgado et al., 2017b).

3.4.3.1.1.2 Study Quality

There are 48 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cardiovascular effects. Study quality evaluations for these 48 studies are shown in Figure 3-31, Figure 3-32, and Figure 3-33.

Of the 48 studies identified since the 2016 assessment, 3 studies were *high* confidence, 26 were *medium* confidence, 12 were considered *low* confidence, 3 were considered *mixed* confidence, and 4 studies were considered *uninformative* (Jain, 2020a, b; Leary et al., 2020; Seo et al., 2018). The main concerns with the *low* confidence studies included the possibility of outcome misclassification (e.g., reliance on self-reporting) in addition to potential for residual confounding or selection bias (e.g., unequal recruitment and participation among subjects with outcome of interest, lack of consideration and potential exclusion due to medication usage). Residual confounding was possible due to SES, which can be associated with both exposure and the cardiovascular outcome. Although PFOA has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further, temporality of PFOA exposure could not be established for several *low* confidence studies due to their cross-sectional design. Several of the *low* confidence studies also had sensitivity issues due to limited sample sizes (Girardi and Merler, 2019; Graber et al., 2019; Khalil et al., 2018; Christensen et al., 2016). Two studies were rated *adequate* for all domains, indicating lower risk of bias; however, both studies treated PFOA as the dependent variable, resulting in both studies being considered *uninformative* (Jain, 2020a, b). Analyses treating PFOA as a dependent variable support inferences for characteristics (e.g., kidney function, disease status, race/ethnicity) that affect PFOA levels in the body, but it does not inform the association between exposure to PFOA and incidence of cardiovascular disease. Small sample size (n = 45) and missing details on exposure measurements were the primary concerns about the remaining *uninformative* study (Leary et al., 2020).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

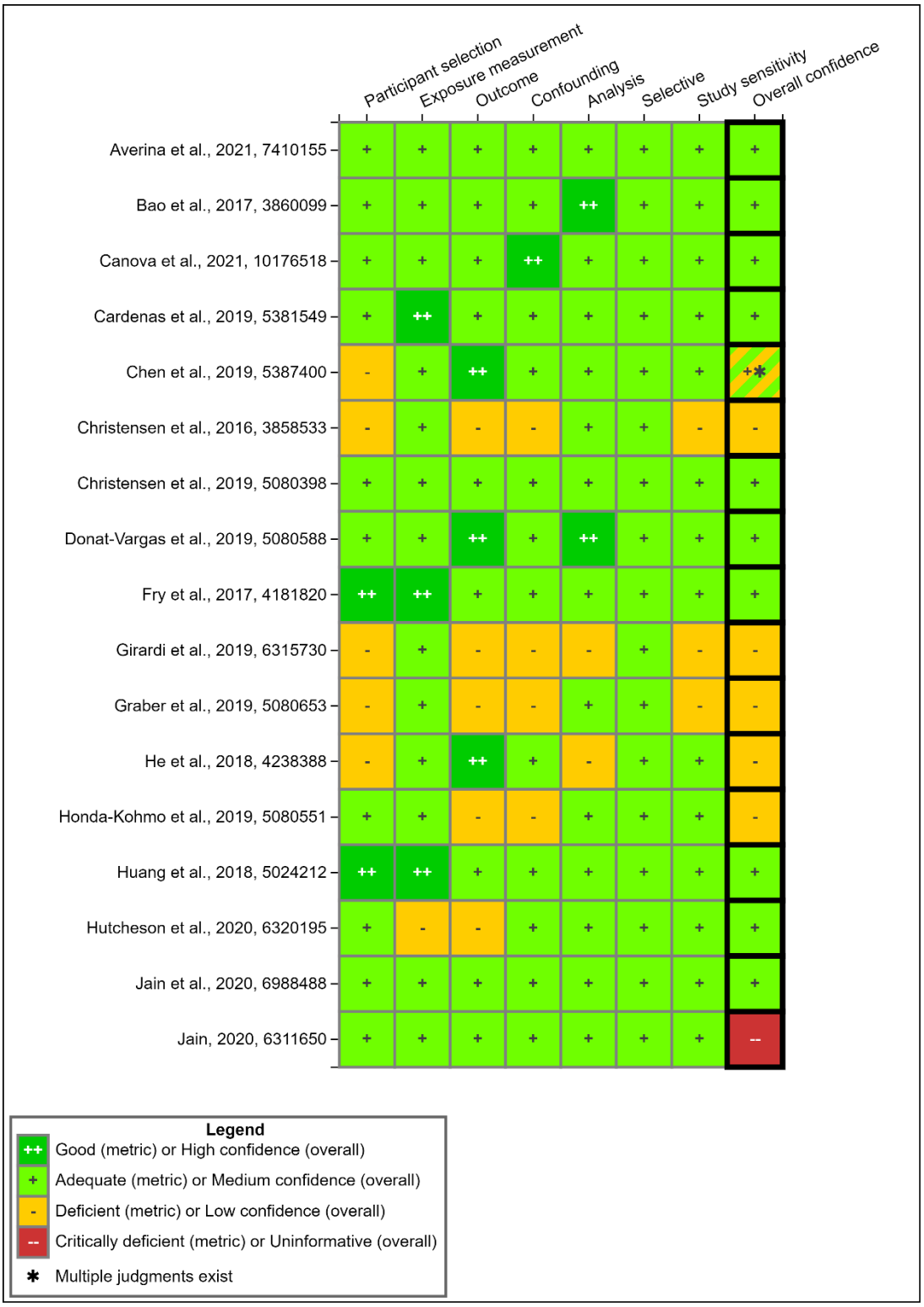


Figure 3-31. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

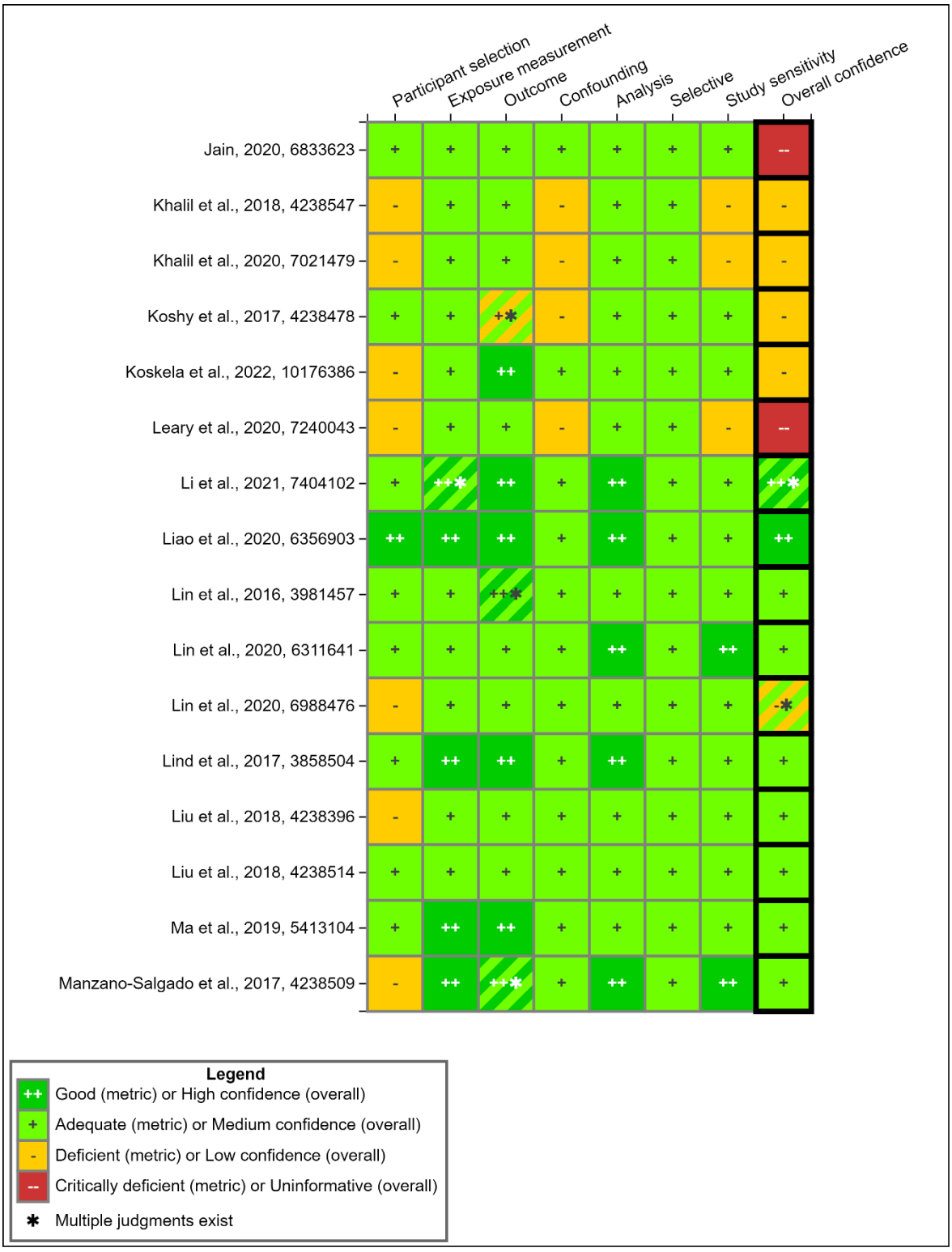


Figure 3-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

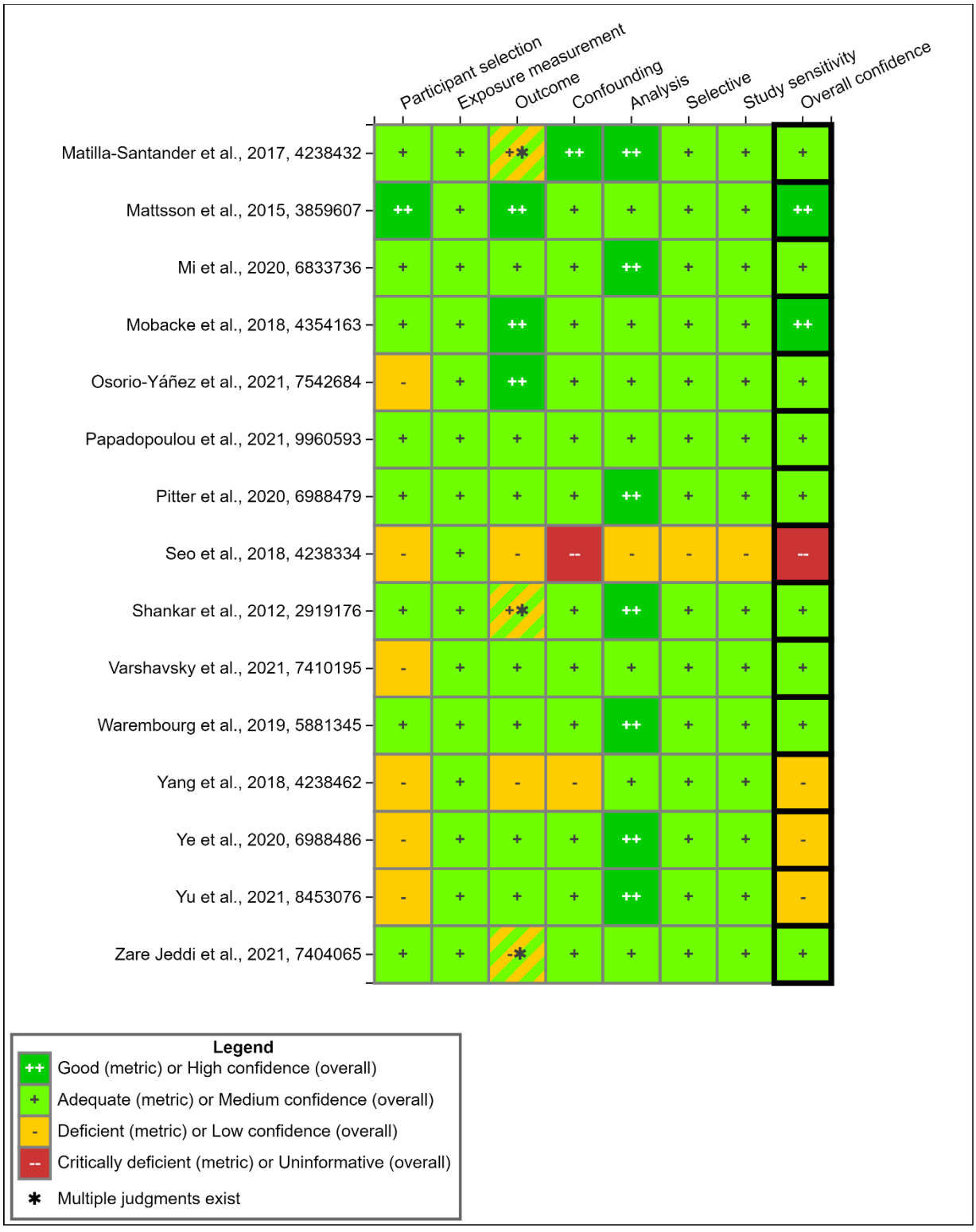


Figure 3-33. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.1.3 Findings From Children and Adolescents

One *high* confidence study (Li et al., 2021) and six *medium* confidence studies (Averina et al., 2021; Canova et al., 2021; Papadopoulou et al., 2021; Ma et al., 2019; Warembourg et al., 2019; Manzano-Salgado et al., 2017b) examined blood pressure in children and adolescents and reported no associations (see Appendix, (U.S. EPA, 2024a)). No association was observed in a *high* confidence study in infants from the Health Outcomes and Measures of the Environment (HOME) Study (Li et al., 2021) between PFOA in maternal serum and child blood pressure measured at 12 years of age. In a cross-sectional analysis, Ma et al. (2019) did not observe an association between serum PFOA and blood pressure among 2,251 NHANES (2003–2012) participants (mean age 15.5 years). Similarly, Manzano-Salgado et al. (2017b) did not observe an association between maternal PFOA and child blood pressure in combined or in gender-stratified analyses at age 4 and 7 years.

In a cohort of 1,277 children (age 6–11 years), PFOA measured both in maternal blood during the pre-natal period and in plasma during the postnatal period were not associated with blood pressure in single-pollutant models (Warembourg et al., 2019). However, the association was significantly positive for systolic blood pressure (SBP) after co-adjustment for organochlorine compounds (i.e., dichlorodiphenyldichloroethane (DDE) and hexachlorobenzene (0.9; 95% CI: 0.1, 1.6; p = 0.021)). An overlapping study (Papadopoulou et al., 2021) examined the association for z-scores of blood pressure in children in a model mutually adjusted for other PFAS and did not find an association. In a cross-sectional study of children and adolescents in a high-exposure community (Canova et al., 2021), blood pressure was lower among adolescents with increasing serum PFOA, but none of the associations reached significance. An increased risk of hypertension (SBP ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg) was observed in a *medium* confidence cross-sectional study (Averina et al., 2021) on Norwegian adolescents taking part in the Fit Futures. The magnitude of the association was larger among increasing quartiles of PFOA exposure, reaching significance for those in the fourth quartile of exposure (OR: 2.08; 95% CI: 1.17, 3.69, p = 0.013). Two *low* confidence studies did not observe associations between serum PFOA and blood pressure (Khalil et al., 2018; Lin et al., 2013).

Other cardiovascular conditions reported in children and adolescents include carotid intima-media thickness test (CIMT) and brachial artery distensibility. Two *medium* confidence studies that examined CIMT among adolescents and young adults from the Young Taiwanese Cohort Study (Lin et al., 2016; Lin et al., 2013) reported no associations. A *low* confidence study of children and adolescents from the World Trade Center (WTC) Health Registry reported PFOA was significantly associated with increased brachial artery distensibility (0.45; 95% CI: 0.04, 0.87; p = 0.03), but was not associated with pulse wave velocity (Koshy et al., 2017). However, concerns for residual confounding by age and SES contributed to the *low* confidence.

3.4.3.1.1.4 Findings From the General Adult Population

Most of the studies identified since the last assessment were conducted among general population adults (see Appendix, (U.S. EPA, 2024a)). A total of 15 studies examined PFOA in association with SBP, diastolic blood pressure (DBP), hypertension, and elevated blood pressure (Zare Jeddi et al., 2021; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017; Christensen et al., 2016).

Of the 10 studies that examined blood pressure as a continuous measure, six reported statistically significant positive associations (Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Pitter et al., 2020; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017). However, the results were not always consistent between SBP and DBP.

A *high* confidence study in 6,967 NHANES (2003–2012) participants 20 years and older reported a statistically significant positive association with SBP (β per 10-fold change in PFOA: 1.83; 95% CI: 0.40, 3.25) in the fully adjusted model (Liao et al., 2020). No association was observed for DBP.

A *high* confidence study (Mitro et al., 2020) conducted among 761 women that examined associations between PFOA concentrations measured during pregnancy and blood pressure assessed at 3 years post-partum reported a positive but nonsignificant association with SBP (β per doubling of PFOA: 0.8; 95% CI: −0.3, 1.8). No association was observed with DBP.

Two *medium* confidence cross-sectional studies with overlapping data from the "Isomers of C8 Health Project," a highly exposed population of Shenyang, China (Mi et al., 2020; Bao et al., 2017), also reported positive associations for blood pressure. In 1,612 participants with elevated PFOA levels (median 6.19 ng/mL), Bao et al. (2017) reported large increases in DBP (β: 2.18; 95% CI: 1.38, 2.98) and SBP (β: 1.69; 95% CI: 0.25, 3.13). After stratification by sex, a positive association was observed in men only for DBP (β: 1.48; 95% CI: 0.58, 2.37) and in women only for SBP (β: 6.65; 95% CI: 4.32, 8.99). In participants with high PFOA levels (median 4.8 ng/mL), Mi et al. (2020) observed statistically significant increases in DBP (β: 1.49; 95% CI: 0.34, 2.64). No association was observed for SBP.

Similar findings were observed in another *medium* confidence study in a high-exposure community in Italy (Pitter et al., 2020). Adults (20–39 years old) included in a regional (i.e., Vento Region) surveillance program were included in a cross-sectional analysis of blood pressure and PFOA exposure. Significant positive associations were reported for DBP (β: 0.34; 95% CI: 0.21, 0.47) and SBP (β: 0.37; 95% CI: 0.19, 0.54) in the overall (n = 15,380) population. Results were generally consistent after stratification by sex. Minor sex differences were observed, such as slightly larger increases in SBP among men (β: 0.46; 95% CI: 0.19, 0.73) and larger increases in DBP among women (β: 0.39; 95% CI: 0.21, 0.57). Monotonic trends were observed in all quartile analyses, although significance was not reported.

Lin et al. (2020c), a *medium* confidence study using data from the Diabetes Prevention Program, a randomized controlled health intervention trial, reported that an increase in baseline PFOA concentration was significantly associated with higher SBP (β: 1.49; 95% CI: 0.29, 2.70); no association was observed with DBP or pulse pressure. In a *medium* confidence weight loss-controlled trial population (the POUNDS Lost Study), Liu et al. (2018b) observed that baseline PFOA was positively correlated with DBP ($p < 0.05$), but at 6- and 24-month follow-up assessments, no associations were observed with SBP or DBP (Liu et al., 2018b).

The findings from three *low* confidence studies (Chen et al., 2019; He et al., 2018; Yang et al., 2018) of PFOA and blood pressure were mixed. Yang et al. (2018) reported a statistically significant positive increased risk of high SBP (≥140 mmHg) for n-PFOA (linear isomers), but no association for SBP as a continuous measure. Two additional studies reported no associations

for SBP (Chen et al., 2019; He et al., 2018), and three studies reported no associations for DBP (Chen et al., 2019; He et al., 2018; Yang et al., 2018).

Of the 11 studies that examined risk of elevated blood pressure (hypertension), six reported statistically significant associations (Ye et al., 2021; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Pitter et al., 2020; Bao et al., 2017). Hypertension was defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed anti-hypertensive medication. Using a generalized additive model and restricted cubic splines, Liao et al. (2020) reported a non-linear (J-shaped) relationship with hypertension, with the inflection point of PFOA at 1.80 ng/mL. Each 10-fold increase in PFOA was associated with a 44% decrease (OR: 0.56; 95% CI: 0.32, 0.99) in the risk of hypertension on the left side of the inflection point, and an 85% increase (OR: 1.85; 95% CI: 1.34, 2.54) on the right side of the inflection point. A significant association with hypertension was observed for the highest (>4.4 ng/mL) versus lowest (≤2.5 ng/mL) tertile (OR: 1.32; 95% CI: 1.13, 1.54), and the test for trend was significant ($p < 0.001$). Additionally, positive associations were observed among women (OR: 1.42; 95% CI: 1.12, 1.79) and in participants 60 years and older (OR: 1.32; 95% CI: 1.03, 1.68). The studies (Ye et al., 2021; Mi et al., 2020; Bao et al., 2017) with overlapping data on highly exposed Isomers of C8 Health Project participants reported significant associations. An overlapping *low* confidence study (Ye et al., 2021) on metabolic syndrome observed a moderate increase (OR: 1.31; 95% CI: 1.11, 1.56) in the risk of elevated blood pressure (SBP ≥130 and/or DBP ≥85; or medication use). Mi et al. (2020) reported higher risk of hypertension overall (OR: 1.72; 95% CI: 1.27, 2.31) and among women (OR: 2.32; 95% CI: 1.38, 3.91), but not in men. Bao et al. (2017) did not observe an association between total PFOA and hypertension. However, in isomer-specific analysis, a natural-log unit (ng/mL) increase of 6-m-PFOA was significantly associated with higher risk of hypertension among all participants (OR: 1.24; 95% CI: 1.05, 1.47) and among women (OR: 1.86; 95% CI: 1.25, 2.78). These results suggest branched PFOA isomers have a stronger association with increased risk of hypertension compared with linear isomers (n-PFOA).

Increased risk of hypertension was observed in a pair of overlapping studies on another high exposure community located in Italy (Zare Jeddi et al., 2021; Pitter et al., 2020). Pitter et al. (2020), a *medium* confidence study, observed a significant association (OR: 1.06; 95% CI: 1.01, 1.12) between PFOA exposure and hypertension in a large cross-sectional sample of adults (n = 15,786). The association remained significant in men (OR: 1.08; 95% CI: 1.02, 1.15), but was not significant in women (OR: 1.06; 95% CI: 0.97, 1.15). A similar increased risk of hypertension was observed among all participants in the overlapping study (Zare Jeddi et al., 2021).

A *medium* confidence study, Lin et al. (2020c), reported in a cross-sectional analysis that the association with hypertension was not statistically significant but was modified by sex. Among males, a doubling of baseline plasma PFOA was associated with a significantly higher risk of hypertension (RR: 1.27; 95% CI: 1.06, 1.53); no association with hypertension was observed among females. In a prospective analysis, among participants who did not have hypertension at baseline, there was no association with hypertension at the approximately 15 years of follow-up (Lin et al., 2020c). In addition, three *medium* confidence studies (Christensen et al., 2019; Donat-Vargas et al., 2019; Liu et al., 2018d) and a *low* confidence study (Christensen et al., 2016) did not observe associations with hypertension.

Ten studies examined other CVD-related outcomes including CHD, stroke, carotid artery atherosclerosis, angina pectoris, C-reactive protein, CHF, peripheral artery disease (PAD), microvascular disease, CIMT, and mortality.

Among the four studies that examined CHD, the findings were mixed. A *high* confidence study (Mattsson et al., 2015), a *medium* confidence study of 10,850 NHANES participants from 1999–2014 (Huang et al., 2018), and a *low* confidence study (Christensen et al., 2016) all reported no associations with CHD. A *low* confidence study from the C8 Health Project (Honda-Kohmo et al., 2019) reported a significant inverse association between PFOA and CHD among adults with and without diabetes. However, study limitations that may have influenced these findings include the reliance on self-reporting of a clinician-based diagnosis for CHD outcome classification and residual confounding by SES.

Among the two NHANES-based studies that examined CVD (Huang et al., 2018; Shankar et al., 2012), the findings were mixed. Using data from NHANES 1999–2000 and 2003–2004 cycles, Shankar et al. (2012) reported significant associations with CVD. The analysis by PFOA quartiles reported significantly higher odds for the presence of CVD in the third (OR: 1.77; 95% CI: 1.04, 3.02) and the highest (OR: 2.01; 95% CI: 1.12, 3.60) quartiles compared with the lowest quartile, with a significant trend ($p = 0.01$). In contrast, using a larger dataset from NHANES 1999–2014 cycles, Huang et al. (2018) did not observe an association with total CVD by quartiles of exposure, nor a positive trend.

Shankar et al. (2012) also observed a significant association with PAD. The analysis by PFOA quartiles reported significantly higher odds for the presence of PAD (OR: 1.78; 95% CI: 1.03, 3.08) in the highest compared with the lowest quartile, with a significant trend ($p = 0.04$).

Among the two studies that examined stroke, the findings also were mixed. A borderline positive association ($p = 0.045$) was observed by Huang et al. (2018). In contrast, Hutcheson et al. (2020) observed a significant inverse association with history of stroke in adults with and without diabetes participating in the C8 Health Project (OR: 0.90; 95% CI: 0.82, 0.98, $p = 0.02$). However, a borderline-significant inverse association was observed among non-diabetics (OR: 0.94; 95% CI: 0.88, 1.00; $p = 0.04$) but not among those with diabetes, although the interaction was not significant.

In addition, a *low* confidence study of adults and children did not observe an association between serum PFOA and self-reported cardiovascular conditions, including high blood pressure, CAD, and stroke (Graber et al., 2019). However, potential selection bias is a major concern for this study owing to the recruitment of volunteers who already knew their PFAS exposure levels and were motivated to participate in a lawsuit.

Huang et al. (2018) also reported significantly higher odds of heart attack for the third quartile (OR: 1.62; 95% CI: 1.04, 2.53) and second quartile (OR: 1.57; 95% CI: 1.06, 2.34), compared with the first quartile. No associations were observed with CHF and angina pectoris.

No associations with microvascular diseases (defined as the presence of nephropathy, retinopathy, or neuropathy) were observed (Cardenas et al., 2019).

One *medium* confidence study (Osorio-Yáñez et al., 2021) examined changes in atherosclerotic plaque in a sample of participants enrolled in the Diabetes Prevention Program. A nonsignificant

positive association (OR: 1.17; 95% CI: 0.91, 1.50) was observed for the odds of having a mild to moderate coronary artery calcium Agatston score (11– 400). Two studies examined changes in heart structure (Mobacke et al., 2018) and carotid atherosclerosis (Lind et al., 2017b) in participants 70 years and older. Mobacke et al. (2018) examined alterations of left ventricular geometry, a risk factor for CVD, and reported that serum PFOA was significantly associated with a decrease in relative wall thickness (β: −0.12; 95% CI: −0.22, −0.001; p = 0.03), but PFOA was not observed to be associated with left ventricular mass or left ventricular end diastolic diameter. Lind et al. (2017b) examined markers of carotid artery atherosclerosis including atherosclerotic plaque, the intima-media complex, and the CIMT (a measure used to diagnose the extent of carotid atherosclerotic vascular disease) and observed no associations.

The association between exposure to PFOA and apolipoprotein B, a protein associated with LDL and increased risk of arthrosclerosis, was examined in a *medium* confidence study (Jain, 2020b) on NHANES participants (2007–2014). Serum apolipoprotein B was significantly increased (β per log10-unit increase PFOA: 0.03878; $p < 0.01$) with increasing PFOA exposure in non-diabetic participants who did not take lipid-lowering medication. No significant associations were observed among lipid-lowering medication users and participants with diabetes. No association between PFOA and C-reactive protein levels (a risk factor for CVD) were observed in two studies, one in women from Project Viva (Mitro et al., 2020) and the other in pregnant women from the Spanish Environment and Childhood (Infancia y Medio Ambiente, INMA) study (Matilla-Santander et al., 2017). One *medium* confidence study examined mortality due to heart/cerebrovascular diseases in 1,043 NHANES (2003–2006) participants 60 years and older and observed no associations (Fry and Power, 2017).

Overall, the findings from one *high* confidence study and several *medium* confidence studies conducted among the general population provide consistent evidence for an association between PFOA and blood pressure. The evidence for an association between PFOA and increased risk of hypertension/elevated blood pressure, overall and in gender-stratified analyses was inconsistent. Evidence for other CVD-related outcomes was more limited, and similarly inconsistent.

3.4.3.1.1.5 Findings From Occupational Studies

Two *low* confidence studies examined occupational PFOA exposure and cardiovascular effects (see Appendix, (U.S. EPA, 2024a)). Steenland et al. (2015) examined 1,881 workers with high serum PFOA levels (median 113 ng/mL) from a subset of two prior studies conducted by the C8 Science Panel. No trend was observed in the exposure-response gradient for stroke, CHD, and hypertension and. In analysis of PFOA levels by quartiles, a significantly higher risk of stroke (no lag) was observed for the 2nd quartile versus the 1st quartile (Rate Ratio (RR): 2.63; 95% CI: 1.06, 6.56). No association was observed with 10-year lag stroke, CHD, and hypertension, respectively. For the assessment of stroke, this study had *low* confidence because of concerns for selection bias, specifically survival bias. For other chronic diseases examined, this study is of *low* confidence due to concerns about outcome misclassification, particularly for hypertension due to lack of medical record validation. In another occupational study of 120 male workers with very high PFOA serum levels (GM: 4,048 ng/mL), Girardi et al. (2019) reported no association with increased risk of mortality due to cardiovascular causes, including hypertensive disease, ischemic heart disease, stroke, and circulatory diseases. However, the potential for selection bias, outcome misclassification, and limited control for confounding may have influenced the reported results.

Overall, the limited evidence available from occupational studies was inconsistent for an association with risk of stroke and indicated PFOA is not associated with an increased risk of CHD, hypertension, and mortality due to cardiovascular causes. However, the findings based on two *low* confidence studies should be interpreted with caution due to potential biases arising from the selection of participants and outcome misclassification.

3.4.3.1.2 Serum Lipids

3.4.3.1.2.1 Introduction

Serum cholesterol and triglycerides are well-established risk factors for CVDs. Major cholesterol species in serum include LDL and HDL. Elevated levels of TC, LDL, and triglycerides are associated with increased cardiovascular risks, while higher levels of HDL are associated with reduced risks. Evidence for changes in serum lipids was synthesized by population (i.e., children, pregnant women, adults, occupational populations), and there may be differences in the interpretation of an effect depending on age. For example, while elevated levels of TC, LDL, and triglycerides are associated with increased cardiovascular risks in adults, serum lipid changes in children are age-dependent and fluctuate during puberty (Daniels et al., 2008). There are 22 epidemiological studies (24 publications)[14] from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and serum lipid effects. Study quality evaluations for these 23 studies are shown in Figure 3-34. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-11 and below.

In the 2016 Health Assessment (U.S. EPA, 2016c) for PFOA, there was relatively consistent and strong evidence of positive associations between PFOA and TC and LDL in occupational (Costa et al., 2009; Sakr et al., 2007a; Sakr et al., 2007b; Olsen et al., 2003) and high-exposure community settings (Winquist and Steenland, 2014; Fitz-Simon et al., 2013; Frisbee et al., 2010; Steenland et al., 2009). Two of the studies were cross-sectional, however, Fitz-Simon (2013) reported positive associations for LDL and TC in a longitudinal analysis of the change in lipids seen in relation to a change in serum PFOA. General population studies (Geiger et al., 2014a; Nelson et al., 2010; Lin et al., 2009) in children and adults using NHANES reported positive associations for TC and increased risk of elevated TC. Other general population studies were generally consistent, reporting positive associations for TC in adults (Eriksen et al., 2013; Fisher et al., 2013) and pregnant women (Starling et al., 2014). Positive associations between PFOA and HDL were also observed in most studies in the general population (Fisher et al., 2013; Frisbee et al., 2010; Lin et al., 2009; Steenland et al., 2009). Positive associations were observed for triglycerides and LDL in high-exposure community studies (Frisbee et al., 2010; Steenland et al., 2009), but associations for triglycerides and LDL were less consistent in other general population studies (Geiger et al., 2014a; Fisher et al., 2013; Lin et al., 2009).

[14] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b).

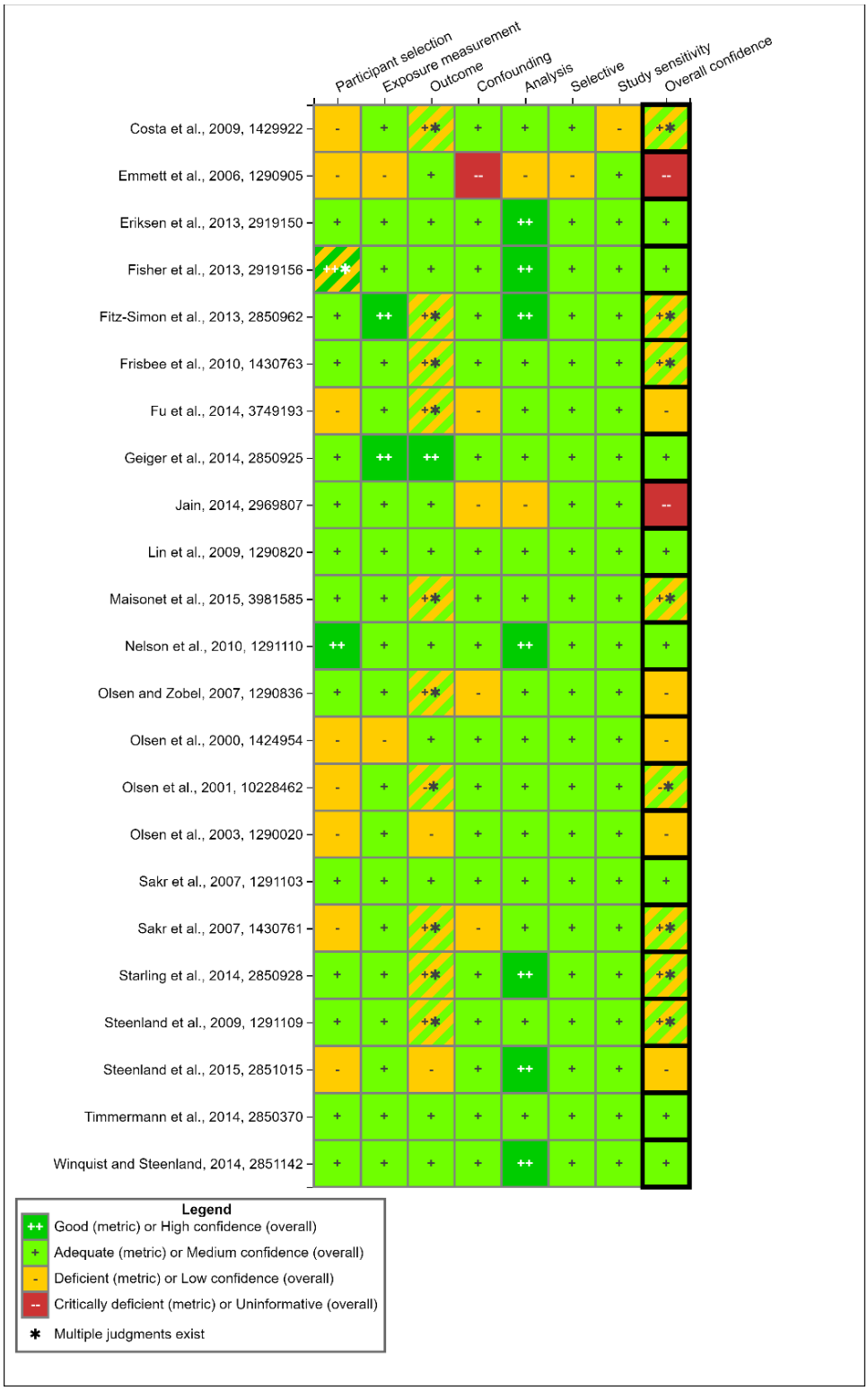


Figure 3-34. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Serum Lipids Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Table 3-11. Associations Between Elevated Exposure to PFOA and Serum Lipids from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]	High Cholesterol[b]
Costa, 2009, 1429922 *Mixed*[c]	Cohort	Occupational	↑↑	↓	NA	↑	NA
Eriksen, 2013, 2919150 *Medium*	Cross-sectional	Adults	↑↑	NA	NA	NA	NA
Fisher, 2013, 2919156 *Medium*	Cross-sectional	Adults	–	–	–	–	NA
Fitz-Simon, 2013, 2850962 *Mixed*[c]	Cohort	Adults	↑	↓	↑	–	NA
Frisbee, 2010, 1430763 *Mixed*[c]	Cross-sectional	Children	↑↑	–	↑↑	↑↑	NA
Fu, 2014, 3749193 *Low*	Cross-sectional	Adults and children	↑↑	–	↑	↑	NA
Geiger, 2014, 2850925 *Medium*	Cross-sectional	Adolescents	↑↑	↓↓	↑↑	–	NA
Lin, 2009, 1290820 *Medium*	Cross-sectional	Adults	NA	↑	NA	–	NA
Maisonet, 2015, 3981585 *Mixed*[c]	Cohort	Children	↓	–	–	↓	NA
Nelson, 2010, 1291110 *Medium*	Cross-sectional	Adults	↑↑	↓	↑	NA	NA
Olsen, 2000, 1424954 *Low*	Cross-sectional	Occupational	↑	↓↓	–	NA	NA
Olsen, 2003, 1290020 *Low*[c]	Cohort	Occupational	↑↑	NA	NA	↑↑	NA
Olsen and Zobel, 2007, 1290836 *Low*	Cross-sectional	Occupational	↑	↓↓	↑	↑↑	NA

Reference, Confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]	High Cholesterol[b]
Sakr, 2007, 1291103 *Medium*	Cross-sectional	Occupational	↑↑	↓	↑↑	↑	NA
Sakr, 2007, 1430761 *Mixed*[c]	Cohort	Occupational	↑↑	↓↓	↑	–	NA
Starling, 2014, 2850928 *Mixed*[c]	Cohort	Children	↑	↑	↑	–	NA
Steenland, 2009, 1291109 *Mixed*[c]	Cross-sectional	Adults	↑↑	↑	↑	↑	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	NA	NA	–
Timmerman, 2014, 2850370 *Medium*	Cohort	Children	NA	NA	NA	↑	NA
Winquist and Steenland, 2014, 2851142 *Mixed*[c]	Cohort	Occupational	NA	NA	NA	NA	↑↑

Notes: HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein; NA = no analysis was for this outcome was performed; TC = total cholesterol; TG = triglycerides; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Olsen (2001a) and Olsen (2001b) report data overlapping with Olsen (2003), which was considered the most updated information.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

3.4.3.1.2.2 Study Quality

All studies were evaluated for risk of bias, selective reporting, and sensitivity following the EPA IRIS protocol. Three considerations were specific to evaluating the quality of studies on serum lipids. First, because lipid-lowering medications strongly affect serum lipid levels, unless the prevalence of medication use is assumed to be low in the study population (e.g., children), studies that did not account for the use of lipid-lowering medications by restriction, stratification, or adjustment were rated as *deficient* in the *participant selection* domain. Second, because triglyceride levels are sensitive to recent food intake (Mora, 2016), outcome measurement error is likely substantial when triglyceride is measured without fasting. Thus, studies that did not measure triglycerides in fasting blood samples were rated *deficient* in the *outcome measures* domain for triglycerides. The o*utcome measures* domain for LDL was also rated *deficient* if LDL was calculated based on triglycerides. Fasting status did not affect the *outcome measures* rating for TC, directly measured LDL, and HDL because the serum levels of these lipids change minimally after a meal (Mora, 2016). Third, measuring PFOA and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 years) (Li et al., 2018c), current blood concentrations are expected to correlate well with past exposures. Furthermore, although reverse causation due to hypothyroidism (Dzierlenga et al., 2020b) or enterohepatic cycling of bile acids (Fragki et al., 2021) has been suggested, there is not yet clear evidence to support these reverse causal pathways.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 64 new epidemiological studies (65 publications)[15] report on the association between PFOA exposure and serum lipids. Except for 10 studies (Blomberg et al., 2021; Li et al., 2021; Liu et al., 2020a; Sinisalu et al., 2020; Tian et al., 2020; Donat-Vargas et al., 2019; Lin et al., 2019; Liu et al., 2018b; Domazet et al., 2016; Olsen et al., 2012), all studies were cross-sectional. Some cohort studies provided additional cross-sectional analyses (Blomberg et al., 2021; Li et al., 2021; Sinisalu et al., 2020). Most studies assessed exposure to PFOA using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but a few studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on clinical cut-points, medication use, doctor's diagnosis, or criteria for metabolic syndrome. Study quality evaluations for these 65 studies are shown in Figure 3-35, Figure 3-36, Figure 3-37.

On the basis of the considerations mentioned, one study was classified as *high* confidence*,* one study was classified as *high* confidence for prospective analyses and *medium* confidence for cross-sectional analyses, 21 studies were classified *medium* confidence for all lipid outcomes, nine studies were rated *medium* confidence for TC or HDL, but *low* confidence for triglycerides or LDL, 26 studies were rated *low* confidence for all lipid outcomes, and 7 studies were rated *uninformative* for all lipid outcomes (Sinisalu et al., 2021; Abraham et al., 2020; Leary et al., 2020; Sinisalu et al., 2020; Huang et al., 2018; Seo et al., 2018; Predieri et al., 2015). Notably, 10 studies (Blomberg et al., 2021; Canova et al., 2021; Dalla Zuanna et al., 2021; Canova et al., 2020; Lin et al., 2020e; Tian et al., 2020; Yang et al., 2020b; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015) were rated *low* confidence specifically for triglycerides and/or LDL because these studies measured triglycerides in non-fasting blood

[15] Dong et al. (2019) counted as two studies, one in adolescents and one in adults.

samples. The *low* confidence studies had deficiencies in participant selection (Cong et al., 2021; Kobayashi et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020a; Chen et al., 2019; Fassler et al., 2019; Graber et al., 2019; He et al., 2018; Khalil et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Christensen et al., 2016; Rotander et al., 2015; Lin et al., 2013; Wang et al., 2012), outcome measures (Kobayashi et al., 2021; Graber et al., 2019; Yang et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Kishi et al., 2015; Rotander et al., 2015), confounding (Liu et al., 2021; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020a; Sinisalu et al., 2020; Fassler et al., 2019; Graber et al., 2019; Convertino et al., 2018; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012), analysis (He et al., 2018; Liu et al., 2018b; Sun et al., 2018), sensitivity (Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Christensen et al., 2016; Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012), or selective reporting (adolescent portion) (Dong et al., 2019).

The most common reason for a *low* confidence rating was potential for selection bias, including a lack of exclusion based on use of lipid-lowering medications (Cong et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Li et al., 2020b; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Wang et al., 2012), potential for self-selection (Li et al., 2020b; Graber et al., 2019; Christensen et al., 2016; Rotander et al., 2015), highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOA and lipids (Lin et al., 2013), and missing key information on the recruitment process (Khalil et al., 2020; Fassler et al., 2019; Khalil et al., 2018; Yang et al., 2018). Another common reason for *low* confidence was a serious risk for residual confounding by SES (Li et al., 2020b; Lin et al., 2020a; Sinisalu et al., 2020; Fassler et al., 2019; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012). Frequently, deficiencies in multiple domains contributed to an overall *low* confidence rating. The *uninformative* studies had *critical deficiencies* in at least one domain or were *deficient* in several domains. These *critical deficiencies* include a lack of control for confounding (Abraham et al., 2020; Huang et al., 2018; Seo et al., 2018), convenience sampling (Sinisalu et al., 2021), and treating PFOA as an outcome of all lipids instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination (Predieri et al., 2015). Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study (Leary et al., 2020).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

Figure 3-35. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids

Interactive figure and additional study details available on HAWC.

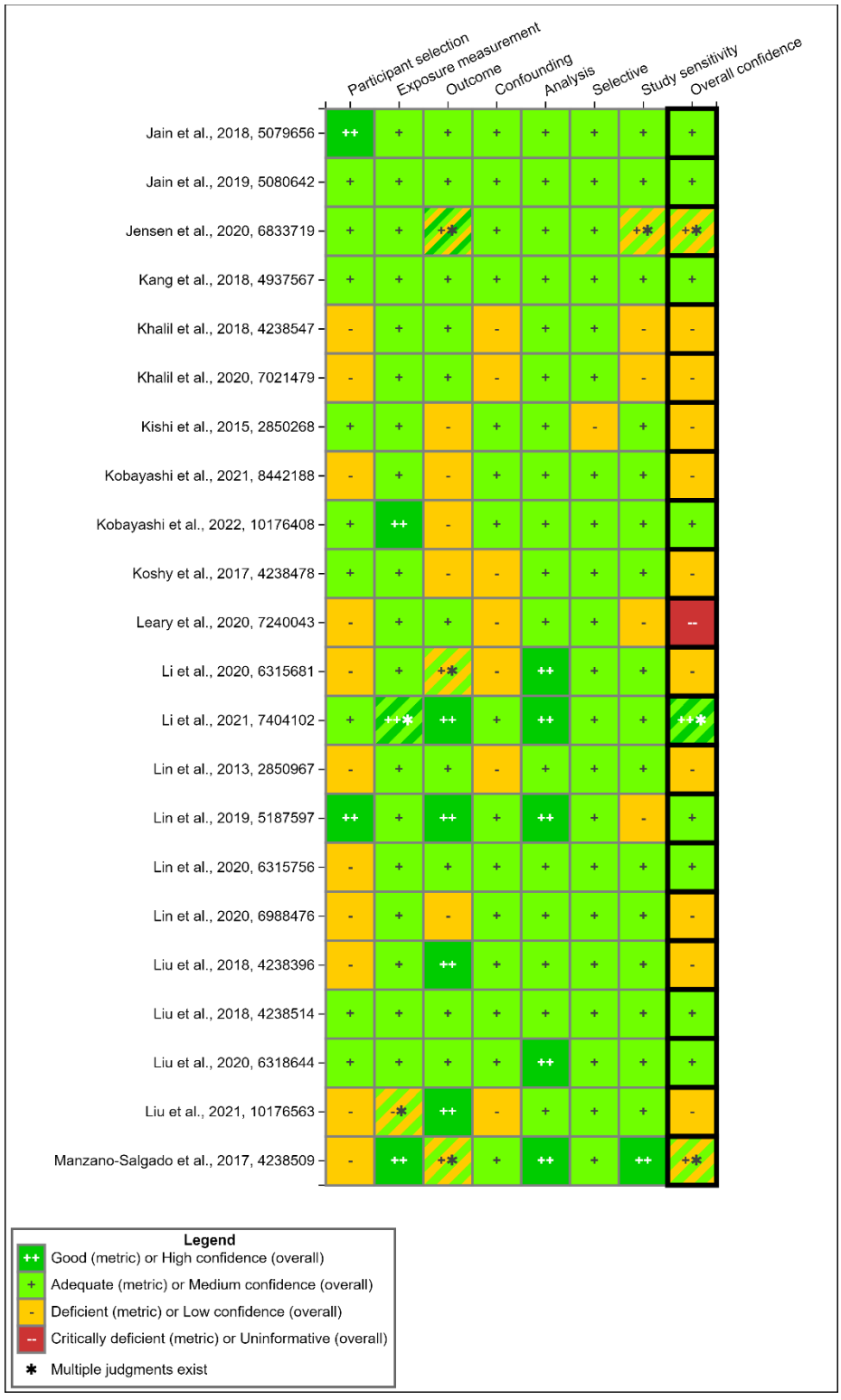


Figure 3-36. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

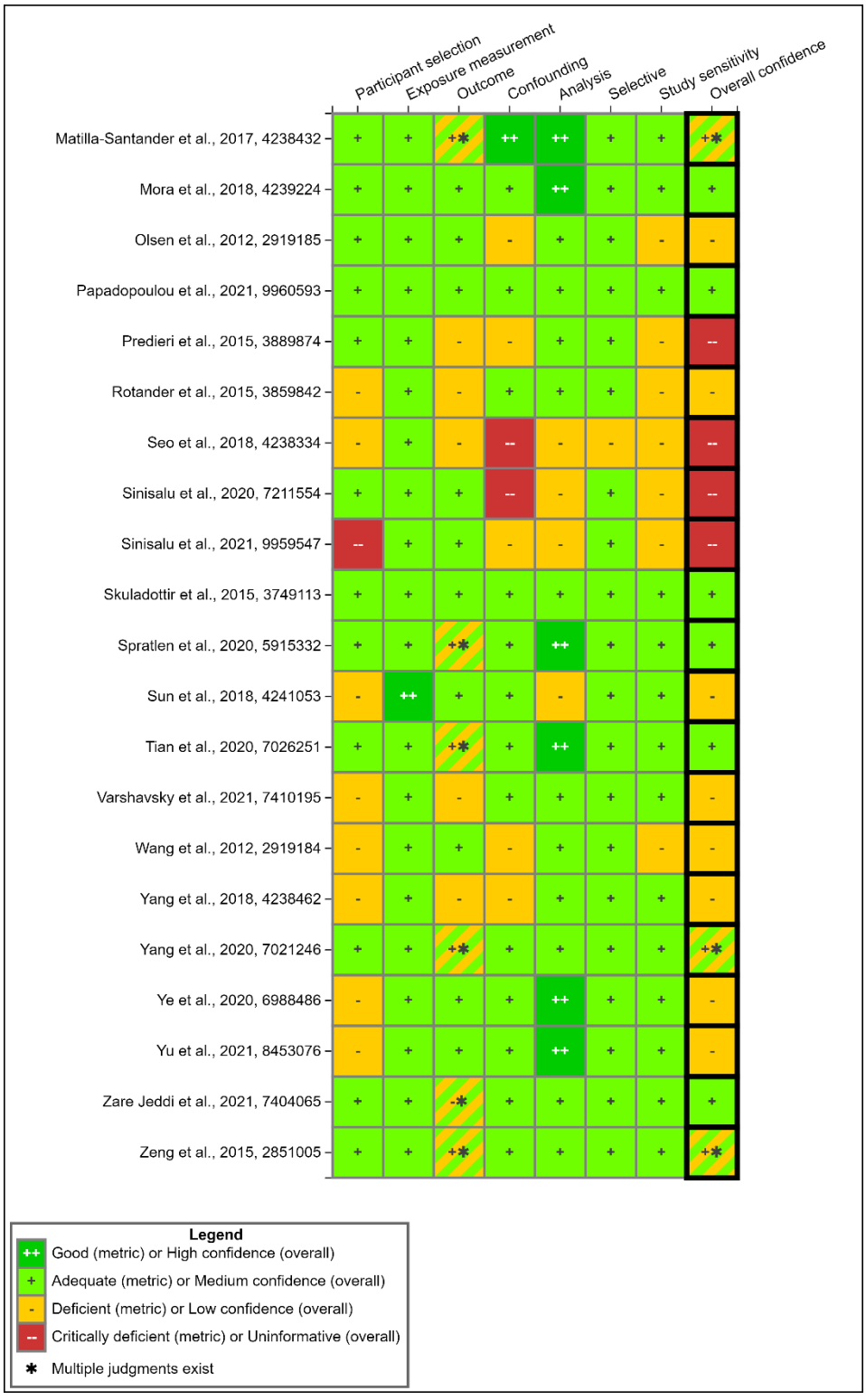


Figure 3-37. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.2.3 Findings From Children

Results for the studies that examined TC in children are presented in the Appendix (U.S. EPA, 2024a). Eleven *medium* confidence and four *low* confidence studies examined the association between PFOA and TC in children. Of these, five studies examined the association between prenatal PFOA exposure and TC in childhood (Averina et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 10 examined the association between childhood PFOA exposure and concurrent TC (Blomberg et al., 2021; Canova et al., 2021; Dong et al., 2019; Fassler et al., 2019; Jain and Ducatman, 2018; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). Positive associations between PFOA and TC were reported in seven *medium* confidence studies (Blomberg et al., 2021; Canova et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but the direction of association sometimes differed by age and sex (Blomberg et al., 2021; Jensen et al., 2020; Manzano-Salgado et al., 2017b). Of all the positive associations observed in *medium* confidence studies, only three were significant, including: all children (age 12–15 years) in Zeng (2015), among girls in mid-childhood in Mora (2018), and children and adolescents in the highest quartile of exposure from Canova (2021).

In three out of four *low* confidence studies, PFOA was positively associated with TC (Fassler et al., 2019; Khalil et al., 2018; Koshy et al., 2017). However, residual confounding by SES may have positively biased these findings. Taken together, these studies suggest a positive association between PFOA and TC in children. However, the true association between PFOA and TC remains uncertain given the heterogeneity by age and sex and the imprecise findings in most *medium* confidence studies.

Seven *medium* confidence and five *low* confidence studies examined the association between PFOA and LDL in children. Of these, five examined prenatal exposure (Papadopoulou et al., 2021; Jensen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and eight examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Dong et al., 2019 adolescent portion; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). The *medium* studies generally reported small, positive associations between PFOA and LDL, but most of the associations were not statistically significant (see Appendix, (U.S. EPA, 2024a)) (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). In one *medium* study, the association was inverse among 3-month old infants and 18-month old boys (Jensen et al., 2020).

One *low* confidence study (Canova et al., 2021) on children and adolescents in a high-exposure community located in Italy observed significantly increased LDL among adolescents (beta per ln-unit increase in PFOA: 1.03; 95% CI: 0.39, 1.66). Most *low* confidence studies reported a positive association between PFOA and LDL (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but residual confounding by SES (Khalil et al., 2018; Koshy et al., 2017) and the use of non-fasting samples (Canova et al., 2021; Manzano-Salgado et al., 2017b; Zeng et al., 2015) were concerns in these studies. Overall, increases in LDL with increasing PFOA were observed in children, though less consistently.

One *high* confidence, nine *medium* confidence and four *low* confidence studies examined the association between PFOA and HDL in children. Of these, six examined prenatal exposure

(Blomberg et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 12 examined childhood exposure (Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Dong et al., 2019 adolescent portion; Fassler et al., 2019; Jain and Ducatman, 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). Prenatal PFOA exposure was inversely associated with HDL, but most associations were not statistically significant (Blomberg et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) (see Appendix, (U.S. EPA, 2024a)). Sex-stratified analyses showed that the inverse association occurred mainly in boys (Mora et al., 2018; Manzano-Salgado et al., 2017b). Results on childhood exposure were less consistent (see Appendix, (U.S. EPA, 2024a)). One *medium* study reported a statistically significant, positive association between PFOA and HDL in mid-childhood (Mora et al., 2018), but another *medium* study reported an inverse, though statistically nonsignificant association (Zeng et al., 2015). One *medium* confidence study (Canova et al., 2021) in a high-exposure community observed a significant increase in HDL in children, but results were less consistent in adolescents. Most *low* confidence studies reported a positive association between childhood PFOA exposure and HDL (Fassler et al., 2019; Khalil et al., 2018; Koshy et al., 2017). In summary, PFOA was not consistently associated with lower HDL in children. Effect modification by exposure window may explain this inconsistency.

One *high* confidence, nine *medium* confidence and five *low* confidence studies examined the association between PFOA and triglycerides in children. Of these, seven examined prenatal exposure (Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 11 examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Fassler et al., 2019; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Domazet et al., 2016; Zeng et al., 2015). No association was observed in the only *high* confidence study (Li et al., 2021). PFOA was significantly associated with increased triglycerides in newborns in one *medium* study (Spratlen et al., 2020) (see Appendix, (U.S. EPA, 2024a)). Some *medium* studies also reported positive associations, but they were not statistically significant (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). Results from other *medium* confidence studies were imprecise (Li et al., 2021; Papadopoulou et al., 2021). In one *medium* study that examined the association between PFOA and triglycerides longitudinally, PFOA at age 9 years was associated with lower triglycerides at age 15 years and 21 years, while PFOA at age 15 years was associated with higher triglycerides at age 21 years (Domazet et al., 2016). None of the associations were statistically significant. In most *low* confidence studies, PFOA was positively associated with triglycerides (Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but the use of non-fasting samples and residual confounding by SES may have biased these results upwards. Overall, increased triglycerides with increasing PFOA were observed in children, but results were less consistent and not always statistically significant.

In summary, the association between PFOA and serum lipids in children remains inconclusive. For TC, LDL, and triglycerides, positive associations were generally observed, but few were statistically significant. Differences in the direction of association by age or sex further contributed to inconsistency in findings; it is difficult to determine if the differences were due to effect modification or random error. For HDL, prenatal exposure appeared to be associated with

lower HDL, especially in boys, although childhood exposure was associated with higher HDL. Few findings were statistically significant, however, suggesting caution in interpreting these results.

3.4.3.1.2.4 Findings From Pregnant Women

One *high* confidence study (Gardener et al., 2021) and four *medium* confidence studies examined the association between PFOA and TC in pregnant women (Dalla Zuanna et al., 2021; Yang et al., 2020b; Matilla-Santander et al., 2017; Skuladottir et al., 2015) and two reported significantly positive associations between PFOA and TC (see Appendix, (U.S. EPA, 2024a)) (Matilla-Santander et al., 2017; Skuladottir et al., 2015). One *medium* confidence study in a high-exposure community in Italy (Dalla Zuanna et al., 2021) considered PFOA exposure concentrations across trimesters using a generalized additive model (GAM). Authors reported significantly decreased TC with an increasingly inverse trend across all sampled trimesters. Results were consistent for second and third trimester samples in sensitivity analyses, but the direction of effect was positive for first trimester samples (see Appendix, (U.S. EPA, 2024a)). No association between PFOA and TC was observed in a cohort of pregnant women in the United States (Gardener et al., 2021) or in a Chinese study of pregnant women (Yang et al., 2020b). No association was found in the single *low* confidence study (Varshavsky et al., 2021) on total serum lipids after adjustment for race/ethnicity, insurance type, and parity. These findings suggest a consistently positive association between PFOA and TC in pregnant women.

Two studies examined PFOA and LDL in pregnant women (Dalla Zuanna et al., 2021; Yang et al., 2020b) and were considered *low* confidence due to lack of fasting blood samples for LDL measurement. In a high-exposure community (Dalla Zuanna et al., 2021), a decrease in LDL was reported with increasing PFOA concentrations when considering exposure concentrations sampled across trimesters. In individual trimester sensitivity analyses, results were consistently inverse for second and third trimester samples, including a significant finding for the third trimester. However, nonsignificant positive associations were observed for first trimester samples. No associations were observed for LDL in the other *low* confidence study, but a significant decrease was reported for the LDL:HDL ratio (see Appendix, (U.S. EPA, 2024a)).

Three *medium* confidence studies examined PFOA and HDL in pregnant women (Starling, 2017, 3858473; Dalla Zuanna, 2021, 7277682; Yang, 2020, 7021246;) and two observed positive statistically significant associations (see Appendix, (U.S. EPA, 2024a)) (Dalla Zuanna et al., 2021; Starling et al., 2017). Starling et al. (2017) reported a positive association between maternal PFOA serum concentrations (collected during 20 to 34 weeks of pregnancy with a median of 27 weeks) and HDL in a United States cohort. Dalla Zuanna (2021) observed significant positive associations when considering blood samples across all trimesters of pregnancy in a high-exposure community in Italy. The association was consistent, but no longer significant, when trimesters were modeled individually. (Yang et al., 2020b) observed a null association between PFOA exposures and HDL levels measured in early pregnancy.

One *high* confidence, one *medium* confidence, and three *low* confidence studies examined the association between PFOA and triglycerides in pregnant women. The *high* confidence study reported a significant increasing trend for triglycerides with increasing PFOA exposure quartile in a cohort of pregnant women from the United States (Gardener et al., 2021). The *medium* confidence study reported an inverse association between PFOA and triglycerides, but the

association was small and not statistically significant (Starling et al., 2017). The *low* confidence studies each reported inverse (Yang et al., 2020b; Matilla-Santander et al., 2017) or positive associations (Kishi et al., 2015) that were not statistically significant. Each study was limited by their use of non-fasting blood samples. Kishi et al. (2015) additionally examined the association between PFOA and select fatty acids in serum. PFOA was not significantly associated with any fatty acids, but the associations were generally positive except for arachidonic acid, docosahexaenoic acid, and omega 3. Together, these studies suggest PFOA was not associated with triglycerides or fatty acids in pregnancy.

In summary, the available evidence supports a positive association between PFOA and HDL in pregnancy. The available evidence does not support a consistent, positive association between PFOA and TC or triglycerides. Finally, the available evidence is too limited to determine the association between PFOA and LDL in pregnant women.

3.4.3.1.2.5 Findings From the General Adult Population

Ten *medium* confidence and 13 *low* confidence studies examined PFOA and TC or hypercholesterolemia in adults (Figure 3-35, Figure 3-36, and Figure 3-37). All studies examined cross-sectional associations (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020e; Liu et al., 2020a; Chen et al., 2019; Donat-Vargas et al., 2019; Dong et al., 2019; Graber et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Convertino et al., 2018; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016; Wang et al., 2012) and two studies additionally examined the association between baseline PFOA and changes in TC or incident hypercholesterolemia (Liu et al., 2020a; Lin et al., 2019).

Of the 10 *medium* confidence studies, eight reported positive associations (Figure 3-39, Figure 3-40, Figure 3-41, Figure 3-42). In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020) reported statistically significant, positive associations with TC, including an increased risk of high cholesterol (Figure 3-38). Canova et al. (2020) also reported a concentration-response curve when PFOA was categorized in quartiles or deciles, with a higher slope at higher PFOA concentrations, which tended to flatten above around 20–30 ng/mL. Results from another *medium* confidence study (Lin et al., 2020e) on older adults in a high-exposure community in Taiwan also reported positive associations for TC, which was consistent across quartiles of PFOA exposure.

Four of the *medium* confidence studies used overlapping data from NHANES 2003–2014. All four studies reported significant positive associations between PFOA and TC in adults (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) (see Appendix, (U.S. EPA, 2024a)). Stratified analyses in Jain et al. (2019b) suggested that the positive association occurred mainly in obese men. A significantly positive association between PFOA and TC also was observed at baseline in the DPPOS (Lin et al., 2019). This study reported positive associations between PFOA and prevalent, as well as incident, hypercholesterolemia. However, the HR for incident hypercholesterolemia was relatively small and not statistically significant (HR = 1.06, 95% CI: 0.94, 1.19). In contrast to these findings, Liu et al. (2020a) reported no association between PFOA and TC. Further, Donat-Vargas et al. (2019) reported generally inverse associations between PFOA and TC, regardless of whether PFOA was measured

concurrently or averaged between baseline and follow-up. It is noteworthy that all participants in Lin et al. (2019) were prediabetic, all participants in Liu et al. (2020a) were obese and enrolled in a weight loss trial, and all participants in Donat-Vargas et al. (2019) were free of diabetes for at least 10 years of follow-up. It is unclear whether differences in participants' health status explained the studies' conflicting findings.

In *low* confidence studies, positive associations between PFOA and TC or hypercholesterolemia were reported in nine of 13 studies (Cong et al., 2021; Khalil et al., 2020; Li et al., 2020b; Chen et al., 2019; Graber et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016). However, oversampling of persons with potentially high PFOA exposure and health problems was a concern in three of these studies (Li et al., 2020b; Graber et al., 2019; Christensen et al., 2016). Selection bias concerns, including lack of consideration of lipid-lowering medication and convenience sampling, were issues in two of the studies (Cong et al., 2021; Khalil et al., 2020). Further, He et al. (2018) used similar data as the four *medium* NHANES studies and thus added little information.

Contrary to these findings, in one *low* confidence study, participants treated with extremely high levels of ammonium perfluorooctanoate (APFO) in an open-label, nonrandomized, phase 1 trial, were found to have reduced levels of TC with increasing plasma PFOA concentrations (Convertino et al., 2018). This study differed from the other studies in several ways. First, all participants were solid-tumor cancer patients who failed standard therapy and may have distinct metabolic profiles compared with the general population. Second, participants ingested high dose levels of APFO rather than being exposed to PFOA. Third, participants' plasma PFOA concentrations were several orders of magnitude higher than those reported in the general population. Participant serum concentrations were of similar magnitude as serum concentrations resulting in decreased TC serum in rodent studies (see Section 3.4.3.2). It is unclear whether these factors explained the inverse association between PFOA and TC.

Considering *medium* and *low* confidence studies together, increased TC with increasing PFOA was observed in adults. Some inconsistencies in the direction of association across studies were found. Further studies are needed to determine if these inconsistencies reflect effect modification by subject characteristics or PFOA dose levels.

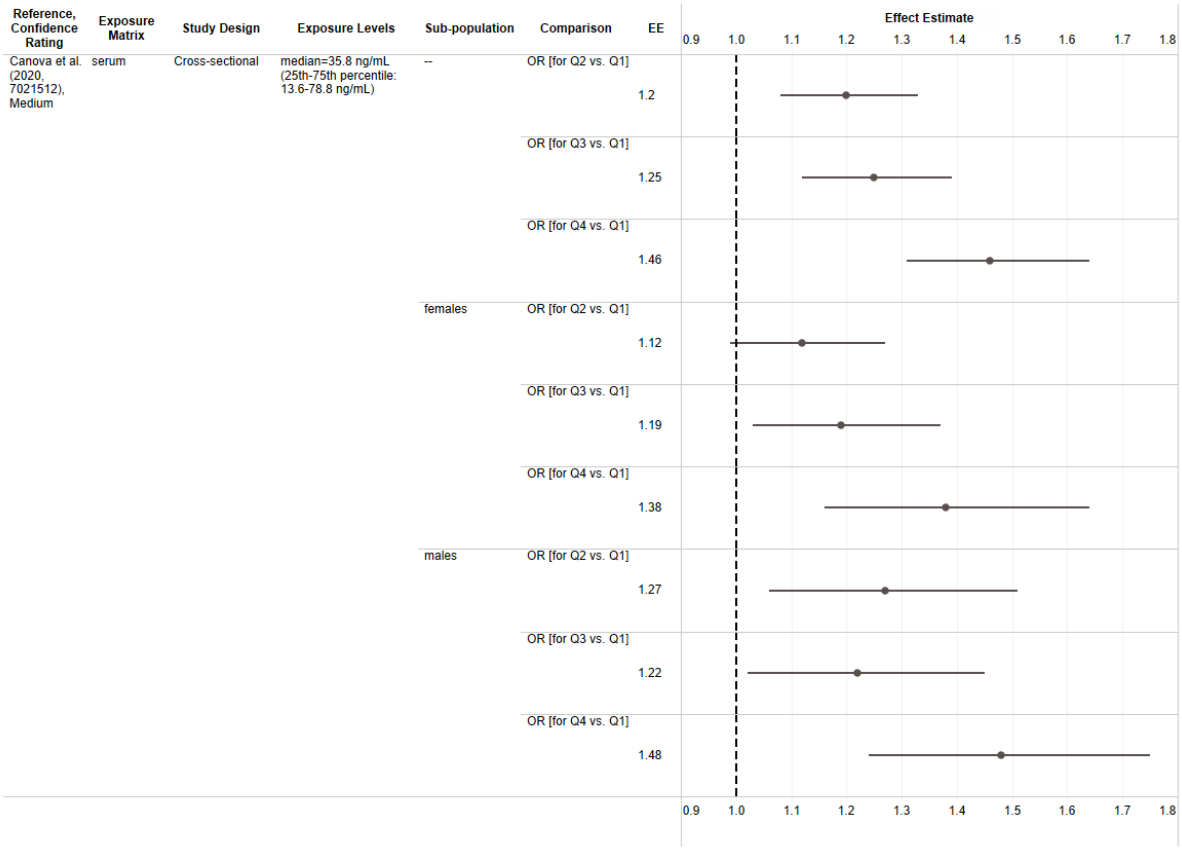


Figure 3-38. Odds of High Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

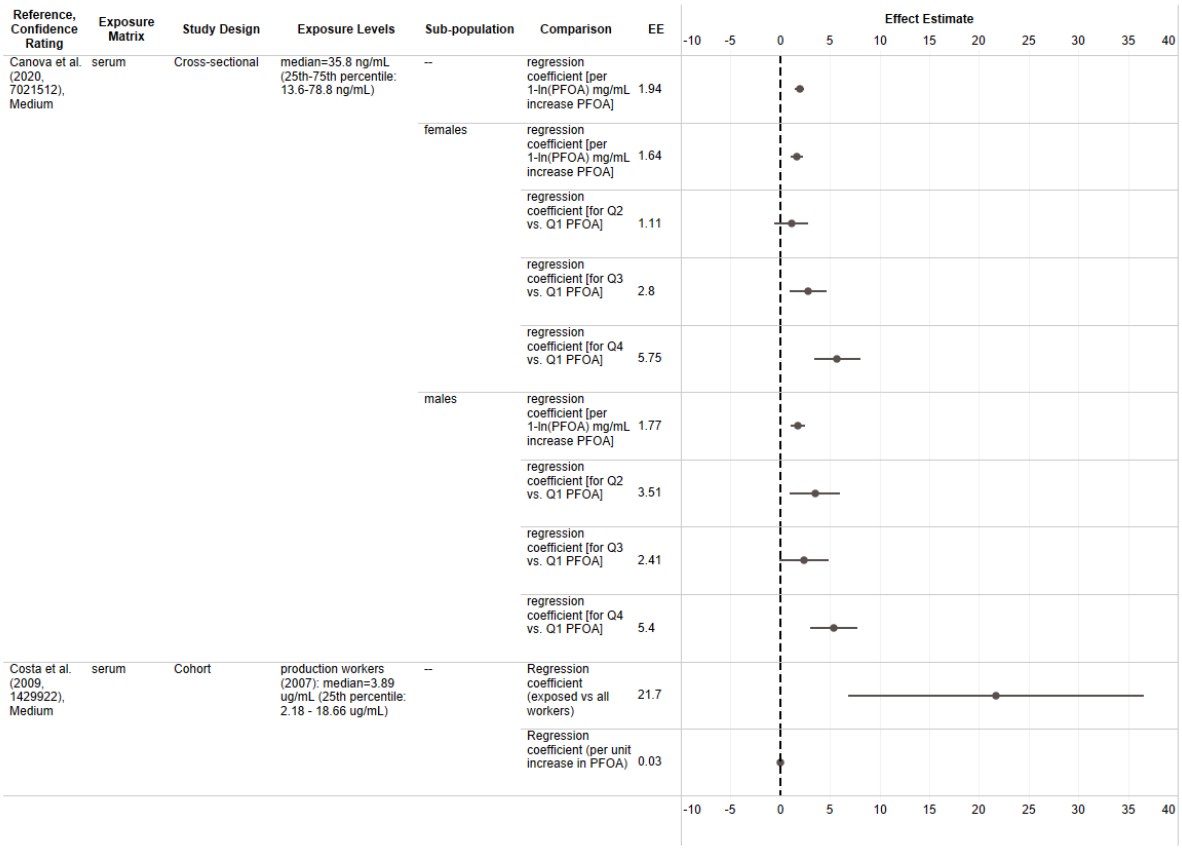
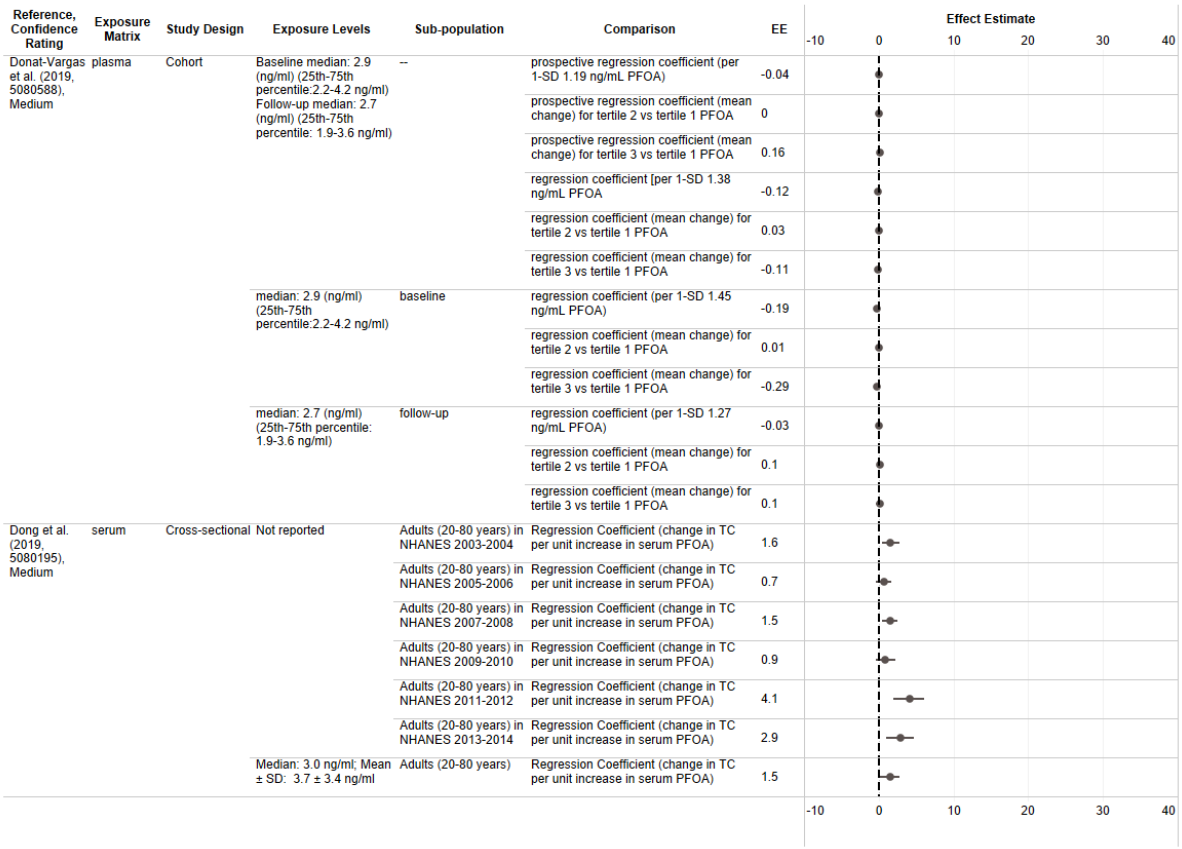


Figure 3-39. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.



Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Donat-Vargas et al. (2019, 5080588), Medium	plasma	Cohort	Baseline median: 2.9 (ng/ml) (25th-75th percentile:2.2-4.2 ng/ml) Follow-up median: 2.7 (ng/ml) (25th-75th percentile: 1.9-3.6 ng/ml)	--	prospective regression coefficient (per 1-SD 1.19 ng/mL PFOA)	-0.04
					prospective regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0
					prospective regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	0.16
					regression coefficient [per 1-SD 1.38 ng/mL PFOA	-0.12
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.03
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	-0.11
			median: 2.9 (ng/ml) (25th-75th percentile:2.2-4.2 ng/ml)	baseline	regression coefficient (per 1-SD 1.45 ng/mL PFOA)	-0.19
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.01
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	-0.29
			median: 2.7 (ng/ml) (25th-75th percentile: 1.9-3.6 ng/ml)	follow-up	regression coefficient (per 1-SD 1.27 ng/mL PFOA)	-0.03
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.1
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	0.1
Dong et al. (2019, 5080195), Medium	serum	Cross-sectional	Not reported	Adults (20-80 years) in NHANES 2003-2004	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.6
				Adults (20-80 years) in NHANES 2005-2006	Regression Coefficient (change in TC per unit increase in serum PFOA)	0.7
				Adults (20-80 years) in NHANES 2007-2008	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.5
				Adults (20-80 years) in NHANES 2009-2010	Regression Coefficient (change in TC per unit increase in serum PFOA)	0.9
				Adults (20-80 years) in NHANES 2011-2012	Regression Coefficient (change in TC per unit increase in serum PFOA)	4.1
				Adults (20-80 years) in NHANES 2013-2014	Regression Coefficient (change in TC per unit increase in serum PFOA)	2.9
			Median: 3.0 ng/ml; Mean ± SD: 3.7 ± 3.4 ng/ml	Adults (20-80 years)	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.5

Figure 3-40. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Fan et al. (2020, 7102734), Medium	serum	Cross-sectional	median=2.05 ug/L (25th-75th percentile: 1.31-3.10 ug/L)	--	regression coefficient [per 1-log(10) increase in PFOA]	6.74
Fisher et al. (2013, 2919156), Medium	plasma	Cross-sectional	geometric mean (SD) = 2.46 ug/L (1.83)	--	regression coefficient (per ln unit increase PFOA)	0.03
Han et al. (2021, 7762348), Medium	serum	Case-control	Cases: median=10.05 ng/mL (25th - 75th percentile: 6.75 - 17.05 ng/mL); Controls: median=11.40 ng/mL (25th - 75th percentile: 9.20 - 17.40 ng/mL)	--	regression coefficient (per log10 ng/mL increase in PFOA)	0.01
Jain et al. (2019, 5080642), Medium	serum	Cross-sectional	Geometric mean=2.5 ng/ml; 25th - 75th percentiles: 1.5 - 4.1; SD 2.1	Non-obese females	regression coefficient (per 1-log10 unit change in PFOA)	0.01
				Obese females	regression coefficient (per 1-log10 unit change in PFOA)	0
			Geometric mean=3.4 ng/ml; 25th - 75th percentiles: 2.3 - 5.3; SD 1.9	Non-obese males	regression coefficient (per 1-log10 unit change in PFOA)	-0.01
				Obese males	regression coefficient (per 1-log10 unit change in PFOA)	0.05
Lin et al. (2019, 5187597), Medium	plasma	Cohort and cross-sectional	median=4.9 ng/ml (25th-75th percentile: 3.5 - 6.7)	participants who were not on lipid-lowering medication	Regression coefficient (mean difference) (per doubling of baseline plasma PFOA)	6.09
					Regression coefficient (mean difference) (for Q2 vs Q1)	2
					Regression coefficient (mean difference) (for Q3 vs Q1)	10.13
					Regression coefficient (mean difference) (for Q4 vs Q1)	13.36
Lin et al. (2020, 6988476), Medium	serum	Cross-sectional	median=8.6 ng/mL (25th-75th percentile: 6.2-11.6)	Participants not taking lipid lowering medication	Regression Coefficient (for Q2 vs Q1)	2.48
					Regression Coefficient (for Q3 vs Q1)	2.88
					Regression Coefficient (for Q4 vs Q1)	4.04
Nelson et al. (2010, 1291110), Medium	serum	Cross-sectional	median: 3.9 ug/L (range: 0.1-37.3 ug/L)	20- to 80-year-olds	regression coefficient (per ug/L increase in PFOA)	1.22
					regression coefficient [for Q4 (5.5-37.3 ug/L) vs. Q1 (0.1-2.7 ug/L) PFOA]	9.8

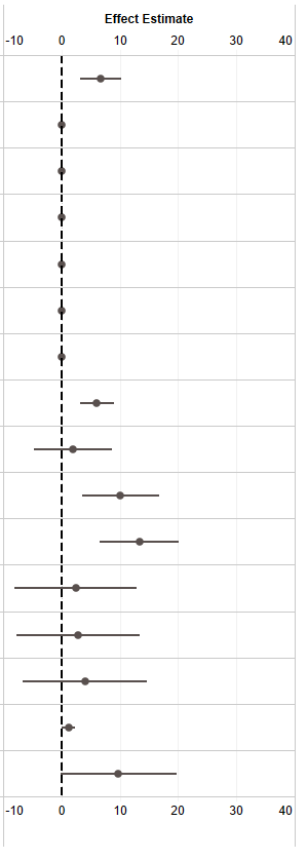


Figure 3-41. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

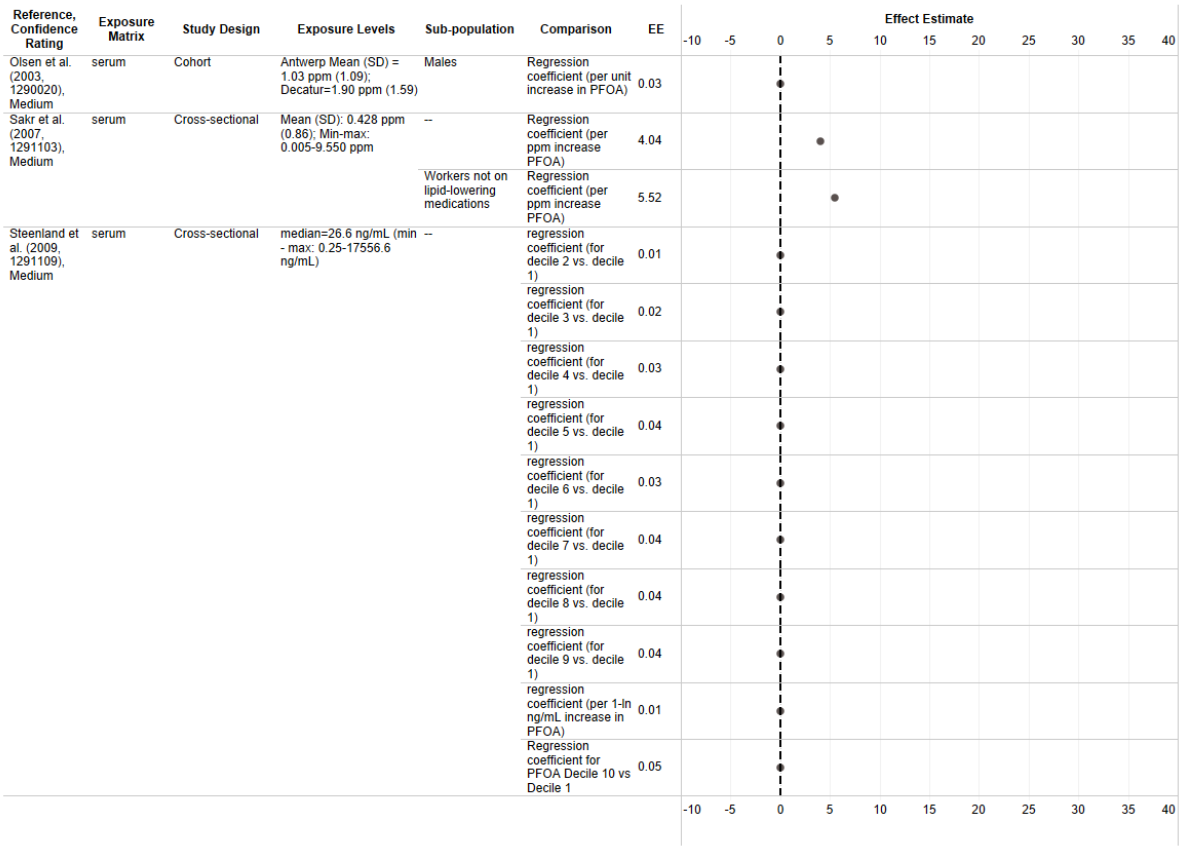


Figure 3-42. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

Six *medium* confidence studies examined PFOA and LDL in adults, and all reported positive associations (Figure 3-35, Figure 3-36, and Figure 3-37). Higher PFOA was significantly associated with higher LDL at baseline in the DPPOS (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). This study also reported statistically significant, positive associations between PFOA and cholesterol in non-HDL and VLDL, which are lipoprotein fractions related to LDL and associated with increased cardiovascular risks (Lin et al., 2019). A positive association was observed in a cross-sectional analysis of cases and controls in a study on type 2 diabetes (Han et al., 2021). Positive associations between PFOA and LDL were also reported in the four NHANES studies (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d), but statistical significance was observed in obese men only (Jain and Ducatman, 2019b) and in participants from NHANES cycle 2011–2012 (Fan et al., 2020; Dong et al., 2019). Liu et al. (2020a) reported that PFOA was positively associated with cholesterol and apolipoprotein C-III (ApoC-III) in combined fractions of intermediate-density (IDL) and LDL that contained ApoC-III; the association with ApoC-III was statistically significant. IDL and LDL containing ApoC-III and ApoC-III itself are strongly associated with increased cardiovascular risks. Thus, the positive associations with cholesterol and ApoC-III in ApoC-III-containing fractions of IDL and LDL were consistent with the positive associations reported for LDL.

Consistent with these findings, nine of the 13 *low* confidence studies report positive associations between PFOA and LDL (Cong et al., 2021; Liu et al., 2021; Canova et al., 2020; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020e; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b). Altogether, the available evidence supports a relatively consistent positive association between PFOA and LDL in adults, especially those who are obese or prediabetic. Associations with other lipoprotein cholesterol known to increase cardiovascular risks were also positive, which increased confidence in the findings for LDL.

Eleven *medium* confidence and 13 *low* confidence studies examined PFOA and HDL or clinically defined low HDL in adults (). All studies examined cross-sectional associations (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020e; Lin et al., 2020a; Liu et al., 2020a; Chen et al., 2019; Christensen et al., 2019; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Convertino et al., 2018; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Wang et al., 2012). Two studies also examined the association between baseline PFOA and changes in HDL (Liu et al., 2020a; Liu et al., 2018b). In a population of young adults aged 20 to 39 years in the Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020) reported statistically significant, positive associations with HDL. Canova et al. (2020) also reported a concentration-response curve when PFOA was categorized in deciles. PFOA was inversely associated with HDL at baseline in the DPPOS, but the association was not statistically significant (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). Four studies used overlapping data from NHANES 2003–2014 and reported associations with HDL that were sometimes positive (Fan et al., 2020; Christensen et al., 2019; Liu et al., 2018d) and sometimes inverse (Dong et al., 2019). The direction of association differed by survey cycles. Few associations in this set of NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was not associated with HDL at baseline or changes in HDL over two years (Liu et al., 2020a). Similarly, *low* confidence studies also reported a mix of positive (Li et al., 2020b; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Yang et al., 2018) associations with changes in HDL in the 6–24 months of the study), inverse (Chen et al., 2019; Liu et al., 2018b) associations with concurrent HDL or changes in HDL in the first 6 months of the study (Ye et al., 2021 positive finding for reduced HDL), or essentially null (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Khalil et al., 2020; Convertino et al., 2018; Wang et al., 2012) associations, with few being statistically significant. Given the inconsistent findings in both *medium* and *low* confidence studies, the available evidence suggests PFOA is not associated with HDL in adults.

Nine *medium* confidence and 16 *low* confidence studies examined the association between PFOA and triglycerides or hypertriglyceridemia. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020e; Lin et al., 2020a; Liu et al., 2020a; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Convertino et al., 2018; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Lin et al., 2013; Wang et al., 2012); three studies additionally examined the association between baseline PFOA and changes in triglycerides or incident hypertriglyceridemia (Liu et al., 2020a; Lin et al., 2019; Liu et al., 2018b). Higher PFOA was significantly associated with higher levels of triglycerides in the DPPOS (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). This study also reported that

PFOA was significantly associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively (Lin et al., 2019). Similarly, PFOA was associated with slightly higher levels of triglycerides in Liu et al. (2020a). The association was stronger and statistically significant for triglycerides in the apoC-III-containing combined fractions of IDL and LDL and apoC-III-negative HDL (Liu et al., 2020a). In contrast, the four *medium* studies using overlapping data from NHANES 2005–2014 reported positive (Christensen et al., 2019; Jain and Ducatman, 2019b) or inverse associations (Fan et al., 2020; Jain and Ducatman, 2019b; Liu et al., 2018d) between PFOA and triglycerides/hypertriglyceridemia. The direction of association appeared to differ by survey cycle, sex, and obesity status. No associations in these NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was inversely associated with triglycerides, regardless of whether PFOA was measured concurrently or averaged between baseline and follow-up (Donat-Vargas et al., 2019). All participants in this study were free of diabetes for over 10 years, as opposed to the obese or prediabetic adults in Liu et al. (2020a) and Lin et al. (2019). It is unclear whether participants' different health status explained differences in the findings across *medium* studies.

In *low* confidence studies, a mix of positive (Liu et al., 2021; Ye et al., 2021; Canova et al., 2020; Khalil et al., 2020; Lin et al., 2020e in women; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b association with concurrent triglycerides or changes in triglycerides in the first 6 months of the study; Sun et al., 2018; Yang et al., 2018), inverse (Li et al., 2020b; Lin et al., 2020e in men; Liu et al., 2018b association with changes in triglycerides in the 6–24 months of the study; Lin et al., 2013), and essentially null (Cong et al., 2021; Convertino et al., 2018; Wang et al., 2012) associations with triglycerides or hypertriglyceridemia were reported. Some associations were statistically significant. Overall, the available evidence suggests that PFOA was associated with elevated triglycerides in some adults. Whether PFOA increases triglycerides in all adults is unclear given inconsistency in reported associations.

In summary, in the general adult population, a relatively consistent, positive association was observed between PFOA and LDL or TC. Increased triglycerides with increasing PFOA exposure were also observed, but less consistently. HDL was not associated with PFOA.

3.4.3.1.2.6 Findings From Occupational Studies

Workers are usually exposed to higher levels of PFOA, in a more regular manner (sometimes daily), and potentially for a longer duration than adults in the general population. At the same time, according to the "healthy worker effect," workers tend to be healthier than non-workers, which may lead to reduced susceptibility to toxic agents (Shah, 2009). Because of these potential differences in exposure characteristics and host susceptibility, occupational studies are summarized separately from studies among adults in the general population.

Three *low* confidence studies examined the association between PFOA and TC or hypercholesterolemia in workers. Two of these studies examined the cross-sectional association between PFOA and TC in fluorochemical plant workers or firefighters exposed to aqueous film-forming foam (AFFF) (Rotander et al., 2015; Wang et al., 2012). One investigated the association between baseline PFOA and changes in TC over the course of a fluorochemical plant demolition project (Olsen et al., 2012). The cross-sectional studies reported positive (Wang et al., 2012) or inverse (Rotander et al., 2015) associations between PFOA and TC; neither association

was statistically significant. Olsen et al. (2012) reported that over the course of the demolition project, changes in PFOA were inversely associated with changes in TC; this association was not statistically significant (Olsen et al., 2012). Taken together, these studies suggest no association between PFOA and TC in workers.

Two studies examined PFOA and LDL in workers. One study examined PFOA and non-HDL, of which LDL is a major component. All studies were considered *low* confidence. The two studies on LDL reported positive (Wang et al., 2012) or inverse (Rotander et al., 2015) association between PFOA and concurrent LDL; neither association was statistically significant. The study examining non-HDL reported that changes in PFOA during the fluorochemical plant demolition project were inversely associated with changes in non-HDL, but the association was not statistically significant (Olsen et al., 2012). Overall, these studies suggest no association between PFOA and LDL in workers.

The studies that examined LDL or non-HDL also examined the association between PFOA and HDL (Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012). The two cross-sectional studies in this set of studies reported inverse association between PFOA and HDL, including a statistically significant finding in Wang (2012) (Rotander et al., 2015). Contrary to these findings, Olsen et al. (2012) reported that changes in PFOA over the demolition project were positively associated with changes in HDL (Olsen et al., 2012). This association was not statistically significant. When changes in TC to HDL ratio were examined as an outcome, however, a statistically significant, inverse association was observed. This suggests that increasing PFOA exposure was associated with decreases in TC/HDL over time, potentially partly due to a positive association between changes in PFOA and changes in HDL. Together, the occupational studies reported a consistently inverse association between PFOA and concurrent HDL, but this cross-sectional association was not coherent with longitudinal findings.

Two *low* confidence cross-sectional studies examined PFOA and triglycerides in workers and reported inverse associations between PFOA and triglycerides (Rotander et al., 2015; Wang et al., 2012). Neither association was statistically significant.

In summary, among workers, the available evidence suggests no association between PFOA and TC or LDL. Inverse, cross-sectional associations between PFOA and HDL and triglycerides were found, but these associations were small, often not statistically significant, and were not coherent with longitudinal findings. Overall, the associations between PFOA and serum lipids among workers are different from those in the general adult population. It is unclear whether well-known biases in occupational studies such as “healthy worker effect” may have attenuated the association between PFOA and an unfavorable serum lipid profile. Additional higher-quality occupational studies are needed to improve hazard identification among workers.

3.4.3.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and seven studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and cardiovascular effects in animal models. Study quality evaluations for these 10 studies are shown in Figure 3-43.

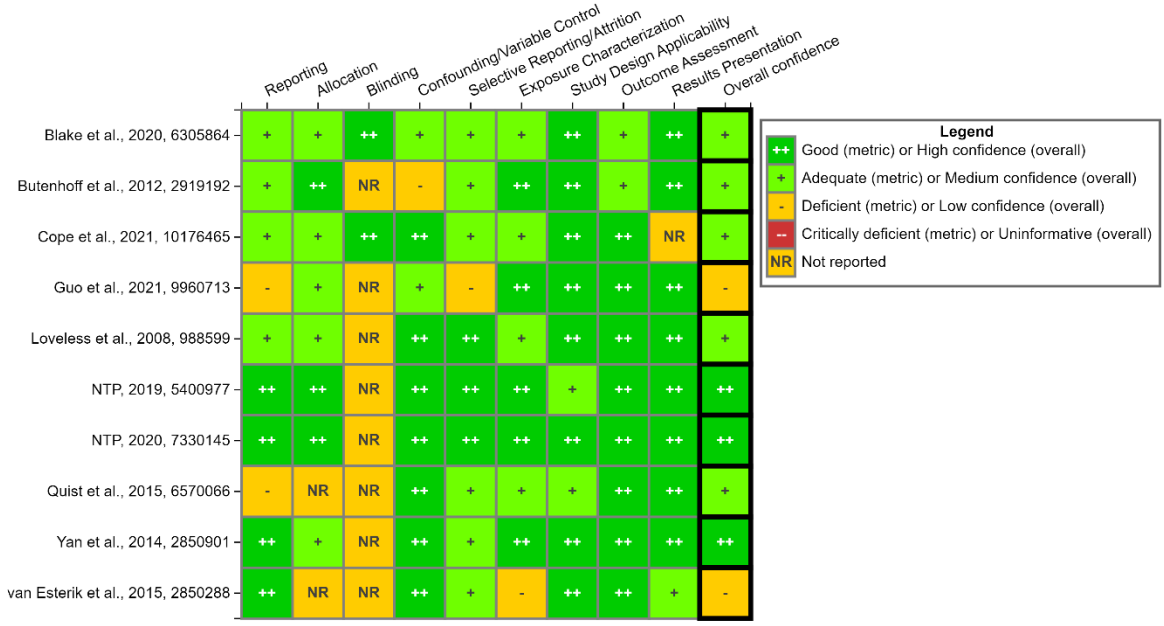


Figure 3-43. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

Cardiovascular effects following exposure to PFOA were minimal according to two chronic studies with doses between 1.1–14.2 mg/kg/day (NTP, 2020; Butenhoff et al., 2012) and one short-term 28-day study with doses between 0.312–5 mg/kg/day (NTP, 2019). No toxicologically relevant changes were observed for heart weight (NTP, 2020, 2019; Butenhoff et al., 2012), minimal changes were observed for heart histopathology (NTP, 2020, 2019; Butenhoff et al., 2012), and no changes were observed for aorta histopathology (NTP, 2019; Butenhoff et al., 2012) following exposure to PFOA in male and female Sprague-Dawley rats.

PFOA has been observed to cause perturbations in lipid homeostasis, which may have effects on the cardiovascular system. Alterations in serum lipid levels have been observed in mice and rats in subchronic, chronic, and developmental studies of oral exposure to PFOA (Figure 3-44). Overall, studies have generally reported consistent decreases in serum lipids including TC, triglycerides, LDL cholesterol, HDL cholesterol, and/or non-HDL cholesterol in rats (NTP, 2020, 2019; Elcombe et al., 2010; Loveless et al., 2008; Martin et al., 2007) and mice (Cope et al., 2021; Blake et al., 2020; Quist et al., 2015; Yan et al., 2014; Minata et al., 2010; Yahia et al., 2010; Dewitt et al., 2009; Loveless et al., 2008).

In a developmental study of female CD-1 P_0 mice exposed to PFOA (0, 1, and 5 mg/kg/day) by oral gavage from either GD 1.5–11.5 or GD 1.5–17.5, authors reported maximum decreases in serum triglyceride levels of 58% and 66%, respectively, at the highest dose of 5 mg/kg/day. No changes were observed for serum TC, HDL cholesterol, or LDL cholesterol (Blake et al., 2020). In a secondary developmental study of gestational PFOA exposure (0.1 and 1.0 mg/kg/day), female CD-1 P_0 mice were exposed via gavage from GD 1.5 to GD 17.5 (Cope et al., 2021). Male and female F_1 offspring were fed either a low-fat diet (LFD) or high-fat diet (HFD) at PND

22 and serum cholesterol markers were evaluated at PND 22 and at postnatal week (PNW) 18. At PND 22, there was a significant reduction in serum triglycerides in males and females and a significant reduction in LDL in males only but no effects in TC or HDL. At PNW 18, LFD female mice exhibited nonsignificant decreases in TC, HDL, LDL, and triglycerides. However, animals that were given a HFD no longer exhibited decreased levels of TC, HDL, or triglycerides and developed significantly higher levels of LDL (1.0 mg/kg/day) when compared with HFD control. Males fed the LFD exhibited nonsignificant increases in TC, HDL, LDL, and triglycerides; however, this trend was lost when animals were fed the HFD.

Male BALB/c mice exposed to PFOA by gavage for 28 days had significant decreases in serum TC and HDL levels at concentrations as low as 1.25 mg/kg/day (Yan et al., 2014). For serum triglyceride levels, significant increases were observed at lower exposure concentrations of PFOA (0.31 and 1.25 mg/kg/day) while significant decreases were seen following exposure to higher PFOA concentrations (5 and 10 mg/kg/day); no changes were observed in serum LDL cholesterol levels. In a study conducted by NTP, sex differences were observed in Sprague-Dawley rats exposed to PFOA by gavage for 28 days (NTP, 2019). Males had significantly decreased serum TC and triglyceride levels at exposure concentrations as low as 0.625 mg/kg/day. Female rats in the same study were exposed to 10-fold higher doses than their male counterparts due to sex differences in PFOA excretion (see Appendix, (U.S. EPA, 2024a)). Females had significant increases in both serum TC and triglyceride levels at the two highest doses (50 and 100 mg/kg/day). In the available chronic study (NTP, 2020), F_1 male and female Sprague-Dawley rats were exposed during gestation and lactation (perinatal exposure with postweaning exposure) or postweaning exposure only until animals were 19 weeks of age (e.g., 16-week interim time point; see further study design details in Section 3.4.4.2.1.2). Serum TC levels were significantly decreased only in males exposed during both the perinatal and postweaning phases (at postweaning doses of approximately 1 and 4.6 mg/kg/day); serum triglyceride levels were decreased in all exposure groups. Serum TC levels were significantly decreased only in the mid-dose F_1 females exposed during both perinatal and postweaning phases; TG levels were not altered in F_1 females.

Conclusions from these studies are met with limitations as the difference in serum lipid composition between humans and commonly used rodent models may impact the relevance to human exposures (Oppi et al., 2019; Getz and Reardon, 2012). It should be noted that human population-based PFOA exposure studies have consistently found that as PFOA exposure increases both serum cholesterol and serum triglycerides also increase. Some rodent studies (Yan et al., 2014) exhibit a biphasic dose response where low exposure concentrations lead to increased serum lipid levels while high exposure concentrations lead to decreased serum lipid levels. This has called in the validity of using rodent models to predict human lipid outcomes. The relatively high exposure and PFOA serum concentrations that produce these inverse effects are generally beyond the scope of human relevance, though there is some evidence in humans that similarly high serum PFOA serum concentrations result in decreased serum total cholesterol (e.g., Convertino et al. (2018)). This suggests that rodent models may be utilized accurately if the tested doses are within human health relevant exposure scenarios. Additionally, food consumption and food type may confound these results (Cope et al., 2021; Fragki et al., 2021; Schlezinger et al., 2020), as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection and laboratory diets contain a lower fat content

compared with typical Westernized human diets. More research is needed to understand the influence of diet on the response of serum cholesterol levels in rodents treated with PFOA.

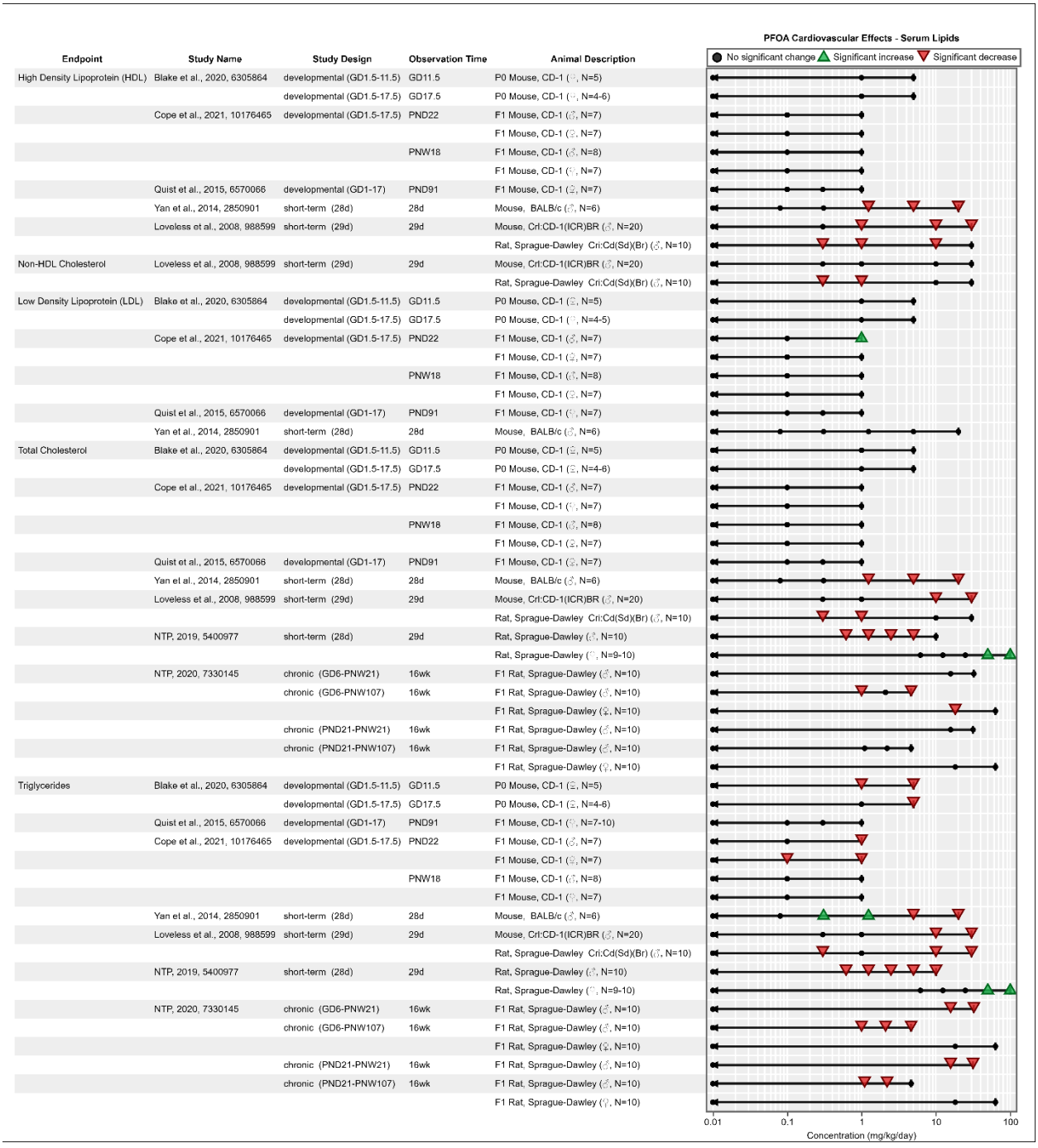


Figure 3-44. Serum Lipid Levels in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; PNW = postnatal week; F_1 = first generation; PND = postnatal day; d = day; wk = week.

3.4.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cardiovascular outcomes is discussed in Sections 3.1.1.1 and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to cardiovascular effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-45.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	0	1	0	1
Atherogenesis And Clot Formation	0	1	3	4
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	1	1	3
Cell Signaling Or Signal Transduction	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	0	1
Inflammation And Immune Response	0	0	1	1
Oxidative Stress	0	2	0	2
Grand Total	2	3	3	8

Figure 3-45. Summary of Mechanistic Studies of PFOA and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

3.4.3.3.1 Lipid Transport and Metabolism

Blood lipid levels are associated with risk factors for cardiovascular disease. Pouwer et al. (2019) investigated how PFOA influences plasma cholesterol and triglyceride metabolism using a transgenic mouse model of human-like lipoprotein metabolism (APOE*3-Leiden.CETP mice, which express the human CETP gene), human plasma samples, and in silico predictions. In the animal toxicological study, mice were fed a semisynthetic Western-type diet (0.25% cholesterol (wt/wt), 1% corn oil (wt/wt), and 14% bovine fat (wt/wt)) with varying levels of PFOA added (10, 300, or 30,000 ng/g/d). At the end of 4 or 6 weeks, mice were sacrificed and levels of triglycerides, TC, free fatty acids (FFA), ALT, glycerol, VLDL, HDL, and CETP were measured. The authors found that administration of PFOA at the 30,000 ng/g/d levels "reduced plasma TG and TC levels by affecting VLDL-TG production through decreased apoB synthesis and by increasing VLDL clearance." The authors also observed that PFOA at the highest dose decreased hepatic VLDL production rate, increased plasma VLDL clearance through enhanced LPL activity and affected gene expression of TG and cholesterol metabolism markers. Upon further analysis. PPARα was determined to be the major transcription factor affecting gene expression and fatty acid oxidation that regulates triglyceride and TC levels.

One study summarized in the 2016 PFOA HESD (U.S. EPA, 2016c) evaluated a subset of 290 individuals in the C8 Health Project for evidence that PFOA exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al., 2013). Inverse associations were found between PFOA levels and expression of genes involved in cholesterol transport including Nuclear Receptor Subfamily 1 Group H Member 2 (NR1H2), Niemann-Pick disease type C (NPC1), and ATP Binding Cassette Subfamily G Member 1 (ABCG1). When males and females were analyzed separately, PFOA serum concentrations were negatively associated with expression of genes involved in cholesterol transport in both males and females, although the genes themselves differed between sexes (males: NPC1, ABCG1, PPARα; females: Nuclear Receptor Subfamily 1, Group H, Member 1 (NCEH1)). For additional information on the disruption of lipid metabolism, transport, and storage in the liver following PFOA exposure, please see Section 3.4.1.3.2.

3.4.3.3.2 Apoptosis and Cell Cycle Regulation

To elucidate the mechanisms involved in PFOA-induced vascular tissue apoptosis and CIMT, the levels of endothelial microparticles (CD62E, CD31+/CD42a−) and platelet microparticles (CD62P, CD31+/CD42a+) were measured in the serum of adolescents and young adults in another epidemiological study (Lin et al., 2016). The results showed that there was no association between PFOA serum levels and markers of apoptosis, endothelial activation, or platelet activation. This study also measured the relationship between oxidative stress and PFOA by measuring levels of 8-hydroxydeoxyguanosine (8-OHdG) in the urine. Similar to the markers of apoptosis, no association was found between PFOA and 8-OHdG. Another study by the same researchers also found that there was no association between PFOA and oxidative/nitrative stress markers 8-OHdG and 8-nitroguanine (8-NO_2Gua) in Taiwanese adults (Lin et al., 2020a).

One study evaluated the potential for PFOA to affect cell cycle regulation in the heart and other tissues (Cui et al., 2019). Male mice were orally dosed with 5 mg/kg/day PFOA for 28 days, and microRNA-34 (miR-34), a marker of tissue damage, was measured in the heart at the end of the exposure period. To further study the role of cardiovascular miR-34a under PFOA treatment, the authors also dosed miR-34a-knockout and wild-type mice for 28 days. In the wild-type mice, the expression of miR-34a in the heart was not significantly different in the treatment group compared with the control group. There were also no detectible levels miR-34b or miR-34c in the heart for either the treatment group or the control group.

3.4.3.3.3 Mechanisms of Atherogenesis and Clot Formation

Four groups of researchers published studies on the mechanism of atherogenesis and clot formation. The first two studies investigated how the structure of PFOA and other PFAS leads to activation of the plasma kallikrein-kinin system (KKS) using in vitro and ex vivo activation assays and in silico molecular docking analysis. KKS is a key component of plasma that plays a role in regulation of inflammation, blood pressure, coagulation, and vascular permeability. Activation of the plasma KKS can release the inflammatory peptide bradykinin (BK), which can lead to dysfunction of vascular permeability. The cascade activation of KKS includes the autoactivation of Hageman factor XII (FXII), cleavage of plasma prekallikrein (PPK), and activation of high-molecular-weight kininogen (HK) (Liu et al., 2018e). Results from the ex vivo mouse plasma study by Liu et al. (2017b) revealed that the addition of PFOA (5 mM) at the highest dose binds with FXII in a structure dependent manner and triggers the cascade to the rest

of the system. Liu et al. (2018e) observed no activation of the KKS cascade when mouse plasma was incubated with up to 500 μM PFOA.

Bassler et al. (2019) focused on several disease biomarkers, including plasminogen activator ihhibitor-1 (PAI-1), an indicator of clot formation that may lead to atherosclerosis. Human serum was collected from 200 patients as part of the larger C8 Health Project and analyzed for PFOA content. The authors found that there was no statistically significant difference in PAI-1 concentration in association with high exposure to PFOA concentrations.

The final study among the four groups of researchers, conducted by De Toni et al. (2020), investigated the effect of PFOA on platelet function, a key factor in atherosclerosis. Whole blood and peripheral blood samples were taken from healthy males that lived in low exposure areas and incubated with 400 ng/mL of PFOA. After isolating erythrocytes, leukocytes, and platelets and quantifying the amount of PFOA present, platelets were found to be the cell target of PFOA accumulation. The authors then used the platelets in an in vitro system and inoculated them with 400 ng/mL of PFOA and found that substantially more PFOA accumulated in the membrane of platelets versus the cytoplasm. Using molecular docking analysis, they were able to target the specific binding sites of PFOA to phosphatidylcholine, a major platelet phospholipid, suggesting that the accumulation of PFOA in the platelet may alter the activation process of platelets by impairing membrane stability.

3.4.3.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and cardiovascular effects in humans based on consistent positive associations with serum lipids, particularly LDL, and TC (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020e; Donat-Vargas et al., 2019; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Winquist and Steenland, 2014; Eriksen et al., 2013; Fitz-Simon et al., 2013; Nelson et al., 2010; Steenland et al., 2009). Additional evidence of positive associations with blood pressure and hypertension in adult populations supported this classification. The lack of evidence of consistent or precise effects for CVD or atherosclerotic changes raise uncertainty related to cardiovascular health effects following PFOA exposure. The available data for CVD and atherosclerotic changes was limited and addressed a wider range of outcomes, resulting in some residual uncertainty for the association between PFOA exposure and these outcomes.

On the basis of this systematic review of 43 epidemiologic studies, the available evidence revealed positive associations between PFOA exposure and TC, LDL, and triglycerides effects in some human populations. For TC, the association was consistently positive in adults from the general population, positive but less consistently so in children and pregnant women, and generally null in workers. For LDL, the association was generally positive among adults, positive but less consistently so in children, and generally null in workers. Data were not available for PFOA and LDL in pregnant women. For triglycerides, positive, often nonsignificant associations were observed in some adults and children, but not pregnant women and workers. Except for workers, these results are consistent with findings from the 2016 PFOA HESD. Differences in findings from occupational studies between the 2016 PFOA HESD and this review may be attributable to limitations of occupational studies in this review. Similar to the 2016 PFOA HESD, the available evidence in this review does not support an inverse association between PFOA and HDL in any populations. The positive associations with TC are also supported by the

recent meta-analysis restricted to 14 general population studies in adults (U.S. EPA, 2022b). Similarly, a recent meta-analysis including data from 11 studies reported consistent associations between serum PFOA or a combination of several PFCs including PFOA and PFOS, and increased serum TC, LDL, triglyceride levels in children and adults (Abdullah Soheimi et al., 2021).

The epidemiological studies identified since the 2016 assessments do not provide additional clarity on the association between PFOA and CVD. Most of the CVD evidence identified in this review focused on blood pressure in the general adult population (13 studies). The findings from a single *high* confidence study and five *medium* confidence studies conducted in the general adult population did not provide consistent evidence for an association between PFOA and blood pressure. The evidence for an association between PFOA and increased risk of hypertension overall and in gender-stratified analysis was inconsistent. Evidence in children and adolescents also is less consistent. Five studies in children and adolescents, and one study in pregnant women suggest no associations with elevated blood pressure in these populations. Evidence for other CVD-related outcomes across all study populations was more limited, and similarly inconsistent. Consequently, the evidence for these CVD outcomes is broadly consistent with the conclusions of the C8 Science Panel and in the 2016 PFOA assessment, which found no probable link between PFOA exposure and multiple other conditions, including high blood pressure and CAD. It is challenging to compare findings on CVD-related mortality in the current assessment to the prior assessment due to differences in how this outcome was defined. Findings from the prior assessment were mixed, with one study reporting an increased risk of cerebrovascular disease mortality observed in the highest PFOA exposure category among occupationally exposed subjects. However, no association was reported with IHD mortality. The current evidence from a single study indicated PFOA was not associated with an increased risk of mortality due to cardiovascular causes, including hypertensive disease, IHD, stroke, and circulatory diseases. Future analyses of cause-specific CVD mortality could help elucidate whether there is a consistent association between PFOA and cerebrovascular disease mortality. No studies or endpoints were considered for the derivation of PODs since findings for an association between PFOA and CVD outcomes are mixed.

The animal evidence for an association between PFOA exposure and cardiovascular toxicity is *moderate* based on effects on serum lipids observed in animal models in six *high* or *medium* confidence studies. The most consistent results are for TC and triglycerides, although direction of effect can vary by dose. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism. No effects or minimal alterations were noted for heart weight and histopathology in the heart and aorta.

The underlying mechanisms for the observed cardiovascular effects related to PFOA exposure are likely related to changes in lipid metabolism, as described in detail in Section 3.4.1.3. Specifically, alterations in lipid metabolism lead to alterations in serum levels of triglycerides and cholesterol, as evidenced by in vivo in animal models. The events that precede and result in the alterations in serum levels have been proposed as the following, based on experimental evidence: (1) PFOA accumulation in liver activates nuclear receptors, including PPARα; (2) expression of genes involved in lipid homeostasis and metabolism is altered by nuclear receptor activation; (3) gene products (translated proteins) modify the lipid content of liver to favor

triglyceride accumulation and potentially cholesterol accumulation; (4) altered lipid content in the liver leads to accumulation of lipid droplets, which can lead to the development of steatosis and liver dysfunction. It should be noted that the results for PFOA-induced changes to serum lipid levels contrast between rodents (generally decreased) and humans (generally increased). Evidence is ultimately limited regarding a clear mechanism of alterations to serum lipid homeostasis caused by PFOA exposure. In humans, as discussed in the 2016 PFOA HESD (U.S. EPA, 2016c) data from the C8 Health Project indicated that PFOA exposure can influence expression of genes involved in cholesterol metabolism, mobilization, or transport. Specifically, an inverse association was found between PFOA levels and expression of genes involved in cholesterol transport, with sex-specificity for some of the individual gene expression changes. The authors of the study suggested that exposure to PFOA may promote a hypercholesterolaemic environment. Results were inconsistent regarding effects of PFOA on indicators or mechanisms related to atherosclerosis, including a lack of effect on an indicator of clot formation in human serum samples, and dose-dependent effects on the plasma kallikrein-kinin system in mouse plasma. A single study found that PFOA accumulates in platelets in human blood samples exposed in vitro, which may alter the activation process of platelets, although it was not directly evaluated. PFOA did not induce apoptosis or oxidative stress in vascular tissue in humans, as evidenced in two studies that evaluated serum levels of endothelial microparticles and platelet microparticles, and urinary 8-hydroxydeoxyguanosine (8-OHdG) in relation to PFOA levels.

3.4.3.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause adverse cardiovascular effects, specifically serum lipid effects, in humans under relevant exposure circumstances (Table 3-12). The hazard judgment is driven primarily by consistent evidence of serum lipid responses from epidemiological studies at median PFOA exposure levels representative of the NHANES population (median = 3.7 ng/mL). The evidence in animals showed coherent results for perturbations in lipid homeostasis in rodent models in developmental, subchronic, and chronic studies following exposure to doses as low as 0.3 mg/kg/day PFOA. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence studies.

Table 3-12. Evidence Profile Table for PFOA Exposure and Cardiovascular Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.3.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Serum lipids 2 *High* confidence studies 27 *Medium* confidence studies 22 *Low* confidence studies 19 *Mixed*[a] confidence studies	Examination of serum lipids included measures of TC, LDL, HDL, TG, and VLDL. In studies of serum lipids in adults from the general population (29), there is evidence of positive associations with TC (13/15) in *medium* confidence studies. Positive associations were also observed for LDL (6/8) in *medium* confidence studies, and mostly null, but some positive associations with TG (4/11) in *medium* confidence studies. Evidence from studies of children (19) was mixed, and observed associations often failed to reach significance, but findings were mostly positive for TC (10/19). In studies of pregnant women (6), evidence indicated positive associations with TC (3/4) and HDL (2/4) but no other serum lipid	• *High* and *medium* confidence studies • *Consistent* findings of positive associations with serum lipid measures in adults from the general population • *Coherence* of findings across serum lipids serum lipid effects	• *Low* confidence studies • *Inconsistent* findings in children, likely due to variations in measured exposure windows • *Inconsistent* findings by sex or health status	⊕⊕⊙ *Moderate* Evidence for cardiovascular effects is based on numerous *medium* confidence studies reporting positive associations with serum lipids, such as TC, LDL, and TG in adults from the general population. Results from occupational studies were generally consistent with studies of general population adults, particularly for increases in TC. Results from some studies of children and pregnant women also observed positive effects for TC and TG, however, interpretations of changes in serum lipids for children are less clear. *High* and *medium* confidence studies of adults reported positive associations with blood pressure and risk of	*Primary basis and cross-stream coherence*: Human evidence indicated consistent evidence of serum lipids response and animal evidence showed coherent results for perturbations in lipid homeostasis in rodent models in developmental, subchronic, and chronic studies following exposure to PFOA. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence studies *Human relevance and other inferences:* No specific factors are noted.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	measures. In occupational studies (10), positive associations or increased risks were observed for TC and high cholesterol (8/10), LDL (3/5), and TG (4/8). Findings on HDL in occupational studies were mixed.			hypertension, though other *medium* and *low* confidence studies reported nonsignificant associations. Observed effects were inconsistent for CVD and imprecise for atherosclerotic changes across all study populations.	
Blood pressure and hypertension 2 *High* confidence studies 18 *Medium* confidence studies 7 *Low* confidence studies	Studies examining changes in blood pressure, including DBP and SBP, and risk for hypertension in general population adults (15), showed consistent positive associations for SBP (5/6), DBP (6/6), combined BP (2/2), and hypertension (9/10) in *high* and *medium* confidence studies. In studies of children (9), mixed results were observed for SBP (7), DBP (5), and general BP (3). The only study examining hypertension in children reported a positive, dose-dependent association. In occupational studies, one study reported a positive association for hypertension (1/3). In the	• *High* and *medium* confidence studies • *Consistent* findings of effects for blood pressure measures, including hypertension, among adults • *Consistent* findings of effects observed in studies of children for blood pressure measures and hypertension	• *Low* confidence studies • *Imprecision* of findings • *Inconsistent findings* in children, likely due to variation in measured exposure window*s*		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	only study of pregnant women (1), a positive association was reported with hypertension. Hypertension analyses provided evidence of modification by sex, with males having higher risk in some studies.				
Cardiovascular disease 1 *High* confidence study 6 *Medium* confidence studies 6 *Low* confidence studies	CVD measures included CHD, stroke, angina, heart attack, MVD, IHD, PAD, and arrhythmia. Studies of general population adults (9) reported mixed results. The most commonly investigated endpoints were CHD (5), general CVD (5), and stroke (3); in all cases, positive and inverse associations were observed. A significant positive association for risk of heart attack was observed in a *medium* confidence study (1/1). Observations for other outcomes were limited to nonsignificant, imprecise findings by singular studies. In occupational studies (4), consistent inverse associations were observed for IHD (3/3),	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent* findings for CVD-related outcomes • *Imprecision* of findings		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	but results remained mixed for stroke (1/2).				
Atherosclerotic changes 1 *High* confidence study 3 *Medium* confidence studies 3 *Low* confidence studies	In studies of children (2), one study reported significantly increased associations in brachial artery distensibility (1/1). No significant associations were observed for CIMT among Taiwanese children (1/1) or pulse wave velocity among American children (1/1). Studies of adults (4) reported mixed results for measures of atherosclerotic changes. Most studies did not report associations that reached significance, however, one study reported decreased left ventricular relative wall thickness (1/3).	• *High* and *medium* confidence studies	• *Low confidence studies* • *Imprecision* of findings across children and adult study populations		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.3.2)					
Serum lipids 3 *High* confidence studies 4 *Medium* confidence studies	Significant decreases in serum TC were observed in 4/7 studies that examined this endpoint, regardless of species, sex, or study design. In three developmental studies, no changes were observed in mice. Similar decreases	• *High* and *medium* confidence studies • *Consistency of* findings across species, sex, or study design • *Dose-response* relationship	• *Incoherence* of findings in other cardiovascular outcomes • *Biological significance* of the magnitude of effect is unclear	⊕⊕⊙ *Moderate* Evidence based on six *high* or *medium* confidence studies observed that PFOA affects serum lipids in	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	were observed in serum TG (6/7). In a developmental study, decreased serum TG were observed in mice at PND 22 but not during adulthood. In a short-term exposure study, female rats were given 10-fold higher doses of PFOA than males due to sex differences in excretion, and it was found that serum TC and TG were decreased in males but increased in females. Fewer studies examined HDL and LDL, with decreases found in HDL (2/5). Three studies found no changes in LDL, but one developmental study in mice observed increased LDL in males at PND 22 but no changes during adulthood.	observed within multiple studies		animal models. The most consistent results are for total cholesterol and triglycerides, although direction of effect can vary by dose. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects indicate a disruption in lipid metabolism. No effects or minimal alterations were noted for heart weight and histopathology in the heart and aorta. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system.	
Histopathology 2 *High* confidence studies 1 *Medium* confidence study	No changes in heart histopathology were reported in two studies. One chronic study reported decreased incidence of chronic myocarditis in female rats in the mid-dose group	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	only. No changes in aorta histopathology were noted in two studies.				
Organ weight 2 *High* confidence studies, 1 *Medium* confidence study	No changes in absolute or relative heart weights were found in one short-term study and one chronic study in rats. One chronic study in rats reported decreased absolute heart weights in males and females, but those reductions were found to be related to reduced body weights.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Confounding* variables such as decreases in body weights may limit ability to interpret these responses		

Mechanistic Evidence and Supplemental Information (Section 3.4.3.3)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**

Key findings and interpretation:
• Alterations in lipid metabolism results in alterations in serum levels of TG and TC via:
  ○ PFOA accumulation in liver activates nuclear receptors, including PPARα.
  ○ Nuclear receptor activation alters the expression of genes involved in lipid homeostasis and metabolism.
PPARα is a major transcription factor affecting expression of genes that regulate fatty acid oxidation and triglyceride and total cholesterol levels.
Limitations:
• Only a single study demonstrating PFOA accumulation in platelets in vitro.
• Results are inconsistent and conflicting regarding effects on indicators or mechanisms related to atherosclerosis, primarily related to clot formation.
	Findings support plausibility that cardiovascular effects, specifically changes to serum TG and TC levels, can occur through changes in lipid metabolism related to PFOA exposure.

Notes: CHD = coronary heart disease; CIMT = carotid intima-media thickness; CVD = cardiovascular disease; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVD = microvascular disease; PAD = peripheral arterial disease; PPARα = peroxisome proliferator-activated receptor alpha; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride.

[a] *Mixed* confidence studies had split confidence determinations for different serum lipid measures with some measures rated *medium* confidence and others rated *low* confidence.
[b] *Mixed* confidence studies had split confidence determinations for different subgroups of participants with some measures rated *medium* confidence and others rated *low* confidence.

3.4.4 Developmental

EPA identified 100 epidemiological and 19 animal toxicological studies that investigated the association between PFOA and developmental effects. Of the epidemiological studies, 30 were classified as *high* confidence, 39 as *medium* confidence, 19 as *low* confidence, 5 as *mixed* (2 *high/medium*, 1 *medium/low*, 2 *low/uninformative*) confidence, and 7 were considered *uninformative* (Section 3.4.4.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 12 as *medium confidence,* and 4 as *low* confidence, and 1 was considered *mixed* (*medium/low*) (Section 3.4.4.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low confidence* studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.4.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.4.1.1 Introduction

This section describes studies of PFOA exposure and potential in utero and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. The latter includes all studies where exposure is limited to gestation and/or early life up to 2 years of age. Developmental endpoints can include gestational age, measures of fetal growth (e.g., birth weight), birth defects, and fetal loss (i.e., spontaneous abortion/miscarriage and stillbirths), as well as infant/child development.

The 2016 PFOA HESD (U.S. EPA, 2016c) summarized epidemiological studies that examined developmental effects in relation to PFOA exposure. There are 22 studies from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and developmental effects. Study quality evaluations for these 22 studies are shown in Figure 3-46. Studies included ones conducted both in the general population as well as in communities known to have experienced high PFOA exposure (e.g., the C8 population in West Virginia and Ohio). Results from studies summarized in the 2016 PFOA HESD are described in Table 3-13 and below.

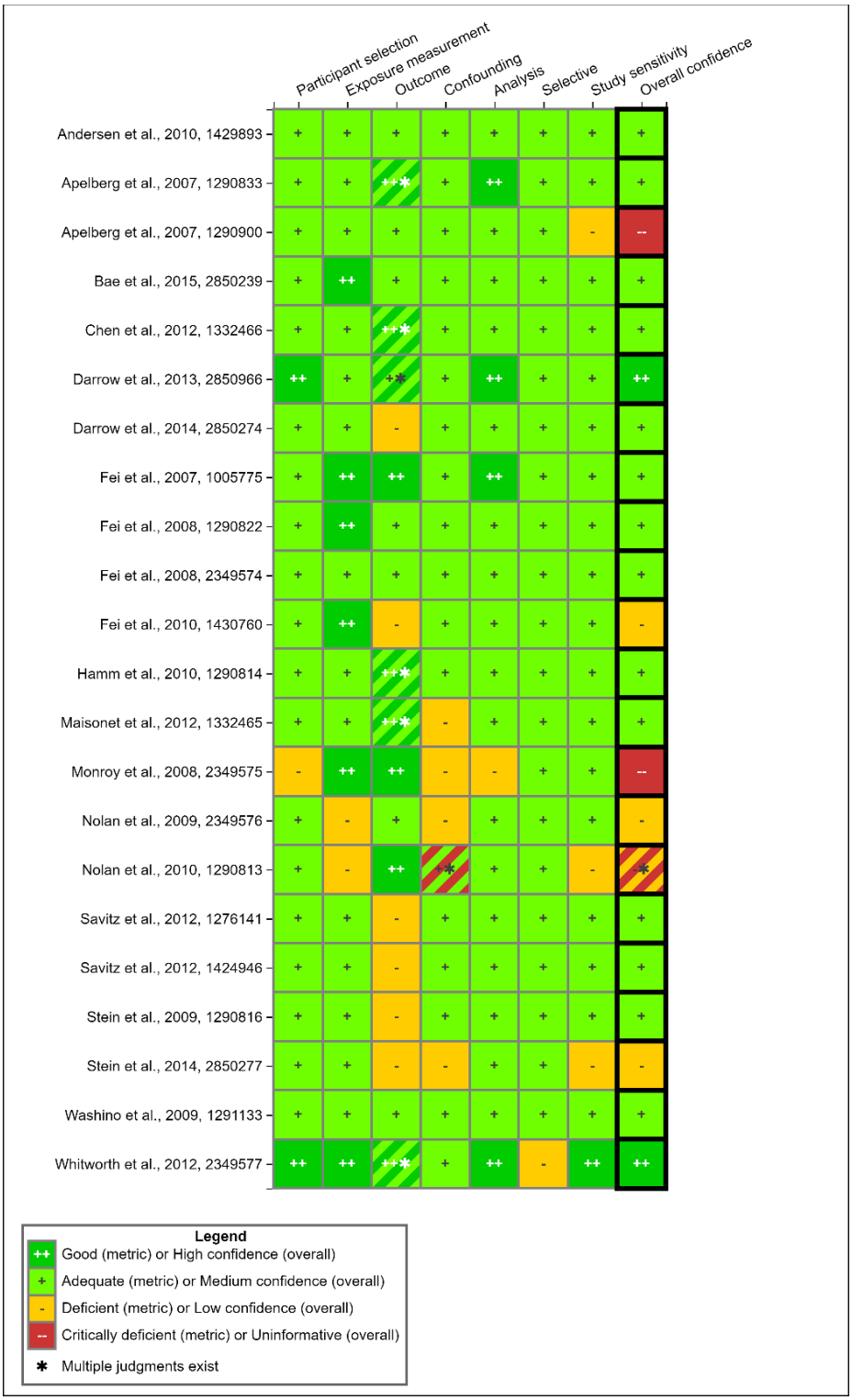


Figure 3-46. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Developmental Effects Published before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

As noted in the 2016 PFOA HESD, several available studies measured fetal growth outcomes. Apelberg et al. (2007b) found that birth weight was inversely associated with umbilical cord PFOA concentration (β per log unit increase: −104 g; 95% CI: −213, –5) in a study of 293 infants born in Maryland in 2004–2005 (mean PFOA concentration of 0.0016 μg/mL). Maisonet et al. (2012) evaluated fetal growth outcomes in 395 singleton female births of participants in the Avon Longitudinal Study of Parents and Children (ALSPAC) and found that increased maternal PFOA concentration (median concentration of 0.0037 μg/mL) was inversely associated with birth weight (β per log unit increase: −34.2 g; 95% CI: −54.8, –13). A study of 252 pregnant women in Alberta, Canada found no statistically significant association between PFOA concentration measured in maternal blood during the second trimester (mean concentration of 0.0021 μg/mL) and birth weight (Hamm et al., 2010). In a Japanese prospective cohort of 428 infants in the Hokkaido Study on Environment and Children's Health (2002–2005), Washino et al. (2009) observed a large nonsignificant association between PFOA concentration in maternal blood during pregnancy (mean PFOA concentration of 0.0014 μg/mL) and birth weight (β per each $\log_{10}$ increase: –75.1 g; 95% CI: –191.8 to 41.6). Chen et al. (2012) examined 429 mother-infant pairs from the Taiwan Birth Panel Study and found no statistically significant association between umbilical cord blood PFOA concentration (geometric mean (GM) of 0.0018 μg/mL) and birth weight (β per each ln-unit increase: –19.2 g; 95% CI: –63.5, 25.1).

Some studies evaluated fetal growth parameters in the prospective Danish National Birth Cohort (DNBC; 1996–2002) (Andersen et al., 2010; Fei et al., 2008b, 2007). Maternal blood samples were taken in the first and second trimester. Fei et al. (2007) found a small, nonsignificant inverse association between maternal PFOA concentration (blood samples taken in the first and second trimester) and birth weight (β per unit increase: −8.7; 95% CI: −19.5, 2.1). Fei et al. (2008b) found an inverse association between maternal PFOA levels and birth length and abdominal circumference in the DNBC. Change in birth length per unit increase was 0.069 cm (95% CI: 0.024, 0.113) and change in abdominal circumference per unit increase was 0.059 cm (95% CI: 0.012, 0.106). Andersen et al. (2010) examined the association between maternal PFOA concentrations and measures of standardized birth weight, birth length, and infant body mass index (BMI) and body weight at 5 and 12 months of age in DNBC participants. Andersen et al. (2010) also reported an inverse association with birth weight, but the study population overlapped with participants reported in Fei et al. (2007). Regarding post-natal growth, they observed a positive association between adiposity and maternal PFOA concentration based on BMI measured at 5 and 12 months in boys, but not girls.

Some studies described in the 2016 PFOA HESD evaluated developmental outcomes in the C8 Health Project study population, which comprises a community known to have been subjected to high PFAS exposure (Darrow et al., 2014; Darrow et al., 2013; Savitz et al., 2012a; Savitz et al., 2012b; Stein et al., 2009). The C8 Health Project included pregnancies within 5 years prior to exposure measurement, and many of the women may not have been pregnant at the time of exposure measurement. As noted in the 2016 PFOA HESD, none of the studies reported statistically significant or large magnitude associations between PFOA and either birth weight or the risk of low birth weight. Darrow et al. (2013) reported a non-statistically significant increased risk (ORs ranging 1.3 to 1.49) for participants in the upper three quintiles of PFOA exposure (PFOA concentrations ≥11.1 ng/mL) compared with the lowest (PFOA concentration >8.6 ng/mL), but results from other C8 studies reported null associations for preterm birth. In the *low* confidence study (Stein et al., 2014) on the C8 Health Project

community population, modeled maternal serum PFOA was associated with brain birth defects (albeit with only 13 cases), but no associations were observed for other birth defects. Additionally, two studies (Nolan et al., 2010, 2009) evaluated birth weight, gestational age of infants, and frequencies of congenital anomalies in this community based on whether participants were supplied with contaminated public drinking water (PFOA concentrations were not measured in participants). The studies found no associations between these developmental effects and water supply status. These two studies were rated *low* confidence for most endpoints and *uninformative for* congenital anomalies in Nolan et al. (2010).

Table 3-13. Associations Between Elevated Exposure to PFOA and Developmental Outcomes in Children from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Andersen, 2010, 1429893[c] *Medium*	Cohort	↓↓	NA	NA	NA	NA	NA	NA	↓
Apelberg, 2007, 1290833 *Medium*	Cross-sectional	↓↓	NA	NA	↑	NA	NA	NA	NA
Chen, 2012, 1332466[d] *Medium*	Cohort	↓	↓	↑	–	↓	NA	NA	NA
Darrow, 2014, 2850274 *Medium*	Cohort	NA	NA	NA	NA	NA	NA	–	NA
Darrow, 2013, 2850966 *High*	Cohort	–	–	NA	–	↑	NA	NA	NA
Fei, 2007, 1005775[c] *Medium*	Cohort	↓	↑	–	NA	↑↑	NA	NA	NA
Hamm, 2010, 1290814 *Medium*	Cohort	↑	NA	↓	↓	↑	NA	NA	NA
Maisonet, 2012, 1332465 *Medium*	Cohort	↓↓	NA	NA	↓	NA	NA	NA	–
Nolan, 2009, 2349576 *Low*	Cross-sectional	–	NA	NA	–	NA	NA	NA	NA
Nolan, 2010, 1290813 *Mixed*[e]	Cross-sectional	NA	NA	NA	–	NA	–	NA	NA
Savitz, 2012, 1276141 *Medium*	Cohort	NA	–	NA	NA	–	–	–	NA
Savitz, 2012, 1424946 *Medium*	Cohort	↓	–	↓	NA	↑	NA	–	NA
Stein, 2009, 1290816	Cohort	NA	↓	NA	NA	–	↑	–	NA

Reference, Confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Medium									
Stein, 2014, 2850277 *Low*	Cohort	NA	NA	NA	NA	NA	–	NA	NA
Washino, 2009, 1291133[f] *Medium*	Cohort	↓	NA	NA	NA	NA	NA	NA	NA
Whitworth, 2012, 2349577 *High*	Cohort	↓	NA	–	NA	↓↓	NA	NA	NA

Notes: LBW = low birth weight; NA = no analysis was for this outcome was performed; PNG = post-natal growth; SGA = small-for-gestational age; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Apelberg et al. (2007a) and Monroy et al. (2008) were not included in the table due to their *uninformative* overall study confidence ratings. Fei et al. (2008a), Fei et al. (2008b), and Fei et al. (2010a) were not included in the table because the studies only analyzed other developmental outcomes that were more prone to measurement error (see Study Evaluation Considerations in Section 3.4.4.1.2) or were not as heavily studied (i.e., other measures of fetal growth restriction such as birth length and head circumference and breastfeeding duration or developmental milestones, respectively).

[a]Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b]Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Fei (2007) reports results from a population overlapping with Meng et al. (2018), which was considered the most updated data.

[d] Chen (2012) reports results from a population overlapping with Chen et al. (2017b), which was considered the most updated data.

[e] Nolan (2010) was rated *uninformative* for congenital abnormalities and *low* confidence for all other outcomes.

[f] Washino et al. (2009) reports results from a population overlapping with Kashino et al. (2020), which was considered the most updated data.

3.4.4.1.2 Study Evaluation Considerations

There were multiple developmental outcome-specific considerations that informed domain-specific ratings and overall study confidence. For the Confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Some hemodynamic factors related to physiological changes during pregnancy were also considered in this domain as potential confounders (e.g., GFR and blood volume changes over the course of pregnancy) because these factors may be related to both PFOA levels and the developmental effects examined here. More confidence was placed in the epidemiologic studies that adjusted for GFR in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, this was considered in the study quality evaluations and as part of the overall weight of evidence determination. Further discussion of considerations for potential confounding by co-occurring PFAS can be found in Section 5.1.

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFOA in humans noted in Section 3.3.1.4.5, samples collected during all three trimesters, before birth or shortly after birth were considered adequately representative of the most critical in utero exposures for fetal growth and gestational duration measures. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where in utero perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in (De Boo and Harding, 2006) and (Perng et al., 2016). There is some evidence that birth weight (BWT) deficits can be followed by increased weight gain that may occur especially among those with rapid growth catch-up periods during childhood (Perng et al., 2016). Therefore, the primary critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero for study evaluation purposes, and studies of this outcome were downgraded in the exposure domain if exposure data were collected later during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (e.g., for sex-stratified results). In the Outcome domain, specific considerations address validation and accuracy of specific endpoints and adequacy of case ascertainment for some dichotomous (i.e., binary) outcomes. For example, BWT measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the Outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias toward the null if ascertainment of fetal loss is not associated with PFOA exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null can manifest as an apparent protective effect. Fetal and

childhood growth restriction were examined using several endpoints including low BWT, small for gestational age (SGA), ponderal index (i.e., BWT grams)/birth length (cm^3) × 100), abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher quality endpoints (i.e., classified as good in the Outcome domain) that were available in multiple studies to allow for an evaluation of consistency and other considerations across studies. However, even when databases were more limited, such as for spontaneous abortions, the evidence was evaluated for its ability to inform developmental toxicity more broadly, even if available in only one study.

Overall, mean BWT and BWT-related measures are considered very accurate and were collected predominately from medical records; therefore, more confidence was placed in these endpoints in the Outcome domain judgments. Some of the adverse endpoints of interest examined here included fetal growth restriction endpoints based on BWT such as mean BWT (or variations of this endpoint such as standardized BWT z-scores), as well as binary measures such as SGA (e.g., lowest decile of BWT stratified by gestational age and other covariates) and low BWT (i.e., typically <2500 grams; 5 pounds, 8 ounces) births. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary to be classified as good for these endpoints in this domain. In contrast, other measures of fetal growth that are subject to more measurement error (e.g., head circumference and body length measures such as ponderal index) were given a rating of adequate (Shinwell and Shlomo, 2003). These sources of measurement error are expected to be non-differential with respect to PFOA exposure status and, therefore, would not typically be a major concern for risk of bias but could impact study sensitivity.

Gestational duration measures were presented as either continuous (i.e., per each gestational week) or binary endpoints such as preterm birth (PTB, typically defined as gestational age <37 weeks). Although changes in mean gestational age may lack some sensitivity (especially given the potential for measurement error), many of the studies were based on ultrasound measures early in pregnancy, which should increase the accuracy of estimated gestational age and the ability to detect associations that may be present. Any sources of error in the classification of these endpoints would also be anticipated to be non-differential with respect to PFOA exposure. While they could impact precision and study sensitivity, they were not considered a major concern for risk of bias.

3.4.4.1.3 Study Inclusion for Updated Literature Search

There are 79 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and developmental effects. Although every study is included in the endpoint-specific study quality evaluation heat maps for comprehensiveness, six developmental epidemiological studies identified in the literature search were excluded from this synthesis due to study population overlap with other included studies (i.e., were considered duplicative). The Li et al. (2017c) Guangzhou Birth Cohort Study overlaps with a more recent study by Chu et al. (2020). Four other studies (Kobayashi et al., 2022; Kobayashi et al., 2017; Minatoya et al., 2017; Kishi et al., 2015) were also not considered in this synthesis, because they provided overlapping data from the same Hokkaido Study on Environment and Children's Health birth cohort as Kashino et al. (2020). For those studies with the same endpoints analyzed across different

subsets from the same cohort, such as mean BWT, the analysis with the largest sample size was used in forest plots and tables (e.g., (Kashino et al., 2020) for the Hokkaido birth cohort study). Although the Kobayashi et al. (2017) study included a unique endpoint called ponderal index, this measure is more prone to measurement error and was not considered in any study given the wealth of other fetal growth restriction data. Similarly, the Costa et al., (2019) study that examined a less accurate in utero growth estimate was not considered in lieu of their more accurate birth outcomes measures reported in the same cohort (Manzano-Salgado et al., 2017a). One study by Bae et al. (Bae et al., 2015) was the only study to examine sex ratio and was not further considered here. In general, to best gauge consistency and magnitude of reported associations, EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Three additional studies with overlapping cohorts were all included in the synthesis, as they provided some unique data for different endpoints. For example, the Woods et al. (2017) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018) but the authors provided additional mean BWT data. The mean BWT results for singleton and twin births from Bell et al. (2018) are included in forest plots here, while the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019) are also included as they target different developmental endpoints. The Bjerregaard-Olesen et al. (2019) study from the Aarhus birth cohort also overlaps with Bach et al. (2016). The main effect results are comparable for head circumference and birth length in both studies despite a smaller sample size in the Aarhus birth cohort subset examined in Bjerregaard-Olesen et al. (2019). Given that additional sex-specific data are available in the Bjerregaard-Olesen et al. (2019) study, the synthesis for head circumference and birth length are based on this subset alone. Chen et al., (2021) reported an implausibly large effect estimate for head circumference. After correspondence with study authors, an error was identified, and the study was not considered for head circumference.

Following exclusion of the seven studies above, 72 developmental epidemiological studies were available for the synthesis. One study by Bae et al. (2015) was the only study to examine sex ratio and was not further considered here. Six additional studies (Gundacker et al., 2021; Jin et al., 2020; Maekawa et al., 2017; Alkhalawi et al., 2016; Lee et al., 2016; Lee et al., 2013) were considered *uninformative* due to critical deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies and are not further examined here. Thus, 66 studies were included across various developmental endpoints for further examination and synthesis. Forty-six of the 66 studies examined PFOA in relation to fetal growth restriction measured by the following fetal growth restriction endpoints: SGA, low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Twenty studies examined different measures of gestation duration, five examined fetal loss, four examined birth defects, and 13 examined post-natal growth.

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

3.4.4.1.4 Growth Restriction: Fetal Growth

3.4.4.1.4.1 Birth Weight

Of the 43 studies examining different BWT measures in relation to PFOA exposures, 37 examined mean birth weight differences. Fifteen studies examined standardized BWT measures (e.g., z-scores) with nine of these reporting results for mean and standardized BWT (Eick et al., 2020; Wikström et al., 2020; Wang et al., 2019; Workman et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016). Twenty-six of the 37 mean BWT were prospective birth cohort studies, and the remaining 11 were cross-sectional analyses defined here as if biomarker samples were collected at birth or post-partum (Yao et al., 2021; Gao et al., 2019; Wang et al., 2019; Xu et al., 2019a; Bell et al., 2018; Gyllenhammar et al., 2018; Shi et al., 2017; Callan et al., 2016; de Cock et al., 2016; Kwon et al., 2016; Wu et al., 2012).

Eight of the 37 studies with data on the overall population relied on umbilical cord measures (Wang et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016), and one collected blood samples in infants 3 weeks following delivery (Gyllenhammar et al., 2018). Results from the Bell et al. (2018) study were based on infant whole blood taken from a heel stick and captured onto filter paper cards at 24 hours or more following delivery, and one study used both maternal serum samples collected 1–2 days before delivery and cord blood samples collected immediately after delivery (Gao et al., 2019). One of the prospective birth cohort studies examined pre-conception maternal serum samples (Robledo et al., 2015). Twenty-four studies had maternal exposure measures that were sampled during trimesters one (Sagiv et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), two (Buck Louis et al., 2018; Lauritzen et al., 2017), three (Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Kashino et al., 2020; Valvi et al., 2017; Callan et al., 2016; Wang et al., 2016; Wu et al., 2012), or across multiple trimesters (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Marks et al., 2019; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016). The study by Meng et al. (2018) pooled exposure data from two study populations, one which measured PFOA in umbilical cord blood and one which measured PFOA in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean BWT, only one biomarker measure was used (e.g., preferably maternal samples when collected in conjunction with umbilical cord samples or maternal only when more than the parent provided samples). In addition, other related publications (e.g., Gyllenhammar et al. (2017)) or additional information or data provided by study authors were used.

Sixteen of the 37 studies reporting mean BWT changes in relation to PFOA in the overall population were rated *high* in overall study confidence (Luo et al., 2021; Chu et al., 2020; Eick et al., 2020; Wikström et al., 2020; Bell et al., 2018; Buck Louis et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016; Govarts et al., 2016; Wang et al., 2016), while 13 were rated *medium* (Chang et al., 2022; Chen et al., 2021; Yao et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Woods et al., 2017; de Cock et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015), and eight were classified as *low* (Gao et al., 2019; Marks et al.,

2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016; Wu et al., 2012) as shown in Figure 3-47, Figure 3-48, and Figure 3-49.

Of the 29 *high* or *medium* confidence studies highlighted in this synthesis, two had deficient study sensitivity (Bell et al., 2018; de Cock et al., 2016). Nine studies (Chen et al., 2021; Yao et al., 2021; Wikström et al., 2020; Lauritzen et al., 2017; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016; Wang et al., 2016; Robledo et al., 2015) were considered to have good study sensitivity, and 18 studies (Chang et al., 2022; Luo et al., 2021; Chu et al., 2020; Eick et al., 2020; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Bach et al., 2016; Govarts et al., 2016; Kwon et al., 2016) were considered adequate. The median exposure values across all studies ranged from 0.86 ng/mL (Callan et al., 2016) to 42.8 ng/mL (Yao et al., 2021).

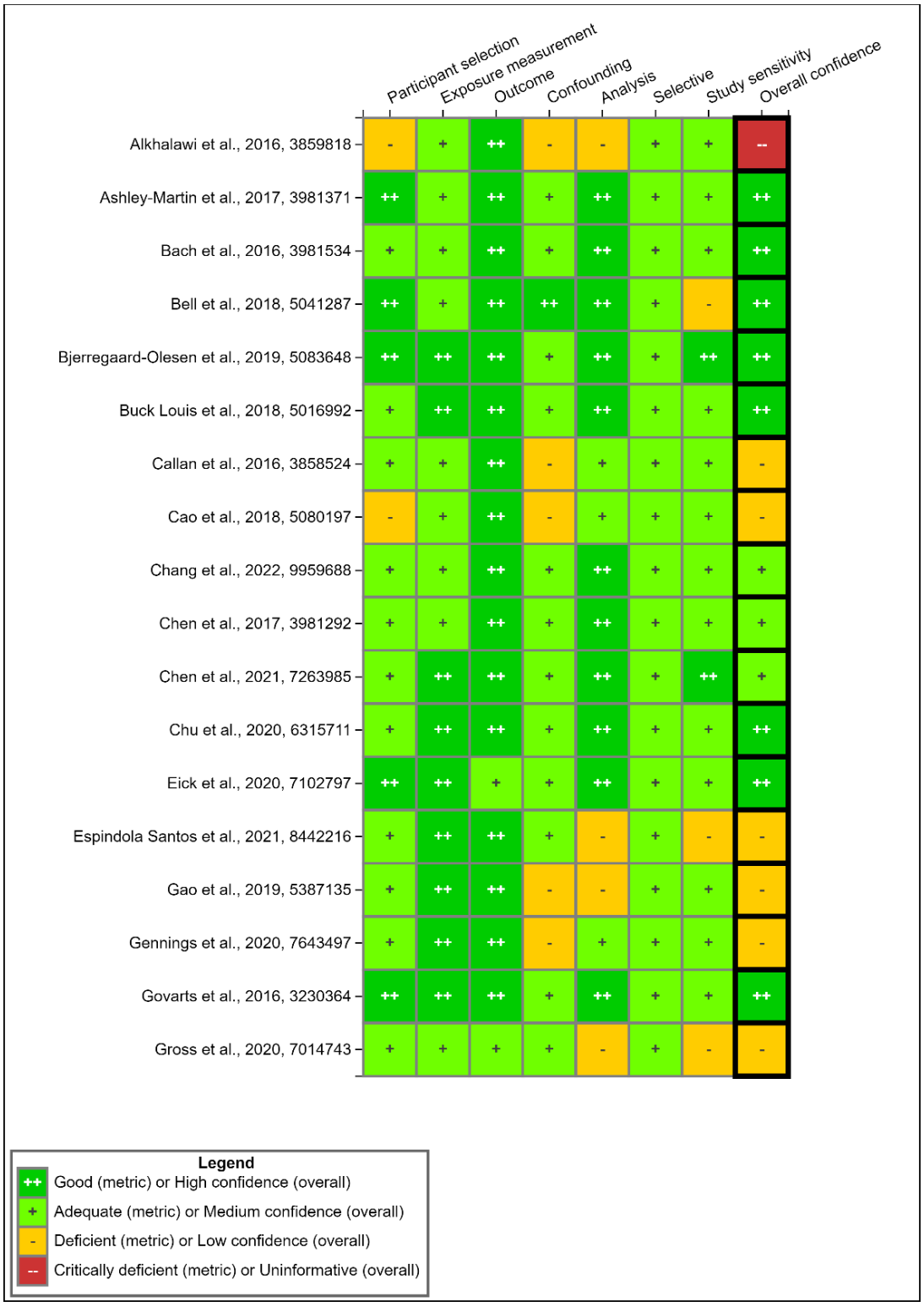


Figure 3-47. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Weight Effects

Interactive figure and additional study details available on HAWC.

Figure 3-48. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Birth Weight Effects (Continued)

Interactive figure and additional study details available on HAWC.

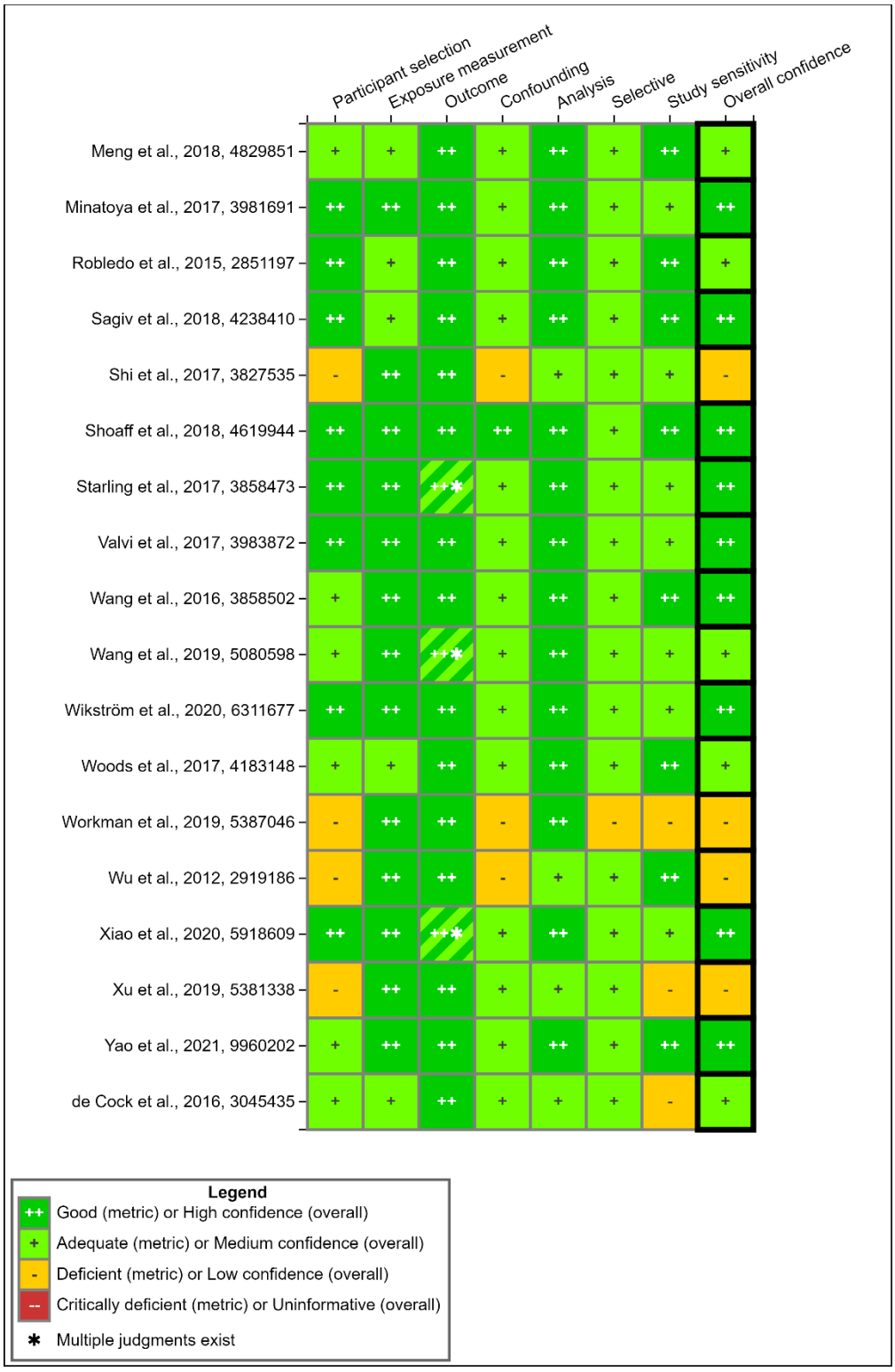


Figure 3-49. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Birth Weight Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.1.1 Mean Birth Weight Study Results: Overall Population Studies

Thirty-two of the 37 included studies with mean BWT data that examined data in the overall population (Chang et al., 2022; Chen et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Kashino et al., 2020; Wikström et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Xu et al., 2019a; Bell et al., 2018; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Shi et al., 2017; Starling et al., 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Callan et al., 2016; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Wang et al., 2016; Robledo et al., 2015; Wu et al., 2012), while five reported sex-specific data only (Marks et al., 2019; Ashley-Martin et al., 2017; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015). Twenty-one of the 32 PFOA studies reported some mean BWT deficits in the overall population, albeit these were not always statistically significant (see Appendix, (U.S. EPA, 2024a)). Five of these mean BWT studies in the overall population reported null associations (Bell et al., 2018; Buck Louis et al., 2018; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016), while six reported increased mean BWT deficits with increasing PFOA exposures (Chen et al., 2021; Eick et al., 2020; Gao et al., 2019; Xu et al., 2019a; Shi et al., 2017; de Cock et al., 2016). Seventeen of the 25 *medium* and *high* confidence studies reported some BWT deficits in relation to PFOA exposures. Among the 10 studies presenting results based on categorical data, two studies (Meng et al., 2018; Starling et al., 2017) showed inverse monotonic exposure-response relationships (Figure 3-50, Figure 3-51, Figure 3-52, and Figure 3-53).

Among the 21 studies showing some inverse associations in the overall population, there was a wide distribution of deficits ranging from –14 to –267 grams across both categorical and continuous exposure estimates with results based on a per unit (continuous measure) when studies presented both. Among those with continuous PFOA results in the overall population, 14 of 20 studies reported deficits from –27 to –82 grams with increasing PFOA exposures. There were no clear patterns were observed by confidence level, but there was a preponderance of inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) including 15 of the overall 21 studies and 6 of the 9 *high* confidence studies. The two largest associations (one *medium* and one *low* confidence study) expressed per each PFOA change were detected in studies with later pregnancy samples, while three of the four smallest associations were based on earlier biomarker samples. Thus, some of these reported results may be related to pregnancy hemodynamic influences on the PFOA biomarkers during pregnancy. For example, 11 of the 12 largest mean BWT deficits (–48 grams or larger per unit change) in the overall population were detected among studies with either later pregnancy samples (i.e., maternal samples during trimesters 2, 3, or post-partum or umbilical cord samples). However, five (Chang et al., 2022; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018) of nine *medium* and *high* confidence studies still reported some evidence of reductions in mean BWT based on early pregnancy biomarker samples.

Sampling Period	Reference, Confidence Rating	Study design	Exposure Matrix	Sub-population	Exposure levels	Comparison	EE
Early pregnancy	Bach et al. (2016, 3981534), High	Cohort	maternal serum	Term births (GA >= 37 weeks)	median=2.0 ng/mL (25th-75th percentile: 1.5-2.6 ng/mL)	Regression coefficient for Q2 (1.54-2.02 ng/mL) vs. Q1 (<1.54 ng/mL)	-36
						Regression coefficient for Q3 (2.03-2.64 ng/mL) vs. Q1 (<1.54 ng/mL)	-7
						Regression coefficient for Q4 (2.65-15.10 ng/mL) vs. Q1 (<1.54 ng/mL)	4
						Regression coefficient per IQR (1.1 ng/mL) increase	13
	Buck Louis et al. (2018, 5016992), High	Cohort	maternal blood	--	Median (25th-75th percentiles): 1.985 ng/mL (1.297-3.001 ng/mL)	Regression coefficient (change in birth weight per SD increase in log-PFOA)	-5.9
Later pregnancy	Bell et al. (2018, 5041287), High	Cross-sectional	blood	Singleton	median=1.10 ng/mL (25th-75th percentile: 0.69-1.63 ng/mL)	Regression coefficient (per log(PFOA+1) unit increase)	-11.6
	Chu et al. (2020, 6315711), High	Cohort	maternal serum	--	median=1.538 ng/mL (25th percentile=0.957 ng/mL, 75th percentile=2.635 ng/mL)	Regression coefficient (per 1 ln change in PFOA)	-73.6
	Eick et al. (2020, 7102797), High	Cohort	serum	full-term births	median= 0.76 ng/mL (25th-75th percentile= 0.46 - 1.12 ng/mL)	Regression Coefficient [for T2 (1.40-1.96 ng/ml) vs. T1 (<1.40 ng/ml)]	62.9
						Regression Coefficient [for T3 (>1.96 ng/ml) vs. T1 (<1.40 ng/ml)]	86.1
	Govarts et al. (2016, 3230364), High	Cohort	cord blood	--	geometric mean = 1.52 ug/L (25th-75th percentile = 1.10-2.10 ug/L)	Regression coefficient (per IQR change in PFOA z-score)	-34.5

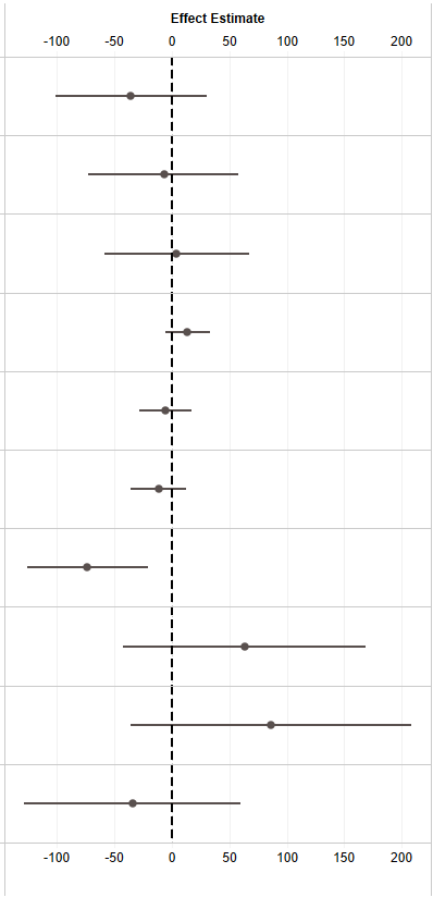


Figure 3-50. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

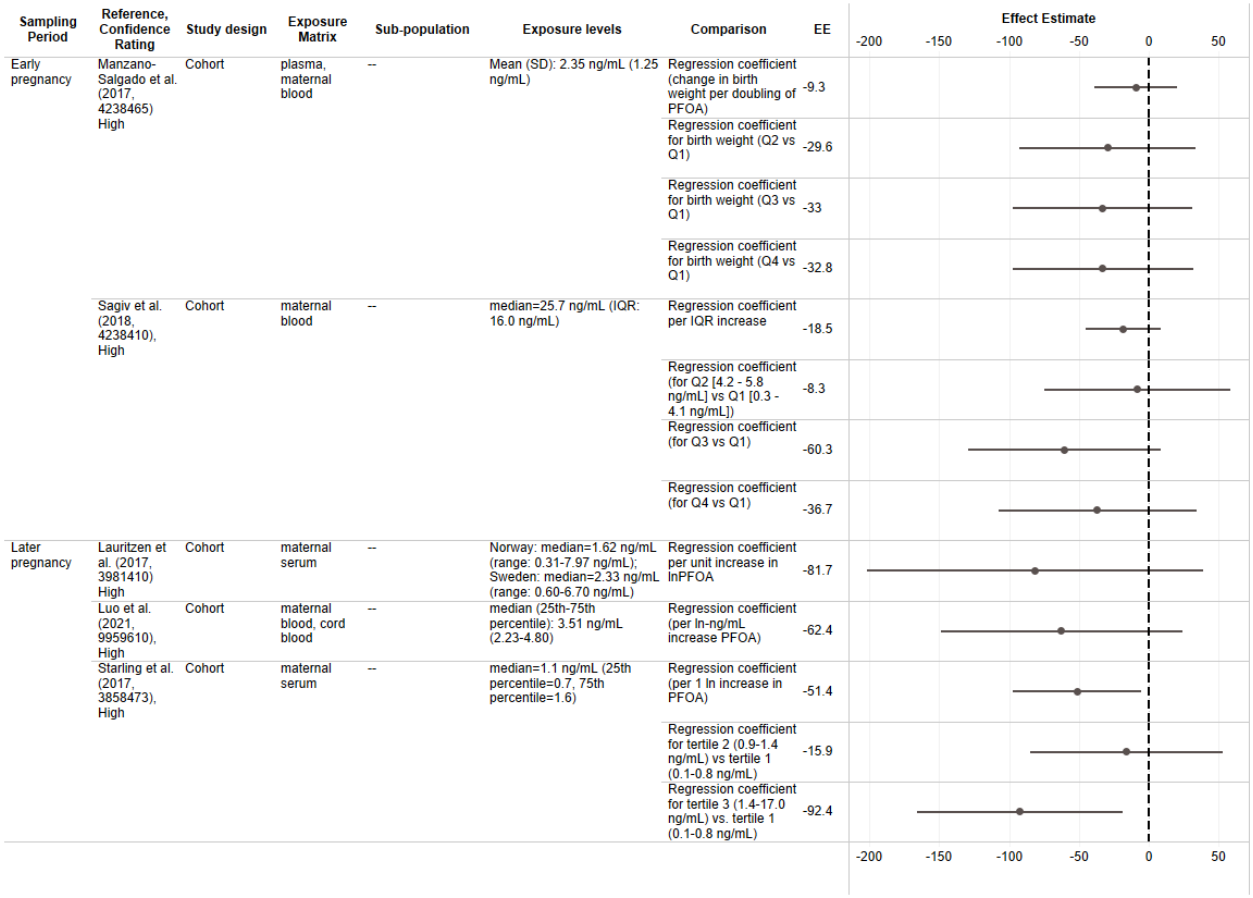


Figure 3-51. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

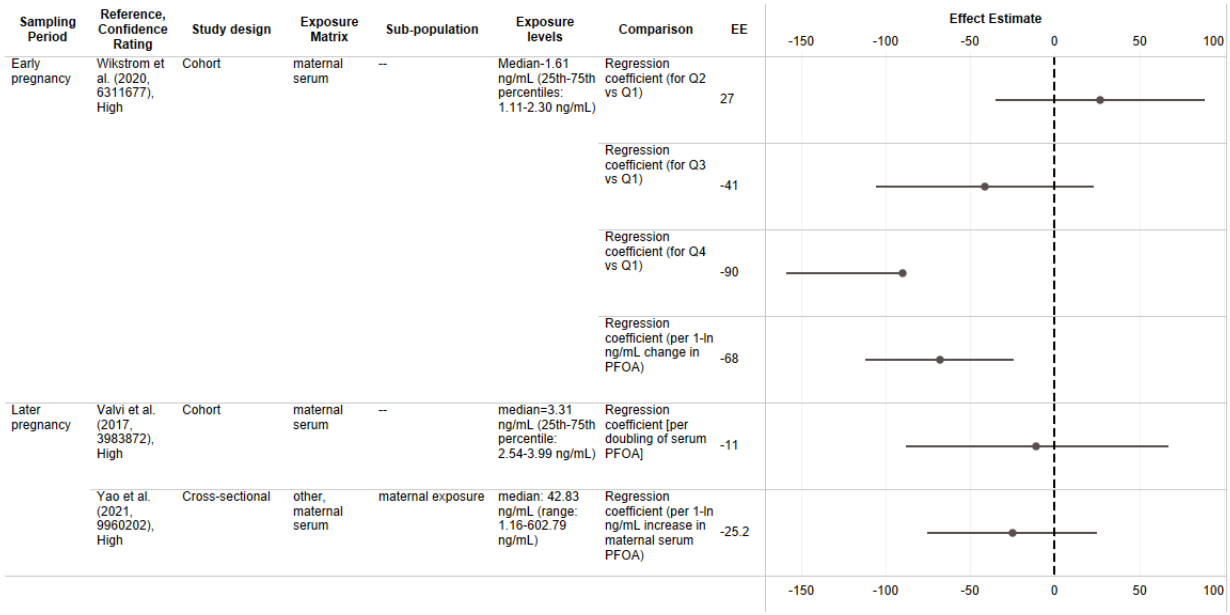


Figure 3-52. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.
Wikström et al. (2020) has a manuscript error in the regression coefficient for Q4 vs. Q1.

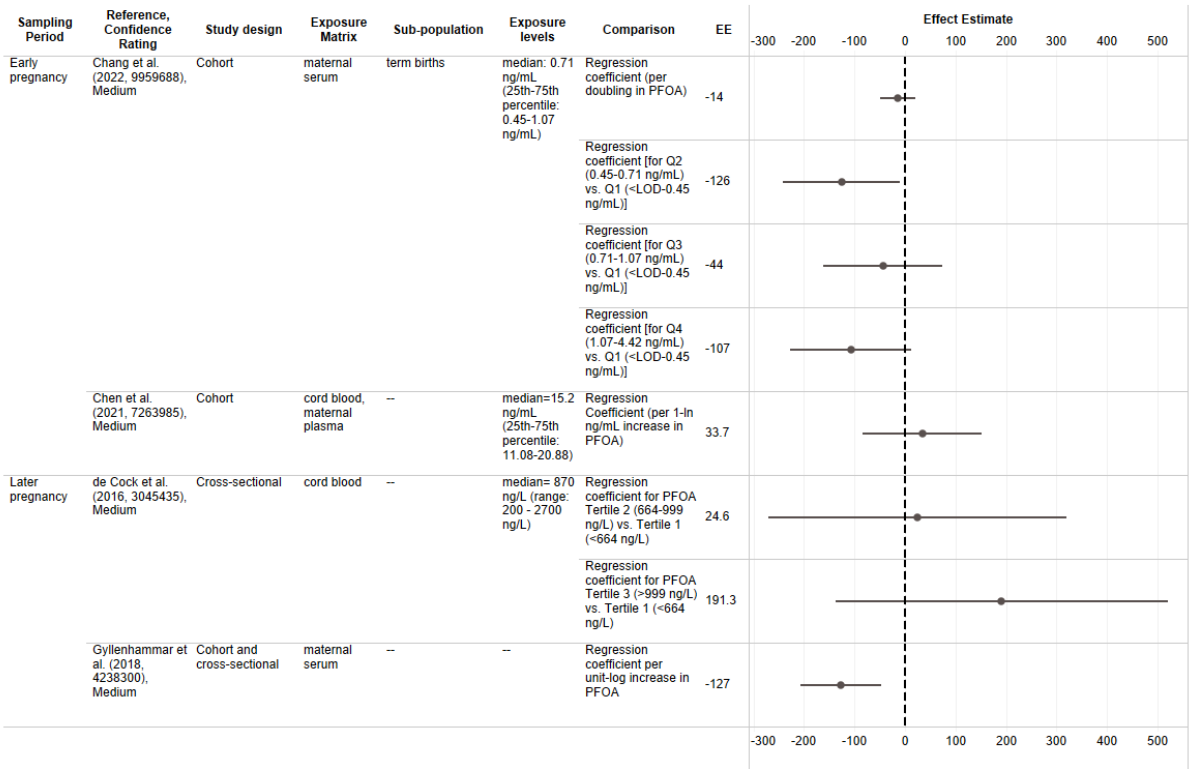


Figure 3-53. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.1.2 Mean BWT-Overall Population Summary

Overall, 21 of the 32 PFOA studies reported some mean BWT deficits in the overall population with limited evidence of exposure-response relationships. Seventeen of the 21 studies were *medium* or *high* confidence (out of 25 in total), but the majority of studies that showed inverse associations were based on later biomarker sampling timing (i.e., trimester two onward). While some of the changes were relatively large in magnitude (most were from –27 to –82 grams per each unit PFOA change), there was also a pattern of stronger associations detected amongst studies with later pregnancy biomarker samples. These patterns may be indicative of pregnancy hemodynamic influences on the PFOA biomarkers during pregnancy.

3.4.4.1.4.1.3 Mean Birth Weight Study Results: Sex-Specific Studies

Mean BWT findings were reported for 18 and 19 studies in female and male neonates, respectively. Eleven of 18 epidemiological studies examining sex-specific results in female neonates showed some BWT deficits including 10 of 16 *medium* and *high* confidence studies. Twelve of 19 *medium* and *high* confidence epidemiological studies examining sex-specific results in male neonates showed some BWT deficits. The remaining 7 studies (Hjermitslev et al., 2019; Wang et al., 2019; Lind et al., 2017a; Shi et al., 2017; Bach et al., 2016; de Cock et al., 2016; Robledo et al., 2015) in male neonates were either null or showed larger birth weights with increasing PFOA exposures. The *low* confidence study by Marks et al. (2019) of boys only reported large deficits in the upper two PFOA tertiles (−53 and −46 grams, respectively) with no exposure-response relationship. None of the other five studies with categorical data in either girls or boys showed evidence of monotonic exposure-response relationships.

Nine of the 18 studies examining mean BWT associations in both boys and girls detected some deficits in both sexes with one of these reporting comparable BWT deficits (Lenters et al., 2016). Five of the 9 studies showed larger deficits in girls (Wikström et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Cao et al., 2018; Ashley-Martin et al., 2017) and 3 showed larger deficits among boys (Chu et al., 2020; Meng et al., 2018; Lauritzen et al., 2017). One study showed comparable results irrespective of sex (Lenters et al., 2016). Three additional studies each reported mean BWT deficits either only in boys (Kashino et al., 2020; Manzano-Salgado et al., 2017a; Valvi et al., 2017) or girls (Hjermitslev et al., 2019; Wang et al., 2016; Robledo et al., 2015).

Overall, no consistent patterns in magnitude of deficits were observed with the sex-specific studies by sample timing and other study characteristics; however, the three largest deficits in male studies were later pregnancy sampled studies. Although other studies based on different exposure measures were more variable, some consistency in the magnitude of deficits (range: −80 to −90 g) was observed among four studies in girls (Wikström et al., 2020; Wang et al., 2019; Ashley-Martin et al., 2017; Wang et al., 2016) including three *high* confidence studies based on analyses of continuous PFOA measurements (i.e., per each ln or log10 PFOA exposures increase). The magnitude of deficits in boys across 7 studies (Kashino et al., 2020; Wikström et al., 2020; Wang et al., 2019; Meng et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Lenters et al., 2016) was fairly consistent per each continuous unit PFOA change (range: −21 to −49 g), although 3 studies (Chu et al., 2020; Lauritzen et al., 2017; Valvi et al., 2017) reported larger deficits in excess of –71 grams.

3.4.4.1.4.1.4 Standardized Birth Weight Measures

Fifteen studies examined standardized BWT measures including 14 studies reporting changes in standardized BWT scores on a continuous scale per each PFOA comparison. Eight of the 15 were *high* confidence studies (Gardener et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016), 4 were *medium* (Wang et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Chen et al., 2017b) and 3 were *low* confidence (Espindola-Santos et al., 2021; Gross et al., 2020; Workman et al., 2019).

Eight out of 15 studies with standardized BWT scores in the overall population showed some inverse associations and 5 of these were *high* confidence. The *high* confidence study by Gardener et al. (2021) reported that participants in PFOA quartiles 2 (OR = 0.84; 95% CI: 0.40–1.80) and 3 (OR = 0.91; 95% CI: 0.41–2.02) had a lower odds of being in the lowest standardized birth weight category (vs. the top 3 birth weight z-score quartiles). They also reported that there were no statistically significant interactions for their BWT z-score measures by sex.

Among the 14 studies examining continuous standardized BWT measures in the overall population, 8 showed some inverse associations of at least –0.1. The ranges of deficits were –0.1 (Wang et al., 2019; Sagiv et al., 2018; Ashley-Martin et al., 2017), –0.2 (Wikström et al., 2020; Shoaff et al., 2018; Chen et al., 2017b), and –0.3 (Gross et al., 2020; Xiao et al., 2019). More associations were detected among the *high* confidence studies (5/8), compared with 2 of the 4 *medium*, and 1 of the 3 *low* confidence studies. None of the 5 studies (Eick et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016) showed any evidence of exposure-response relationships. Overall, four out of six studies in boys (Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Chen et al., 2017b) and 3 of 5 in girls (Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019) showed lower BWT z-scores with increasing PFOA exposures. For example, the *low* confidence study by Gross et al. (2020) reported BWT z-score deficits in both sexes (males β: –0.17; SE = 0.29; p-value = 0.57; females β: –0.38; SE = 0.26; p-value = 0.16) for PFOA levels greater than the mean level. Gardener et al. (2021) only reported that there were no statistically significant interactions for standardized BWT measures by sex in their analysis.

3.4.4.1.4.1.5 Standardized BWT summary

Eight out of 15 studies with standardized BWT scores in the overall population showed some inverse associations with PFOA exposures. Seven of these 8 studies were either *medium* or *high* confidence studies (of 17 in total), and most of these had moderate or large exposure contrasts. Although some studies may have been underpowered to detect associations small in magnitude relative to PFOA exposure, there was consistent lower BWT z-scores reported across all confidence levels. There was no apparent pattern related to magnitude of deficits across study confidence, but more associations were evident across *high* confidence levels in general. Many studies (5 of 8) showing inverse associations were based on later(Gross et al., 2020; Wang et al., 2019; Xiao et al., 2019; Shoaff et al., 2018; Chen et al., 2017b) versus early (i.e., at least some trimester one maternal samples) pregnancy sampling (3 of 9); this might be reflective of some impact of pregnancy hemodynamics on biomarker concentrations over time. There was no evidence of exposure-response relationships in the 5 studies reporting categorical data. There were also few evident patterns and minimal differences seen across sexes. Overall, 9 out of 15

overall studies in the overall population showed some suggestion of inverse associations with the same studies showing associations in 4 out of 5 studies of male neonates and 3 of 5 studies in females.

3.4.4.1.4.2 Small for Gestational Age/Low Birth Weight

Eleven informative and two *uninformative* non-overlapping epidemiological studies examined associations between PFOA exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), low birth weight (LBW), or both (i.e., (Manzano-Salgado et al., 2017a)) (Figure 3-54). Five studies were rated *high* confidence (Chu et al., 2020; Wikström et al., 2020; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016), three were rated *medium* confidence (Govarts et al., 2018 Hjermitslev, 2020, 5880849; Meng et al., 2018), three were low confidence studies (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a) and two were *uninformative* (Gundacker et al., 2021; Arbuckle et al., 2013). Of the informative studies, four studies had good study sensitivity (Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016), four were considered adequate (Chang et al., 2022; Chu et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019) and three were deficient (Souza et al., 2020; Xu et al., 2019a; Govarts et al., 2018).

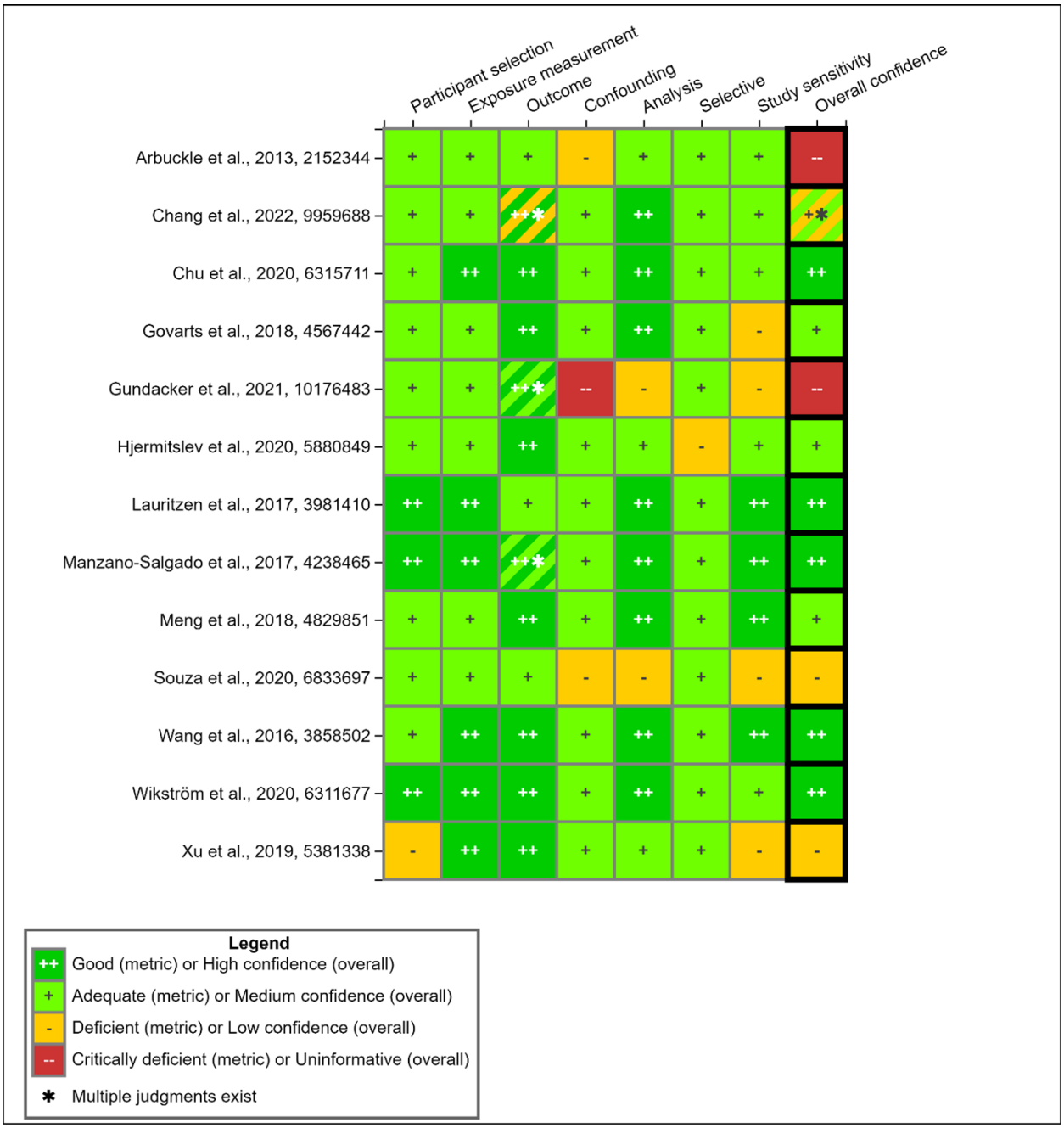


Figure 3-54. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Small for Gestational Age and Low Birth Weight Effects[a]

Interactive figure and additional study details available on HAWC.
[a] Manzano-Salgado et al. (2017a): *High* confidence for SGA; *medium* confidence for LBW.

Six of eight SGA studies (Chang et al., 2022; Souza et al., 2020; Wikström et al., 2020; Govarts et al., 2018; Lauritzen et al., 2017; Wang et al., 2016) showed some increased risk, while two studies were entirely null (Xu et al., 2019a; Manzano-Salgado et al., 2017a) (Figure 3-55, Figure 3-56, Figure 3-57). Although they were not always statistically significant, the relative risks reported in the five studies examining the overall population based on either categorical or

continuous exposures (per each unit increase) were fairly consistent in magnitude (odds ratio (OR) range: 1.21 to 2.81). The *medium* confidence study by Govarts et al. (2018) reported an increased risk (OR = 1.64; 95% CI: 0.97, 2.76) per each PFOA IQR increase. The *high* confidence study by Lauritzen et al. (2017) showed a slight increased risk in the overall population (OR = 1.21; 95% CI: 0.69, 2.11 per each ln-unit PFOA increase), but this was driven by associations only in participants from Sweden (OR = 5.25; 95% CI: 1.68, 16.4) including large risks detected for both girls and boys. One (Souza et al., 2020) of the three studies examining exposure quartiles detected an exposure-response relationship in the overall population (OR range: 1.26–2.81). The *medium* confidence study by Chang et al. (2022) reported nonmonotonic but consistent statistically significant ORs across the upper three quartiles (range: 2.22–2.44) in their study of African American pregnant women. The *high* confidence study by Wikström et al. (2020) reported comparable ORs for the 4th quartile (OR = 1.44; 95% CI: 0.86, 2.40) as well as per each per ln-unit increase (OR = 1.43; 95% CI: 1.03, 1.99). Among females only, they reported a twofold increased risk per each ln-unit increase risk (OR = 1.96; 95% CI: 1.18, 3.28) and nonmonotonic increased risks in the upper two quartiles (OR range: 1.64–2.33). The *high* confidence study by Wang et al. (2016) only reported sex-specific results but also showed an increased risk (OR = 1.48; 95% CI: 0.63, 3.48 per each ln-unit increase) for SGA among girls only. SGA findings from *low* confidence studies are not included in figures.

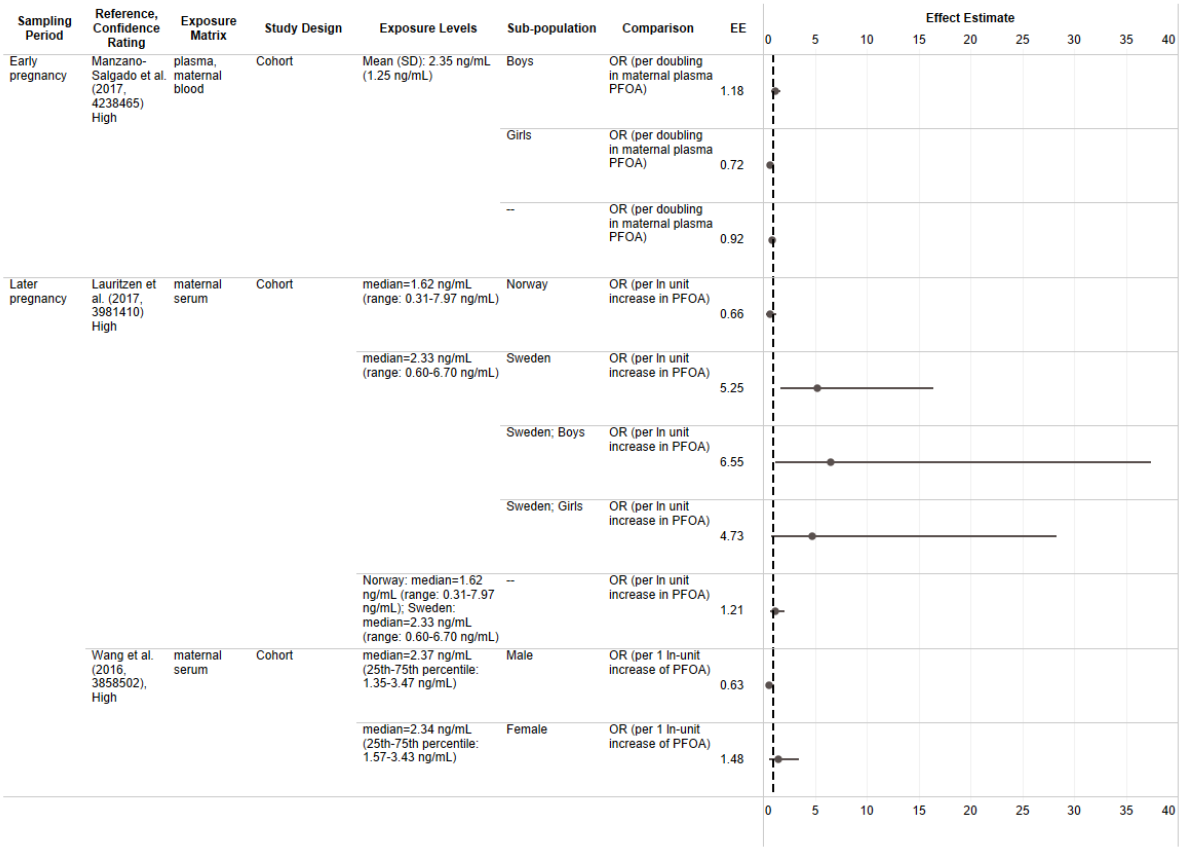


Figure 3-55. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.

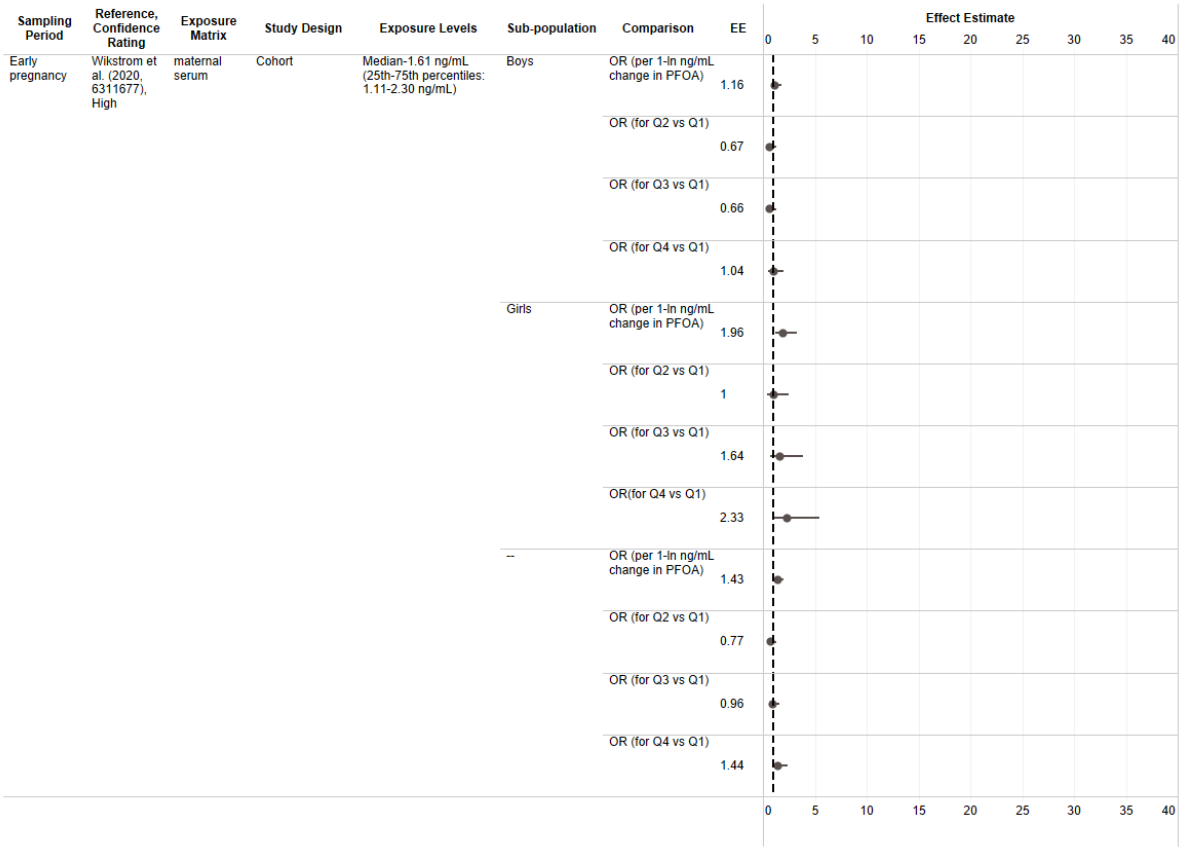


Figure 3-56. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.
Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.

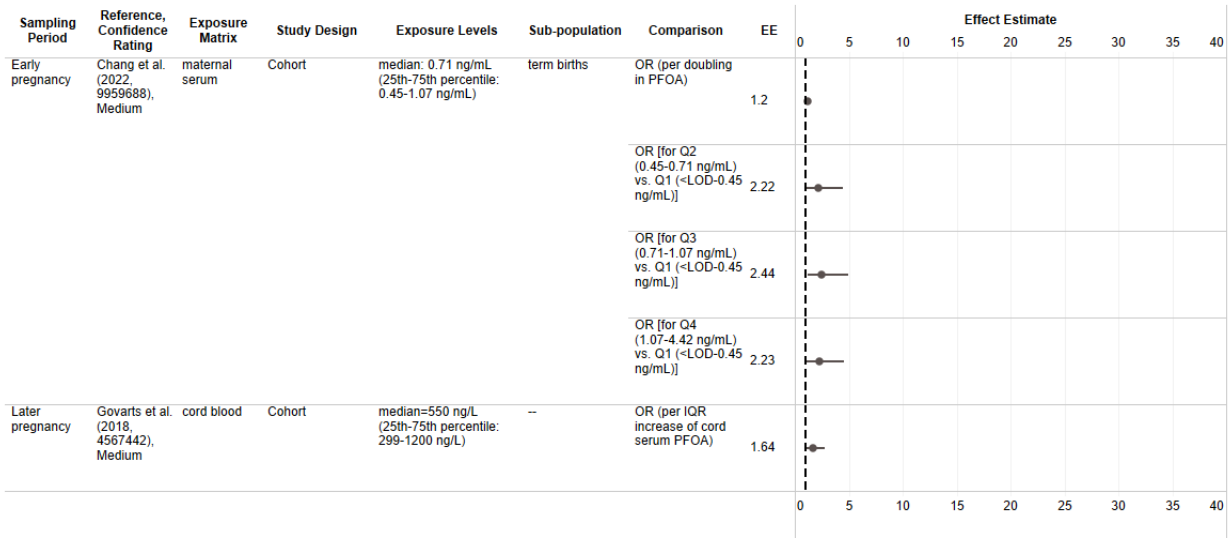


Figure 3-57. Odds of Small for Gestational Age in Children from Medium Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Odds of Small-for-gestational-age in Children from *Medium* Confidence Epidemiology Studies Following Exposure to PFOA

Four studies examined LBW in relation to PFOA including two each that were rated *high* (Chu et al., 2020; Manzano-Salgado et al., 2017a) or *medium* confidence (Hjermitslev et al., 2019; Meng et al., 2018) confidence. Two of four LBW studies (Meng et al., 2018; Manzano-Salgado et al., 2017a) showed some associations within the overall population, and/or in boys or girls (Figure 3-58). The *medium* confidence study by Meng et al. (2018) reported nonsignificant increased ORs (range: 1.2–1.5) across all quartiles but saw no evidence of an exposure-response relationship. The *high* confidence Manzano-Salgado (Manzano-Salgado et al., 2017a) study showed some suggestion of an increased risk (OR = 1.67; 95% CI: 0.72, 3.86) for term LBW in boys only.

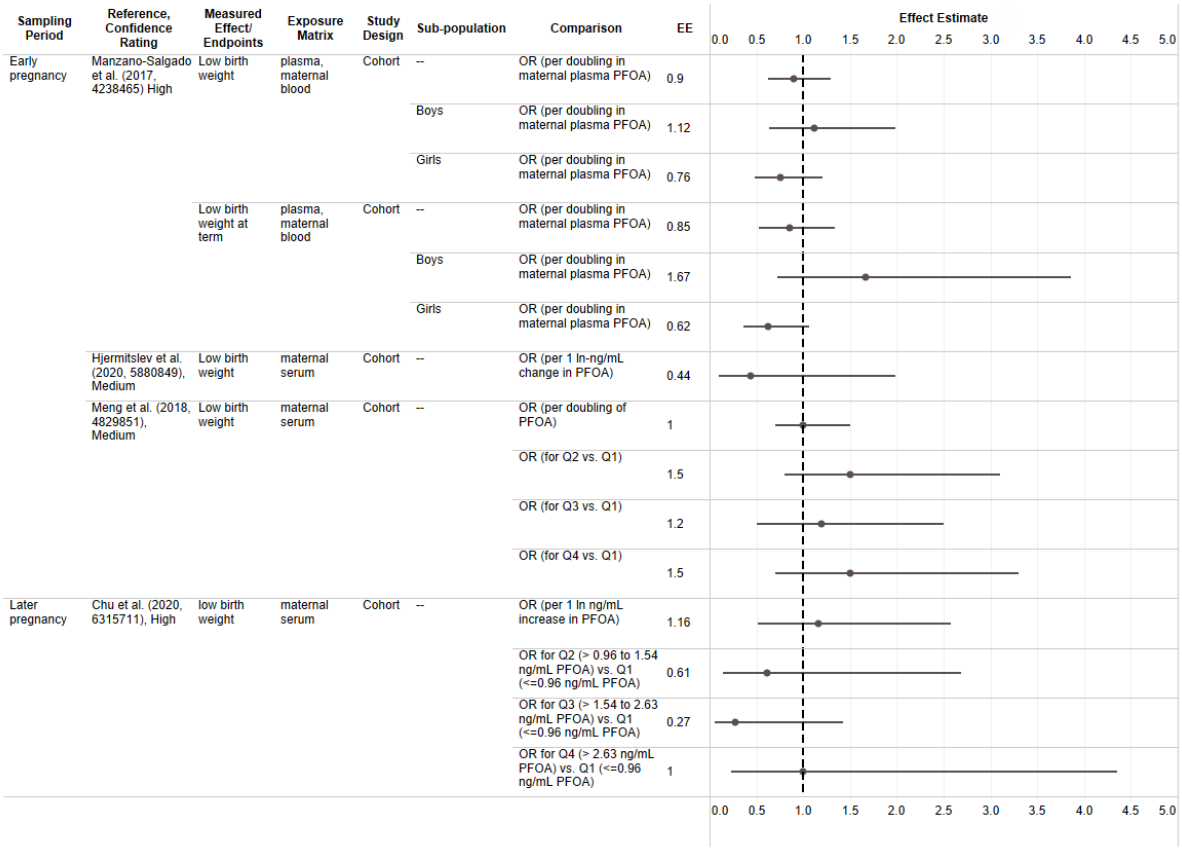


Figure 3-58. Odds of Low Birthweight in Children from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Low birthweight defined as birthweight <2,500 g.

Overall, eight of the 11 informative studies reporting main effects for either SGA or LBW or both showed some increased risks with increasing PFOA exposures. The magnitude of the associations was typically from 1.2 to 2.8 with limited evidence of exposure-response relationships among the studies with categorical data. Although the number of studies was fairly small, few discernible patterns across study characteristics or confidence ratings were evident across the SGA or LBW findings. For example, four (Chang et al., 2022; Wikström et al., 2020; Meng et al., 2018; Manzano-Salgado et al., 2017a) of the eight studies showing increased odds of either SGA or LBW were based on early sampling biomarkers, suggesting the results were not overly influenced by pregnancy hemodynamics. Collectively, the majority (8 of 11) of epidemiological studies were supportive of an increased risk of either SGA or LBW with increasing PFOA exposures.

3.4.4.1.4.3 Birth Length

As shown in Figure 3-59 and Figure 3-60, 34 birth length studies were considered as part of the study evaluation. Four studies were considered *uninformative* (Gundacker et al., 2021; Jin et al., 2020; Alkhalawi et al., 2016; Lee et al., 2013) and four more studies noted above (Kobayashi et al., 2022; Bach et al., 2016; Kishi et al., 2015 Kobayashi, 2017, 3981430) were not further

considered for multiple publications from the same cohort studies. Among the 26 non-overlapping informative studies examined birth length in relation to PFOA, including five studies with standardized birth length measures (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018; Shoaff et al., 2018; Chen et al., 2017b), and one study evaluated standardized and mean birth length changes (Workman et al., 2019). Eighteen studies examined mean birth length differences in the overall study population. 13 studies examined sex-specific data with three studies (Marks et al., 2019; Wang et al., 2016; Robledo et al., 2015) reporting only sex-specific results.

Nine of the 26 studies were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Buck Louis et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016), eight were *medium* (Chen et al., 2021; Luo et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; Chen et al., 2017b; Robledo et al., 2015) and nine were *low* confidence (Espindola-Santos et al., 2021; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016; Wu et al., 2012). Eight PFOA studies had good study sensitivity (Chen et al., 2021; Bjerregaard-Olesen et al., 2019; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016; Robledo et al., 2015; Wu et al., 2012), 14 had adequate (Luo et al., 2021; Kashino et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Wang et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; Chen et al., 2017b; Shi et al., 2017; Valvi et al., 2017; Callan et al., 2016) sensitivity and four (Espindola-Santos et al., 2021; Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018) considered deficient.

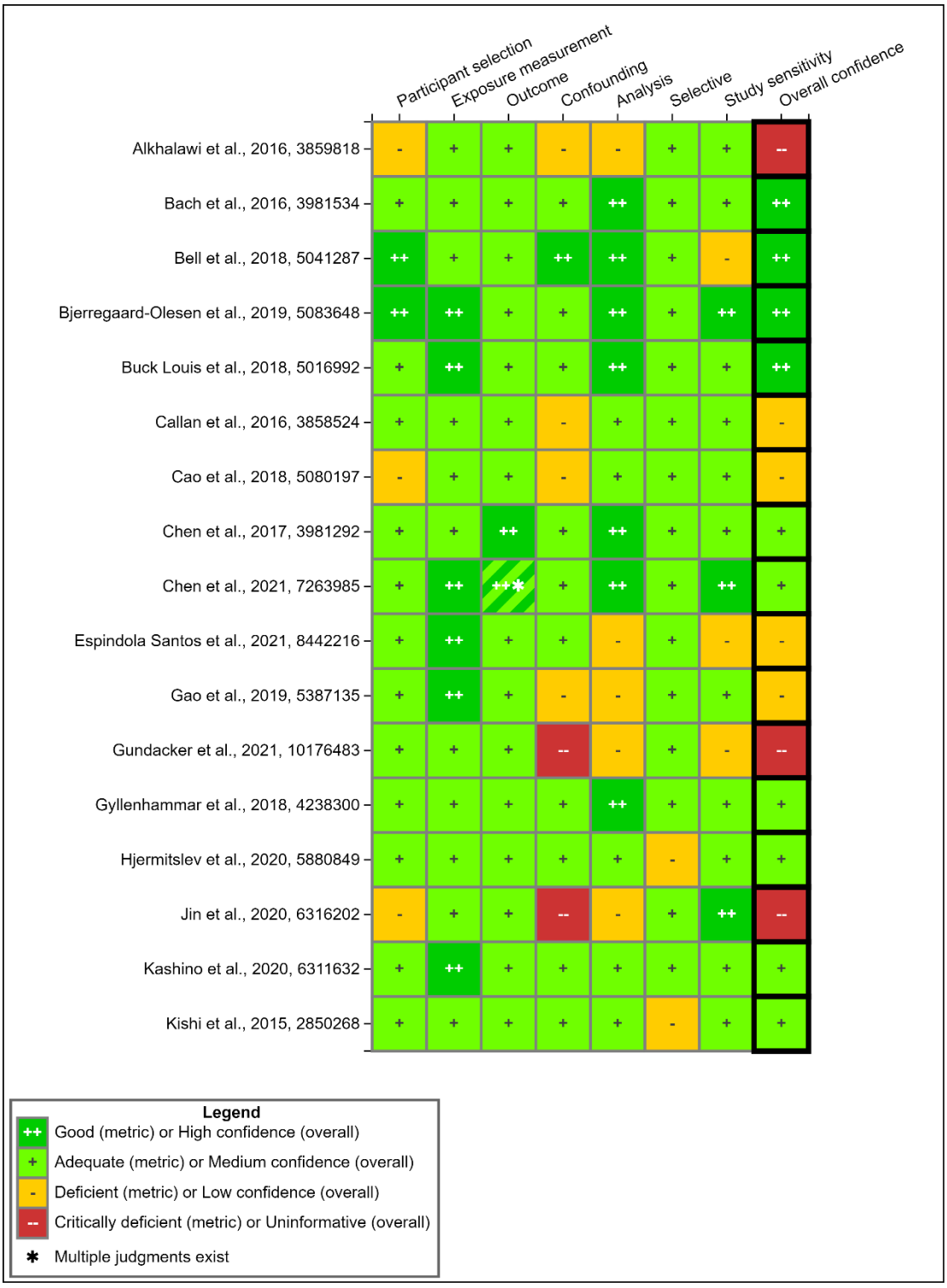


Figure 3-59. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Length Effects

Interactive figure and additional study details available on HAWC.

Figure 3-60. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Length Effects (Continued)

Interactive figure and additional study details available on HAWC.

Amongst the 26 birth length studies (examining mean differences or changes in standardized scores), nine of them reported some inverse associations including three of the six studies that reported standardized birth length data. There was limited evidence of exposure-response relationships in the three studies that examined categorical data. The *high* confidence study by Xiao et al. (2019) reported a reduced birth length z-score (β per log2 increase in PFOA: −0.14; 95% CI: −0.40, 0.13) in the overall population that appeared to be driven by male neonates (β: −0.27; 95% CI: −0.65, 0.10). The *low* confidence Workman et al. (Workman et al., 2019) study reported a nonsignificant deficit similar in magnitude (β: –0.26; 95% CI: −1.13, 0.61). The other study *high* confidence study by Shoaff et al. (2018) of standardized birth length measures showed a deficit only for tertile 3 (β: −0.32; 95% CI: −0.72, 0.07) compared with tertile 1. In contrast, the *low* confidence study by Espindola-Santos et al. (2021) reported a larger birth length z-score (β per $\log_{10}$ PFOA increase: 0.26; 95% CI: -0.21, 0.73).

Among the 21 studies examining mean birth length differences, eight different studies showed inverse associations. This included six different studies (out of 18) based on the overall population as well two out of three studies (Wang et al., 2016; Robledo et al., 2015) reporting only sex-specific results. The *high* confidence study by Wang et al. (2016) only showed deficits among females for only PFOA quartiles 1 (β: −0.39 cm; 95% CI: −1.80, 1.02) and 3 (β: −0.60 cm; 95% CI: −1.98, 0.77). The *medium* confidence study by Chen et al. (Chen et al., 2021) reported similar birth length deficits in the overall population (β per ln-unit PFOA increase: –0.27 cm; 95% CI: –0.61, 0.07), males (β: –0.21; 95% CI: –0.73, 0.32) and females (β: –0.21; 95% CI: –0.74, 0.33). In the *medium* confidence study by Robledo et al. (2015), smaller deficits in birth length were detected for both male and female neonates per each 1 standard deviation (SD) PFOA increase. The *high* confidence study by Lauritzen et al. (2017) showed a deficit in the overall population (β: −0.49 cm; 95% CI: −0.99, 0.02), but detected the strongest association when restricted to the Swedish population (β: −1.2 cm; 95% CI: −2.1, −0.3) and especially Swedish boys (β: −1.6 cm; 95% CI: −2.9, −0.4). Overall, four sex-specific studies showed deficits for both boys and girls with two studies showing larger deficits among boys. One study showed larger deficits amongst girls and the fourth study showed results equal in magnitude.

In the overall population studies showing inverse associations, the reported magnitude of deficits was quite variable (range: −0.16 to −1.91 cm). For example, the low confidence study by Wu et al. (2012) showed the largest deficit (β per log10 increase: −1.91 cm; 95% CI: −3.31, −0.52). The *low* confidence study by Cao et al. (2018) showed consistent results across their overall population (β: −0.45 cm; 95% CI: −0.79, −0.10 per each ln-unit PFOA increase), male (β: −0.36 cm; 95% CI: −0.80, 0.09), and female neonates (β: −0.58 cm; 95% CI: −1.12, −0.04) with evidence of exposure-response relationships in all three of these groups. Overall, 6 of 12 studies in girls and 4 of 13 studies in boys showed some birth length deficits. One of the three studies in either or both boys and girls showed some additional evidence of exposure-response relationships. The same study by Cao et al., (Cao et al., 2018) was the only study in the overall population to show evidence of exposure-response.

Overall, 9 different studies out of 26 studies examining birth length reported deficits in relation to PFOA exposures, including 6 *medium* or *high* confidence studies. There was no apparent relationship between studies showing inverse associations and study confidence ratings. However, seven of these studies sampled PFOA biomarkers later in pregnancy (Workman et al., 2019; Xiao et al., 2019; Cao et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Wang et al.,

2016; Wu et al., 2012) and may be more prone to potential bias from pregnancy hemodynamic changes. Among the mean birth length studies, most showed consistent deficits ranging from –0.21 to –0.49 cm per different PFOA comparisons. An unusually large result (β per log10 PFOA increase = –1.91 cm; 95% CI: –3.21, –0.52) was reported in an earlier study (Wu et al., 2012) that reported the largest exposure range. There was a preponderance of inverse associations among females (6 of 12 studies) compared with males (4 of 13); however, amongst the four studies that reported associations in both sexes, more studies reported larger deficits in male neonates.

3.4.4.1.4.4 Head Circumference at Birth

As shown in Figure 3-61, 21 informative studies examined head circumference at birth in relation to PFOA exposures. Six of the 21 studies were *low* confidence (Espindola-Santos et al., 2021; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Callan et al., 2016), while seven studies were *medium* (Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; Lind et al., 2017a; Robledo et al., 2015) and eight were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Buck Louis et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016). Four studies were deficient in study sensitivity (Espindola-Santos et al., 2021; Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018), while five were good (Chen et al., 2021; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016; Robledo et al., 2015) and 12 had adequate study sensitivity (Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Wang et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; Lind et al., 2017a; Valvi et al., 2017; Callan et al., 2016).

Figure 3-61. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Head Circumference Effects

Interactive figure and additional study details available on HAWC.

Eighteen of the 21 included studies reported PFOA in relation to mean head circumference differences including 17 studies that provided results based on the overall population. Including

the Xiao et al. (2019) z-score data, 13 of these 21 studies reported sex-specific head circumference data with four other studies (Marks et al., 2019; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015) providing sex-specific data only.

Among the 21 studies, 10 reported some inverse associations between PFOA exposures and different head circumference measures in the overall population, in either or both male and female neonates, across different racial strata, or different countries in the same study population. For example, the *high* confidence study by Lauritzen et al. (2017) reported a similar deficit only in their Swedish population (β per ln-unit PFOA increase: −0.4 cm; 95% CI: −1.0, 0.1) ; this was largely due to an association seen in male neonates (β: –0.6 cm; 95% CI: –1.3, 0.1). The *high* confidence study by Buck Louis et al. (2018), reported nonsignificant head circumference differences (β: −0.14 cm; 95% CI: −0.29, 0.02) among Black neonates but no main effect association in the overall population. Six out of 17 studies based on the overall population reported some inverse associations between PFOA exposures and either mean head circumference measures or standardized z-scores. The *high* confidence study by Xiao et al. (2019) reported a reduced head circumference z-score (β: −0.17; 95% CI: −0.48, 0.15) in the overall population per each log2 increase in PFOA that appeared to be driven by female neonates (β: −0.30; 95% CI: −0.74, 0.13) (data not shown on figures). Although it was not statistically significant, the *low* confidence study by Espindola-Santos et al. (2021) reported a larger head circumference z-score (β per log10 PFOA increase: 0.62; 95% CI: –0.06, 1.29). The *medium* confidence study by Gyllenhammar et al. (2018) was null based on their standardized head circumference measure.

Among the 14 studies that examined mean head circumference at birth in the overall population, four of them reported inverse associations. Nine studies were largely null, and one study showed larger mean head circumference in the overall population with increasing PFOA exposures. Of the 11 different studies examining sex-specific results associations were observed 5 of 10 in female neonates (Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Wang et al., 2019; Cao et al., 2018; Robledo et al., 2015) and three (Wang et al., 2019; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a) of 11 studies in male neonates. The *medium* confidence study by Wang et al. (2019) reported an association in the overall population (β: −0.37 cm; 95% CI: −0.70, -0.40) with larger deficits noted in female (β: −0.57 cm; 95% CI: −1.07, −0.08) than in male neonates (β: −0.35 cm; 95% CI: −0.79, −0.10). The *medium* confidence study by Hjermitslev et al. (2019) showed a significant reduction in head circumference for the term births in the overall population (β per ng/mL PFOA increase: −0.30 cm; 95% CI: −0.56, –0.04) which seemed to be driven by results in females (β: −0.25 cm; 95% CI: −0.65, 0.14). The *high* confidence study by Manzano-Salgado et al. (2017a) reported a nonsignificant decrease only in quartile 4 (β: −0.16 cm; 95% CI: −0.38, 0.06) compared with quartile 1 from the overall population and a deficit among male neonates only (β per log2 PFOA increase: −0.13 cm; 95% CI: −0.27, 0.0). In the *medium* confidence study by Robledo et al. (2015), opposite results were seen for male (0.18 cm; 95% CI: –0.25, 0.60) and female neonates (β per 1 SD PFOA increase: −0.18 cm; 95% CI: −0.59, 0.23). In their *low* confidence study, Cao et al. (2018) reported an overall null association, while divergent and large changes were seen for male (β per ln-unit PFOA increase: 0.72 cm; 95% CI: –0.51, 1.94) and female neonates (β: −1.46 cm; 95% CI:

−2.96, 0.05). The *low* confidence study by Callan et al. (2016) reported a −0.40 cm (95% CI: −0.96, 0.16) difference per each ln-unit PFOA change.

Among the 21 epidemiological studies examining PFOA and mean differences and standardized measures of head circumference, 10 different studies reported some evidence of inverse associations in the overall population or across sexes or race. This included 4 of 15 studies in the overall population and 5 of 12 sex-specific studies in either or both sexes. No definitive patterns across sex were observed as deficits were found in four or fewer studies in both male and female neonates. Apart from the Wang et al. (2019) study, no other sex-specific studies reported reduced head circumference in both sexes. Few patterns were seen based on study characteristics or overall confidence levels although nearly all of the *high* and *low* confidence studies were null. Among the nine different studies reporting associations across various populations examined there was no definitive pattern of results by biomarker sample timing as five studies relied on early sampling periods (Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Robledo et al., 2015). This suggests that pregnancy hemodynamics is not fully explaining the inverse asociations detected here.

3.4.4.1.4.5 Fetal Growth Restriction Summary

The majority of studies examining fetal growth restriction showed some evidence of associations with PFOA exposures especially those that included BWT data (i.e., SGA, low BWT, as well as mean and standardized BWT measures). The evidence for two fetal growth measures such as head circumference and birth length were less consistent but still reported many inverse associations. For example, 10 (out of 21) different epidemiological studies of PFOA examining head circumference reported some evidence of inverse associations in either the overall population or across the sexes, which included 8 of 15 *medium* or *high* confidence studies. Nine different studies out of 26 studies reported some birth length deficits in relation to PFOA exposures with limited evidence of exposure-response relationships. This included 6 of 17 *medium* or *high* confidence studies of birth length. Across the fetal growth measures, there was not consistent evidence of sexual dimorphic differences across the fetal growth measures; however, as noted above, many of the individual study results lacked precision and statistical power to detect sex-specific differences that vary considerably in magnitude. There was minimal evidence of exposure-response relationships reported among those examining categorical exposure data, but the categorical data generally supported the linearly expressed associations that were detected.

Among the most accurate fetal growth restriction endpoints examined here, there was generally consistent evidence for BWT deficits across different measures and types of PFOA exposure metrics considered. For example, nearly two-thirds of studies showed BWT deficits based on differences in means or standardized measures. There was limited evidence of exposure-response relationships in either analyses specific to the overall population or different sexes, although the categorical data generally supported the linearly expressed associations that were detected. Associations were also seen for the majority of studies examining SGA and low birth weight measures. The magnitude of some fetal growth measures were at times considered large especially when considering the per unit PFOA increases across the exposure distributions. The range of deficits detected in the overall population across all categorical and continuous exposure estimates ranged from−14 to −267 grams. Among those with continuous PFOA results in the overall population. For example, 14 of the 21 studies reported deficits from −27 to −82 grams in

the overall population based on each unit increase in PFOA exposures. Interestingly, 11 of the 12 largest mean BWT deficits (–48 grams or larger per unit change) in the overall population were detected among studies with later biomarker sampling. However, five (Chang et al., 2022; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018) of nine *medium* and *high* confidence studies still reported some evidence of reductions in mean BWT based on early pregnancy biomarker samples.

The current database (since the 2016 PFOA HESD) is fairly strong given the wealth of studies included here with most of them considered *high* or *medium* confidence (e.g., 17 out of 25 mean BWT studies with data in the overall population) and most of them had adequate or good study sensitivity. As noted earlier, one source of uncertainty is that previous meta-analyses of PFOS by Dzierlenga et al. (2020a) and PFOA by Steenland et al. (2018a) have shown that some measures like mean BWT may be prone to bias from pregnancy hemodynamics especially in studies with later biomarker sampling. For many of these endpoints, such as birth weight measures, there was a preponderance of associations amongst studies with later biomarker samples (i.e., either exclusive trimester 2/3 maternal sample or later, such as umbilical cord or post-partum maternal samples). This would seem to comport with the PFOA meta-analysis by Steenland et al. (2018a) that suggested that results for mean BWT may be impacted by some bias due to pregnancy hemodynamics. Therefore, despite some consistency in evidence across these fetal growth endpoints, some important uncertainties remain mainly around the degree that some of the results examined here may be influenced by sample timing. This source of uncertainty and potential explanation of different results across studies may indicate some bias due to the impact of pregnancy hemodynamics.

3.4.4.1.5 Postnatal Growth

Thirteen studies examined PFOA exposure in relation to postnatal growth measures. The synthesis here is focused on postnatal growth measures including body mass index (BMI)/adiposity measures (Gross et al., 2020; Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Chen et al., 2017b; de Cock et al., 2014) and rapid growth during infancy (Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b), as well as mean and standardized weight (all 13 studies except Gross et al. (2020), Tanner et al. (2020), and Jensen et al. (2020) depicted in Figure 3-62), and height (Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018; Lee et al., 2018; Shoaff et al., 2018; Chen et al., 2017b; Wang et al., 2016; de Cock et al., 2014) measures.

Six postnatal growth studies were *high* confidence (Jensen et al., 2020; Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Wang et al., 2016), four were *medium* confidence (Gyllenhammar et al., 2018; Chen et al., 2017b; Manzano-Salgado et al., 2017b; de Cock et al., 2014) and three were *low* confidence (Gross et al., 2020; Cao et al., 2018; Lee et al., 2018). Five postnatal growth studies had good study sensitivity (Tanner et al., 2020; Lee et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017b; Wang et al., 2016), six were adequate (Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018; Chen et al., 2017b) and two were considered deficient (Gross et al., 2020; de Cock et al., 2014). The synthesis here is focused on postnatal body mass index (BMI)/adiposity measures, head circumference and mean and standardized weight and height measures. Rapid growth during infancy is also included as it was examined in five studies (Tanner et al.; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et

al., 2017b). The *medium* confidence study by de Cock et al. (2014) did not report effect estimates for postnatal infant height (p-value = 0.045), weight (p-value = 0.35), and BMI (p-value = 0.81) up to 11 months of age. But their lack of reporting of effect estimates precluded consideration of magnitude and direction of any associations and are not further considered below in the summaries.

The *medium* confidence study by Manzano-Salgado et al. (2017b) had null associations for their overall population and female neonates measured at 6 months but reported an increased weight gain z-score for males (0.13; 95% CI: 0.01, 0.26) per each log2 PFOA increases. The *medium* confidence study by Chen et al. (2017b) did not report associations between each per ln-unit PFOA exposure increase and height z-score measures up to 24 months of age. The sex-specific data were not always consistent across time. For example, nonsignificant increases small in magnitude for boys (0.11; 95% CI: −0.04, 0.27) and decreases in greater height per each ln-unit PFOA increase in the 12- to 24-month window. The *low* confidence study by Lee et al. (2018) reported statistically significant associations detected for mean height differences at age 2 years (−0.91 cm; 95% CI: −1.36, −0.47 for each PFOA ln-unit increase), as well as height change from birth to 2 years (−0.86 cm; 95% CI: −1.52, −0.20). Large differences were seen for mean weight differences at age 2 years (−210 g; 95% CI: −430, 0.20) but not for weight change from birth to 2 years. An exposure-response relationship was detected when examined across PFOA categories with the highest exposure associated with smaller statistically significant height increases at age 2 compared with lower exposures.

In the *medium* confidence study by Gyllenhammar et al. (2018), no associations were detected for infant height deficits among participants followed from 3 months to 60 months of age per each IQR PFOA change. They also did not report statistically significant standardized BWT deficits per each IQR PFOA change, but they did show slight weight deficits (approximately −0.2) at 3 months that gradually decreased over time (to approximately −0.1) at 60 months of age. Compared to the PFOA tertile 1 referent, the *low* confidence study by Cao et al. (2018) reported slight increases (1.37 cm; 95% CI: −0.5, 3.28) in postnatal length (i.e., height) amongst infants (median age of 19.7 months), while large postnatal weight deficits were reported for tertile 2 (−429.2 g; 95% CI: −858.4, −0.12) and tertile 3 (−114.9 g; 95% CI: −562.0, 332.1). These height increases were predominately due to female infants, while the weight deficits were driven by males. Few differences were observed in the overall population for postnatal head circumference with slight nonsignificant deficits seen amongst females only.

In their *high* confidence study, Wang et al. (2016) reported statistically significant childhood weight (−0.14; 95% CI: −0.39, 0.11) and height (−0.15; 95% CI: −0.38, 0.08) z-scores for female neonates when averaged over the first 11 years and per 1-ln-unit PFOA increase. Results were null for male neonates for childhood average weight (0.03; 95% CI: −0.11, 0.18) and height (0.01; 95% CI: −0.24, 0.25) z-scores. However, when they examined the first 2 years only, statistically significant deficits in both height and weight z-scores were only seen for male neonates. These weight deficits dissipated in males later during childhood, while the height deficits detected at age 2 years continued through age 11. In contrast, the height deficits in female children that were detected at birth were no longer evident in older kids until later ages (i.e., 11 years). The weight deficits in female children detected at birth did not persist.

In their *high* confidence study, Yeung et al. (2019) reported statistically significant negative growth trajectories for weight-for-length z-scores in relation to each log SD increase in PFOA

exposures among singletons followed for three years. In contrast, the authors showed positive infant length (i.e., height) growth trajectory across two different measures. Some sex-specific results were detected with larger associations seen in singleton females for weight-for-length z-score (−0.13; 95% CI: −0.19, –0.06). An infant weight deficit of –12.6 g (95% CI: –49.5, 24.3 per each 1 log SD PFOA increase) was also observed and appeared to be driven by results in females (–30.2 g; 95% CI: –84.1, 23.6). In their *high* confidence study of repeated measures at 4 weeks, 1 year and 2 years of age, Shoaff et al. (2018) detected statistically significant deficits for weight-for-age (−0.46; 95% CI: −0.78, −0.14) z-score, and weight-for-length z-score (−0.34; 95% CI: −0.59, −0.08) in PFOA tertile 3 compared with tertile 1 with exposure-response relationships detected for infant weight-for-length z-score. Deficits comparable in magnitude that were not statistically significant were observed in tertile 3 for height measured as length for age z-score (−0.32; 95% CI: −0.72, 0.07). No associations were found in the overall population from the *high* confidence study by Starling et al. (2019) for postnatal measures at 5 months of age, but an exposure-response relationship of increased adiposity was seen among male neonates with increasing PFOA tertiles (2.81; 95% CI: 0.79, 4.84 for tertile 3). Similarly, no associations were found in the overall population for weight-for-age or weight-for-length z-scores and PFOA exposures, but both measures were increased among male neonates.

Overall, seven of nine studies with quantitative estimates (including six *high* and *medium* confidence studies) showed some associations between PFOA exposures and different measures of infant weight. Two of four studies with categorical data showed some evidence of inverse monotonic exposure-response relationships. Three (two *high* and one *low* confidence) of seven studies with quantitative estimates examining different infant height measures showed some evidence of inverse associations with PFOA. Study quality ratings, including study sensitivity and overall confidence, did not appear to be explanatory factors for heterogeneous results across studies.

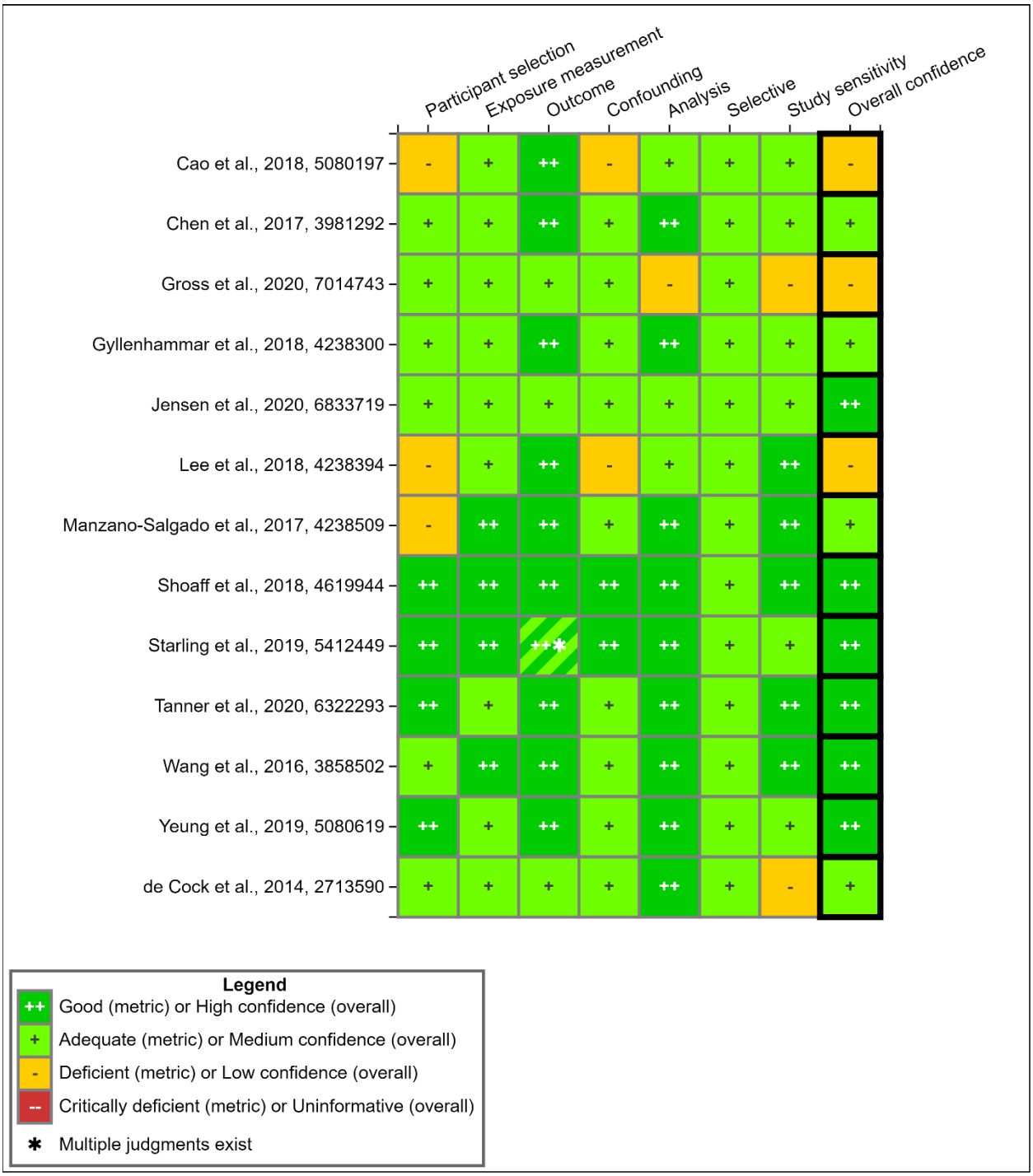


Figure 3-62. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Postnatal Growth

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1 Adiposity/BMI

The *medium* confidence study by Chen et al. (2017b) reported lower BMI z-scores (−0.16; 95% CI: −0.37, 0.05) per each ln-unit PFOA increase in the birth to 6–months window. In their *high*

confidence study of repeated measures at 4 weeks, 1 year, and 2 years of age, Shoaff et al. (2018) detected statistically significant deficits for infant BMI z-score (−0.36; 95% CI: −0.60, −0.12) in PFOA tertile 3 compared with tertile 1 with exposure-response relationships detected for infant BMI z-score. The *high* confidence study by Yeung et al. (2019) reported statistically significant negative growth trajectories for BMI, BMI z-score in relation to each log SD increase in PFOA exposures among singletons followed for three years. Some sex-specific results were detected with larger associations seen in singleton females for BMI (−0.18 kg/m^2; 95% CI: –0.27, –0.09) and BMI z-scores (−0.13; 95% CI: −0.19, −0.07). An exposure-response relationship was evident with decreasing BMI z-scores across PFOA quartiles in the overall population and for female neonates. An exposure-response relationship of increased adiposity was seen among male neonates with increasing PFOA tertiles (2.81; 95% CI: 0.79, 4.84 for tertile 3) in the *high* confidence study by Starling et al. (2019). The *high* confidence study by Jensen et al. (2020) reported null associations between adiposity and per each 1-unit increase in PFOA measured at 3 and 18 months. The *low* confidence study by Gross et al. (2020) reported a null association (OR = 0.91; 95% CI: 0.36 to 2.29) of being overweight at 18 months for PFOA levels greater than the mean level. They showed discordant sex-specific results with higher odds of being overweight at 18 months in males (OR = 2.62; p-value = 0.22) and lower odds among females (OR = 0.41; p-value = 0.27).

Overall, there was very limited evidence of adverse associations between PFOA exposures and either increased BMI or adiposity measures. Only one out of seven studies in the overall population showed evidence of increased adiposity or BMI changes in infancy in relation to PFOA. One of these studies did report increased odds of being overweight at 18 months for higher PFOA levels in males only. Only one of two studies showed an inverse monotonic relationship between either BMI or adiposity with increasing PFOA exposures.

3.4.4.1.5.2 Rapid Weight Gain

Five studies (Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) examined rapid infant growth, with all five considered *high* confidence. Limited evidence of associations was reported with these studies, as only one (Starling et al., 2019) of four studies (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) showed increased odds of rapid weight gain with increasing PFOA. For example, Starling et al. (2019) reported small increased ORs (range: 1.25 to 1.43) for rapid growth in the overall population based on either weight-for-age-based z-scores or weight-for-length-based z-scores. The most detailed evaluation by Tanner et al. (2020) also showed some adverse associations including higher prenatal PFOA concentrations related to a longer duration of time needed to complete 90% of the infant growth spurt (Δtertile 1: 0.06; 95% CI: 0.01, 0.11). Higher prenatal PFOA concentrations were also significantly related to delayed infant peak growth velocity (δ1: 0.58; 95% CI: 0.17, 0.99) and a higher post-spurt weight plateau (α1: 0.81; 95% CI: 0.21, 1.41).

3.4.4.1.5.3 Postnatal Growth Summary

Seven of the nine studies reporting quantitative results for different infant weight measures showed some evidence of adverse associations with PFOA exposures, with two of these studies showing adverse results predominately in females and one in males only. Two other studies showed increased weight among males only and lack of reporting of effect estimates in one study precluded further consideration of adversity. Two (Starling et al., 2019; Manzano-Salgado et al.,

2017b) of three studies did not report adverse associations in either the overall population or females, but did detect increased infant weight measures among males. Three of the seven studies reporting quantitative results showed some evidence of inverse associations between PFOA exposures and infant height. Only one out of seven studies in the overall population showed evidence of increased adiposity or BMI changes in infancy in relation to PFOA. One study showed increased adiposity amongst males only, while four studies each were null or reported some inverse associations (i.e., lower adiposity/BMI with increasing PFOA). Two of the studies showed exposure-response relationships for PFOA and decreased BMI scores, while a third showed the opposite exposure-response for increased adiposity. Although the data across different endpoints was not entirely consistent, the majority of infant weight studies indicated that PFOA may be associated with post-natal growth measures up to 2 years of age.

3.4.4.1.6 Gestational Duration

Twenty-two different studies examined gestational duration measures (i.e., PTB or gestational age measures) in relation to PFOA exposures. Nine of these studies examined both PTB and gestational age measures, while two studies only examined PTB (Gardener et al., 2021; Liu et al., 2020c). Two of these studies were *uninformative* and not considered further below (Gundacker et al., 2021; Lee et al., 2013).

3.4.4.1.6.1 Gestational Age

Eighteen different informative studies examined the relationship between PFOA and gestational age (in weeks) (Figure 3-63). Seventeen of these examined associations in the overall population and one study reported sex-specific findings only (Lind et al., 2017a). Ten of these 18 studies were *high* confidence (Chu et al., 2020; Eick et al., 2020; Huo et al., 2020a; Bell et al., 2018; Buck Louis et al., 2018; Sagiv et al., 2018; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), four were *medium* (Yang et al., 2022In Press; Hjermitslev et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018) and four were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a; Wu et al., 2012). Six of the studies had good study sensitivity (Huo et al., 2020a; Meng et al., 2018; Sagiv et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wu et al., 2012), nine were adequate (Yang et al., 2022In Press; Chu et al., 2020; Eick et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Lind et al., 2017a; Bach et al., 2016) and three (Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018) were deficient.

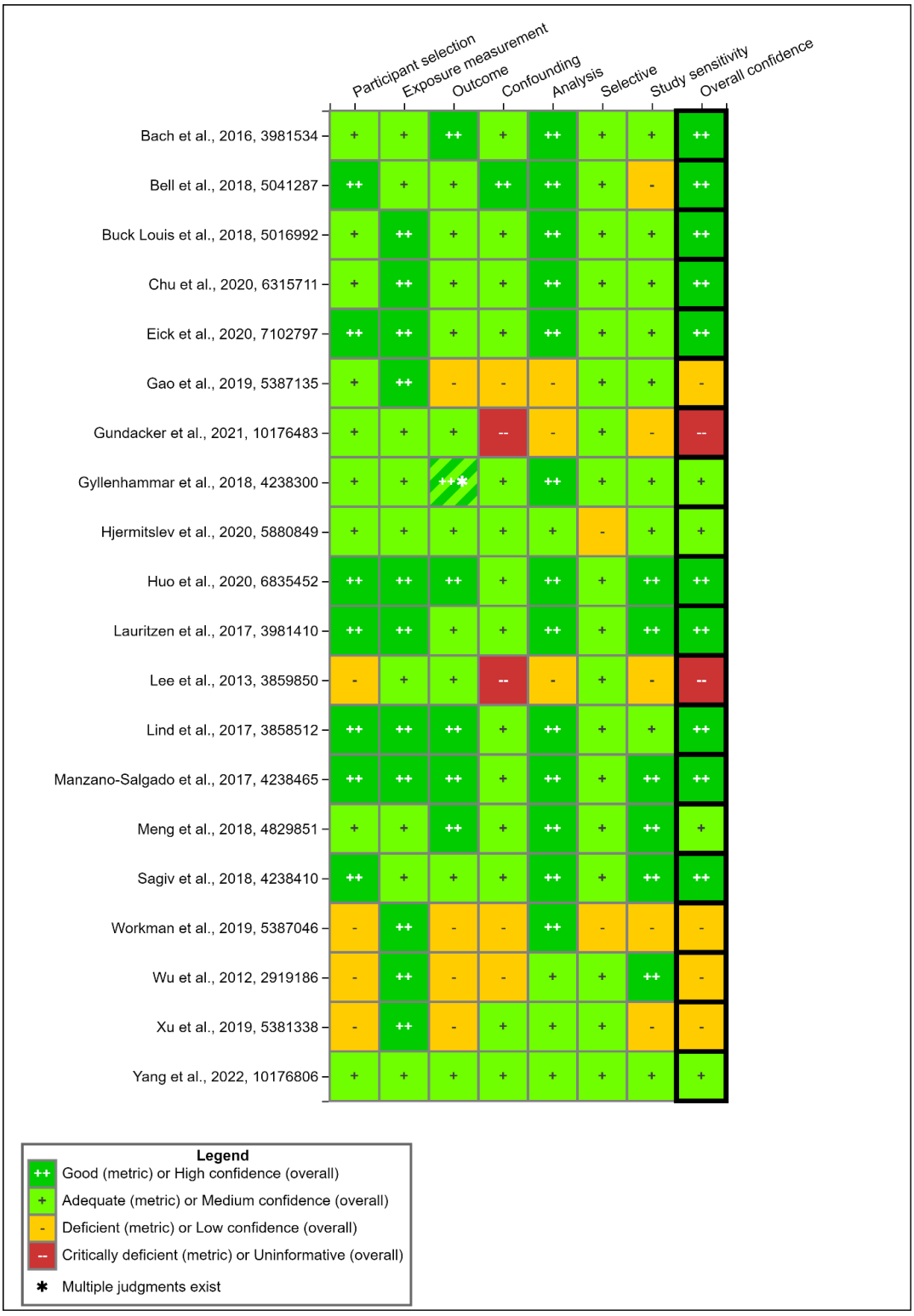


Figure 3-63. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Gestational Age

Interactive figure and additional study details available on HAWC.

Five (3 *low* confidence and 1 each *medium* and *high* confidence) of the 18 studies showed some evidence of increased gestational age (Gao et al., 2019; Hjermitslev et al., 2019; Workman et al., 2019; Xu et al., 2019a; Bach et al., 2016) in relation to PFOA while six others were largely null (Huo et al., 2020a; Bell et al., 2018; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a). The remaining seven studies showed some evidence of adverse impacts on gestational age either in the overall population or either. The *high* confidence study by Lind et al. (2017a) examined only sex-specific data and reported larger deficits in female (−0.21 cm; 95% CI: −0.61, 0.19 per each ln-unit PFOA increase) than male neonates (−0.10 cm; 95% CI: −0.41, 0.21). Among the other six studies with results based on the overall population, three were *high* confidence, two were *medium*, and one was *low* confidence. The *low* confidence study by Wu et al. (2012) study reported an extremely large difference (−2.3 weeks; 95% CI: −4.0, −0.6) in gestational age per each log10 unit PFOA change. The *medium* confidence study by Yang et al. (Yang et al., 2022In Press) reported a larger (–1.04 weeks; 95% CI: –3.72, 1.63 per each PFOA IQR increase) difference in gestational age among preterm births than among term births (–0.38 weeks; 95% CI: –1.33, 0.57 per each PFOA IQR increase). The *medium* confidence study by Meng et al. (2018) reported statistically significant gestational age deficits (range: −0.17 to −0.24 weeks) across all quartiles but no evidence of an exposure-response relationship. The *high* confidence study by Lauritzen et al. (2017) reported a slight decrease in the overall population (−0.2 weeks; 95% CI: −0.34, 0.14). They also showed larger deficits in their Swedish population (−0.3 weeks; 95% CI: −0.9, 0.3) which was predominately driven by results among male neonates (−0.4 weeks; 95% CI: −1.2, 0.5). The *high* confidence study by Chu et al. (2020) showed larger deficits in the overall population (−0.21 weeks; 95% CI: −0.44, 0.02) which was driven by female neonates (−0.83 weeks; 95% CI: −0.53, −0.23). The *high* confidence study by Eick et al. (Eick et al., 2020) reported decreased gestational age only among tertile 2 only in the overall population (–0.29 weeks; 95% CI: –0.74, 0.17), males (–0.24 weeks; 95% CI: –0.91, 0.43) and females (–0.31 weeks; 95% CI: –0.95, 0.34) relative to tertile 1.

Overall, seven of the 18 studies showed some evidence of adverse impacts on gestational age. Six of the seven studies were either *medium* or *high* confidence studies. Few patterns emerged based on study confidence or other study characteristics. For example, three of the null studies were rated as having good sensitivity, along with two studies with adequate and one with deficient ratings. There was a preponderance of associations related to sample timing possibly related to pregnancy hemodynamic influences on the PFOA biomarkers, as five of the seven studies reporting inverse associations were sampled later in pregnancy (i.e., exclusively trimester two or later).

3.4.4.1.6.2 Preterm Birth

As shown in Figure 3-64, eleven studies examined the relationship between PFOA and PTB; all of the studies were either *medium* (Yang et al., 2022In Press; Liu et al., 2020c; Hjermitslev et al., 2019; Meng et al., 2018) or *high* confidence (Gardener et al., 2021; Chu et al., 2020; Eick et al., 2020; Huo et al., 2020b; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016). Nine of the 11 studies were prospective birth cohort studies, and the two studies by Liu et al. (2020c) and Yang et al. (Yang et al., 2022In Press) were case-control studies nested with prospective birth cohorts. Four studies had maternal exposure measures that were sampled either during trimester one (Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016) or trimester three (Gardener et al., 2021). The *high* confidence study by Chu et al. (Chu et al., 2020)

sampled during the late third trimester or within three days of delivery. Four studies collected samples across multiple trimesters (Eick et al., 2020; Huo et al., 2020b; Liu et al., 2020c; Hjermitslev et al., 2019). The *medium* confidence study by Meng et al. (2018) pooled exposure data from two study populations, one which measured PFOA in umbilical cord blood and one which measured PFOA in maternal blood samples collected in trimesters 1 and 2. The *medium* confidence study by Yang et al. (2022In Press) collected umbilical cord blood samples. Four studies (Huo et al., 2020b; Meng et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a) were considered to have *good* sensitivity and one was *deficient* (Liu et al., 2020c). The other six studies were rated *adequate* in this domain. The median exposure values across all studies ranged from 0.76 ng/mL (Eick et al., 2020) to 11.85 ng/mL (Huo et al., 2020b).

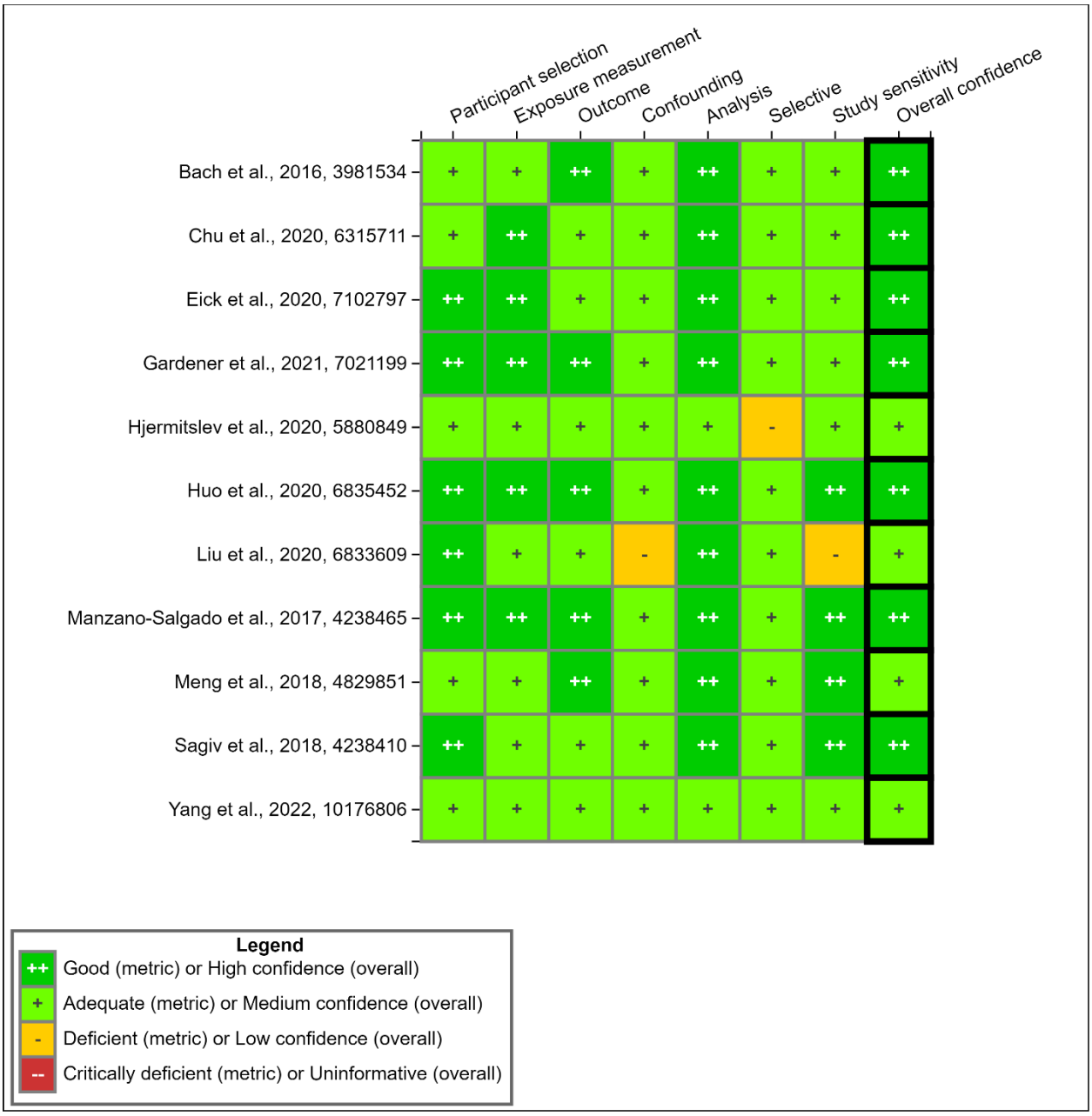


Figure 3-64. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Preterm Birth Effects

Interactive figure and additional study details available on HAWC.

Six of the 11 studies reported an increased risk of PTB with elevated exposure to PFOA. Null or inverse associations were reported by Bach et al. (2016), Hjermitslev et al. (2019), Liu et al. (2020c), Manzano-Salgado et al. (2017a) and Yang et al. (2022In Press). The *medium* confidence study by Meng et al. (2018) reported consistently elevated nonmonotonic ORs for PTB in the upper three PFOA quartiles (OR range: 1.7–3.2), but little evidence was observed per each doubling of PFOA exposures (OR = 1.1; 95% CI: 0.8, 1.5). Although they were not statistically significant, the *high* confidence study by Chu et al. (2020) reported increased ORs of similar

magnitude per each ln ng/mL increase (OR = 1.49; 95% CI: 0.94, 2.36) and when quartile 3 (OR = 1.60; 95% CI: 0.60, 4.23) and quartile 4 (OR = 1.84; 95% CI: 0.72, 4.71) exposures were compared with the referent. ORs similar in magnitude were detected in the *high* confidence study by Eick et al. (2020) study albeit in a more monotonic fashion across all quantiles (tertile 2: OR = 1.48; 95% CI: 0.66, 3.31); 95% CI: tertile 3: OR = 1.63; 95% CI: 0.74, 3.59). Associations between PFOA and overall PTB near or just below the null value were consistently detected for either categorical or continuous exposures in the *high* confidence Huo et al. (2020b) study. Few patterns emerged across PTB subtypes in that study, although there was an increase in clinically indicated PTBs (OR = 1.71; 95% CI: 0.80, 3.67 per each ln-unit PFOA increase) which seemed to be largely driven by results in female neonates (OR = 2.64; 95% CI: 0.83, 8.39). The *high* confidence study by Sagiv et al. (2018) reported increased nonsignificant risks (OR range: 1.1–1.2) for PTB across all PFOA quartiles. Relative to the referent, the *high* confidence study by Gardener (Gardener et al., 2021) showed higher odds of PTB in PFOA quartiles 2 and 3 (range: 3.1–3.2) than that found in quartile 4 (OR = 1.38; 95% CI: 0.32–5.97). Outside of the aforementioned Eick et al. (2020) study, none of the other seven studies with categorical data showed evidence of exposure-response relationships.

Overall, 6 of the 11 studies showed increased risk of PTB with PFOA exposures with limited evidence of exposure-response relationships. Although small numbers limited the confidence in many of the sub-strata comparisons, there were few apparent patterns by study evaluation ratings or other characteristics that explained the heterogeneous results across studies. However, there were more associations amongst studies with later sample timing data collection, as three of the five studies with later PFOA biomarker sampling showed some increased odds of preterm birth compared with two of six studies with earlier sampling.

3.4.4.1.6.3 Gestational Duration Summary

Overall, there was mixed evidence of exposure to PFOA and both inverse associations with gestational age and increased risk of preterm birth. Most of the associations for either gestational duration measures were reported in *medium* or *high* confidence studies. Few other patterns were evident that explained any between study heterogeneity.

3.4.4.1.7 Fetal Loss

Five (two *high*, two *medium* and one *low* confidence) studies examined PFOA exposure and fetal loss with limited evidence as only one study showing increased risks of miscarriage. Two studies had good study sensitivity (Wang et al., 2021; Wikström et al., 2021), while three had adequate sensitivity (Liew et al., 2020; Buck Louis et al., 2016; Jensen et al., 2015) (Figure 3-65).

The *high* confidence study by Wikström et al. (2021) showed a statistically significant association between PFOA and miscarriages (OR = 1.48; 95% CI: 1.09, 2.01 per doubling of PFOA exposures. The authors also reported a monotonic exposure-response relationship across PFOA quartiles (ORs/95% CIs: Q2: 1.69; 0.8, 3.56; Q3: 2.02; 0.95, 4.29; Q4: 2.66; 1.26, 5.65). The *medium* confidence study by Liew et al. (2020) detected a 40% increased risk of miscarriage (OR = 1.4; 95% CI: 1.0, 1.9) per each PFOA doubling with increased risks detected for quartiles three (OR = 1.4; 95% CI: 0.8, 2.6) and four (OR = 2.2; 95% CI: 1.2, 3.9) only. No associations were detected in the *high* confidence study by Wang et al. (Wang et al., 2021) for preclinical spontaneous abortion (OR = 0.99; 95% CI: 0.94, 1.05) or in the *medium* confidence study by Buck Louis et al. (2016) (hazard ratio (HR) =0.93; 95% CI: 0.75, 1.16 per each SD PFOA

increase). In the *low* confidence study by Jensen et al. (Jensen et al., 2015), a decreased risk of miscarriages was reported (OR = 0.64; 95% CI: 0.36, 1.18 per each ln-unit PFOA increase).

Overall, there was positive evidence for fetal loss with increased relative risk estimates in two out of five studies. In those two studies, the magnitude of associations detected ranged from 1.4 to 2.7 with an exposure-response relationship detected in one study. No patterns in the results were detected by study confidence ratings including sensitivity.

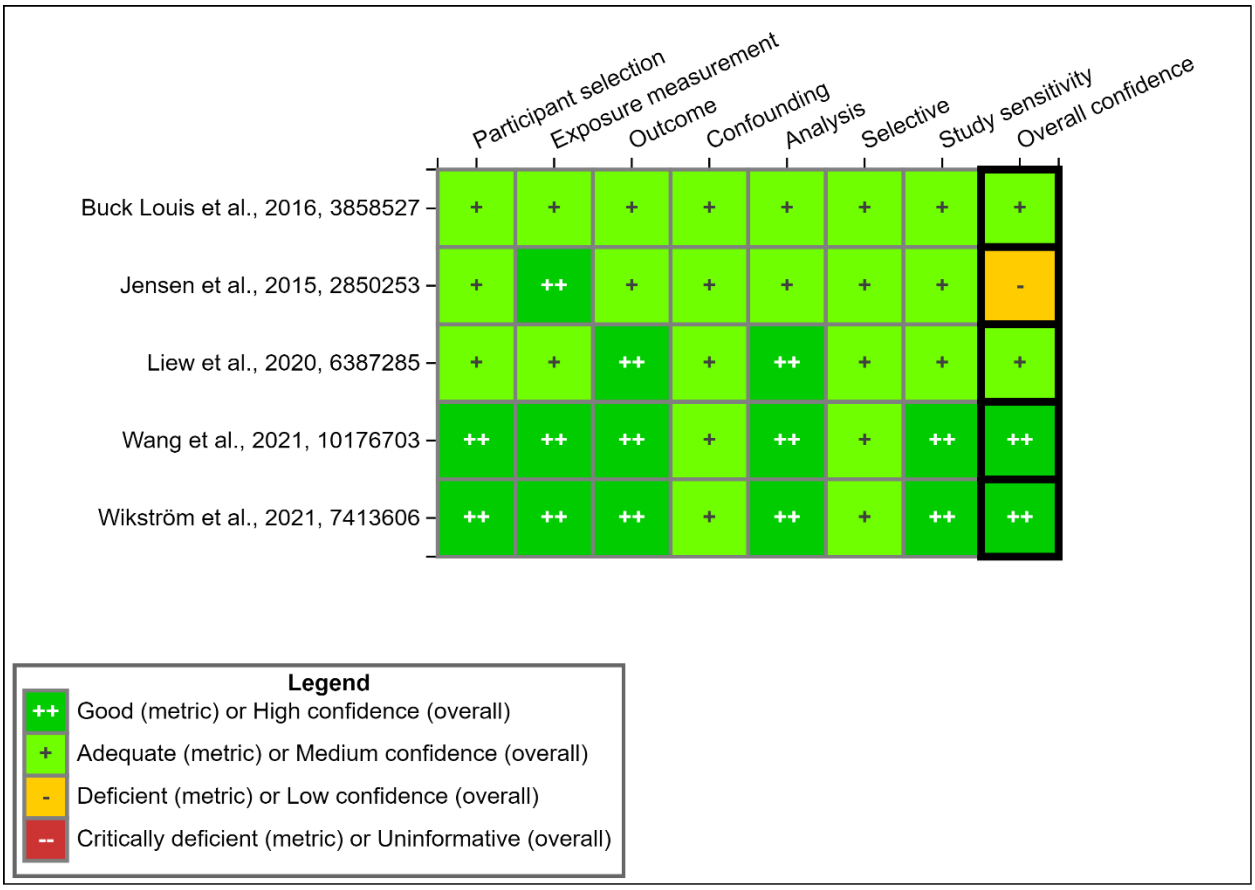


Figure 3-65. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Fetal Loss

Interactive figure and additional study details available on HAWC.

3.4.4.1.8 Birth Defects

Four birth defect studies examined PFOA exposure with three of these four having adequate study sensitivity (one was deficient) as shown in Figure 3-66. This included a *medium* confidence study by Vesterholm Jensen et al. (2014) that reported no increased risk for cryptorchidism (OR = 0.83; 95% CI: 0.44, 1.58 per each ln-unit PFOA increase). A *medium* confidence study by Ou et al. (2021) reported decreased risks for septal defects (OR = 0.54; 95% CI: 0.18, 1.62), conotruncal defects (OR = 0.28; 95% CI: 0.07, 1.10), and total congenital heart defects (OR = 0.64; 95% CI: 0.34, 1.21) among participants with maternal serum levels over >75th PFOA percentile (relative to those <75th percentile). A *low* confidence study (Cao et al., 2018) of a nonspecific all birth defect grouping reported limited evidence of an association

(OR = 1.24; 95% CI: 0.57, 2.61), but interpretation of an all-birth defect grouping is challenging given that etiological heterogeneity may occur across individual defects. Compared to the referent group of no Little Hocking Water Association supplied water, no associations (both ORs were 1.1) were reported in a *low* confidence study from Washington County, Ohio among infants born to women partially or exclusively supplied in part by the Little Hocking Water Association (Nolan et al., 2010). The study was considered *uninformative* for examination of individual defects given the lack of consideration of confounding and other limitations in those analyses.

Overall, there was negligible evidence of associations between PFOA and birth defects based on the four available epidemiological studies including two *medium* confidence studies which reported decreased odds of birth defects relative to exposures. As noted previously, there is considerable uncertainty in interpreting results for broad any defect groupings which are anticipated to have decreased sensitivity to detect associations.

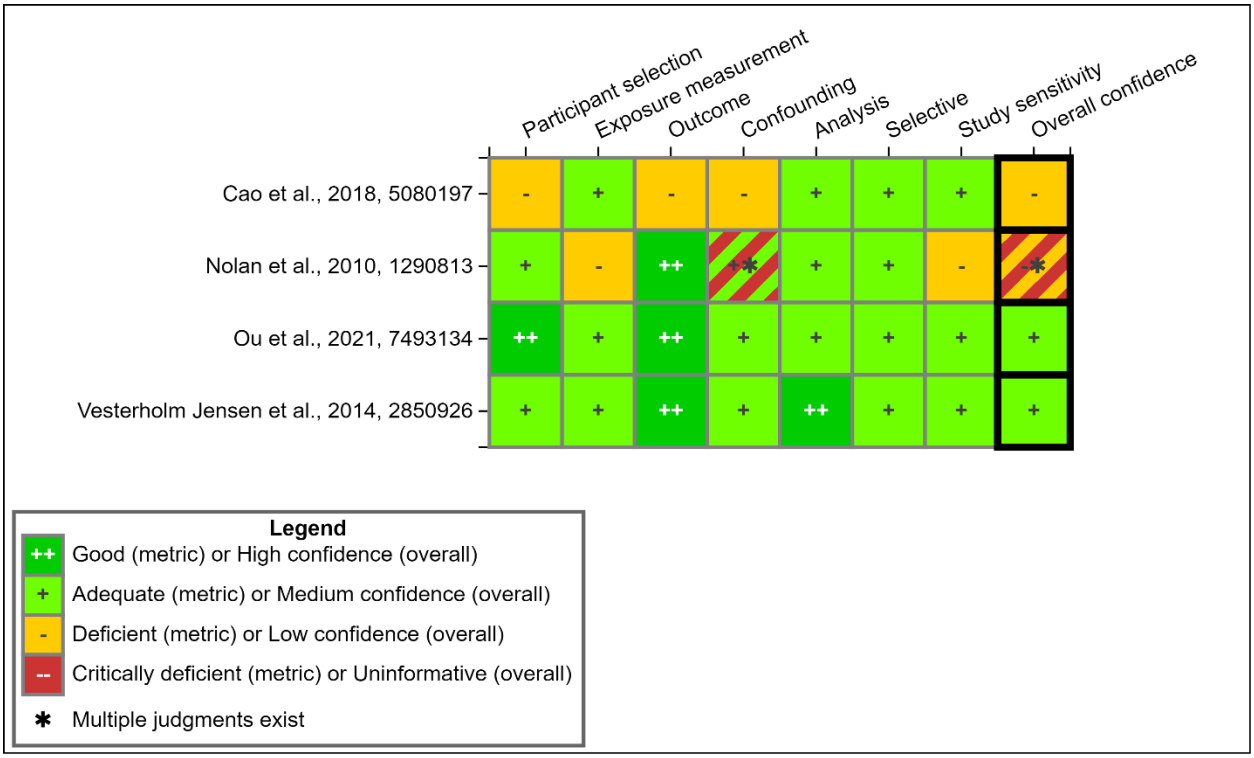


Figure 3-66. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Defects

Interactive figure and additional study details available on HAWC.

3.4.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 13 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and developmental effects in animal models. Study quality evaluations for these 19 studies are shown in Figure 3-67.

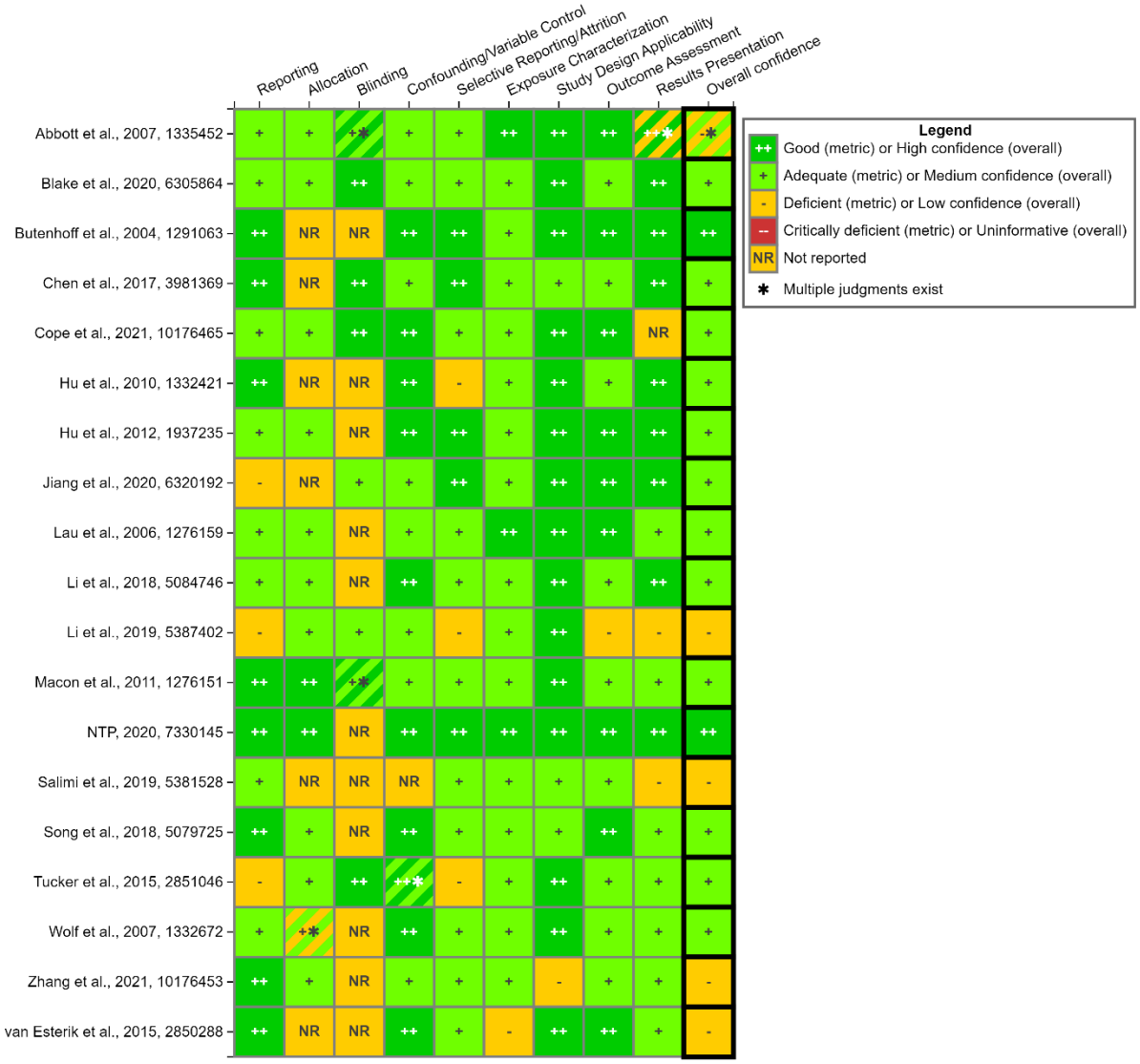


Figure 3-67. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Developmental Effects

Interactive figure and additional study details available on HAWC.

Evidence suggests that PFOA exposure can adversely affect development. Oral studies in mice and rats report effects in offspring including decreased survival, decreased body weights, structural abnormalities (e.g., reduced skeletal ossification), delayed eye opening, and altered mammary gland development. Doses that elicited responses were generally lower in mice than in rats. Additionally, three studies of gestational PFOA exposure to mice reported effects on placental weight and histopathological changes in placental tissue, suggesting that the placenta may be a target of PFOA. In some cases, adverse developmental effects of PFOA exposure that relate to other health outcomes may be discussed in the corresponding health outcome section (e.g., neurodevelopmental effects are discussed in the Appendix (U.S. EPA, 2024a)).

3.4.4.2.1 Maternal Effects

Exposure to PFOA resulted in significant decreases in maternal body weight and/or weight gain at doses ≥10 mg/kg/day in multiple strains of pregnant mice (Li et al., 2018a; Yahia et al., 2010; Lau et al., 2006) and at doses ≥30 mg/kg/day in pregnant Sprague-Dawley rats (Hinderliter et al., 2005; Butenhoff et al., 2004a). The effect followed a dose-related trend in some studies. PFOA exposure was also associated with significantly delayed parturition at doses ≥3 mg/kg/day in CD-1 mice (Lau et al., 2006) and at 10 mg/kg/day in ICR mice (Yahia et al., 2010).

3.4.4.2.1.1 Studies in Mice

Li et al. (2018a) reported marked, dose-related decreases in maternal body weight gain at ≥10 mg/kg/day in pregnant Kunming mice exposed from gestation day 1 to 17 (GD 1 to GD 17; no statistical tests performed). Dose-related decreases in body weight gain were also seen in pregnant CD-1 mice exposed to 10, 20, or 40 mg/kg/day (significant at 20 and 40 mg/kg/day) by Lau et al. (2006); significantly delayed time to parturition was also seen at 3, 10, and 20 mg/kg/day in this study (all litters at 40 mg/kg/day were resorbed). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD 0 to GD 17 (sacrificed on GD 18) or GD 0 to GD 18 (allowed to give birth), and at 10 mg/kg/day, observed significant decreases in body weight gain from GD 12 onward in dams allowed to give birth as well as significantly decreased terminal body weight in dams sacrificed on GD 18. In the same study, a significant decrease in food intake during early gestation was also reported for the dams allowed to give birth, but data were not shown. Delayed parturition was also observed at 10 mg/kg/day (data not shown). Pregnant CD-1 mice exposed to 25 mg/kg/day from GD 11 to GD 16 exhibited significantly decreased body weight from GD 13 to GD 16 (Suh et al., 2011). Hu et al. (2010) exposed pregnant C57BL/6N mouse dams to 0.5 or 1.0 mg/kg/day PFOA and found no significant differences relative to controls on GD 19. No significant effects on maternal body weight were noted in C57BL/6N mouse dams exposed to 0.02, 0.2, or 2 mg/kg/day PFOA from time of mating through PND 21 (Hu et al., 2012). In contrast to the above-described findings, two studies in pregnant CD-1 mice reported significantly increased maternal body weight gain after exposure to 5 mg/kg/day (Blake et al., 2020) or 3 or 5 mg/kg/day PFOA (Wolf et al., 2007) from GD 1 to GD 17. Abbott et al. (2007) found no effects of 0.1, 0.3, 0.6, or 1 mg/kg/day PFOA on maternal weight changes in 129S1/SvlmJ wild-type mice (exposure to 5, 10, and 20 mg/kg/day PFOA led to increased maternal death) (Figure 3-68).

3.4.4.2.1.2 Studies in Rats

A two-generation oral gavage reproductive toxicity study in Sprague-Dawley rats reported no effect on parental generation (P_0) maternal body weight or food consumption but found significantly decreased body weight in first-generation (F_1) parental females at 30 mg/kg/day during pre-cohabitation, gestation (GD 0–GD 14), and lactation day 5 to 15 (LD 5–LD 15). Decreased absolute food consumption was reported, but data were not shown; relative feed consumption was unaffected (Butenhoff et al., 2004a). In pregnant Sprague-Dawley rats dosed with 30 mg/kg/day from GD 4 to LD 21, body weight gain was decreased during gestation and body weight was 4% lower than controls during lactation (statistical significance not indicated) (Hinderliter et al., 2005).

In a two-year chronic toxicity/carcinogenicity assay conducted by the NTP (2020), female Sprague-Dawley (Hsd:Sprague-Dawley® SD®) rat dams were exposed to 0, 150, or 300 parts per million (ppm) PFOA in feed during the perinatal period. In study 1, F_1 male rats were

administered 0, 150, or 300 ppm PFOA and F_1 female rats were administered 0, 300, or 1,000 ppm PFOA in feed during the postweaning period. For study 2, lower postweaning exposure levels (0, 20, 40, or 80 ppm) were utilized for males due to unexpected toxicity in male offspring using the original exposure regime. Exposure for all F_1 generations in both studies occurred for 107 weeks or until the 16-week interim necropsy. The perinatal and postweaning exposure regimes for females and males for both studies are presented in Table 3-14. Dose groups for this study are referred to as "[perinatal exposure level]/[postweaning exposure level]" (e.g., 300/100).

Table 3-14. Study Design for Perinatal and Postweaning Exposure Levels for F_1 Male and Female Rats for the NTP (2020) Study

Perinatal Dose	Postweaning Dose						
	0 ppm	20 ppm	40 ppm	80 ppm	150 ppm	300 ppm	1,000 ppm
Study 1 Females							
0 ppm	X	–	–	–	–	X	X
150 ppm	–	–	–	–	–	X	
300 ppm	–	–	–	–	–	–	X
Study 1 Males							
0 ppm	X	–	–	–	X	X	–
150 ppm	–	–	–	–	X		–
300 ppm	–	–	–	–	–	X	–
Study 2 Males							
0 ppm	X	X	X	X	–	–	–
300 ppm	X	X	X	X	–	–	–

Notes: F_1 = first generation; X = exposure level used.

In pregnant Sprague-Dawley rats exposed to 150 or 300 ppm via diet (equivalent to approximately 11 and 22 mg/kg/day during gestation and 22 and 45 mg/kg/day from LD 1 to LD 14), no consistent effects were observed on body weight or body weight gain during gestation or lactation (Figure 3-68). Food consumption was marginally but significantly decreased (up to 4%) at one or both dose levels at various intervals. In a repeat of this study that tested a single dose level of 300 ppm (approximately 21.8 mg/kg/day during gestation and 48.3 mg/kg/day from LD 1 to LD 14), no effects were observed on maternal body weight or body weight gain during gestation; from LD 1 to LD 14, there was a marginal but significant decrease (2%–3%) in maternal body weight and body weight gain and a significant decrease (5%) in food consumption (NTP, 2020).

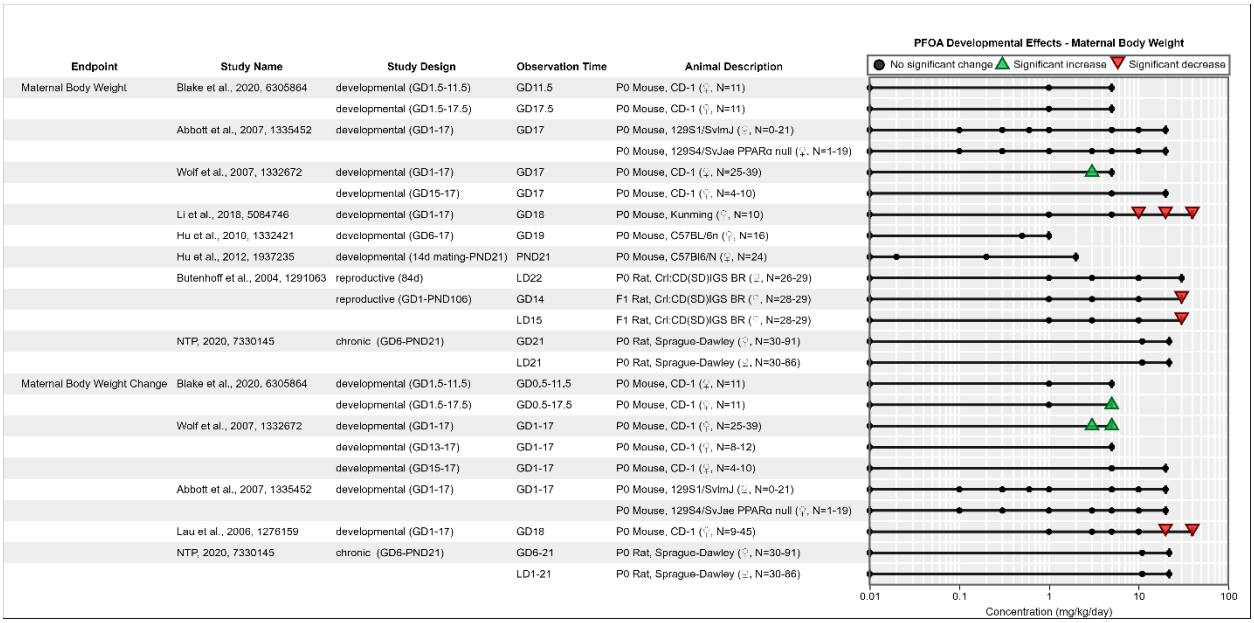


Figure 3-68. Maternal Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactation day; P_0 = parental generation; F_1 = first generation.

3.4.4.2.2 Placenta Effects

Two oral gavage studies in CD-1 mice reported significant decreases in embryo to placenta weight ratio at 5 mg/kg/day PFOA (Blake et al., 2020) or doses ≥2 mg/kg/day (Suh et al., 2011), as well as treatment-related histopathological lesions at 5 mg/kg/day (Blake et al., 2020) or doses ≥10 mg/kg/day (Suh et al., 2011). A third study in Kunming mice reported decreased placenta to body weight ratio at PFOA doses ≥5 mg/kg/day and histopathological changes in placental tissue at doses ≥2.5 mg/kg/day (Jiang et al., 2020) (Figure 3-69).

Blake et al. (2020) administered 0, 1, or 5 mg/kg/day to pregnant CD-1 mice from GD 1.5 through sacrifice on GD 11.5 or GD 17.5, Suh et al. (2011) administered 0, 2, 10, or 25 mg/kg/day to CD-1 mice from GD 11 through sacrifice on GD 16, and Jiang et al. (2020) administered 0, 2.5, 5, or 10 mg/kg/day to Kunming mice from GD 1 through sacrifice on GD 13. The embryo to placental weight ratio was significantly decreased at 5 mg/kg/day in Blake et al. (2020) and at doses ≥2 mg/kg/day in Suh et al. (2011). Blake et al. (2020) observed significantly increased placental weight at 5 mg/kg/day at GD 17.5 and no changes in the numbers of viable fetuses or resorptions, whereas Suh et al. (2011) observed significantly decreased placental weight and increased numbers of resorptions and dead fetuses at ≥2 mg/kg/day. Jiang et al. (2020) observed significantly decreased relative placental weight at ≥5 mg/kg/day (decreases were also seen at lower dose levels, but they did not reach statistical significance). Histopathological changes in placental tissue were also observed at PFOA doses ≥2.5 mg/kg/day (increased area of spongiotrophoblast, decreased blood sinusoidal area in labyrinth), ≥5 mg/kg/day (increased interstitial edema of spongiotrophoblast), or 10 mg/kg/day (decreased labyrinth area, increased ratio of spongiotrophoblast to labyrinth area). Jiang et al.

(2020) found no effect on fetus to maternal body weight ratio. Viable fetus weight was significantly decreased in Blake et al. (2020) at 5 mg/kg/day and in Suh et al. (2011) at ≥10 mg/kg/day and corresponded with treatment-related lesions in the placenta. The incidence of GD 17.5 placentas within normal limits was significantly lower in mice exposed to 5 mg/kg/day (Blake et al., 2020), and the lesions observed in placentas from that group included labyrinth atrophy (3/40 placentas), labyrinth congestion (23/40), and early fibrin clot (1/40). In dams treated with 1 mg/kg/day, labyrinth necrosis was observed in 1/32 placentas and placental nodules were observed in 2/32 placentas. Histopathologic examination by Suh et al. (2011) showed normal placental tissue in 0 and 2 mg/kg/day groups and dose-dependent necrotic changes in placentas from the 10 and 25 mg/kg/day groups (incidences of specific lesions and statistical significance not reported).

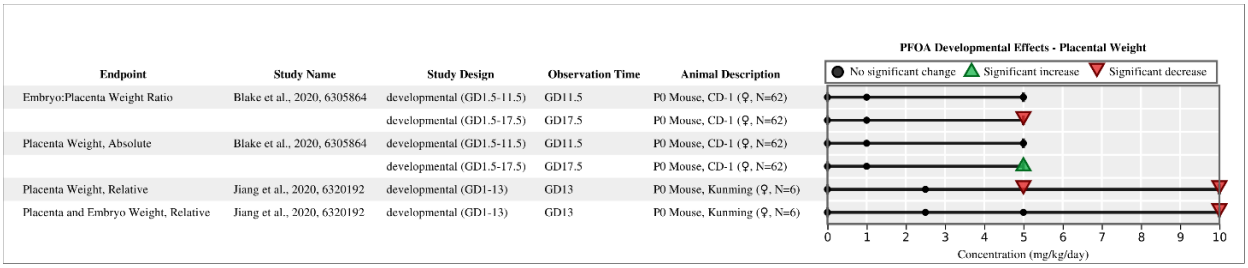


Figure 3-69. Placental Weights in Mice Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation.

3.4.4.2.3 Offspring Mortality

Studies of oral PFOA exposure in mice reported significant increases in resorptions and dead fetuses with PFOA dose levels as low as 2 mg/kg/day in prenatal evaluations (Li et al., 2018a; Suh et al., 2011; Lau et al., 2006). Stillbirths, pup mortality, and total litter loss were observed in several strains of mice at doses ≥5 mg/kg/day (Song et al., 2018; White et al., 2011; Yahia et al., 2010; Wolf et al., 2007; Lau et al., 2006); increased litter loss was seen as low as 0.6 mg/kg/day PFOA in one study in 129S1/SvImJ mice (Abbott et al., 2007). Comparatively, rat pup mortality (pre- and post-weaning) was reported at a higher dose of 30 mg/kg/day (Butenhoff et al., 2004a). Maternal effects observed in some of these studies were not sufficient to explain effects observed in the offspring, as some studies reported effects on offspring survival at dose levels that did not produce maternal effects.

3.4.4.2.3.1 Mice, Prenatal Evaluations

In two studies of gestational PFOA exposure in pregnant Kunming mice, Li et al. (2018a) reported significantly decreased GD 18 fetal survival at 10 and 20 mg/kg/day and total fetal resorption at 40 mg/kg/day (fetal survival was also decreased at 5 mg/kg/day, but the effect did not reach statistical significance), and Chen et al. (2017c) reported a significant increase in the number of resorbed fetuses at GD 13, but not GD 7, after exposure to 10 mg/kg/day PFOA beginning on GD 1 (there were no effects on the number of implantation sites). Suh et al. (2011) exposed pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD 11 to GD 16 (dams were sacrificed on GD16) and observed significant increases in the number of resorptions and dead fetuses at all dose levels; post-implantation loss was 3.87%, 8.83%, 30.98%, and 55.41% at 0, 2, 10, and 25 mg/kg/day, respectively. In pregnant CD-1 mice exposed from GD 1 to GD 17, Lau et

al. (2006) reported significant increases in the number of full-litter resorptions at PFOA doses ≥5 mg/kg/day, with complete loss of all pregnancies at the high dose of 40 mg/kg/day (no effect was observed on the number of implantation sites in litters that were fully resorbed). At 20 mg/kg/day, a significant increase in the percentage of prenatal loss per live litter was observed. White et al. (2011) reported significantly fewer implants in F_1-generation CD-1 mouse dams that had been exposed to 5 mg/kg/day PFOA (Figure 3-70).

3.4.4.2.3.2 Mice, Postnatal Evaluations

Wolf et al. (2007) reported a significant increase in total litter loss following oral PFOA exposure of pregnant CD-1 mice to 5 mg/kg/day (no effect on the number of implantation sites). In offspring exposed to 5 mg/kg/day PFOA in utero and throughout lactation, significantly decreased pup survival was observed from postnatal day (PND) 4 to 22; this effect was not seen in cross-fostered offspring exposed during gestation only or during lactation only. In a separate study, these authors exposed pregnant CD-1 mice to 5 mg/kg/day PFOA for different lengths of time (GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17) and to 20 mg/kg/day from GD 15–17. Control mice received deionized water from GD 7 to GD 17. Although gestational PFOA exposure from GD 1 to GD 6 was not required to elicit adverse developmental responses in pups, the severity of postnatal responses, including decreased pup weight during lactation and delayed eye opening, increased with earlier and longer exposure durations (i.e., GD 7–GD 17 exposure resulted in more severe decreases in pup body weight when compared with pups exposed from GD 15 to GD 17). The authors could not attribute the observed adverse effects to a sensitive window of development as the pups exposed for longer durations had higher serum PFOA levels than pups exposed for shorter durations. Notably, significantly decreased offspring survival was observed in pups exposed to 20 mg/kg/day with the shortest exposure duration from GD 15 to GD 17.

Lau et al. (2006) reported significant increases in the incidence of stillbirths and pup mortality at 5, 10, and 20 mg/kg/day PFOA in CD-1 mice exposed from GD 1 to GD 18 and allowed to deliver naturally. Complete loss of all pregnancies was observed at the high dose of 40 mg/kg/day, though there were no effects on the number of implantation sites. At 10 and 20 mg/kg/day, most of the pups died on PND 1. After exposure of pregnant Kunming mice to 1, 2.5, or 5 mg/kg/day from GD 1 to GD 17, Song et al. (2018) reported a significant decrease in the number of surviving pups per litter on PND 7, 14, and 21 at 5 mg/kg/day (a dose-related trend was observed, but statistical significance was achieved only at the high dose). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day PFOA from GD 0 to GD 18, and the dams were allowed to give birth naturally. Approximately 58% of pups born to high-dose dams were stillborn, and the remaining pups died within 6 hours of birth. Mean PND 4 survival rate was 98%, 100%, 84.4%, and 0% at 0, 1, 5, and 10 mg/kg/day, respectively (with significant decreases at 5 and 10 mg/kg/day). In the same study, some of the pregnant mice were exposed to the same dose levels from GD 0 to GD 17 and sacrificed on GD 18, and the number of live GD 18 fetuses from these dams was not significantly affected at any dose level. White et al. (2011) conducted a multigenerational study and dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD 1 to GD 17. Exposure to 5 mg/kg/day significantly increased prenatal loss, significantly decreased the number of live pups born, and significantly reduced postnatal survival. In adult female F_1 animals, no effects were observed on the prenatal loss or postnatal pup survival of the second generation (F_2) offspring.

Abbott et al. (2007) exposed pregnant 129S1/SvImJ wild-type and PPARα-null mice from GD 1 to GD 17 to dose levels ranging from 0.1 to 20 mg/kg/day and allowed the mice to deliver naturally. There were no treatment-related effects on the number of implantation sites, but wild-type dams exposed to ≥0.6 mg/kg/day PFOA and PPARα-null dams exposed to ≥5 mg/kg/day PFOA had significantly increased litter loss compared with their respective controls. At doses ≥5 mg/kg/day in wild-type dams and 20 mg/kg/day in PPARα-null dams, 100% litter loss occurred. The percentage of dams with full litter resorptions significantly increased in the 5, 10, and 20 mg/kg/day groups, with 100% full litter resorption in the 20 mg/kg/day group. When excluding dams with full litter resorptions, wild-type dams exposed to 1 mg/kg/day had a significant increase in litter loss. Pup survival from birth to weaning was significantly decreased in wild-type litters exposed to PFOA doses ≥0.6 mg/kg/day. No effect was seen in PPARα-null litters. Survival was significantly decreased for wild-type and heterozygous pups born to wild-type dams dosed with 1 mg/kg/day and for heterozygous pups born to PPARα-null dams dosed with 3 mg/kg/day. In the wild-type mice, the number of live and dead pups per litter were not affected by PFOA. Similarly, the number of pups per litter in CD-1 mice exposed to 0.1 or 1 mg/kg/day PFOA from GD 1.5 to GD 17.5 did not significantly differ from control groups (Cope et al., 2021) (Figure 3-70).

3.4.4.2.3.3 Rats, Postnatal Evaluations

The NTP two-year carcinogenicity studies in Sprague-Dawley rats found no effects on offspring survival (NTP, 2020), but Butenhoff et al. (2004a) reported an increase in the total number of dead F_1 rat pups during lactation (26/388 deaths at 30 mg/kg/day and 10/397 in the control group; statistically significant only on LD 6–LD 8) and a significant increase in F_1 female pup deaths with 30 mg/kg/day on post-weaning days 2–8. F_2 generation pup survival was unaffected. In pregnant Sprague-Dawley rats dosed with 0, 3, 10, or 30 mg/kg/day from GD 4 to LD 21, one dam at 3 mg/kg/day and two dams at 30 mg/kg/day delivered small litters (3–6 pups/litter compared with 12–19 pups/litter in the control group); however, statistical significance was not indicated, and given the small sample size (5 dams/group), the biological significance of this finding is unclear (Hinderliter et al., 2005) (Figure 3-70).

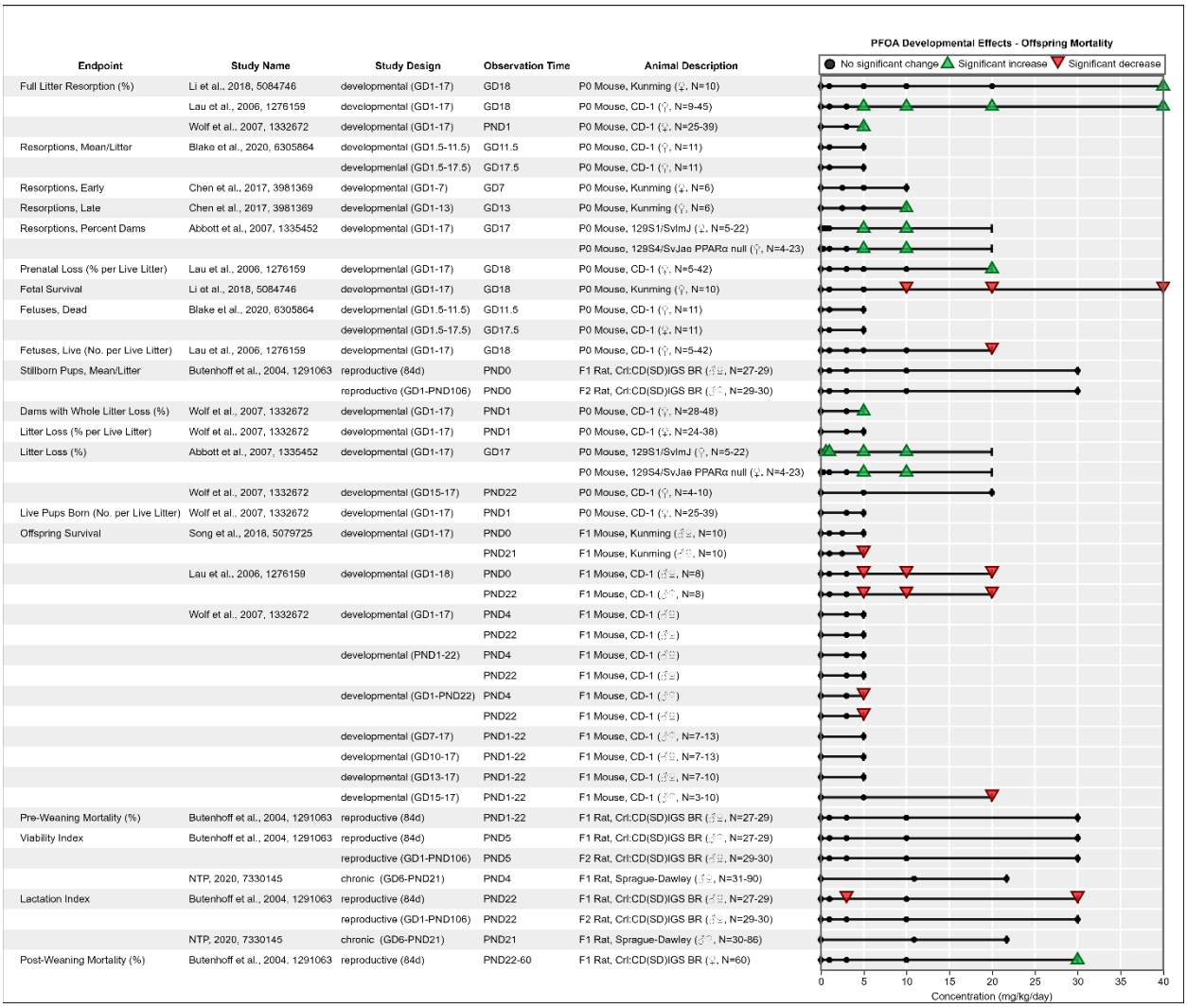


Figure 3-70. Offspring Mortality in Rodents Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; P_0 = parental generation; F_1 = first generation; F_2 = second generation; d = day.
[a] Lau et al. (2006) exposed pregnant mice from GD 1 to GD 19, but some of the mice were sacrificed and examined on GD 18. Based on data from the pregnant mice sacrificed on GD 18, all litters from dams administered 40 mg/kg/day were resorbed, and therefore no offspring were available for postnatal assessments.

3.4.4.2.4 Offspring Body Weight

Available studies of oral gestational PFOA exposure to mice report significant decreases in offspring body weight in prenatal evaluations at doses ≥5 mg/kg/day and postnatal evaluations at dose levels as low as 0.5 mg/kg/day (Blake et al., 2020; Li et al., 2018a; Tucker et al., 2014; Hu et al., 2012; Suh et al., 2011; White et al., 2011; Hu et al., 2010; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006). Offspring weight deficits in pups were observed to extend beyond weaning in three studies in CD-1 mice (at 1, ≥3, and 5 mg/kg/day, respectively) (Tucker et al., 2014; White et al., 2011; Lau et al., 2006) and in a multigeneration rat study at doses of 30 mg/kg/day (Butenhoff et al., 2004a). In some studies, decreased fetal and/or pup body weight was observed in the absence of maternal body weight effects.

3.4.4.2.4.1 Mice, Prenatal Evaluations

Blake et al. (2020) reported significantly decreased GD 17.5 fetal weight with 5 mg/kg/day PFOA following gestational exposure in CD-1 mice, despite significantly increased maternal body weight gain. Lau et al. (2006) reported a significant decrease in GD 18 fetal body weights after gestational exposure of CD-1 mice to 20 mg/kg/day PFOA. In pregnant Kunming mice, gestational exposure was associated with significantly decreased GD 18 fetal weights at 5–40 mg/kg/day (Li et al., 2018a). Suh et al. (2011) reported a significant decrease in GD 16 fetal weights at doses ≥10 mg/kg/day after exposure of pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD 11 to GD 16. Body weights of GD 18 ICR mouse fetuses were significantly decreased following gestational exposure to 5 or 10 mg/kg/day PFOA (Yahia et al., 2010).

3.4.4.2.4.2 Mice, Postnatal Evaluations

Wolf et al. (2007) reported that CD-1 mouse pup body weights were significantly decreased after gestational exposure to 5 mg/kg/day PFOA from GD 1 to GD 17. The authors also exposed pregnant mice to 20 mg/kg/day from GD 15 to GD 17 and to 5 mg/kg/day for different lengths of time (GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17). After exposure to 5 mg/kg/day from GD 7 to GD 17 or GD 10 to GD 17 and to 20 mg/kg/day from GD 15 to GD 17, male pup body weights were significantly decreased. Additionally, with 5 mg/kg/day PFOA, male and female pup body weights were significantly decreased throughout lactation in all exposure groups, and the magnitude of the effect increased with increasing number of exposure days. Body weight deficits in male pups that had been exposed from GD 7 to GD 17 or GD 10 to GD 17 persisted for 10–11 weeks.

Hu et al. (2010) exposed C57BL/6N pregnant mice with 0.5 or 1.0 mg/kg/day PFOA in drinking water from GD 6 through GD 17. At PND 2, litter weights were significantly reduced in the PFOA treatment groups (7%—12% less than the controls). At PND 7 and 14, the 0.5 mg/kg/day group litter weight was equivalent to the controls, but the 1.0 mg/kg/day group was still significantly less than the controls (14% and 5%, respectively, by time point).

Body weights of live pups born to pregnant ICR mice dosed with 5 or 10 mg/kg/day during gestation were significantly reduced (Yahia et al., 2010). At ≥3 mg/kg/day, a dose-related trend in growth retardation (body weight reductions of 25%–30%) was observed in neonates at weaning; body weights reached control levels by 6 weeks of age for females and by 13 weeks of age for males (Lau et al., 2006). Exposure of pregnant C57BL/6N mice to 2 mg/kg/day from mating through lactation resulted in significantly decreased pup weights (32.6% lower than controls, on average) from PND 1 to PND 21 (there were no effects on maternal body weights) (Hu et al., 2012). Song et al. (2018) observed significantly increased body weights in PND 21 male offspring after gestational exposure to 2.5 or 5 mg/kg/day PFOA (female data not provided). However, the authors did not report controlling for litter size in this study; the significantly decreased litter size in the 5 mg/kg/day group could potentially result in increased body weight in those pups due to reduced competition for maternal resources.

In a study in which pregnant 129S1/SvImJ wild-type and PPARα-null mice were orally exposed from GD 1 to GD 17 to dose levels ranging from 0.1 to 20 mg/kg/day (Abbott et al., 2007), decreased offspring body weight was seen in wild-type mice at 1 mg/kg/day (highest dose level at which this effect was measured due to extensive litter loss at higher doses) beginning around

PND 6, and this effect achieved statistical significance on PND 9, PND 10, and PND 22 (males) and PND 7–PND 10 and PND 22 (females). No effects were observed on PPARα-null offspring body weights. White et al. (2011) exposed pregnant CD-1 mice to 0, 1, or 5 mg/kg/day from GD 1 to GD 17. A separate group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD 1 to GD 17 and received drinking water containing 5 ppb PFOA beginning on GD 7. F_1 females and F_2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F_1 breeding and early gestation, to simulate a chronic low-dose exposure. F_1 offspring body weight at PND 42 was significantly reduced at 5 mg/kg/day; at PND 63, body weight was significantly reduced for offspring from dams given 1 mg/kg/day plus 5 ppb in the drinking water compared with offspring from dams given only 1 mg/kg/day. For the F_2 pups, a significant reduction in body weight was observed in control plus 5 ppb drinking water PFOA offspring on PND 1, but there was no difference by PND 3. F_2 offspring from the 1 mg/kg/day and 1 mg/kg/day plus 5 ppb drinking water PFOA groups had increased body weights compared with controls on PND 14, PND 17, and PND 22. Female CD-1 mice that had been exposed gestationally to 1 mg/kg/day had significantly decreased "net" body weights (i.e., absolute body weight minus absolute liver weight) at PND 21 and PND 35 but not at PND 56 (Tucker et al., 2014); the absolute body weights of female offspring were not altered due to gestational PFOA treatment. Macon et al. (2011) found no effects on offspring body weights following exposure of pregnant CD-1 mice to PFOA from GD 1 to GD 17 with doses up to 1 mg/kg/day or from GD 10 to GD 17 with doses up to 3 mg/kg/day. Similarly, Cope et al. (2021) exposed CD-1 dams to 0.1 or 1.0 mg/kg/day PFOA via oral gavage from GD 1.5 to GD 17.5 and did not find treatment-related changes in pup weight at PND 0.5, PND 5, or PND 22.

3.4.4.2.4.3 Rats, Postnatal Evaluations

In two NTP 2-year carcinogenicity studies (NTP, 2020), dietary exposure of pregnant Sprague-Dawley rats to 300 ppm PFOA (approximately 22 mg/kg/day during gestation and 45 mg/kg/day from LD 1 to LD 14) resulted in significantly decreased pup weights throughout lactation (3%–8% lower than controls). In both studies, there were minimal to no effects on maternal body weight.

Significantly decreased F_1 pup weight (8%–11% lower than controls) during lactation was observed following exposure of pregnant Sprague-Dawley rats to 30 mg/kg/day, in the absence of effects on maternal body weight; F_2 pup weight was slightly decreased at 30 mg/kg/day, but the effect was not statistically significant (Butenhoff et al., 2004a). At 30 mg/kg/day, significant decreases in body weight and body weight gain were seen in F_1 male offspring during the juvenile and peripubertal phases and in F_1 female offspring beginning on day 8 postweaning and continuing through pre-cohabitation, gestation, and lactation (along with decreased food consumption) (Figure 3-71).

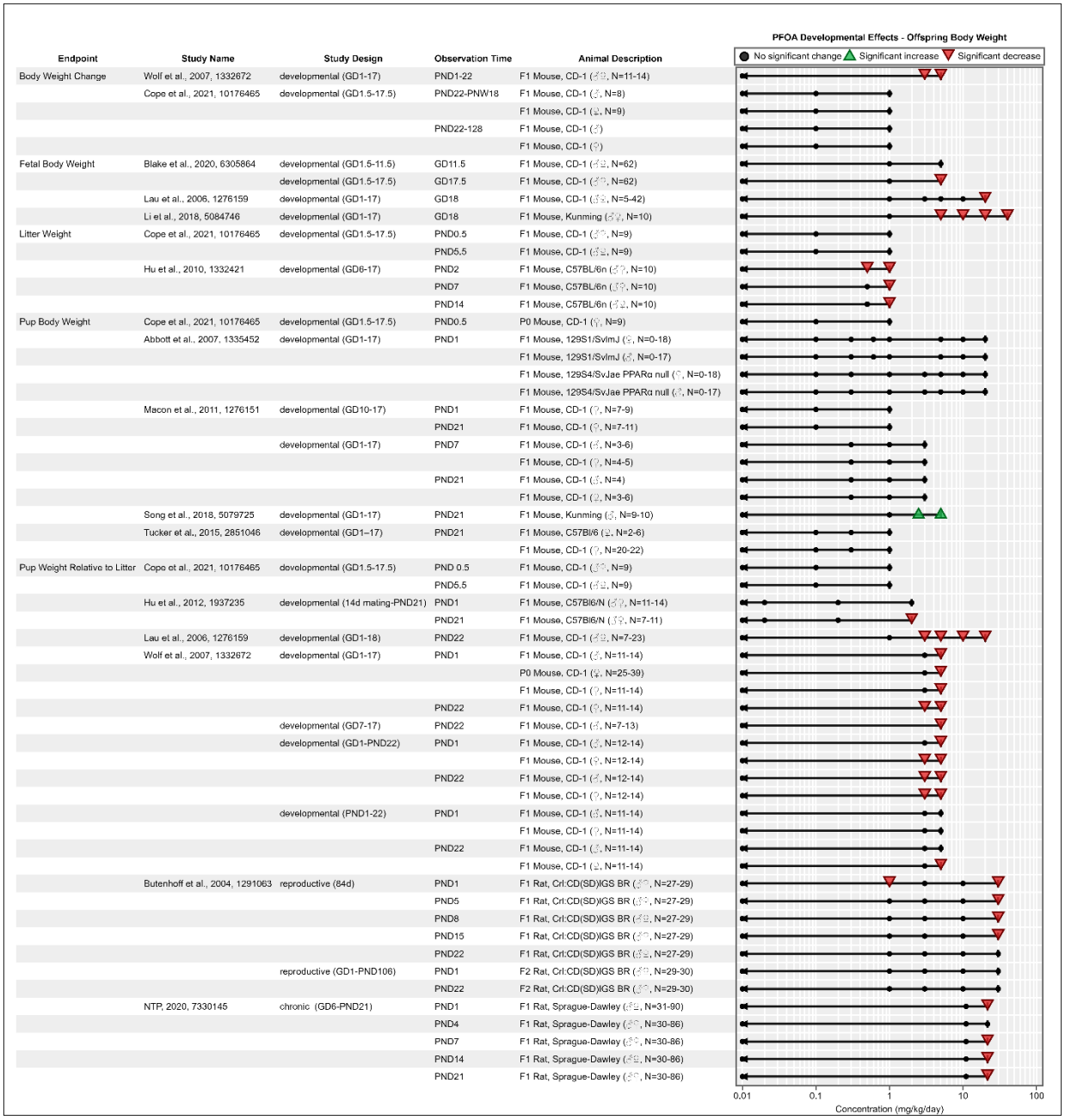


Figure 3-71. Offspring Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)[a]

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.

GD = gestation day; PND = postnatal day; P_0 = parental generation; F_1 = first generation; F_2 = second generation; d = day.

[a] Lau et al. (2006) exposed pregnant mice from GD 1 to GD 19, but some of the mice were sacrificed and examined on GD 18. Based on data from the pregnant mice sacrificed on GD 18, all litters from dams administered 40 mg/kg/day were resorbed, and therefore no offspring were available for postnatal assessments.

3.4.4.2.5 Skeletal and Visceral Alterations

Following exposure of pregnant CD-1 mice to 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA during gestation, Lau et al. (2006) reported decreases in ossification of the forelimb proximal phalanges (significant at all dose levels except 5 mg/kg/day), hindlimb proximal phalanges (significant at all dose levels except 3 and 5 mg/kg/day), calvaria (significant at 1, 3, and 20 mg/kg/day), enlarged fontanel (significant at 1, 3, and 20 mg/kg/day), and supraoccipital bone (significant at 10 and 20 mg/kg/day). Significantly reduced ossification of caudal vertebrae, metacarpals, metatarsals, and hyoid was observed at 20 mg/kg/day. Significant increases in minor limb and/or tail defects were observed in fetuses at ≥5 mg/kg/day (no defects were observed at 0, 1, or 3 mg/kg/day) and significantly increased incidence of microcardia was observed at 10 and 20 mg/kg/day (no incidences were observed in any other groups). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD 0 to GD 17 (sacrificed on GD 18) and reported a significant increase in the incidence of cleft sternum and ossification delays (phalanges) in GD 18 fetuses at 10 mg/kg/day. In the same study, some dams were dosed from GD 0 to GD 18 and allowed to give birth, and pup lungs and brains were examined at PND 4; no abnormalities were reported.

3.4.4.2.6 Altered Developmental Timing

Reduced postnatal growth leading to developmental delays was observed in mice. Lau et al. (2006) and Wolf et al. (2007) reported delayed eye opening in CD-1 mice offspring after gestational exposure to ≥5 mg/kg/day PFOA. Additionally, Wolf et al. (2007) observed delayed eye opening following gestational plus lactational exposure to 3 or 5 mg/kg/day. Wolf et al. (2007) also observed delayed body hair emergence following gestational exposure to 5 mg/kg/day or gestational plus lactational exposure to 3 or 5 mg/kg/day. In pregnant 129S1/SvImJ wild-type and PPARα-null mice orally exposed from GD 1 to GD 17 to 0.1–20 mg/kg/day PFOA (Abbott et al., 2007), offspring born to wild-type dams showed a dose-related trend for delayed eye opening compared with controls at 0.6 and 1 mg/kg/day (significant at 1 mg/kg/day; however, extensive litter loss was observed at the higher doses). In PPARα-null offspring, none of the litters from dams exposed to 3 mg/kg/day had eyes open on PND 13, but no significant difference between this group and the control was observed by PND 14. Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day PFOA from GD 0 to GD 17 (sacrificed on GD 18) and reported a significant decrease in the percentage of GD 18 fetuses with erupted incisors at 10 mg/kg/day.

3.4.4.2.7 Mammary Gland Development

Altered mammary gland development has been shown to result in later-life functional reproductive consequences, such as reduced lactational efficacy and subsequent pup loss, and has been linked to increased incidence of mammary and breast cancers (Macon and Fenton, 2013; Fenton, 2006; Birnbaum and Fenton, 2003). Studies examining effects of PFOA exposure on mammary gland development in CD-1 mice reported delayed mammary gland development at dose levels as low as 0.01 mg/kg/day (Tucker et al., 2014; Macon et al., 2011). However, no differences in response to a lactation challenge were seen in PFOA-exposed CD-1 mouse dams with delayed mammary gland development, and no significant effects on body weight gain were seen in pups nursing from dams with less fully developed mammary glands (White et al., 2011).

Macon et al. (2011) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17 (full gestation) or GD 10 to GD 17 (late gestation) to examine effects of PFOA exposure on mammary gland morphology. Mammary gland whole mounts were scored on a 1 to 4 subjective, age-adjusted, developmental scale. Quantitative measures also were made of longitudinal growth, lateral growth, and number of terminal end buds. At all PFOA exposure levels in both experiments (≥0.3 mg/kg/day in the full gestation study and ≥0.01 mg/kg/day in the late-gestation study), significantly stunted mammary epithelial growth was observed in female offspring in the absence of effects on offspring body weight. Additionally, there were significant differences from controls in quantitative measures of longitudinal and lateral growth and numbers of terminal end buds at 1 mg/kg/day in the late-gestation experiment. The delayed development was characterized by reduced epithelial growth and the presence of numerous terminal end buds. Photographs of the mammary gland whole mounts at PND 21 and PND 84 from the full-gestation experiment showed differences in the duct development and branching pattern of offspring from dams given 0.3 and 1 mg/kg/day PFOA (offspring from high-dose dams not pictured). At PND 21, mammary glands from the 1 mg/kg/day late-gestation group had significantly less longitudinal epithelial growth and fewer terminal end buds compared with controls. In the late-gestation experiment, mammary gland development was delayed by exposure to PFOA, especially longitudinal epithelial growth. At PND 21, all treatment groups had significantly lower developmental scores. At the highest dose, poor longitudinal epithelial growth and decreased number of terminal end buds were observed. The quantitative measures were statistically significant only for the high dose compared with the controls, whereas the qualitative scores at all doses were significantly different from controls.

CD-1 mice were dosed with 5 mg/kg/day on GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17 or with 20 mg/kg/day on GD 15–GD 17 (controls were dosed GD 7–GD 17) and mammary gland effects of this study were published by White et al. (2009). Mammary gland developmental scores for all offspring of dams exposed to PFOA were significantly lower at PND 29 and PND 32. Delayed ductal elongation and branching and delayed appearance of terminal end buds were characteristic of delayed mammary gland development at PND 32. At 18 months of age, mammary tissues were not scored (due to the lack of a protocol applicable to mature animals) but dark foci (composition unknown) in the mammary tissue were observed at a higher frequency in exposed animals compared with controls. There was no consistent response with respect to dosing interval. Qualitatively, mammary glands from treated dams on LD 1 appeared immature compared with control dams (White et al., 2009). The authors also exposed pregnant CD-1 mice to 0, 3, or 5 mg/kg/day from GD 1 to GD 17 and offspring were cross-fostered at birth to create seven treatment groups: control, in utero exposure only (3U and 5U), lactational exposure only (3L and 5L), and in utero + lactational exposure (3U + L and 5U + L). Mammary gland whole mounts from female offspring between PND 22 and PND 63 were scored. With the exception of females of the 3L group, all female offspring of PFOA-exposed dams had reduced mammary gland developmental scores at PND 22. At PND 42, mammary gland scores from females in the 3U + L group were the only ones not statistically different from control scores. This might have been due to inter-individual variance and multiple criteria used to calculate mammary gland development scores. All offspring of dams exposed to PFOA exhibited delayed mammary gland development at PND 63, including those exposed only through lactation (3L and 5L).

White et al. (2011) dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD 1 to GD 17. A second group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD 1 to GD 17 and also received drinking water containing 5 ppb PFOA beginning on GD 7. The F_1 females and F_2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F_1 breeding and early gestation, to simulate a chronic low-dose exposure. Only the P_0 dams were given PFOA by gavage. P_0 females were sacrificed on PND 22. F_1 offspring were weaned on PND 22 and bred at 7–8 weeks of age. F_2 litters were maintained through PND 63. Groups of F_1 and F_2 offspring were sacrificed on PND 22, PND 42, and PND 63. A group of F_2 offspring was also sacrificed on PND 10. A lactational challenge experiment was performed on PND 10 with F_1 dams and F_2 offspring to estimate the volume of milk produced during a discrete period of nursing. Mammary glands were evaluated from P_0 dams on PND 22, from F_1 dams on PND 10 and PND 22, and from F_1 and F_2 female offspring on PND 10 (F_2 only), PND 22, PND 42, and PND 63. Mammary gland whole mounts were scored qualitatively. At PND 22, control P_0 dams displayed weaning-induced mammary involution. At PND 22, the mammary glands of all PFOA-exposed P_0 dams, including the dams receiving 5 ppb PFOA via drinking water only, resembled glands of mice at or near the peak of lactation (~PND 10). The F_1 dams examined on PND 10 and PND 22 had significantly lower developmental scores on PND 10, but that was no longer evident at PND 22, except for those exposed in utero to 5 mg/kg/day. In the F_1 female offspring not used for breeding, the mammary glands of all PFOA-exposed mice were significantly delayed in development on PND 22, 42, and 63. For the F_2 female offspring, some differences in mammary gland scores were observed between the groups, but most were not significantly different from controls. No differences in response to a lactational challenge were seen in PFOA-exposed dams with morphologically delayed mammary gland development.

Tucker et al. (2014) orally exposed pregnant CD-1 and C57BL/6 mice to 0, 0.01, 0.1, 0.3, or 1 mg/kg/day from GD 1 to GD 17. After parturition, the number of pups was reduced so that there were ultimately four to eight CD-1 litters and three to seven C57BL/6 litters per treatment. Different treatment blocks monitored for different endpoints at different times. There was a dose-related trend toward decreasing mammary gland developmental scores for both strains of mice. In CD-1 mice, scores were significantly reduced at PFOA doses ≥0.01 mg/kg/day on PND 35 and ≥0.1 mg/kg/day on PND 21. In C57BL/6 mice, scores were significantly reduced at 0.3 and 1.0 mg/kg/day on PND 21. The authors suggest that these differences in responses between strains may be due to increased serum PFOA levels of the CD-1 mice (Tucker et al., 2014). At 5 mg/kg/day, in mammary glands of C57BL/6 mice, there was a significant increase in the number of terminal end buds and stimulated terminal ducts; ductal length was not affected. Mammary gland development was inhibited in C57BL/6 mice dosed with 10 mg/kg/day, with no terminal end buds or stimulated terminal ducts present and very little ductal growth.

In a study of direct peripubertal exposure, Yang et al. (2009a) orally dosed 21-day-old female BALB/c or C57BL/6 mice with 0, 1, 5, or 10 mg/kg/day PFOA for 5 days/week for 4 weeks. Mammary glands of BALB/c mice treated with 5 or 10 mg/kg/day had reduced ductal length, decreased number of terminal end buds, and decreased stimulated terminal ducts; injection with bromo-2′-deoxyuridine, a marker of cell proliferation, into the mammary gland revealed a significantly lower number of proliferating cells in the ducts and terminal end buds/terminal ducts at 5 mg/kg/day (not examined at 10 mg/kg/day).

3.4.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse developmental outcomes is discussed in Sections 3.2.6, 3.2.7, 3.3.4, 3.4.1, and 3.4.5 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 19 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to developmental effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-72.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	0	6	1	7
Cell Growth, Differentiation, Proliferation, Or Viability	5	1	2	8
Cell Signaling Or Signal Transduction	2	1	0	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	0	1	5
Hormone Function	2	0	0	2
Inflammation And Immune Response	0	1	0	1
Oxidative Stress	2	1	0	3
Xenobiotic Metabolism	3	0	1	4
Other	0	0	1	1
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	8	7	4	19

Figure 3-72. Summary of Mechanistic Studies of PFOA and Developmental Effects

Interactive figure and additional study details available on HAWC.

Mechanistic data available from in vitro, in vivo, and epidemiological studies were evaluated to inform the mode of action of developmental effects of PFOA. The mechanistic data are organized by the following outcomes: early survival, general development, and gross morphology; fetal growth and placental effects; metabolism; hepatic development; cardiac development; and neurological development.

3.4.4.3.1 Early Survival, General Development, Gross Morphology

Mechanisms through which PFOA exposure may alter survival and development were studied in several in vivo experimental animal models. In an in vivo mouse developmental study, pregnant NMRI dams exposed to PFOA from GD 5 to GD 9 via intraperitoneal (IP) injection showed increased fetal death in the offspring at the highest dose (20 mg/kg/day) of PFOA, as well as histopathological abnormalities in the brain, liver, and heart, possibly due to the observed

mitochondrial toxicity/dysfunction (e.g., increased mitochondrial swelling, increased mitochondrial membrane potential (MMP) collapse) or oxidative stress (e.g., increased mitochondrial ROS formation) (Salimi et al., 2019). In another mouse developmental study examining lower doses in the dams, embryo survival was not affected at up to 10 mg/kg/day PFOA exposure in dams exposed from GD 1.5 to GD 11.5 or GD 1.5 to 17.5 via oral gavage (Blake et al., 2020). However, 5 and 10 mg/kg exposure via oral gavage from GD 1 GD 17 decreased survival rate in 5-day old pups, possibly due to hepatotoxicity; the authors observed significantly increased liver index in pups and increased reactive oxygen species and changes in liver enzyme function, mediated by the PPARα pathway (Li et al., 2019b).

Several studies using zebrafish as a model organism that were identified in the current assessment were included in a recent review of developmental effects of PFOA (Lee et al., 2020). In general, PFOA exposure was associated with developmental delays, reductions in measures of embryo survival, and increased malformations in the head and tail that may be related to perturbations in gene expression during critical windows of organism development.

The review by Lee et al. (2020) included a zebrafish multigenerational study by Jantzen et al. (2017), in which embryos were exposed to PFOA from 3 to 120 hours post-fertilization (hpf). Embryos were allowed to reach adulthood and breed. Although exposure to PFOA did not decrease survival in the first exposed generation (P_0), there were significantly fewer eggs and viable embryos than the controls in the P_0. Further, F_1 embryos had significant developmental delays and delayed hatching. Gene expression analysis of four solute carrier organic anion transporter family members (*slco1d1*, *slco2b1*, *slco3a1*, and *slco4a1*) and the growth factor transforming growth factor beta 1a (*tgfb1a)* in the P_0 generation showed that PFOA exposure led to decreased expression in *slco2b1*, *slco3a1*, and *slco4a1* and increased expression in *slco1d1*. In the F_1 embryos, there was a significant increase in expression of the protein transporter adaptor related protein complex 1 subunit sigma 1 (*ap1s1).* The authors concluded that alterations in the expression of these genes during development likely contributed to the delayed development and morphologic and toxic effects observed (Jantzen et al., 2017). The elevations in *ap1s1* were in conflict with a prior publication from the same research group that reported decreased *ap1s1* at 120 hpf, which coincided with alterations in morphometric parameters in zebrafish embryos, including increased interocular distance (a metric of cranio-facial development), reduced total body length, and reduced yolk sac area (Jantzen et al., 2016a). Other alterations in gene expression at 120 hpf included elevations in *slco2b1* (transport protein) and transcription factor 3a (*tfc3a;* involved in muscle development), and *c-fos* (transcription factor complex). Altogether, results suggest that alterations in *ap1s1* are unlikely the result of a global upregulation or downregulation of genes and that PFOA may differentially influence genes at certain points in development. However, the current data cannot rule out the possibility that the observed alterations in gene expression are due to a delay or acceleration in development.

In another zebrafish study by Bouwmeester et al. (2016), embryos that were exposed to 10–320 μM PFOA were examined for developmental toxicity and morphological effects. PFOA did not induce embryotoxic effects at the exposure levels in the experiment; however, some epigenome modifications were noted. When locus-specific methylation was assessed, PFOA exposure was associated with hypomethylation on the CpG region of *vasa*, and hypermethylation at CpG1 in *vitellogenin 1* (*vtg1). Vasa* is expressed in the germline and is active during development, and *vtg1* is expressed in the liver of egg-laying vertebrates and encodes for the

estrogen responsive egg-yolk protein vitellogenin, although, interestingly, PFOA was included in this study to demonstrate a "non-estrogenic PPARγ/RXR agonist." These epigenetic modifications early in life and development may play a role in the development of later life adverse health outcomes (Bouwmeester et al., 2016).

In humans, epigenetic modification during development of the fetus can be measured via cord blood at birth. Several human studies evaluated cord blood DNA methylation patterns to understand the epigenetic effects of PFOA exposure. Miura et al. (2018) found that increased PFOA in the cord blood was associated with global hypermethylation in a cohort from Japan; however, two other cord blood studies of global methylation found no associations between PFOA exposure and global methylation changes (Leung et al., 2018; Liu et al., 2018a). Similarly, Kingsley et al. (2017) did not observe associations between PFOA exposure in cord blood and epigenome-wide changes in global methylation status. However, for the high PFOA exposure group, the authors found hypomethylation in seven CpG sites located in several genes, including *RAS P21 protein Activator 3 (RASA3)* and Opioid Receptor Delta 1 *(OPRD1). OPRD1* is involved in weight and obesity, as well as morphine and heroin dependence, and could potentially be a mechanistic pathway linking PFOA and obesity, an association that has previously been reported (Kingsley et al., 2017). Cord blood samples from a prospective cohort in China were used by Liu et al. (2018c) to evaluate potential associations between PFOA exposure and leukocyte telomere lengths (LTLs). There was no association between PFOA exposure and LTLs in this study.

3.4.4.3.2 Fetal Growth and Placental Effects

Fetal growth was assessed in four mouse developmental studies. Blake et al. (2020) found decreased embryonic weights in CD-1 mice at GD 17.5, with concurrent increases in placental weights and placental lesions consistent with labyrinth congestion (Section 3.4.4.2.4.1). Placentas also had higher thyroxine (T4) levels relative to controls, suggesting a possible endocrine mechanistic pathway of effect. In NMRI mice exposed to 0, 1, 10, or 20 mg/kg/day PFOA from GD 5 to 9, Salimi et al. (2019) observed reduced fetal length and weight, and decreased placental diameter at the highest dose group (20 mg/kg/day). The authors note that toxicity was likely mediated through mitochondrial toxicity in the liver (described below), which appeared to be isolated to the mouse fetus rather than the placenta. Li et al. (2019b) reported a dose-dependent reduction in growth and weight gain in Kunming mouse pups exposed to PFOA during gestation (GD 0–17). The authors attribute the stunted growth to hepatotoxicity consequent to increased ROS and changes in liver enzyme function mediated by the PPARα pathway (Li et al., 2019b).

Perturbations in growth and corresponding changes in gene expression of key developmental genes have been observed in several studies in zebrafish. In the multigenerational zebrafish study by Jantzen et al. (2017), P_0 generation fish exposed to PFOA had significantly shorter body length and reduced body weight compared with controls. Offspring of PFOA-exposed fish were significantly developmentally delayed and had increased expression in the protein transport gene *ap1s1* at 48 hpf, possibly leading to the changes in growth (Jantzen et al., 2017). In Jantzen et al. (2016a), several morphometric endpoints were measured in zebrafish embryos exposed to 0.02, 0.2, or 2.0 μM PFOA, including interocular distance, total body length, and yolk sac area. The size of all three parameters was reduced in groups exposed to PFOA, indicating slowed embryonic development) at values 5- to 25-fold below previously calculated median lethal

concentration (LC_{50}) values. The authors also evaluated gene expression at 120 hpf and 14 days post-fertilization (dpf). At 120 hpf, *slco2b1* (transport protein), *tfc3a* (involved in muscle development), and *c-fos* (transcription factor complex) were upregulated, while *ap1s* (involved in protein transport) was downregulated. At 14 dpf, *slco2b1* and *Tcf3a* (involved in muscle development) were upregulated (Jantzen et al., 2016a).

Gorrochategui et al. (2014) evaluated cytotoxicity and aromatase activity in a placental cell line (JEG-3 cells). PFOA exposure was found to induce cytotoxicity and inhibit aromatase (CYP19) activity (Gorrochategui et al., 2014). In a rhesus monkey trophoblast cell line, PFOA treatment showed significant differences in gene expression, with possible affected diseases/biological functions including cell movement, epithelial tissue growth, and vasculogenesis. Pathways included cysteine metabolism, interleukin signaling, Toll-like receptor, TGF-β, PDGF, PPAR, NFKB, MAPK, Endothelin 1, TNRF2, tight junctions, cytokines including IFNY and IFNα, and possible FOS signaling (Midic et al., 2018). A result from the Kingsley et al (2017) study in human cord blood mentioned above was methylation changes to the *RASA3* gene associated with exposure to PFOA (high exposure group, which could result in impaired cell growth and differentiation, contributing to reduced fetal growth and birth weight.

Lastly, a longitudinal study by Ouidir et al. (2020) examined global methylation in the placenta at birth in women for whom PFOA levels in the plasma were determined in the first trimester. The authors did not find any associations between PFOA exposure and DNA methylation status of the placenta (Ouidir et al., 2020).

3.4.4.3.3 Metabolism

van Esterik et al. (2015) examined metabolic effects of developmental exposure to 3–3,000 µg/kg PFOA exposure in C57BL/6JxFVB hybrid mice. The authors found that PFOA exposure during gestation and lactation resulted in reduction in weight that persisted to adulthood. The weight loss was attenuated by a high-fat diet (from 21—25 days) in males, but not females, suggesting that the weight reductions were mediated through metabolic mechanisms that may exhibit a female bias. There were no significant changes in metabolic parameters (i.e., glucose homeostasis, basal glucose, energy expenditure, uncoupling protein 1 (*ucp1;* also known as *thermogenin*) expression in brown adipose tissue) in either sex. However, in females, cholesterol and triglycerides showed a dose-dependent decrease. The authors suggest that these changes in lipid metabolism could be mediated by PPARα activation (van Esterik et al., 2015). Li et al. (2019b) examined PPARα activation pathways as a mechanism of PFOA-induced liver and metabolic toxicity during development in mice. The authors found that female mice exposed gestationally to PFOA had significantly downregulated gene expression of *PPARα* in the 2.5 and 5 mg/kg/day groups, but not the highest dose group (i.e., 10 mg/kg/day). PFOA exposure also increased gene expressions of *Acot1* and *Acox1* (downstream regulatory genes of PPARα), indicating that early PFOA exposure causes lasting changes in the PPARα pathway. PPARα regulates fatty acid oxidative metabolism and energy consumption, through peroxisome and mitochondrial β-oxidation and microsome ω-oxidation (Li et al., 2019b). PFOA has been described as a weak PPARα ligand, but the role of PPARα in mediating the developmental toxicity associated with PFOA exposure is not yet clear (Peraza et al., 2006).

Metabolomic profiles in relation to PFOA exposure were analyzed in a human study. In a cross-sectional study in 8-year-old children in Cincinnati, OH, the authors conducted untargeted, high-

resolution metabolomic profiling in relation to serum PFOA concentrations. They found that PFOA exposure was associated with several lipid and amino acid metabolism pathways, including that of arginine, proline, aspartate, asparagine, and butanoate (Kingsley et al., 2019).

3.4.4.3.4 Hepatic Development

Three developmental mouse studies examined the effect of PFOA on liver development and function. van Esterik et al. (2015) found that developmental exposure to PFOA resulted in increased liver weights and abnormal liver histopathology, with toxicity possibly mediated through the PPARα pathway. Salimi et al. (2019) exposed pregnant mice to PFOA from GD 5 to 9 and observed mitochondrial disruption in the fetal liver, including mitochondrial swelling and mitochondrial membrane potential collapse. These effects significantly increased at the highest (20 mg/kg/day) exposure group. Measures of oxidative stress (hydrogen peroxide production) in the liver were also significantly higher in groups exposed to 10 or 20 mg/kg/day PFOA in comparison to control animals. Li et al. (2019b) hypothesized that PFOA accumulation in pup liver may promote oxidative stress via PPARα activation pathways that contribute to liver and metabolic toxicity in mice. The authors found that female mice exposed gestationally to PFOA had increased liver weight and dose-responsive morphological changes in the liver including swollen hepatocytes, blurred architecture, and vacuolar degeneration. Liver enzymes (AST and ALT) were increased in the serum, and oxidative stress biomarkers (Catalase (CAT), Superoxide dismutase (SOD), and 8-OHdG) were increased. Liver histone acetyltransferase (HAT) activity was reduced, and histone deacetylase (HDAC) activity was increased. Further, histone acetylation in the liver was reduced. These effects suggest that PFOA can alter the epigenetic regulation of liver responses which may contribute to adverse hepatic health outcomes (Section 3.4.1).

3.4.4.3.5 Cardiac Development

Data from one study in mice, one study in zebrafish, and one in vitro study provide insight into the mechanism by which PFOA perturbs cardiac development. In a recent review that covered PFOA toxicity in zebrafish, Lee et al. (2020) reported that PFOA exposure has been consistently associated with increases in pericardial edema and altered heart rates at various stages of development in embryos. An in vivo mouse developmental study by Salimi et al. (2019) also found that PFOA exposure was associated with cardiotoxicity in offspring. In this study, pregnant dams were treated with PFOA, and fetuses were studied for tissue abnormalities. Groups treated with PFOA showed increased histopathological abnormalities in the fetal heart, including hepatomegaly. Mitochondrial swelling in mitochondrial suspension of fetal heart tissue was also observed along with increased mitochondrial membrane potential collapse. Measures of oxidative stress in the fetal heart were also significantly higher in exposed versus control animals (Salimi et al., 2019). An in vitro experiment by Zhou et al. (2017a) examined the ability of mouse embryonic stem cells to differentiate into myocardiocytes following exposure to 2.5, 5, 10, 20, 40, 80, or 160 μg/mL PFOA. Differentiation was determined by the contractility (i.e., contract rate) of the cells, as well as the upregulation of *myh6*, which is a regulatory gene that is essential for cardiac muscle development. No effects on differentiation or *myh6* expression were observed below 20 μg/mL.

3.4.4.3.6 Neurological Development

Salimi et al. (2019) also reported teratogenic effects in the brain of fetal mice following maternal exposures up to 20 mg/kg/day PFOA via IP injection from GD 5 to 9. The histopathological abnormalities in the brain included anencephaly, microcephaly, and hydrocephaly, all at the highest (20 mg/kg/day) exposure. Mitochondrial swelling in mitochondrial suspension of fetal brain tissue was also observed along with increased mitochondrial membrane potential collapse. Higher mitochondrial disruption was observed at lower concentrations in the brain tissue than other fetal tissues (i.e., heart and liver), suggesting that the brain was more susceptible to mitochondrial toxicity/dysfunction. Measures of oxidative stress in the brain were also significantly higher in exposed animals in comparison to controls.

The effects of PFOA on neurodevelopment and behavior in zebrafish were examined in two studies. In the aforementioned zebrafish embryo assay by Jantzen et al. (2016a), embryonic exposure to 0.02, 0.2, or 2.0 micromolar (μM) PFOA during the first five dpf resulted in hyperactive locomotor activity in larvae as evidenced by increased swimming velocity, possibly mediated through altered expression of development-associated genes (*c-fos, tfc3a*, *slco2b1*, and *ap1s*). Stengel et al. (2018) developed a neurodevelopmental toxicity test battery using zebrafish embryos. PFOA did not produce any changes in acetylcholinesterase (AChE) inhibition, nor the neuromast assay, olfactory, or retinal toxicity assays (Stengel et al., 2018).

3.4.4.3.7 Conclusion

In the context of the available mechanistic studies, it appears that several mechanisms may be involved in PFOA-driven developmental toxicity. In general, the observed effects suggest that the developing liver, developing heart, and placenta may be affected by PFOA at the molecular level (e.g., differential methylation of genes, gene expression changes), which may be reflected in developmental health effects described in Section 3.4.4. The effects tend to vary by sex and developmental timepoint of outcome evaluation. More research is needed to strengthen the association between PFOA exposure to any one of the several possible contributing factors, including fluctuations in transporter gene expression, epigenetic changes, oxidative stress, and PPARα pathway activation, particularly in the placenta.

3.4.4.4 Evidence Integration

The evidence of an association between PFOA and developmental effects in humans is *moderate* based on the recent epidemiological literature. As noted in the fetal growth restriction summary, there is evidence that PFOA may impact fetal growth restriction across a variety of BWT-related measures. Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catch-up periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT. Collectively, the majority of LBW studies were supportive of an increased risk with increasing PFOA exposures. Five *medium* or *high* confidence studies on LBW showed increased risks with increased PFOA levels. Several meta-analyses also support evidence of associations between maternal or cord blood serum PFOA and BWT or BWT-related measures (Steenland et al., 2018a; Negri et al., 2017; Verner et al., 2015; Johnson et al., 2014) (see Appendix A, (U.S. EPA, 2024a)).

Overall, there was mixed evidence of inverse associations between PFOA and both gestational age (7 of the 18 studies) and preterm birth (6 of 11 studies). Most of the associations for either of these gestational duration measures were reported in *medium* or *high* confidence studies. For example, five of six studies were increased odds of PTB were *high* confidence. Few other patterns were evident that explained any between study heterogeneity. For example, five of the null studies were rated as having adequate sensitivity, and one was rated deficient. There was a preponderance of associations related to sample timing possibly related to pregnancy hemodynamic influences on the PFOA biomarkers, as five of the seven studies reporting inverse associations were sampled later in pregnancy (i.e., trimester two onward).

There was less consistent evidence of PFOA impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available for other endpoints such as fetal loss and no evidence of associations in recent studies of PFOA and birth defects such as cryptorchidism or hypospadias. Similarly, there was less consistent evidence of an impact of PFOA exposure on gestational duration measures i.e., as many of studies did not show inverse associations for gestational age measures or for an increased risk of preterm birth.

However, as noted previously, considerable uncertainty remains as to what degree the evidence may be impacted by pregnancy hemodynamics factors related to sample timing may result in either confounding or reverse causality and explain some of the observed birth weight deficits (Steenland et al., 2018a). Additional uncertainty exists due to the potential for confounding by other PFAS, and considerations for potential confounding by co-occurring PFAS are described in Section 5.1. Very few of the existing studies performed multipollutant modeling in comparison with single-pollutant estimates of PFOA associations. The multipollutant modeling results were often mixed from single-pollutant estimates with some estimates increasing and some decreasing. Unlike other PFAS, PFOA was chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants adjusted for two different studies (Starling et al., 2017; Lenters et al., 2016). Although these results are smaller in magnitude, they appear coherent with single exposure model results. There is some concern that controlling for other highly correlated co-exposures in the same model may amplify the potential confounding bias of another co-exposure rather than removing it (Weisskopf et al., 2018). Given these interpretation difficulties and potential for this co-exposure amplification bias, it remains unclear whether certain mutually adjusted models give a more accurate representation of the independent effect of specific pollutants for complex PFAS mixture scenarios.

The animal evidence of an association between PFOA and developmental toxicity is *robust* based on 13 *high* or *medium* confidence animal toxicological studies, in concordance with the data in humans, supporting that the developing fetus is a target of PFOA toxicity. Specifically, several studies in rodents show decreased fetal and pup weight with gestational PFOA exposure, similar to the evidence of LBW seen in infants. Oral studies in rodents consistently show that gestational PFOA exposure results in pre- and postnatal effects on offspring, as well as maternal effects in dams. Notably, mice appear to be more sensitive to developmental toxicity as a result of gestational exposure compared with rats. In addition, studies in both rats and mice show that effects on offspring (e.g., decreases in body weight, survival) occur at lower dose levels than those that produce maternal body weight effects.

Evidence from mechanistic studies that relates to observed developmental effects of PFOA is limited. Decreased survival in the offspring of pregnant mice exposed to PFOA was potentially related to hepatotoxicity induced by PPARα activation, as discussed in detail in Section 3.4.1.3. In human cord blood samples, evidence of epigenetic alterations within genes that are involved in cell growth and differentiation and obesity was observed; however, these epigenetic alterations were not evaluated in the context of postnatal outcomes and are inconsistent; two other studies found no association between PFOA exposure and changes to the epigenome. In zebrafish studies, the expression of several genes that are related to growth and development (e.g., *tfc3a*, which is involved in muscle development) was altered by PFOA exposure, with variable magnitude and, in some cases, the direction of change according to the timepoint measured. Oxidative stress was observed in the developing brain and heart of mice exposed to PFOA in utero, suggesting toxicity of PFOA during development. Overall, the data demonstrate that PFOA may alter the expression of genes involved in growth and development, although additional studies in mammals are needed to confirm such. Additionally, evidence exists that PFOA can alter the epigenome, although the functional effects of the epigenetic effects are not clear.

3.4.4.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause developmental toxicity in humans under relevant exposure circumstances (Table 3-15). This conclusion is based primarily on evidence of decreased birth weight from epidemiologic studies in which PFOA was measured during pregnancy, primarily with median PFOA ranging from 1.1 to 5.2 ng/mL. The conclusion is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth, birth length), as well as consistent findings of dose-dependent decreases in fetal weight and other developmental effects observed in animal models gestationally exposed to PFOA at doses as low as 0.5 mg/kg/day. Although there is available mechanistic information that provides support for the biological plausibility of the phenotypic effects observed in exposed animals, the data are too limited to sufficiently support the human relevance of the animal findings.

Table 3-15. Evidence Profile Table for PFOA Exposure and Developmental Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.4.1)					⊕⊕⊙ *Evidence Indicates (likely)* *Primary basis and cross-stream coherence*: Evidence consisted of decreased birth weight from epidemiologic studies in which PFOA was measured during pregnancy. This is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth, birth length) and consistent findings of dose-dependent decreases in fetal weight observed in animal models gestationally exposed to PFOA. *Human relevance and other inferences:* Although there is available mechanistic information that provides support for the biological plausibility of the phenotypic effects observed in exposed animals, the data are too limited to sufficiently support the human relevance of the animal findings.
Fetal growth restriction 26 *High* confidence studies 25 *Medium* confidence studies 13 *Low* confidence studies 3 *Mixed* confidence studies	Some deficits in mean birth weight were observed in most studies (30/42) in the overall population. The majority of studies on changes in standardized birth weight measures reported inverse associations (10/18), with most (7/10) of these being *high* and *medium* confidence. Similarly, most studies (12/17) observed either an increased risk of low birth weight or SGA. Deficits in birth weight were supported by adverse findings for related FGR outcomes such as decreased birth length and head circumference in the overall population or across sexes.	• *High* and *medium* confidence studies • *Consistent* direction of effects for most outcomes • *Coherence* of findings across different measures of FGR	• *Limited* evidence of exposure-response relationships based on categorical data • *Potential bias* due to hemodynamic differences noted in studies using samples from later pregnancy	⊕⊕⊙ *Moderate* Epidemiological evidence for developmental effects is based on consistent adverse effects for FGR and post-natal growth. Consistent deficits in birth weight and standardized birth weight were observed in many *high* and *medium* confidence cohort studies. Birth weight findings were supported by adverse results reported for other measures of FGR, including birth length and head circumference, and adverse effects on gestational duration. Some uncertainties remain regarding the	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Gestational duration 13 *High* confidence studies 13 *Medium* confidence studies 7 *Low* confidence studies	In *medium* and *high* confidence studies, inverse effects were observed on gestational age (10/20). An increased risk of preterm birth was also observed in *medium* and *high* confidence studies (9/18).	• *High* and *medium* confidence studies	• *Potential bias* due to hemodynamic difference noted in studies using samples from later pregnancy	shape of the exposure-response relationship, and the potential impact of hemodynamics in later pregnancy due to use of biomonitoring samples from the second and third trimester or post-partum.	
Fetal Loss 2 *High* confidence studies 6 *Medium* confidence studies 1 *Low* confidence study	A significantly increased risk of fetal loss was reported in one *high* (1/2) and one *medium* (1/6) confidence study. The response in the *high* confidence study was monotonic across exposure quartiles. Other *medium* confidence studies (5/6) reported mixed results, differing by the exposure comparison. One study reported a	• *High* and *medium* confidence studies • *Good* or *adequate* sensitivity • *Consistent* magnitude of effect • *Exposure-response* relationship	• No factors noted		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	decreased risk of fetal loss, but the study was considered *low* confidence.				
Post-natal growth 6 *High* confidence studies 7 *Medium* confidence studies 3 *Low* confidence studies	Five *medium* and *high* confidence studies (5/11) reported inverse associations with infant weight and two studies (2/11) reported positive associations, while the remaining studies were mixed by sex or timepoint. Similarly, inverse associations with BMI were observed in five *medium* and *high* confidence studies (5/8),	• *High* and *medium* confidence studies • *Good* or *adequate sensitivity* for most studies	• *Inconsistent* timing of follow-up evaluation		
	and increased risk of rapid growth rate was observed in only one study (1/5). Two *medium* and *high* confidence studies (2/8) observed increased infant length or height and one study reported an inverse association, while				

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	other studies were null or mixed by sex.				
Birth Defects 4 *Medium* confidence studies 2 *Low* confidence studies	Two *low* confidence studies and two *medium* confidence studies reported mixed results for total or combined birth defects. No association with cryptorchidism was reported in one study; one study reported decreased odds of septal defects, conotruncal defects, and total congenital heart defects.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining individual defects		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.4.2)					
Maternal body weight 2 *High* confidence studies 6 *Medium* confidence studies	Many rodent studies observed a change in maternal body weight or weight gain following PFOA exposure (5/8). The direction of this change was not consistent among studies, with some rodent studies observing a decrease in weight (3/5), and some mouse studies	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects	⊕⊕⊕ *Robust* Evidence based on 13 *high* or *medium* confidence animal toxicological studies indicates that the developing fetus is a target of PFOA toxicity. Several studies in rodents show decreased fetal	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	observing an increase (2/5).			and pup weight with gestational PFOA exposure, similar to the evidence of FGR seen in human infants. Oral studies in rodents consistently show that gestational PFOA exposure results in pre- and postnatal effects on offspring, as well as maternal effects in dams. Notably, mice appear to be more sensitive to developmental toxicity as a result of gestational exposure compared with rats. In addition, studies in both rats and mice show that effects on offspring (e.g., decreases in body weight, survival) occur at lower dose levels than those that produced maternal body weight effects.	
Offspring body weight 2 *High* confidence studies 10 *Medium* confidence studies	Many rodent studies observed changes in fetal or pup body weight following PFOA exposure (9/12). Most of these show a decrease in offspring weight (8/9). One study observed an increase in offspring body weight, but only in male mice. Three mouse studies showed no change in offspring body weight (3/12).	• *High* and *medium* confidence studies • *Consistent direction* of effects	• No factors noted		
Offspring mortality 2 *High* confidence studies 7 *Medium* confidence studies	Many rodent studies observed increases in offspring mortality following PFOA exposure (6/9). A rat study observed increased post-weaning mortality in female pups but no pre-weaning mortality or change in stillborn pups. Five mouse studies found increased offspring mortality	• *High* and *medium* confidence studies • *Consistent* direction of effects	• No factors noted		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	including increased resorption (4/4), decreased live fetuses or live pups born (2/4), and decreased postnatal survival (2/3). Two studies found no change in offspring mortality or survival (2/8). No change in litter size was observed in any rat or mouse study (3/3).				
		•	•		
Placenta effects 2 *Medium* confidence studies	Two mouse studies noted a decrease in relative placenta weight following gestational PFOA exposure. In these studies, lesions on the placenta and	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	other histopathological changes were observed including changes to the labyrinth (e.g., atrophy, decreased area, congestion, necrosis) and early fibrin clot. Fewer placentas were determined to be within normal limits (1/1).				
Offspring liver weight 3 *Medium* confidence studies	Increases in offspring relative liver weight were noted in three mouse studies following gestational PFOA exposure (3/3).	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		
Developmental timing 2 *Medium* confidence studies	Delayed eye opening (2/2) and delayed body hair development (1/1) were observed in both sexes of mice.	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		
Structural abnormalities 1 *Medium* confidence study	One mouse study found structural abnormalities (e.g., reduced skeletal ossification) after developmental exposure to PFOA.	• *Medium* confidence study	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Mammary gland development 2 *Medium* confidence studies	Two mouse studies (2/2) found abnormal mammary gland development in animals exposed to PFOA during gestation (e.g., decreases in terminal end buds, mammary gland developmental score).	• *Medium* confidence study	• *Limited number* of studies examining outcomes		
Lactation index 2 *High* confidence studies	Of the two rat studies that evaluated lactation index, one noted a decrease following PFOA (1/2).	• *High* confidence studies	• *Limited number* of studies examining outcomes		

Mechanistic Evidence and Supplemental Information (Section 3.4.4.3)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
Key findings and interpretation: • Decreased survival in mice offspring exposed to PFOA in utero related to PPARα-related hepatotoxicity. • Alterations to the expression of genes related to growth and development in vivo in zebrafish. • Inconsistent results for PFOA-related alterations to DNA methylation in human cord blood. **Limitations:** • Very limited database. • The role of epigenetic mechanisms in changes at the mRNA level is not clear, nor is the relationship between molecular changes and apical developmental outcomes.	The limited evidence demonstrates that PFOA exposure during development can alter the epigenome and the expression of genes that control regular growth and development; it is possible that such changes are related,

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
				although the relationship has not been directly measured.	

Notes: DNA = deoxyribonucleic acid; FGR = fetal growth restriction; mRNA = messenger ribonucleic acid; PPARα = peroxisome proliferator-activated receptor alpha; SGA = small-for-gestational-age.

3.4.5 Evidence Synthesis and Integration for Other Noncancer Health Outcomes

Consistent with the SAB's recommendation (U.S. EPA, 2022e), EPA concluded that the noncancer health outcomes with the strongest evidence are hepatic, immune, cardiovascular, and developmental. For all other health outcomes (e.g., reproductive and endocrine), EPA concluded that the epidemiological and animal toxicological evidence available from the preliminary scoping considered in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* is either *suggestive* of associations or *inadequate* to determine associations between PFOA and the health effects described (U.S. EPA, 2021c). Based on this analysis, these outcomes were not prioritized for the subsequent literature search update efforts; the evidence synthesis and integration for these outcomes are presented in Appendix C (U.S. EPA, 2024a). In addition, Section 5.5 further describes rationale for evidence integration judgments for health outcomes which EPA determined had *evidence suggestive* of associations between PFOA and related adverse health effects, though the databases for those health outcomes shared some characteristics with the *evidence indicates* judgment.

3.5 Cancer Evidence Study Quality Evaluation, Synthesis, Mode of Action Analysis and Weight of Evidence

EPA identified 28 (29 publications[16]) epidemiological and 5 animal toxicological studies that investigated the association between PFOA and cancer. Of the epidemiological studies, 12 were classified as *medium* confidence, 12 as *low* confidence, 2 were considered *uninformative,* and 2 were *mixed* confidence (1 *medium*/*low* and 1 *low/uninformative* confidence) (Section 3.5.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 1 as *medium* confidence, and 2 as *low* confidence (Section 3.5.2). Though *low confidence* studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.5.1 Human Evidence Study Quality Evaluation and Synthesis

3.5.1.1 Introduction

There are 10 epidemiological studies (11 publications[17]) from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cancer effects. Study quality evaluations for these 10 studies are shown in Figure 3-73.

The 2016 PFOA HESD (U.S. EPA, 2016c) concluded there was suggestive evidence of carcinogenic effects of PFOA for kidney and testicular cancer, based on two C8 Health Project studies and two occupational cohorts (Figure 3-73). Specifically, two studies involving participants in the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 24 ng/mL) and kidney and testicular cancers (Barry et al., 2013; Vieira et al., 2013). There is some overlap in the cases included in these studies. As part of the C8 Health

[16] Ghisari, 2014, 2920449 analyzes interactions between gene polymorphisms and PFOA exposure on breast cancer risk in the same population analyzed in Bonefeld-Jørgensen, 2011, 2150988.
[17] Ghisari, 2014, 2920449 analyzes interactions between gene polymorphisms and PFOA exposure on breast cancer risk in the same population analyzed in Bonefeld-Jørgensen, 2011, 2150988.

Project, the C8 Science Panel (C8 Science Panel, 2012b) concluded that a probable link existed between PFOA exposure and testicular and kidney cancer. Two occupational cohorts in Minnesota and West Virginia (Raleigh et al., 2014; Steenland and Woskie, 2012) also examined cancer mortality. Raleigh et al. (2014) reported no evidence of elevated risk for kidney cancer. In the West Virginia occupational cohort, Steenland and Woskie (2012) observed significantly elevated risk of kidney cancer deaths in the highest quartile of modeled PFOA exposure (>2,384 ng/mL-years). However, each of these studies is limited by a small number of observed cases (six kidney cancer deaths, 16 incident kidney cancer cases, and five incidence testicular cancer cases in Raleigh et al. (2014); 12 kidney cancer deaths and one testicular cancer death in Steenland and Woskie (2012)). None of the general population studies reviewed for the 2016 PFOA HESD examined kidney or testicular cancer, and no associations were observed in the general population between exposure to PFOA (mean serum PFOA levels up to 86.6 ng/mL) and colorectal, breast, prostate, bladder, or liver cancer (Bonefeld-Jørgensen et al., 2014; Hardell et al., 2014; Innes et al., 2014; Eriksen et al., 2009). In the C8 Health Project cohort, Barry et al. (2013) observed a significant inverse association with breast cancer for both unlagged and 10-year lagged estimated cumulative PFOA serum concentrations. Barry et al. (2013) also observed positive and significant associations between PFOA and thyroid cancer in DuPont workers at the Washington, West Virginia plant, but not in community residents. However, Vieira et al. (2013) found no association between estimated serum concentrations of PFOA with thyroid cancer risk among residents living near the DuPont Teflon-manufacturing plant in Parkersburg, West Virginia.

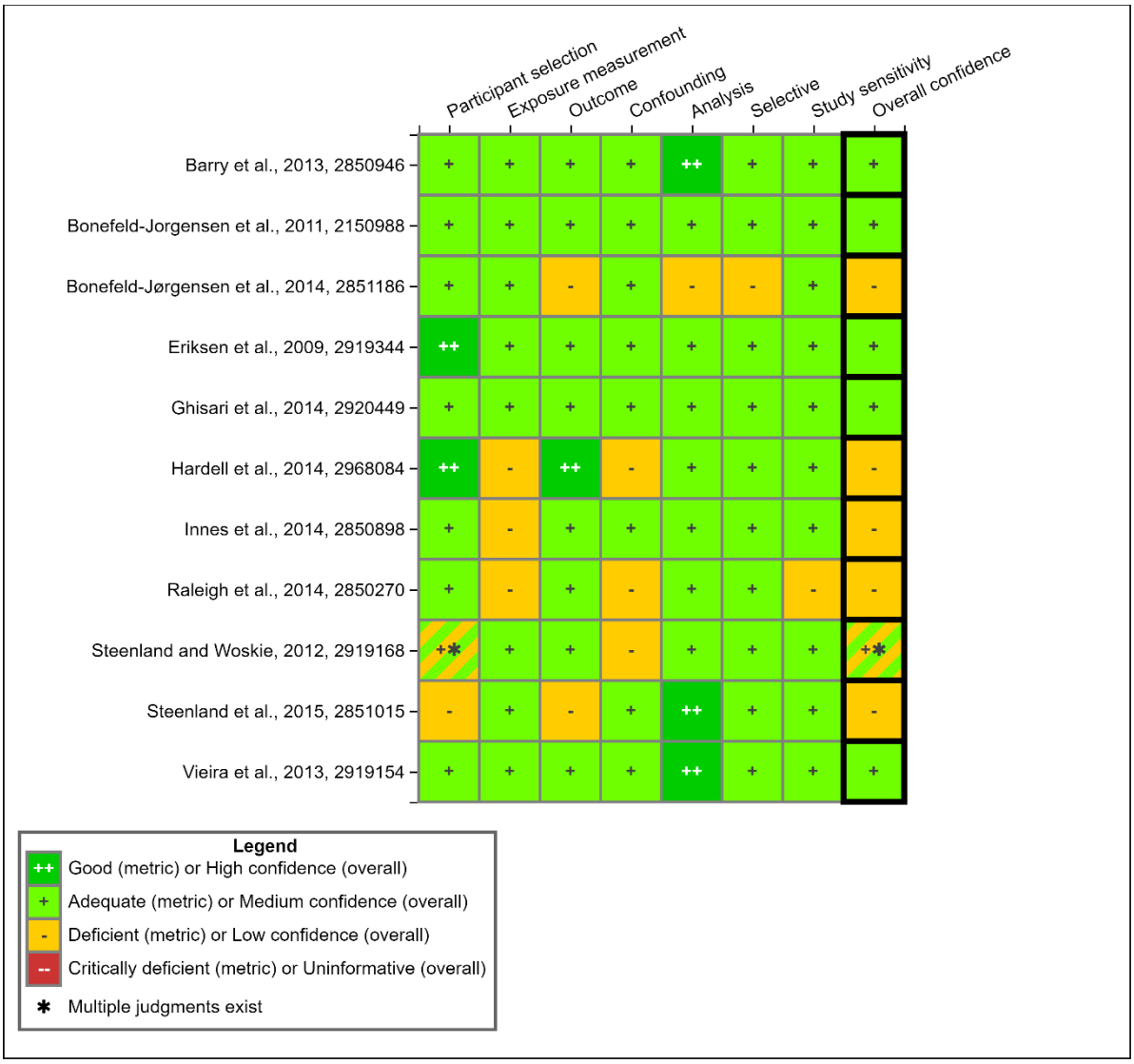


Figure 3-73. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cancer Effects Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 18 epidemiological studies have been published that investigated the association between PFOA and cancer (see Appendix, (U.S. EPA, 2024a)). One of the publications (Girardi and Merler, 2019) was an occupational study and the remainder were conducted on the general population, with one in a high-exposure community (C8 Health Project). Different study designs were also used including four cohort studies (Li et al., 2022; Girardi and Merler, 2019; Fry and Power, 2017; Steenland et al., 2015), six case-control studies (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Wielsøe et al., 2017), six nested case-control studies (Goodrich et al., 2022; Shearer et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Hurley et al., 2018; Ghisari et al., 2017), and three cross-sectional studies (Omoike et al., 2021; Christensen et al., 2016; Ducatman et al., 2015).

The studies were conducted in different study populations including populations from China (Cao et al., 2022; Liu et al., 2021; Lin et al., 2020b), Denmark (Ghisari et al., 2017), France (Mancini et al., 2020), Greenland (Wielsøe et al., 2017), Italy (Girardi and Merler, 2019), Japan (Itoh et al., 2021), Sweden (Li et al., 2022), Taiwan (Tsai et al., 2020), and the United States (Goodrich et al., 2022; Omoike et al., 2021; Shearer et al., 2021; Cohn et al., 2020; Hurley et al., 2018; Fry and Power, 2017; Christensen et al., 2016; Ducatman et al., 2015; Steenland et al., 2015). All studies measured PFOA in study subjects' blood components (i.e., serum or plasma) with two exceptions: one study measured PFOA in the maternal serum (Cohn et al., 2020) and one study categorized exposure to any PFAS based on residence near highly contaminated sources of drinking water (Li et al., 2022). Cancers evaluated included bladder (Li et al., 2022; Steenland et al., 2015), breast (Li et al., 2022; Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017; Wielsøe et al., 2017), colorectal (Li et al., 2022; Steenland et al., 2015), germ cell tumors (Lin et al., 2020b), kidney (Li et al., 2022; Shearer et al., 2021), liver (Cao et al., 2022; Goodrich et al., 2022; Li et al., 2022; Girardi and Merler, 2019), lung (Li et al., 2022; Girardi and Merler, 2019), lymphatic or hematopoietic tissue (Li et al., 2022; Girardi and Merler, 2019), melanoma (Li et al., 2022; Steenland et al., 2015), ovarian (Omoike et al., 2021), prostate (Omoike et al., 2021; Ducatman et al., 2015; Steenland et al., 2015), thyroid (Liu et al., 2021) uterine (Omoike et al., 2021), and any cancer (Li et al., 2022; Girardi and Merler, 2019; Fry and Power, 2017; Christensen et al., 2016).

3.5.1.2 Study Quality

There are 18 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cancer effects. Study quality evaluations for these 18 studies are shown in Figure 3-74.

Of the 18 studies identified since the 2016 PFOA HESD (U.S. EPA, 2016c), eight were considered *medium* confidence, and eight were *low* confidence (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Girardi and Merler, 2019; Christensen et al., 2016). One study conducted in the high exposure to PFAS Ronneby Register Cohort in Sweden was *uninformative* (Li et al., 2022) because of concerns about exposure assessment and lack of data on important covariates. One study conducted in Greenland was *uninformative* (Wielsøe et al., 2017) because of concerns about selection bias and exposure assessment. One study included a liver cancer biomarker analysis which was *uninformative* due to lack of information on biomarker measurement methods (Cao et al., 2022). As a result, these two studies and the biomarker analysis will not be further considered in this review. Concerns with the *low* confidence studies included the possibility of outcome misclassification, confounding, or participation selection methods. Residual confounding was also a concern, including lack of considering co-exposures by other PFAS, and lack of appropriately addressing SES and other lifestyle factors, which could be associated with both exposure and cancer diagnosis. The two *low* confidence occupational studies (Girardi and Merler, 2019; Steenland et al., 2015) had several potential sources of bias including potential selection bias, outcome measurement limitations which may lead to survival bias, and poor/insufficient study sensitivity due to a small number of deaths. Girardi et al. (2019) had the potential for residual confounding because of use of standardized mortality ratios (SMRs), which

only account for gender, age, and calendar year. Confounders specific for cancer outcomes, besides age and gender, including factors such as smoking or socioeconomic factors were not addressed in the study and behavioral risk factors could have differed by outcome. Although PFOA has a long half-life in the blood, concurrent measurements may not be appropriate for cancers with long latencies. Temporality of exposure in terms of cancer development was noted to be an issue in several *low* confidence studies (Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Tsai et al., 2020). Many of the *low* confidence studies also had sensitivity issues due to limited sample sizes. Limited details or reporting issues were also a concern for some *low* confidence studies which resulted in difficulty in quantitatively interpreting analysis results (Cao et al., 2022).

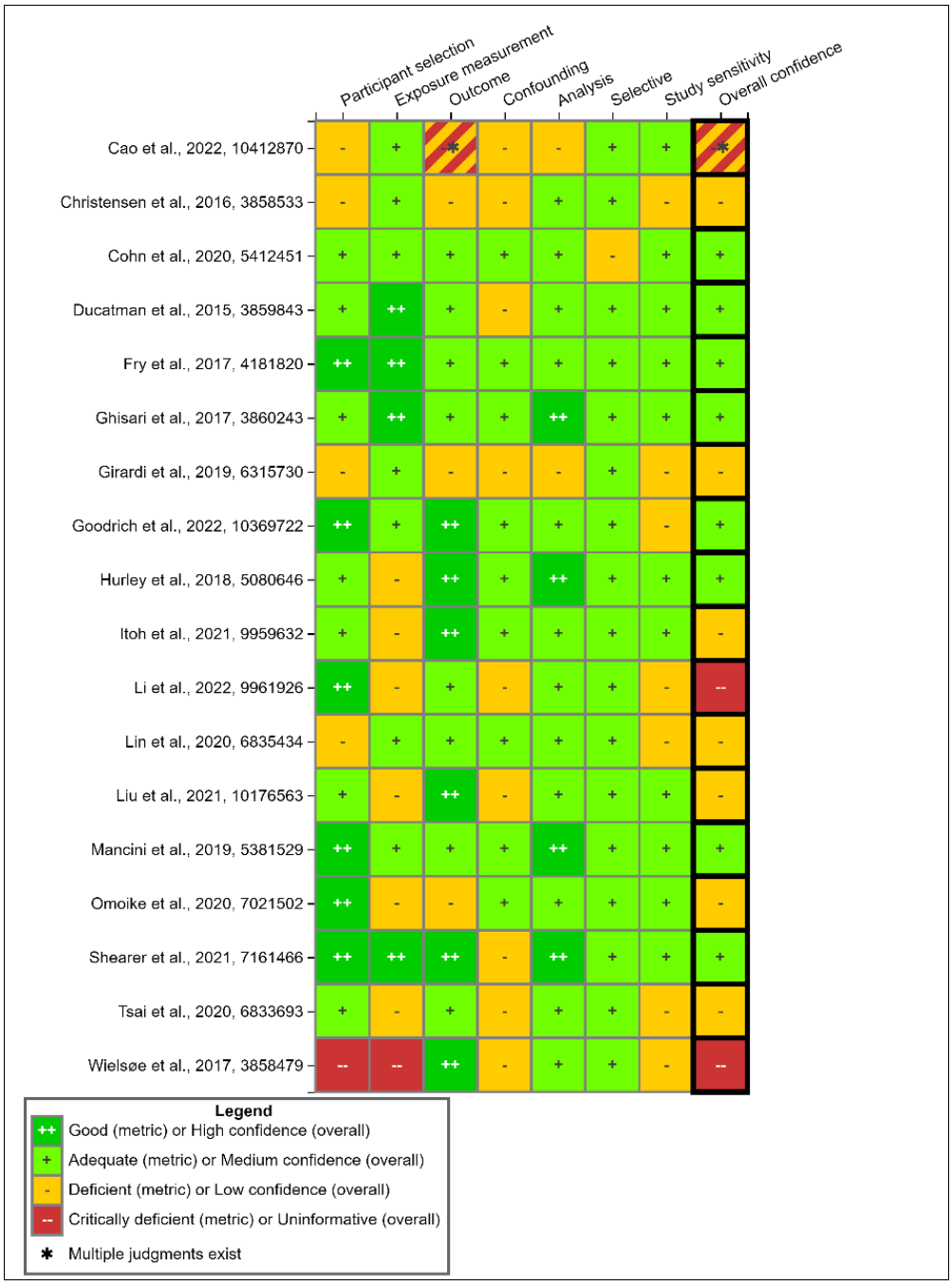


Figure 3-74. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

3.5.1.3 Findings From Children

One *low* confidence study examined cancers in children (Lin et al., 2020b) and reported a statistically significant higher median PFOA concentration in 42 pediatric germ cell tumor cases compared with 42 controls in blood samples collected from the children one week after

diagnosis. However, the study did not observe an increase in cancer risk when evaluated on a per ng/mL increase in blood PFOA.

3.5.1.4 Findings From the General Adult Population

PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) (Shearer et al., 2021). This large *medium* confidence case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of RCC with pre-diagnostic serum levels of PFOA (OR = 2.63; 95% CI: 1.33, 5.20 for the highest vs. lowest quartiles; p-trend = 0.007, or per doubling of PFOA: OR: 1.71; 95% CI: 1.23, 2.37) (Shearer et al., 2021). Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in odds remained across the quartiles and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS in the highest vs. lowest quartiles), although it was no longer statistically significant. Statistically significant increased odds of RCC were observed in participants ages 55–59 years, and in men and in women, separately (see Appendix D, (U.S. EPA, 2024a)).

Seven general population studies published since the 2016 assessment examined breast cancer (Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017). Four were considered *medium confidence* (Cohn et al., 2020; Mancini et al., 2020; Hurley et al., 2018; Ghisari et al., 2017) and had mixed results. All studies were case-control studies (with some nested designs), except for one cross-sectional NHANES-based study (Omoike et al., 2021). Two nested case-control studies did not observe an association between breast cancer and PFOA concentrations measured in maternal serum throughout pregnancy and 1–3 days after delivery ((Cohn et al., 2020); 75th percentile PFOA 0.6 ng/mL) or in in serum after case diagnosis and breast cancer ((Hurley et al., 2018); max concentration of 39.1 ng/mL). Both studies were conducted in California and most breast cancer cases were obtained from the cancer registry. Two nested case-control studies found associations between PFOA and breast cancer, but only in specific genotype or estrogen receptive groups of participants (Mancini et al., 2020; Ghisari et al., 2017). Ghisari (2017) reported an increased risk for breast cancer identified from the cancer registry with increasing PFOA concentrations only in participants with a CC genotype (n = 36 cases and 47 controls) in the CYP19 gene (cytochrome P450 aromatase). A nested case-control study (194 pairs of breast cancer cases and controls) within the French E3N cohort found an 86% higher risk of breast cancer in the 2nd quartile of PFOA (4.8–6.8 ng/mL) compared with the first quartile (1.3–4.8 ng/mL) (OR = 1.86; 95% CI: 1.03, 3.36) in a partially adjusted model (Mancini et al., 2020). Mancini et al. (2020) also reported that the risk for breast cancer (93% verified as pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increase in estrogen receptor negative (ER−) and progesterone receptor negative (PR−) breast cancers in the second quartile of PFOA only. The sample size was small with 26 participants having ER − breast cancers and 57 having PR − breast cancers. No association was observed between PFOA and receptor-positive breast cancer risk.

Three studies were considered *low* confidence (Itoh et al., 2021; Omoike et al., 2021; Tsai et al., 2020) because of concerns about temporality of exposure measurements and breast cancer development, lack of confirmation of control status via examination or medical records (Tsai et al., 2020), and potential for residual confounding due to SES, lifestyle factors and other PFAS.

One *low* confidence study (Tsai et al., 2020) conducted in Taiwan observed an increased risk of breast cancer only in women younger than 50 years (OR = 1.14; 95% CI: 0.66, 1.96). Tsai et al. (2020) also reported an increase in risk in ER+ participants aged 50 years or younger and a decrease in risk for ER− breast cancers in participants aged 50 years or younger, but neither achieved statistical significance. Statistically significant increased odds of breast cancer were also observed in a *low* confidence NHANES study (2005–2012) (Omoike et al., 2021) both per ng/mL increase in PFOA (OR = 1.089; 95% CI: 1.089, 1.090) and across quartiles of exposure. One *low* confidence case-control study conducted in Japanese women (Itoh et al., 2021) observed a significant inverse association across serum PFOA quartiles with a significant dose-response trend (p-value < 0.0001) (see Appendix D, (U.S. EPA, 2024a)). Median PFOA levels ranged from 3.2 ng/mL in the lowest quartile to 9.3 ng/mL in the highest quartile. The association was null in pre-menopausal women and remained significantly inverse in postmenopausal women in the highest tertile of exposure, with a significant dose-response trend (p-value for trend = 0.005).

Two general population studies published since the 2016 assessment examined liver cancer (Cao et al., 2022; Goodrich et al., 2022) and observed *mixed* results. One study was considered *medium* confidence (Goodrich et al., 2022) and one study was considered *low* confidence (Cao et al., 2022). The *medium* confidence nested case-control study of U.S. adults observed a nonsignificant increase in risk of liver cancer comparing participants with PFOA exposure concentrations above the 85th percentile (8.6 ng/mL) compared with those at or below (OR = 1.20; 95% CI: 0.52, 2.80). There was no association in analyses of continuous PFOA exposure. However, the sample size was small (n = 50 cases and controls each) which likely limited study sensitivity (Goodrich et al., 2022). Elevated risk of liver cancer was also observed in a *low* confidence Chinese case-control study in adults and children (OR per log-ng/mL increase in PFOA exposure = 1.036; 95% CI: 1.002, 1.070) (Cao et al., 2022). However, the confidence in the study results was considered *low* due to limited information regarding selection of controls, diagnosis method for liver cancer, adjustment for potential confounding, and details on the statistical analysis.

One *medium* confidence study based on the C8 Health Project (Ducatman et al., 2015). examined prostate-specific antigen (PSA) as a biomarker for prostate cancer in adult males over age 20 years who lived, worked, or went to school in one of the six water districts contaminated by the DuPont Washington Works facility. No association was observed between PSA levels in either younger (i.e., 20–49-years-old) or older (i.e., 50–69-years-old) men and concurrent mean serum PFOA concentration up to 46 ng/mL. In an NHANES population, Omoike et al. (2021) observed a significantly inverse association with prostate cancer (OR = 0.944; 95% CI: 0.943, 0.944).

Omoike et al. (2021) also observed statistically significant increased odds of ovarian cancer both per ng/mL increase in PFOA (OR = 1.015; 95% CI: 1.013, 1.017) and for the highest versus lowest quartiles of exposure (OR = 1.77; 95% CI: 1.75, 1.79), although the association was significantly inverse for the second and third quartiles of exposure (see Appendix D, (U.S. EPA, 2024a)). A significantly inverse association was also observed for uterine cancer (OR = 0.912; 95% CI: 0.910, 0.914 per ng/mL increase in PFOA) (Omoike et al., 2021).

One *low* confidence study conducted in Shandong Province, in eastern China (Liu et al., 2021) observed a statistically significant inverse association with thyroid cancer across quartiles of

serum PFOA (p-value for trend < 0.001). The median serum PFOA levels were higher in controls than in cases (10.9 vs. 7.7 ng/mL, p-value < 0.001). However, there is some concern about possible reverse causality. The ability to metabolize PFAS could change when the thyroid becomes cancerous, thereby changing the PFAS concentrations. The abnormality of thyroid hormones may also disturb the PFAS levels.

Two studies examined all cancers together, but collected different information on cancers (i.e., incidence vs. mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was observed with PFOA exposure in a *medium* confidence study among subjects over 60 years of age from NHANES 2003–2006 with median PFOA concentration 23.7 ng/g lipid (Fry and Power, 2017). PFOA was associated with an increase in self-reported cancer incidence in a *low* confidence study on male anglers over 50 years (Christensen et al., 2016). Christensen et al. (2016) was considered *low* confidence due to the potential of self-selection because subjects were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.5.1.5 Findings From Occupational Studies

Two *low* confidence occupational studies examined cancer incidence (Steenland et al., 2015) and mortality (Girardi and Merler, 2019). Issues of population selection, outcome measurement and small number of deaths reducing the sensitivity were noted. In a retrospective occupational cohort study based on the same DuPont cohort from West Virginia reported in the 2016 assessment (Steenland and Woskie, 2012), Steenland et al. (2015) observed no significant associations with incidence of cancers of the bladder, colorectal, prostate, and melanoma when compared with the general population (median serum levels in workers was 113 ng/mL in 2005 compared with 4 ng/mL in the general population). There was modest evidence of a positive nonsignificant trend for prostate cancer (across quartiles) and a statistically significant negative exposure-response trend for bladder cancers (p-value = 0.04).

Girardi et al. (2019) conducted a retrospective cohort study at a factory in Italy where PFOA was produced from 1968–2014 and observed statistically significant increases in liver cancer mortality, malignant neoplasms of the lymphatic and hematopoietic tissue, and in all malignant neoplasms with cumulative serum PFOA exposure of >16,956 ng/mL-years. There was no association observed with lung cancer in this occupational cohort. Mortality from cancers in this cohort was low and supplemental data provided mortality for other cancers including kidney, but no risk estimates were calculated.

3.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and cancer effects in animal models. Study quality evaluations for these five studies are shown in Figure 3-75.

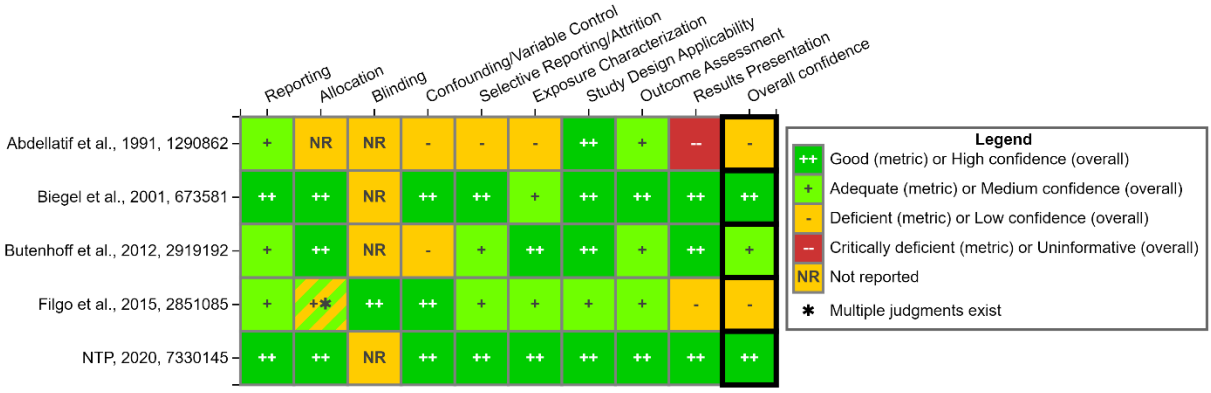


Figure 3-75. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

Three *high* or *medium* confidence animal carcinogenicity studies indicate that PFOA exposure can lead to multiple types of neoplastic lesions including liver adenomas (NTP, 2020; Biegel et al., 2001) or carcinomas (NTP, 2020), Leydig cell tumors (LCTs) (Butenhoff et al., 2012; Biegel et al., 2001), and pancreatic acinar cell tumors (PACTs; adenomas or adenocarcinomas) (NTP, 2020; Biegel et al., 2001) in male Sprague-Dawley rats. Neoplastic lesions were also observed in female Sprague-Dawley rats, but the incidences were not as high as the incidences observed in the males and often did not achieve statistical significance, though there were reported incidences of rare and/or malignant neoplasms of the liver, pancreas, and uterus (NTP, 2020; Butenhoff et al., 2012). Another study (Filgo et al., 2015) assessed hepatic tumor development in three strains of female mice after perinatal exposures to PFOA. This study is not further discussed here because of an inadequate study design to assess lifetime/chronic carcinogenicity (i.e., the study did not include exposure postweaning) and the results were equivocal (i.e., few significant findings that did not display a dose-response relationship) and difficult to interpret due to small sample sizes (n = 6–10 for some strains).

In the three rat carcinogenicity studies (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001), rats were fed diets containing similar concentrations of PFOA for approximately 2 years. Butenhoff et al. (2012) analyzed a variety of tissues collected from male and female Sprague-Dawley rats fed diets containing 0, 30, or 300 ppm PFOA (equivalent to 1.3 and 14.2 mg/kg for males and 1.6 and 16.1 mg/kg for females) for 2 years. Similarly, Biegel et al. (2001) analyzed tissues collected from male Crl:CD® BR (CD) rats fed diets containing 0 or 300 ppm PFOA (equivalent to 13.6 mg/kg/day) for 24 months. Using a matrix-type exposure paradigm, NTP (2020) administered PFOA in feed to pregnant Sprague-Dawley (Hsd:Sprague-Dawley® SD®) rats starting on GD 6 and analyzed tissues of male and female offspring also fed postweaning diets containing PFOA for a total of 107 weeks. Dose groups for this report are referred to as "[perinatal exposure level]/[postweaning exposure level]" (e.g., 300/1,000; see further study design details in Section 3.4.4.2.1.2).

Liver adenomas in male rats were observed in the Biegel et al. (2001) study at an incidence of 10/76 (13%) at 13.6 mg/kg/day, compared with 2/80 (3%) in controls. Liver adenomas in male

rats were also significantly increased in the NTP (2020) in the 0/40, 0/80, and 300/80 ppm groups (Table 3-16). Both the 0/0 and 300/0 ppm control groups had no observed liver adenomas. NTP (2020) reported increases in the incidence of hepatocellular carcinomas in the male 300/80 ppm group only and a statistically significant trend of increased incidence with dose in the groups exposed during both perinatal and postnatal periods. Although no liver adenomas were observed in Butenhoff et al. (2012), carcinomas were identified in the male controls (3/49), males in the low-dose group (1.3 mg/kg/day; 1/50), and male (5/50) and female (1/50) rats in the high-dose group (14.2 and 16.1 mg/kg/day, respectively). The differences in carcinoma incidences from controls were not statistically significant in the Butenhoff et al. (2012) study.

Table 3-16. Incidences of Liver Tumors in Male Sprague-Dawley Rats as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
Hepatocellular Adenomas				
0 ppm	0/50 (0%)***	0/50 (0%)	7/50 (14%)*	11/50 (22%)**
300 ppm	0/50 (0%)***	1/50 (2%)	5/50 (10%)	10/50 (20%)**
Hepatocellular Carcinomas				
0 ppm	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
300 ppm	0/50 (0%)*	0/50 (0%)	0/50 (0%)	4/50 (8%)

Notes:
*Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.
**Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.01$.
***Statistically significant trend of response at $p \leq 0.001$.

Nonneoplastic/preneoplastic liver lesions were identified by Butenhoff et al. (2012) in males and females at the 1- and 2-year sacrifices. An increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis occurred in the high-dose groups. At the 2-year sacrifice, hepatic cystic degeneration (characterized by areas of multilocular microcysts in the liver parenchyma) was observed in males. Hyperplastic nodules in male livers were increased in the 14.2 mg/kg/day group. NTP (2020) similarly reported a variety of nonneoplastic and preneoplastic liver lesions in both male and female rats including increased incidences of liver necrosis and mixed-cell foci, hepatocyte hypertrophy, and focal inflammation. These lesions were more pronounced in males than females and were observed at both the 16-week interim and 107-week final necropsies.

Testicular LCTs were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The tumor incidence reported by Butenhoff et al. (2012) was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the 0, 1.3, and 14.2 mg/kg/day dose groups, respectively. The Biegel et al. (2001) study included one dose group (13.6 mg/kg/day); the tumor incidence was 8/76 (11%) compared with 0/80 (0%) in the control group. LCT incidence at similar dose levels was comparable between the two studies (11% and 14%). NTP (2020) analyzed testicular tissue for LCTs but did not observe increased incidence due to PFOA treatment. The authors noted that this inconsistency with other carcinogenicity studies could be a result of differences in exposure concentrations or stock of Sprague-Dawley rat (i.e., CD vs. Hsd:Sprague-Dawley).

PACTs were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups compared with their respective controls (Table 3-17). NTP (2020) observed increases in pancreatic acinar cell adenocarcinoma incidence in males in multiple dose groups and slight increases in the incidence of combined acinar cell adenoma or carcinoma in females from the 300/1,000 ppm dose group, though these increases did not reach statistical significance (Table 3-17 and Table 3-18). In male rats, the incidence of PACTs in the Biegel et al. (2001) study was 8/76 (11%; 7 adenomas, 1 carcinoma) at 13.6 mg/kg/day while none were observed in the control animals. In a peer-reviewed pathological review of male pancreatic tissue collected by Butenhoff et al. (2012), Caverly Rae et al. (2014) identified 1/47 carcinomas in the 300 ppm group (compared with 0/46 in the control and 30 ppm groups) and no incidence of adenomas with any treatment. Pancreatic acinar hyperplasia was observed in males of the control, 1.3, and 14.2 mg/kg/day groups at incidences of 3/46 (7%), 1/46 (2%), and 10/47 (21%), respectively. Butenhoff et al. (2012) also reported increased incidences of acinar atrophy in males (6/46 (13%), 9/46 (20%), and 11/49 (22%) in 0, 1.3, and 14.2 mg/kg/day dose groups, respectively), though this lesion was not discussed in the peer-reviewed pathology report (Caverly Rae et al., 2014). NTP (2020) similarly reported increased incidences of acinus hyperplasia in males at incidence rates of 32/50 (64%), 37/50 (74%), 31/50 (62%) in the 0/20, 0/40, 0/80, and 27/50 (54%), 38/50 (76%), and 33/50 (66%) in the 300/20, 300/40, and 300/80 groups. The incidences in controls were 18/50 (36%) and 23/50 (46%) in the 0/0 and 300/0 groups, respectively. There were also low occurrences of acinus hyperplasia in the exposed female groups, though not as frequently observed as in males. However, the authors concluded that the incidence of pancreatic acinar cell neoplasms in males increased confidence that the occurrence in females was due to PFOA exposure.

Table 3-17. Incidences of Pancreatic Acinar Cell Tumors in Male Sprague-Dawley Rats as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
Pancreatic Acinar Cell Adenomas				
0 ppm	3/50 (6%)**	28/50 (56%)**	26/50 (52%)**	32/50 (64%)**
300 ppm	7/50 (14%)**	18/50 (36%)*	30/50 (60%)**	30/50 (60%)**
Pancreatic Acinar Cell Adenocarcinomas				
0 ppm	0/50 (0%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
300 ppm	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)

Notes:
*Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.
**Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.001$. Asterisks on the control group denotes a statistically significant trend of response.

Table 3-18. Incidences of Pancreatic Acinar Cell Tumors in Female Sprague-Dawley Rats

as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose		
	0 ppm	**300 ppm**	**1,000 ppm**
Pancreatic Acinar Cell Adenomas			
0 ppm	0/50 (0%)	0/50 (0%)	1/49 (2%)
150 ppm	–	0/50 (0%)	–
300 ppm	–	–	3/50 (6%)
Pancreatic Acinar Cell Adenocarcinomas			
0 ppm	0/50 (0%)	0/50 (0%)	1/49 (2%)
150 ppm	–	0/50 (0%)	–
300 ppm	–	–	2/50 (4%)

NTP (2020) observed increased incidences of uterine adenocarcinomas in female Sprague-Dawley rats during the extended evaluation (i.e., uterine tissue which included cervical, vaginal, and uterine tissue remnants). Incidence rates for this lesion are reported in Table 3-19. The accompanying incidences of uterine hyperplasia did not follow a dose-response relationship. Butenhoff et al. (2012) identified mammary fibroadenomas and ovarian tubular adenomas in female rats, though there were no statistical differences in incidence rates between PFOA-treated dose groups and controls.

Table 3-19. Incidences of Uterine Adenocarcinomas in Female Sprague-Dawley Rats from the Standard and Extended Evaluations (Combined) as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose		
	0 ppm	**300 ppm**	**1,000 ppm**
0 ppm	1/50 (2%)	5/49 (10%)	7/48 (15%)*
150 ppm	–	3/50 (6%)	–
300 ppm	–	–	5/48 (10%)

Notes:
*Statistically significant compared with the control group (0/0 ppm) at p = 0.050.

NTP concluded that under the exposure conditions presented, there was *clear evidence* of carcinogenic activity of PFOA in male Sprague-Dawley rats based on increased incidences of hepatocellular neoplasms (predominately hepatocellular adenomas) and acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas (NTP, 2020). In females, NTP concluded there was *some evidence* of carcinogenic activity of PFOA based on increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The study authors also noted that the higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure (NTP, 2020).

3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cancer outcomes is discussed in Sections 3.1.2, 3.2.9, 3.3.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 42 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to cancer effects. A summary of these studies is shown in Figure 3-76.

Evidence Stream			
Animal	Human	In Vitro	Grand Total
8	5	33	42

Figure 3-76. Summary of Mechanistic Studies of PFOA and Cancer Effects

Interactive figure and additional study details available on HAWC.

In 2016, 10 key characteristics of carcinogens were selected by a multidisciplinary working group of the International Agency for Research on Cancer (IARC), based upon common empirical observations of chemical and biological properties associated with human carcinogens (i.e., Group 1 carcinogens as determined by IARC) (Smith et al., 2016b). In contrast to the "Hallmarks of cancer" as presented by Hanahan and Weinberg (Hanahan, 2022; Hanahan and Weinberg, 2011, 2000), the key characteristics focus on the properties of human carcinogens that induce cancer, not the phenotypic or genotypic traits of cancers. The 10 key characteristics provide a framework to systematically identify, organize, and summarize mechanistic information for cancer hazard evaluations (Smith et al., 2016b).

To aid in the evaluation of the carcinogenic potential of PFOA, the studies containing mechanistic data were organized by the proposed key characteristics of carcinogens for the following section. Evidence related to eight of the 10 key characteristics of carcinogens was identified in the literature included in this assessment: 'Is Genotoxic,' 'Induces Epigenetic Effects,' 'Induces Oxidative Stress,' 'Induces Chronic Inflammation,' 'Is Immunosuppressive,' 'Modulates Receptor Mediated Effects,' 'Alters Cells Proliferation, Cell Death, and Nutrient Supply,' and 'Causes Immortalization.' No studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and recent systematic literature search and review efforts were identified for the following key characteristics: 'Is Electrophilic or Can Be Metabolically Activated to Electrophiles' (key characteristic #1) and 'Alters DNA Repair and Causes Genomic Instability' (key characteristic #3).

3.5.3.1 Key Characteristic #2: Is Genotoxic

Genotoxicity is a well-characterized mode of action for carcinogens, defined as alterations to DNA through single or double strand breaks, alterations to DNA synthesis, and DNA adducts, all of which can result in chromosomal aberrations, formation of micronuclei, and mutagenesis if not effectively repaired. Overall, the evidence suggests that PFOA does not induce mutations or operate through a genotoxic mechanism, with the majority of the study data demonstrating a lack of genotoxic effect of PFOA in both in vitro and in vivo assays. A notable exception is aneuploidy and DNA fragmentation of sperm significantly associated with PFOA exposure in humans. The genotoxicity evidence is detailed below.

3.5.3.1.1 Gene Mutation

All of the studies identified in this assessment that investigated the mutagenic potential of PFOA were conducted in in vitro models. Of the available studies, most found that PFOA exposure did not induce mutagenicity (Table 3-20). Studies involving Chinese hamster ovary (CHO) K-1 cell lines presented primarily negative results. Sadhu (2002) reported PFOA exposure did not induce gene mutations in CHO K-1 cells when tested with or without metabolic activation. Zhao et al. (2011) also observed that PFOA did not induce mutagenesis in human-hamster hybrid (A_L) cells, which contain a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11, at sub-cytotoxic concentrations (<200 μM). A subsequent experiment using DMSO to quench oxidative stress found that PFOA was not mutagenic in the presence of DMSO, suggesting that an increase in reactive oxygen species production may be required for PFOA-induced mutagenicity (Section 3.5.3.3).

Of the six publications that tested PFOA mutagenicity in *Salmonella typhimurium (S. typhimurium)* or *Escherichia coli (E. coli)* (NTP, 2019; Buhrke et al., 2015; Butenhoff et al., 2014; Fernández Freire et al., 2008; Lawlor, 1996, 1995), two reported exposure-associated mutagenicity (NTP, 2019; Butenhoff et al., 2014) (Table 3-20). Mutation was observed in *S. typhimurium* following exposure to cytotoxic concentrations of PFOA in the presence of S9 metabolic activation (Butenhoff et al., 2014). NTP (2019) reported PFOA exposure caused a slight increase in mutation in *S. typhimurium* TA98 cells, and Lawlor (1996) reported that one plate of *S. typhimurium* had a significant amount of mutagenicity in the absence of S9 metabolic activation. However, neither of these results were reproducible.

3.5.3.1.2 DNA Damage

3.5.3.1.2.1 In Vivo Evidence

3.5.3.1.2.1.1 Human Studies

Two studies reported on the genotoxic potential of PFOA exposure in humans (Table 3-21). Franken et al. (2017) measured blood PFOA concentrations in adolescents (14–15 years of age) that resided for >5 years within industrial areas of Belgium (near a stainless-steel plant or a shredder factory). These data were then compared with age-matched controls. A significant increase in DNA damage associated with PFOA exposure was observed, as evidenced by an alkaline comet assay performed on the same blood samples. Urinary 8-hydroxydeoxyguanosine (8-OHdG) was used as a biomarker for oxidative DNA damage. While there was no significant change observed, a positive dose-response relationship with increasing PFOA concentrations was noted. The authors attributed the DNA damage to oxidative stress, but noted that urinary 8-OHdG can also indicate DNA repair. Governini et al. (2015) collected semen samples from healthy nonsmoking men and evaluated aneuploidy, diploidy, and DNA fragmentation. The occurrence of aneuploidy and diploidy in sperm cells, which are normally haploid, was significantly higher in the PFAS-positive samples (PFOA was detected in 75% of the samples) when compared with PFAS-negative samples. This suggests that PFAS exposure is related to errors in cell division leading to aneugenicity. Additionally, fragmented chromatin levels were also significantly increased for the PFAS-positive group compared with the PFAS-negative group.

3.5.3.1.2.1.2 Animal Toxicological Studies

Studies of the genotoxicity related to PFOA exposure were conducted in rat and mouse models (Table 3-21). All of the studies presented data from micronucleus tests of bone marrow, peripheral blood, and splenocytes, with the exception of one study of DNA strand breaks. Quantifying micronuclei formation in rats via optimal and reliable methods has been previously described (WHO & FAO, 2020; WHO and FAO, 2009; Witt et al., 2000). With the exception of one micronucleus assay, there was no evidence for PFOA-induced genotoxic effects after acute or subchronic exposures (Figure 3-16).The single study of DNA strand breakage used a comet assay in tissues from male C57Bl/6 mice administered ≤5 mg/kg/day for five weeks (Crebelli et al., 2019). Analysis of the liver and testis following exposure indicated there was no change in DNA fragmentation. Acute and subchronic PFOA exposures in mouse studies found no evidence of micronuclei formation, a measure of genotoxic damage to DNA in proliferating cells and spindle formation (Hayashi, 2016), in either peripheral blood cells or splenocytes (Crebelli et al., 2019) or within erythrocytes of the bone marrow (Butenhoff et al., 2014; Murli, 1996c, 1995). NTP (2019) reported using flow cytometry to analyze micronuclei formation in immature polychromatic erythrocytes from the peripheral blood of male and female Sprague-Dawley rats.

A subchronic study in Sprague-Dawley rats noted that PFOA exposure induced a slight increase in micronuclei formation in peripheral blood cells of male rats administered 10 mg/kg/day; however, the micronuclei level was within the historical control range, and there was no effect in females) (NTP, 2019).

3.5.3.1.2.2 In Vitro Evidence

3.5.3.1.2.2.1 Chromosomal Aberrations

Measurements of chromosomal aberrations have been performed using human and animal cell lines, and predominantly found that PFOA exposure does not cause alterations (Table 3-22). In human lymphocytes, PFOA did not induce chromosomal aberrations in the presence of S9 activation (3 hours) or without the addition of S9 (≤46 hours) at concentrations up to 600 µg/mL (Butenhoff et al., 2014). This evidence corroborates previous studies of human lymphocyte cells that found similar results using non-cytotoxic concentrations of PFOA (NOTOX, 2000; Murli, 1996b) as reported in the 2016 PFOA HESD (U.S. EPA, 2016c).

In contrast, Butenhoff et al. (2014) observed chromosomal aberrations after PFOA exposure (≥750 µg/ml) with S9 metabolic activation in CHO cells. These results corroborate with previously reported studies in S9 activated CHO cells (Murli, 1996a, d). Butenhoff et al. (2014) and Murli (1996a) also reported PFOA-induced chromosomal aberrations in CHO cells without S9 metabolic activation but were unable to replicate their own results.

3.5.3.1.2.2.2 DNA Double Strand Breaks

Evaluation of DNA strand breakage using comet assays and histological analysis of phosphorylated H2AX (γH2AX) yielded positive results in all of the studies reviewed (Table 3-22). PFOA exposure caused DNA breakage in a dose-dependent manner in human lymphocytes exposed to ≥250 ppm PFOA for two hours (Yahia et al., 2016) and in HepG2 cells exposed to ≥100 µM PFOA for 24 hours in one study (Yao and Zhong, 2005), ≥10 µM PFOA for 24 hours in another study (Wielsøe et al., 2015), and at 10 and 200 µM PFOA (but not 50 or 100 µM PFOA) for 24 hours in a third study (Florentin et al., 2011). *Paramecium caudatum* (*P.*

caudatum), a unicellular protozoa, exhibited DNA damage after exposure to 100 µM PFOA (Kawamoto et al., 2010). Peropadre et al. (2018) observed a 4.5-fold higher level of double strand breaks in human keratinocyte cells (HaCaT) exposed to 50 µM PFOA for 24 hours, compared with controls, as evidenced by γH2AX. Eight days post-exposure, γH2AX levels were twice that of the controls, indicating that double strand breaks were not fully repaired. In contrast, a study conducted in Syrian hamster embryo (SHE) cells demonstrated no change in DNA strand breaks by the comet assay at 4.1×10^{-5} to 300 µM PFOA for 5 or 24 hours (Jacquet et al., 2012).

3.5.3.1.2.2.3 Micronuclei Formation

Three studies measured micronucleus formation in cells exposed to PFOA (Table 3-22). Buhrke et al. (2013) demonstrated that PFOA exposure (10 µM, 24 hours) did not induce micronuclei formation in Chinese hamster lung cells (V79). Studies conducted in human HepG2 cells reported conflicting results: in one study, PFOA induced micronuclei formation at concentration of ≥100 µM after 24 hours (Yao and Zhong, 2005), while another study reported no difference in micronuclei frequency in HepG2 cells exposed to concentrations of PFOA up to 400 µM for 24 hours compared with controls (Florentin et al., 2011). The micronucleus assays were performed according to the same method (Natarajan and Darroudi, 1991).

Table 3-20. Mutagenicity Data from In Vitro Studies

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of exposure)
		S9-Activated	Non-Activated	
NTP (2019)	*Salmonella typhimurium* (TA98, TA100)	Equivocal[a] (Not reproducible)	Equivocal[a] (Not reproducible)	100–5,000 µg/plate
	*Escherichia coli (*WP2*uvr*A/pkM101)	Negative	Negative	100–10,000 µg/plate
Zhao et al. (2011)	Human-hamster hybrid (A_L) cells	N/A	Positive[b]	1–200 µM (1–16 d)
	Mitochondrial DNA-deficient human-hamster hybrid (p^0A_L) cells	N/A	Negative	1–200 µM (1–16 d)
Sadhu (2002)	CHO K-1	Negative	Negative	≤39 µg/mL (5 or 17 hr)
Butenhoff et al. (2014)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Positive[c]	Negative	20–1,000 µg/plate
Buhrke et al. (2015)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Negative	Negative	5 µM
Fernández Friere et al. (2008)	*Salmonella typhimurium* (TA98, TA100, TA102, TA104)	Negative	Negative	100 or 500 µM
Lawlor (1995)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Negative	Negative	100–5,000 µg/plate

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of exposure)
		S9-Activated	Non-Activated	
	Salmonella typhimurium (TA98, TA100, TA1535, TA1537)	Negative	Negative	100–5,000 µg/plate
	*Escherichia coli (*WP2*uvr*A*)*	Negative	Negative	100–5,000 µg/plate
	Escherichia coli (WP2uvrA)	Negative	Negative	6.67–5,000 µg/plate

Notes:
[a] Mutagens were present in 1 of 3 TA98 replicate plates only.
[b] Mutagens were present in cells that were exposed only to 200 µM for 16 days.
[c] Mutagenicity found at cytotoxic concentrations only.

Table 3-21. DNA Damage Data from In Vivo Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOA Concentration (Dosing Regimen)
DNA Strand Breakage				
Franken et al. (2017)	Human (Male and Female)	Peripheral Blood Cells	Positive	Average Blood Concentration of 2.55 µg/L
Governini et al. (2015)	Human (Male)	Semen	Positive	Average Seminal Plasma Concentration of 7.68 ng/g f.w.
Crebelli et al. (2019)	Mouse, C57BL/6 (Male)	Liver, Testis	Negative	0.1–5 mg/kg/day (daily via drinking water for 5 wk)
Micronuclei Formation				
Crebelli et al. (2019)	Mouse, C57BL/6 (Male)	Peripheral Blood Cells, Splenocytes	Negative	0.1–5 mg/kg/day (daily via drinking water for 5 wk)
Butenhoff et al. (2014)	Mouse, Crl:CD-1 (Male and Female)	Bone Marrow	Negative	250–1,000 mg/kg (single dose via gavage)
NTP (2019)	Rat, Sprague-Dawley (Male and Female)	Peripheral Blood Cells	Positive[a]	6.25–100 mg/kg/day (daily via gavage for 28 d)
Murli (1995)	Mouse	Bone Marrow	Negative	1,250–5,000 mg/kg (Single dose delivered via gavage)
	Mouse	Bone Marrow	Negative	498–1,990 mg/kg (Single dose delivered via gavage)

Notes: f.w. = formula weight.
[a] A slight increase in micronuclei in the male 10 mg/kg/day group was within the historical control range. No change in females.

Table 3-22. DNA Damage Data from In Vitro Studies

Reference	In Vitro Model	Results		Concentration (Duration of exposure)
		S9 Activated	Non-Activated	
Chromosomal Aberrations				

Reference	In Vitro Model	Results		Concentration (Duration of exposure)
		S9 Activated	Non-Activated	
Butenhoff et al. (2014)	Human Lymphocytes	Negative	Negative	12.4–600 µg/mL (3–46 hr)
	Chinese Hamster Ovarian Cells	Positive	N/A	50–1,500 µg/mL (3 hr)
	Chinese Hamster Ovarian Cells	N/A	Positive (Not reproducible)	25–1,000 µg/mL (3–41.8 hr)
NOTOX (2000)	Human Lymphocytes	Negative	Negative	≤Cytotoxic concentration[a]
Murli (1996b)	Human Lymphocytes	Negative	Negative	125–4,010 µg/mL (3–43.3 hr)
	Chinese Hamster Ovarian Cells	Positive	Negative	100–2,750 µg/mL (3–41.8 hr)
	Chinese Hamster Ovarian Cells	Positive	Positive (Not reproducible)	125–5,000 µg/mL (3 hr)
Cell Transformation				
Jacquet et al. (2012)	Syrian Hamster Embryo Cells	N/A	Negative	3.7×10^{-4}–37 µM (6 d)
Garry and Nelson (1981)	C3H10T½	N/A	Negative	0.1–200 µg/mL (24 hr)
DNA Strand Breakage				
Peropadre et al. (2018)	Human Keratinocyte HaCaT cells	N/A	Positive	50 µM (24 hr)
Yahia et al. (2016)	Human Lymphocytes	N/A	Positive	125–500 ppm (2 hr)
Florentin et al. (2011)	Human HepG2 Cells	N/A	Positive[b]	5–400 µM (1 or 24 hr)
Wielsøe et al. (2015)	Human HepG2 Cells	N/A	Positive	0.2– 20 µM (24 hr)
Yao and Zhong (2005)	Human HepG2 Cells	N/A	Positive	50–400 µM (24 hr)
Kawamoto et al. (2010)	Paramecia	N/A	Positive	10–100 µM (1–24 hr)
Micronuclei Formation				
Buhrke et al. (2013)	Chinese Hamster Lung Fibroblast Cells	Negative	Negative	10 µM (3 hr)
Florentin et al. (2011)	Human HepG2 Cells	N/A	Negative	5–400 µM (1 or 24 hr)
Yao and Zhong (2005)	Human HepG2 Cells	N/A	Positive[c]	50–400 µM (24 hr)

Notes: N/A = not applicable.
[a] Findings based on the 2016 EPA's Health Effects Support Document (U.S. EPA, 2016c), concentration(s) unknown.
[b] Slight increase was observed at 10 and 200 µM in a non-dose-dependent manner after 24-hour exposure only.
[c] Micronuclei were present in cells that were exposed only to ≥100 µM for 16 days.

3.5.3.2 Key Characteristic #4: Induces Epigenetic Alterations

Epigenetic alterations are modifications to the genome that do not change genetic sequence. Epigenetic alterations include DNA methylation, histone modifications, changes in chromatin structure, and dysregulated microRNA expression, all of which can affect the transcription of

individual genes and/or genomic stability (Smith et al., 2016b). Overall, the evidence demonstrates that PFOA exposure can lead to cancer-relevant changes in DNA methylation at both the global and gene-specific level, across human, animal, and in vitro studies. The evidence related to epigenetic alterations is detailed below.

3.5.3.2.1.1 In Vivo Evidence

3.5.3.2.1.2 Humans

A cohort of singleton term births were recruited from Faroese hospitals over an eighteen-month period from 1986 to 1987 (Leung et al., 2018). At delivery, samples of umbilical cord whole blood and scalp hair from the mothers were collected and used to measure toxicant levels as well as evaluation of DNA methylation. No change in CpG island methylation was correlated with PFOA levels, although changes in this epigenetic alteration were found to be significantly correlated with several other toxicants in the blood samples. Two other studies evaluated global DNA methylation patterns in cord blood. Miura et al. (2018) found that increased PFOA in the cord blood was associated with a global DNA hypermethylation in a cohort from Japan. Kingsley et al. (2017) did not observe associations between PFOA exposure in cord blood and epigenome-wide changes in methylation status. However, the authors found significant changes in methylation in seven CpG sites located in several genes, including *RAS P21 Protein Activator 3 (RASA3)* and Opioid Receptor Delta 1 *(OPRD1).* Three studies reviewed herein found no association between maternal PFOA exposure and global methylation changes in offspring (Leung et al., 2018; Liu et al., 2018a) or placenta (Ouidir et al., 2020).

A subset of adults enrolled in the C8 Health Project between August 1, 2005, and August 31, 2006, were evaluated for exposure to perfluoroalkyl acids (PFAAs) via drinking water (Watkins et al., 2014). The cross-sectional survey consisted of residents within the mid-Ohio River Valley. A second, short-term follow-up study including another sample collection was conducted in 2010 to evaluate epigenetic alterations in relation to serum PFOA concentrations. Serum concentrations of PFOA significantly decreased between enrollment (2005–2006) and follow-up (2010). However, methylation of long interspersed nuclear elements (LINE-1) transposable DNA elements in peripheral blood leukocytes was not associated with PFOA exposure at any timepoint.

Several studies detail the influence of PFOA exposure on the epigenome in humans. Specifically, in prenatal studies, PFOA exposure was associated with mixed results of increased methylation in cord blood but not in placenta. However, consistently, studies found alterations in methylation patterns in genes associated with fetal growth. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.2.1.3 Animals

An in vivo analysis of epigenetic modifications in an oral PFOA study (1–20 mg/kg/day; 10 days) was performed in female CD-1 mice (Rashid et al., 2020). Measurement of 5-methylcytosine (5mc) and 5-hydroxymethylcytosine (5hmc) indicated no alteration of global CpG methylation levels in the kidneys. Downregulation of DNA methyltransferase 1 (*Dnmt1*) mRNA was observed at ≤5 mg/kg/day PFOA, while *Dnmt1* expression increased by 4- and 7-fold at doses of 10 and 20 mg/kg/day, respectively. Levels of *Dmnt3a* decreased at all doses, and *Dnmt3b* expression increased at the highest dose (20 mg/kg/day). mRNA expression of

translocation (Tet) 1/2/3 methylcytosine dioxygenases was decreased at low doses of PFOA exposure compared with controls, with no change at higher doses.

3.5.3.2.2 In Vitro Evidence

In vitro PFOA exposures have yielded mixed results with evidence of both hyper- and hypomethylation of DNA. Data presented here are categorized by global DNA methylation and gene-specific modifications.

3.5.3.2.2.1 Global DNA Methylation

5mC expression can be used to indicate global DNA methylation. Pierozan et al. (2020) treated MCF-10A cells with PFOA (100 μM, 72 hours) and found elevated global methylation levels in the first daughter cell subculture. However, methylation levels returned to baseline after the second passage. This study contrasts with the results of Wen et al. (2020) in a study conducted in HepG2 cells (20–400 μM PFOA, 48 hours), and Liu and Irudayaraj (2020) in a study of MCF7 cells (20–400 μM PFOA, 24–48 hours). Both studies found dose-dependent reductions in 5mC after PFOA exposure.

3.5.3.2.2.2 Modification to Gene Expression

Assays evaluating gene expression modified by enzymes that regulate DNA methylation levels, such as DNMT and TET enzymes, and histone modifications have been used to assess the impact of PFOA on the epigenome. Liu and Irudayaraj (2020) reported significantly lower levels of DNMT1 protein after PFOA exposure in both MCF7 (≥100 μM) and HepG2 (≥200 μM) cells. However, DNMT3A expression was increased in a dose-dependent manner in MCF7 cells (≥200 μM). Authors attributed PFOA-induced global demethylation to alterations of DNMT3A and subsequent enzymatic activity of DNMT. Levels of DNMT3B did not change significantly in either cell line. Wen et al. (2020) found no significant changes to *DNMT1/3A/3B* gene profiles after PFOA exposure (20–400 μM, 48 hours) in HepG2 cells. Further analysis found PFOA (200 μM) decreased *TET1* expression, which is strongly associated with DNA methylation, but increased *TET2 and TET3*. Pierozan et al. (2020) noted that PFOA-exposed MCF-10A cells and the direct daughter cell passages contained decreased levels of histone 3 lysine 9 dimethylation (H3K9me2). H3K9me2 is a silencing epigenetic marker; thus, a decrease in H3K9me2 is indicative of transcriptional activation, and has been associated with altered gene expression in breast cancer transformation.

3.5.3.3 Key Characteristic #5: Induce Oxidative Stress

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are byproducts of energy production that occur under normal physiological conditions. An imbalance in the detoxification of reactive such species can result in oxidative (or nitrosative) stress, which can play a role in a variety of diseases and pathological conditions, including cancer. The primary mechanism by which oxidative stress leads to the carcinogenic transformation of normal cells is by inducing oxidative DNA damage that leads to genomic instability and/or mutations (Smith et al., 2016b). Overall, the evidence supports that oxidative stress can result from PFOA exposure, based on animal and in vitro studies. The evidence related to oxidative stress is detailed below and in the referenced sections.

3.5.3.3.1 In Vivo Evidence

3.5.3.3.1.1 Humans

Franken et al. (2017) measured urinary 8-OHdG to evaluate DNA damage induced by oxidative stress, in adolescents (14–15 years of age) that resided for >5 years in industrial areas of Belgium and compared their findings to blood PFOA concentrations. While no significant change was observed in urinary 8-OHdG in the subjects when compared with that of age-matched controls, a positive dose-response relationship with increasing PFOA concentrations was noted. The authors attributed the DNA damage to oxidative stress but noted that elevated 8-OHdG could also reflect aberrant DNA repair.

3.5.3.3.1.2 Animals

Several in vivo analyses of PFOA exposure in rodents found evidence that PFOA exposure caused increased oxidative stress and markers of oxidative damage in a tissue-specific manner.

Takagi et al. (1991) performed a two-week subchronic study (0.02% powdered PFOA in the diet) in male Fischer 344 rats and evaluated the levels of 8-OHdG in the liver and kidneys after exposure. While a significant increase was noted in liver and kidney weights, elevated levels of 8-OHdG was observed only in the liver. A second subset of animals were given a single IP injection of PFOA (100 mg/kg) and sacrificed at days 1, 3, 5, and 8. Results were comparable to that of the dietary exposure study, as PFOA significantly increased liver (by day 1) and kidney (on days 3 and 8) weights with elevated liver 8-OHdG levels (by day 3).

Minata et al. (2010) exposed wild-type (129S4/SvlmJ) and *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice to PFOA (≤50 µmol/kg/day) for four weeks. Levels of 8-OHdG were evaluated in the liver. No increase in oxidative stress levels was noted in exposed wild-type mice. In contrast, *Pparα*-null mice demonstrated a dose-dependent increase in 8-OHdG levels, with a significant increase at 50 µmol/kg/day when compared with controls. The correlation between PFOA exposure and 8-OHdG was associated with increased tumor necrosis factor α (*TNF-α*) mRNA levels.

In a developmental toxicity study, Li et al. (2019b) exposed pregnant Kunming mice to PFOA (≤10 mg/kg/day) on gestational day (GD) 1–17. Female mice were sacrificed on postnatal day (PND) 21 and livers were assessed for oxidative damage by quantification of 8-OHdG, catalase, and superoxide dismutase (SOD). Findings indicate the PFOA caused a dose-dependent increase in oxidative DNA damage levels, which were significantly elevated after 2.5 mg/kg/day. These results were associated with increased superoxide dismutase and catalase protein levels. Together, these findings suggest that the livers of exposed mice were producing antioxidant enzymes to counteract PFOA-induced elevated oxidative stress.

The testes are particularly susceptible to oxidative stress due to high energy demand and abundance of polyunsaturated fatty acids. Liu et al. (2015) exposed male Kunming mice to ≤10 mg/kg/day of PFOA for 14 days and examined oxidative stress in the testis and epididymis. A dose-dependent increase in lipid peroxidation and oxidative stress was observed with a significant increase at ≥5 mg/kg/day relative to controls. In contrast to the results of Li et al. (2019b), levels of the antioxidant enzymes SOD and carnitine acyltransferase (CAT), and *Nrf2* expression (an oxidative stress response gene) decreased as PFOA exposure doses increased.

Several other studies measuring oxidative stress in the liver have found that PFOA induces damage through hydrogen peroxide production (Salimi et al., 2019) and through PPARα activation pathways (Li et al., 2019b). For additional information that PFOA induces oxidative stress in the liver, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

Evidence that PFOA induces oxidative stress in the immune system has been reported. Wang et al. (2014) observed that the spleens of mice treated with PFOA had mitochondrial swelling and cavitation as well as swollen and ruptured cristae, which suggests impaired oxidative processes. For additional information that PFOA induces oxidative stress in immune cells, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

Mechanistic studies noted PFOA exposure increased oxidative stress in the heart and brain. For additional information, please see the developmental (Section 3.4.4.3) and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC for additional supporting information and study details).

3.5.3.3.2 In Vitro Evidence

The ability of PFOA to induce oxidative stress has been assessed in vitro in several human, nonhuman primate, and animal cell lines.

PFOA exposure caused a dose-dependent increase in 8-OHdG in human lymphoblast cells (TK6), with significant results noted at ≥250 ppm (2 hours) (Yahia et al., 2016). A similar relationship was noted in HepG2 cells with significant increase in 8-OHdG levels found at PFOA concentrations ≥100 µM (3 hours) (Yao and Zhong, 2005). Yao and Zhong (2005) measured ROS using a 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) assay and observed a dose-dependent increase associated with elevated 8-OHdG levels. Peropadre et al. (2018) found 8-OHdG levels were nonsignificantly elevated in human HaCaT cells following 24-hour exposure to PFOA (50 µM). However, measurements taken 8 days following exposure found levels to be significantly elevated by 50%.

Panaretakis et al. (2001) observed the peak in ROS generation three hours following PFOA exposure in HepG2 cells exposed to concentrations of 200 and 400 µM. Both concentrations significantly increased hydrogen peroxide and superoxide anions. Wielsøe et al. (2015) noted nonsignificant elevated levels of ROS after HepG2 cells were exposed to PFOA (0.2–20 µM) for 24 hours. Additionally, total antioxidant capacities were reduced after exposure to 0.02–2,000 µM. These studies contrast with the findings of Florentin et al. (2011), which found no change in ROS using a DCFH-DA test in HepG2 cells exposed to 5–400 µM PFOA for 1 or 24 hours.

Kidney cells isolated from the African green monkey (Vero) were used in a DCFH-DA assay to measure ROS production (Fernández Freire et al., 2008). Authors reported a dose-dependent increase in ROS production that reached significance at 500 µM after 24 hours. Vero cells also displayed fragmentation of mitochondrial reticulum at ≥50 µM, a morphological change consistent with defective metabolism, indicating that irregular metabolic activity may play a role in ROS production in this model and exposure scenario.

ROS production was significantly higher in *Paramecium caudatum* exposed to PFOA (100 µM) for 12 or 24 hours, while 8-OHdG was not affected by PFOA (Kawamoto et al., 2010). Addition of the antioxidant glutathione attenuated the PFOA-induced ROS production but not DNA damage (as measured by a comet assay), indicating that the PFOA-induced DNA damage was not associated with oxidative stress in *P. caudatum*.

Hocevar et al. (2020) exposed mouse pancreatic acinar cells to PFOA (≤100 µg/mL; 6 or 24 hours) and observed an increase in intracellular calcium-induced activation of the unfolded protein response (UPR) in the endoplasmic reticulum at concentrations ≥50 µg/mL. This is a well-established oxidative stress-inducing pathway.

Zhao et al. (2011) exposed human-hamster hybrid (A_L) cells to PFOA (1–200 µM; 1–16 days) and found significantly increased intracellular ROS, NO, and $O_2^{\cdot -}$ levels at all timepoints exposed to ≥100 µM. These increases correlated with cytotoxicity, which was significant at all timepoints at 100 and 200 µM. DNA mutagenicity was only significant at the highest concentration at the longest exposure (16 days). Effects were reversed when previously PFOA-exposed cells were treated with oxidative stress inhibitors dimethyl sulfoxide (DMSO) and NG-methyl-L-arginine (L-NMMA). When repeating the study using a mitochondrial deficient cell line (p^0A_L), authors reported no mutagenesis, indicating that if the increase in DNA mutation after PFOA exposure is related to ROS generation, the association is mitochondria dependent.

3.5.3.4 Key Characteristic #6: Induces Chronic Inflammation

The induction of chronic inflammation includes increased white blood cells, altered chemokine and/or cytokine production, and myeloperoxidase activity (Smith et al., 2016b). Chronic inflammation has been associated with several forms of cancer, and a role of chronic inflammation in the development of cancer has been hypothesized. However, there are biological links between inflammation and oxidative stress and genomic instability, such that the contribution of each in carcinogenic progression is not always clear. Overall, the evidence demonstrates that PFOA exposure is related to increased markers of inflammation in animal and in vitro studies. The evidence related to chronic inflammation is detailed below.

3.5.3.4.1 In Vivo Evidence

Increased inflammation and/or inflammatory markers (i.e., inflammatory cytokines) has been reported in animal toxicological studies of acute, subchronic, and chronic exposures to PFOA. NTP (2020) used a matrix-type exposure paradigm. Pregnant Sprague-Dawley rats were administered PFOA via gavage beginning on GD 6 and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as (perinatal exposure level (ppm))/(postweaning exposure level (ppm)) and ranged from 0/0–300/300 ppm in males and 0/0–300/1,000 ppm in females. At the 16-week interim sacrifice, incidences of chronic active inflammation of the glandular stomach submucosa was significantly higher in the male 0/300 ppm group compared with the control group. No effects were seen in female rats at the interim sacrifice. At the 2-year evaluation, females in the 0/1,000 and 300/1,000 ppm groups exhibited increased incidences of ulcer, epithelial hyperplasia, and chronic active inflammation of the submucosa of the forestomach when compared with controls.

Histopathological analysis of animals exposed to PFOA (0.625–10 mg/kg) by oral gavage for 28 day exhibited nasal respiratory epithelium inflammation in both males and females, though

these effects did not follow a linear dose response (NTP, 2019). Similarly, olfactory epithelial inflammation and degeneration were observed in females. Increases in nasal and olfactory hyperplasia were thought to be a result of the observed epithelial degradation and/or inflammation.

Activation of the NF-κB signaling pathway plays an important role in the regulation of inflammation, including through expression of proinflammatory cytokines (Shane et al., 2020; Zhong et al., 2020; Lee et al., 2017a; Zhang et al., 2014a). Modification to NF-κB expression has been observed in adult zebrafish after 7, 14, and 21 days of PFOA exposure (Zhong et al., 2020; Zhang et al., 2014a) and in female BALB/c mice dermally exposed to PFOA for 14 days (Shane et al., 2020). Additionally, proinflammatory cytokines IL-1β, TNF-α, and others were upregulated by PFOA exposure at doses ranging from 0.002% w/w in the diet and 2.5–10 mg/kg/day by gavage for 10 or 14 days in various tissues across several mouse studies (Liu et al., 2016; Wang et al., 2014; Yang et al., 2014; Qazi et al., 2010).

3.5.3.4.2 In Vitro Evidence

Saejia et al. (2019) noted that PFOA (1 nM, 72 hours) significantly increased activation of NF-κB in FTC133 cells. Furthermore, translocation of the phosphorylated version of NF-κB to the nucleus from the cytosol, a crucial step in inflammation cytokine production, was observed. Inhibition of NF-κB activation was found to reduce invasive characteristics of cells, likely through reduced expression of MMP-2 and MMP-9. PFOA increased the levels of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, in a dose-responsive manner in IgE-stimulated rat mast cells (RBL-2H3 cell line) (Lee et al., 2017a). It is important to note that in vitro models may be used for the evaluation of changes in inflammatory markers and response, they are generally not effective in modeling the events that are associated with chronic inflammation.

Several studies have identified the potential of PFOA to increase inflammation within various testing systems. For additional information, please see the immune (Section 3.4.2.3), hepatic (Section 3.4.1.3), and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.5 Key Characteristic #7: Is Immunosuppressive

Immunosuppression refers to the reduction in the response of the immune system to antigen, which is important in cases of tumor antigens (Smith et al., 2016b). It is important to note that immunosuppressive agents do not directly transform cells, but rather can facilitate immune surveillance escape of cells transformed through other mechanisms (e.g., genotoxicity). Overall, the evidence demonstrates that PFOA exposure can alter and impair immune and inflammatory response and function in both humans and animals, as detailed briefly in the following paragraph and in further detail in the referenced section.

Studies have identified the immunosuppressive potential of PFOA in in vivo and in vitro testing systems. The pleotropic immunomodulatory effects of PFOA, including impaired vaccine response in humans and reduction in B and T cell populations in the thymus and spleen in laboratory animals, may reflect perturbed function of B and/or T cells. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating

apoptosis, participates in the regulation of CD4+ T cell differentiation, and is involved in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the underlying mechanism of PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation. For additional information, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.6 Key Characteristic #8: Modulates Receptor-Mediated Effects

Modulation of receptor-mediated effects involves the activation or inactivation of receptors (e.g., PPAR, AhR) or the modification of endogenous ligands (including hormones) (Smith et al., 2016b). Overall, the animal and in vitro evidence demonstrates that PFOA activates several nuclear receptors: PPARα, CAR/PXR, ERα, and HNF4α, as detailed briefly in the following paragraphs and in detail in the referenced sections.

3.5.3.6.1 In Vivo Evidence

Yan et al. (2015a) exposed adult male Balb/c mice to PFOA (0.08–20 mg/kg/day) via oral gavage for four weeks. Livers were isolated and mRNA levels of several peroxisome proliferator-activated receptors (PPARs) were evaluated using RT-PCR. PPARα was found to be increased by 50% in the 0.08 and 0.31 mg/kg/day dose groups. This trend was not consistent as PPARα levels diminished at higher doses. PPARγ was found to increase in a dose-dependent manner that reached significance at 1.25 mg/kg/day PFOA. No differences were observed in PPARβ/δ mRNA expression after exposure.

Data from studies conducted in rodent models have demonstrated PPARα activation as a mechanism for PFOA-induced hepatotoxicity, due to the association between hepatic lesions and/or increased liver weight and peroxisome proliferation downstream of PPARα activation. There is also growing evidence the PFOA activates other nuclear receptors (e.g., CAR/PXR, ERα, HNF4α) in tandem with PPARα to enact its effects. For additional information, please see the hepatic (Section 3.4.1.3) and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.6.2 In Vitro Evidence

PPARα and PPARγ gene expression was assessed in hepatocellular carcinoma cells (Hepa 1-6) exposed to PFOA (50–200 μM; 72 hours) (Yan et al., 2015a). While no significant changes were observed for these genes, PPARα target genes were significantly increased, indicating that PPARα was activated by PFOA.

Available mechanistic evidence demonstrates that PFOA has the potential to dysregulate hormone levels in hepatic cells, particularly regarding thyroid function. Furthermore, rodent and human hepatocytes treated with PFOA demonstrated a concentration-dependent decrease in lipid accumulation that was associated with PPARα activation. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.7 Key Characteristic #9: Causes Immortalization

Immortalization leads to tumorigenesis when cells continue to divide after sustaining DNA damage and/or shortened telomeres, events that cause cells to cease to divide in healthy or normal states (i.e., the Hayflick limit). Immortalization is a key characteristic typically observed in and associated with human DNA and RNA viruses, such as human papillomaviruses and hepatitis C virus, among others. In vitro cell transformation assays have been historically used to test carcinogenic potential of both genotoxic and non-genotoxic compounds (Creton et al., 2012), and is recognized as an assay related to key characteristic #9 (Smith et al., 2020). Overall, the limited evidence demonstrates that PFOA does not alter cell transformation or cause immortalization, as detailed in the following paragraph.

In the case of PFOA, two studies reported no change in cell transformation in vitro in cells exposed to PFOA relative to controls. Jacquet et al. (2012) exposed SHE cells to PFOA at concentrations ranging from 3.7×10^{-4} to 37.2 µM for 6 days with or without pre-treatment with the tumor initiator benzo-α-pyrene (BaP). PFOA exposure alone did not induce cell transformation, but PFOA did significantly induce transformation in BaP-sensitized cells, indicating that PFOA does not alone initiate cell transformation, but may have tumor promoter-like activity. A second in vitro cell transformation assay reported no evidence of transformation in C3H 10T-1/2 mouse embryo cells exposed to 0.1–200 µg/mL PFOA in a 14-day colony assay for transformation nor in a 38-day foci transformation assay (Garry and Nelson, 1981).

3.5.3.8 Key Characteristic #10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

Aberrant cellular proliferation, cell death, and/or nutrient supply is a common mechanism among carcinogens. This mechanism includes aberrant proliferation, decreased apoptosis or other evasion of terminal programming, changes in growth factors, angiogenesis, and modulation of energetics and signaling pathways related to cellular replication or cell cycle control (Smith et al., 2016b). Overall, the evidence demonstrates that PFOA exposure can increase cell proliferation in animals and in cell models, and results are conflicting on the ability of PFOA to induce or inhibit apoptosis. The evidence related to cell proliferation, cell death, and migration (cancer cell invasiveness) is detailed below.

3.5.3.8.1 In Vivo Evidence

To determine if PFOA exposure induced proliferation in cancer cells, Ma et al. (2016) xenografted human endometrial adenocarcinoma (Ishikawa cell line) cells into the flanks of six-week-old female BALB/c mice. Animals were then treated with PFOA (20 mg/kg/day) by oral gavage daily for three weeks beginning the same day of the xenograft. Tumor volume was measured after five weeks, and data indicated that PFOA caused tumors to nearly triple in size. Additionally, levels of proliferating cell nuclear antigen (PCNA) and vimentin protein were both upregulated by PFOA, suggesting increased cell proliferation and invasion. E-cadherin expression was downregulated after PFOA exposure, indicating that cells were more likely to migrate and form metastases.

Treatment effects on apoptosis and cell cycle have also been observed in immune system cells of animals exposed to PFOA. Wang et al. (2014) exposed BALB/c mice to PFOA (5–20 mg/kg/day, 14 days) via gavage and reported that the percent of apoptotic cells increased in

the spleen (10–20 mg/kg/day) and in the thymus (20 mg/kg/day). Yang et al. (2002b) reported significant reductions in the proportion of thymocytes in the S and G2/M phases and significant increases in the G0/G1 phases of mice treated with PFOA, effects that were PPARα-dependent.

Additional mechanistic studies, detailed elsewhere, noted PFOA exposure alters the number of various B and T cell subsets in primary and secondary lymphoid organs, which may impact immune system development, including dysregulation of proliferation, differentiation, and/or apoptosis. For additional information, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.8.2 In Vitro Evidence

PFOA has been demonstrated to increase cell proliferation and apoptosis evasion in vitro. Evidence presented here is organized into three categories: induced proliferation, apoptosis evasion, and modification of cellular migration.

3.5.3.8.2.1 Proliferation

Exacerbation of proliferation in cancer cell lines is of particular concern to the development and prognosis of cancer. Several studies have utilized MTT assays to measure cellular metabolic activity to determine cell proliferation and cytotoxicity rates.

PFOA exposure (5–50 μM) increased cellular proliferation in MCF-7 human breast cancer cells and HepG2 human hepatoma (nontumorigenic) cells (Liu and Irudayaraj, 2020; Buhrke et al., 2015; Buhrke et al., 2013). However, predictably, proliferation rates decreased at cytotoxic concentrations (≥100 μM PFOA) (Wen et al., 2020; Buhrke et al., 2015; Buhrke et al., 2013). Similar results were observed in the breast epithelial (nontumorigenic) cell line MCF-10A, in which PFOA exposure (50 and 100 μM; 24–72 hours) increased cell proliferation, whereas proliferation rates decreased as the PFOA concentration was increased to a cytotoxic level (250 μM) (Pierozan et al., 2018). A subsequent study by Pierozan et al. (2020) reported that PFOA-induced (100 μM, 72 hours) proliferation persisted in MCF-10A daughter subcultures that were not exposed to PFOA. PFOA exposure (1–100 nM) in colorectal cancer cells (DLD-1) has also been shown to modify the cell cycle by causing more cells to enter S-phase and less in G_1 of mitosis (Miao et al., 2015).

Several studies of the effects of low exposure to PFOA found no evidence of modification to cell proliferation rates. These studies include ovarian cancer cell line A2780 (1–200 nM, 48 hours) (Li et al., 2018b) Ishikawa human endometrial adenocarcinoma cells (50 nM, 48 hours) (Ma et al., 2016), and human colorectal cancer cell line DLD-1 (1–10,000 nM, 72 hours) (Miao et al., 2015).

Insulin growth factor 1 (IGF-1) expression has been implicated in governing proliferation in cancer cells. A series of experiments performed by Gogola et al. (2020a; 2020b, 2019) used COV434 and KGN cells exposed to PFOA (0.02 ng/mL–2 μg/mL; 72 hours). All studies found increased proliferation in both cell lines. Proliferation was highest in COV434 and KGN cells at 0.02 ng/mL and 2 ng/mL, respectively. Interestingly, proliferation returned to baseline levels in both cell lines at PFOA concentration of 2 μg/mL, indicating a bell-shaped dose response. These experiments were repeated after inhibition of IGF-1 caused normalization in both cell lines after

PFOA exposure. Together, these studies demonstrate the potential pathway in which PFOA induces proliferation in cancer cells.

HepG2 cells were exposed to non-cytotoxic concentrations of PFOA for 24 hours before SHP-2, a tumor suppressor protein, was immunoprecipitated from the cell lysates (Yang et al., 2017). PFOA (100 μM) slightly lowered SHP-2 mRNA expression and decreased SHP-2 enzyme activity in a concentration-dependent manner. SHP-2 protein levels were increased only at 140 μM exposure, and unchanged at other concentrations. These results indicate that PFOA inhibits SHP-2 by reducing enzyme activity, not protein content.

Rainieri et al. (2017) evaluated the effects of PFOA on cell proliferation by quantifying the distribution of cells in different stages of the cell cycle in a human macrophage cell line (TLT cells). Significantly more cells were in G2M phase following exposure to PFOA (50–500 mg/L; 12 hours) in parallel with a lower proportion of cells in the G0/G1 phase, suggesting increased cell proliferation. For additional evidence of the effect of PFOA on cell death and cell proliferation in the immune system, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.8.2.2 Apoptosis

Evasion of programmed cell death is a characteristic of cancer cells, allowing them to continue proliferating, which can be enhanced by PFOA exposure. Dairkee et al. (2018) evaluated several human breast cancer cell lines for apoptosis following PFOA exposure (1 or 100 nM; 7 days). Using fluorescence activated cell sorting (FACS) of Annexin V-FITC, PFOA concentrations were found to be inversely correlated with apoptosis rates. However, in HepG2 cells, PFOA exposure was found to increase metabolically induced BAX apoptosis in a dose-dependent manner (Wen et al., 2020). Apoptosis was also found to increase in HepG2 cells after PFOA exposure (200 or 400 μM; ≤24 hours) and was associated with an increase in caspase-9 activation after 5 hours of exposure (Panaretakis et al., 2001). Additionally, the murine spermatogonial cell line GC-1 exhibited a dose-dependent increase in apoptosis after exposure to PFOA (≥250 μM) for 24 hours that reached significance at ≥500 μM (Lin et al., 2020d).

Caspase protease enzymes are essential in apoptotic cell death and are frequently used to assess apoptosis. Gogola at al. (2020a; 2020b) found that PFOA (0.2–20 ng/mL; 72 hours) caused no changes to caspase 3/7 expression in COV434 and KGN cells. Additionally, PFOA (≤100 μM) had no effect on caspase 3/7 activity in HepG2 cells. Lin et al. (2020d) reported a dose-dependent increase in caspase-3 activity that correlated with apoptosis rates in GC-1 cells. Additionally, apoptosis and caspase activity were inversely correlated with Bcl-2/Bax ratios. These results indicate that PFOA may induce apoptosis through an increase in BAX expression. Hu and Hu (2009) also suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing ROS, impacting mitochondria, and changing expression of apoptosis gene regulators, based on their findings in studies with HepG2 cells. Overall, data are conflicting on the ability of PFOA to induce or inhibit apoptosis, with the variation likely dependent upon dose and duration of exposure.

3.5.3.8.2.3 Modulation of Migration

Cancer cells are invasive in nature due to their ability to increase mobility, reduce attachment to neighboring cells, and express proteins that break down the extracellular matrix of tissues. Wound healing assays are a common and reproducible way to inflict a ‘wound’ on a monolayer

plate of cells and measure the time for the cells to re-establish confluency. Two independent studies concluded PFOA exposure increased the rate at which Ishikawa cells (50 nM, 48 hours) (Ma et al., 2016) and A2780 cells (≥100 nM, 72 hours) (Li et al., 2018b) were able to re-establish confluency in a dose-dependent manner.

Assays of migration and invasion measure the ability of a cell to travel either without inhibition or through the extracellular matrix of plated cells, respectively. Two studies investigated cellular migration after PFOA exposure and found no change after FTC133 cells were exposed to 1 nM (72 hours) (Saejia et al., 2019) or 0–1 mM (24–72 hours) (Pierozan et al., 2018), while an increase in migration was found at 100 nM (72 hours) in MCF-10A cells (Pierozan et al., 2018). All studies reviewed found an increase in the invasive nature of cancer cells lines FTC133 (1 nM, 72 hours) (Saejia et al., 2019), Ishikawa (≥50 nM) (Ma et al., 2016), MCF-10A (100 nM, 72 hours) (Pierozan et al., 2018), A2780 (≥100 nM, 72 hours) (Li et al., 2018b), and DLD-1 (1 nM–1 μM, 72 hours) (Miao et al., 2015) after PFOA exposure.

Pierozan et al. (2020) exposed MCF-10A cells to PFOA (100 μM, 72 hours) and found that invasion and migration of daughter cell passages was elevated when compared with control.

Several reports noted cell invasion and upregulated MMP2 and MMP9 expression levels, which help to break down the extracellular matrix allowing cells to move freely, indicating that cancer cells could be more likely to become invasive or metastasize after exposure to PFOA (Saejia et al., 2019; Li et al., 2018b; Miao et al., 2015).

Additional mechanistic studies have identified the potential of PFOA to induce aberrant cellular proliferation rates and increase apoptosis within in vitro testing systems. For additional information, please see the immune (Section 3.4.2.3) and hepatic (Section 3.4.1.3) mechanistic sections (refer to the interactive HAWC visual for additional supporting information and study details).

3.5.4 Weight of Evidence for Carcinogenicity

3.5.4.1 Summary of Evidence

The carcinogenicity of PFOA has been documented in both epidemiological and animal toxicological studies. The evidence from *medium* confidence epidemiological studies is primarily based on the incidence of kidney and testicular cancer, as well as some evidence of increased breast cancer incidence in susceptible subpopulations. Other cancer types have been observed in humans, although the evidence for these is generally limited to *low* confidence studies. The evidence of carcinogenicity in animal models is provided in three *high* or *medium* confidence chronic oral animal bioassays in Sprague-Dawley rats which together identified neoplastic lesions of the liver, pancreas, and testes. The available mechanistic data suggest that multiple MOAs could play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models.

3.5.4.1.1 Evidence From Epidemiological Studies

The strongest evidence of an association between PFOA exposure and cancer in human populations is from studies of kidney cancer. Two *medium* confidence studies of the C8 Health Project population reported positive associations between PFOA levels (mean at enrollment 0.024 μg/mL) and kidney cancer among the residents living near the DuPont plant in

Parkersburg, West Virginia (Barry et al., 2013; Vieira et al., 2013). Vieira et al. (2013) reported elevated risk of kidney cancer in residents of the Little Hocking water district of Ohio (OR: 1.7, 95% CI: 0.4, 3.3; n = 10) and the Tuppers Plains water district of Ohio (OR: 2.0, 95% CI: 1.3, 3.1; n = 23). Barry et al. (2013) extended this work, and found increased risk of kidney cancer (HR: 1.10, 95% CI: 0.98, 1.24; n = 105), though the levels did not reach statistical significance. The high-exposure occupational study by Steenland and Woskie (2012) evaluated kidney cancer mortality in workers from West Virginia and observed significant elevated risk of kidney cancer death in the highest exposure quartile. As part of the C8 Health Project, the C8 Science Panel (2012a) concluded a probable link between PFOA exposure and kidney cancer (Steenland et al., 2020).

The findings of another recently published *medium* confidence study add support to the previous evidence of an association between PFOA and kidney cancer (Shearer et al., 2021). Shearer et al. (2021) is a multicenter case-control study nested within the National Cancer Institute (NCI) Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial (n = 326). The authors reported a statistically significant increase in risk of renal cell carcinoma (RCC) with pre-diagnostic serum levels of PFOA (OR = 2.63; 95% CI: 1.33, 5.20 for the highest vs. lowest quartiles; p-trend = 0.007, or per doubling of PFOA: OR: 1.71; 95% CI: 1.23, 2.37). The association remained significant in analyses on a per doubling increase in PFOA after adjusting for other PFAS. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant. Statistically significant increased odds of RCC were observed in a subgroup of participants ages 55–59 years, and in men and in women, analyzed separately. A recent critical review and meta-analysis of the epidemiological literature concluded that there was an increased risk for kidney tumors (16%) for every 10 ng/mL increase in serum PFOA (Bartell and Vieira, 2021). Although the authors concluded that the associations were likely causal, they noted the limited number of studies and therefore, additional studies with larger cohorts would strengthen the conclusion. Taken together, the recent pooled analysis of the NCI nested case-control study (Shearer et al., 2021) of 324 cases and controls and the C8 Science Panel Study (Barry et al., 2013) of 103 cases and 511 controls provide evidence of concordance in kidney cancer findings from studies of the general population and studies of high-exposure communities (Steenland et al., 2022). CalEPA (2021) similarly concluded, "[t]here is evidence from epidemiologic studies that exposure to PFOA increases the risk of kidney cancer."

There is also evidence of associations between PFOA serum concentrations and testicular cancer in humans, though no new epidemiological studies reporting these associations have been published since the studies described in the 2016 PFOA HESD (U.S. EPA, 2016c). Similar to their results for kidney cancer, Vieira et al. (2013) reported an increased adjusted OR for testicular cancer (OR: 5.1, 95% CI: 1.6, 15.6; n = 8) in residents of the Little Hocking water district of Ohio. Barry et al. (2013) also found significantly increased testicular cancer risk with an increase in estimated cumulative PFOA serum levels (HR: 1.34, 95% CI: 1.00, 1.79; n = 17). The C8 Science Panel (2012a) concluded that a probable link also exists between PFOA exposure and testicular cancer (Steenland et al., 2020). A recent critical review and meta-analysis of the epidemiological literature concluded that there was an increased risk for testicular tumors (3%) for every 10 ng/mL increase in serum PFOA (Bartell and Vieira, 2021) (see Appendix A, (U.S. EPA, 2024a)). In their review of the available epidemiological data, IARC (2016)

concluded that the evidence for testicular cancer was "considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers." Similarly, CalEPA (2021) concluded, "[o]verall, the epidemiologic literature to date suggests that PFOA is associated with testicular cancer."

The majority of epidemiological studies examining the carcinogenicity after PFOA exposure reported on breast cancer risk. Two nested case-control studies found associations between PFOA exposure and breast cancer, but only in participants with known genetic susceptibility (e.g., specific genotype or tumor estrogen receptor (ER) type) (Mancini et al., 2020; Ghisari et al., 2017). In Taiwan, Tsai et al. (2020) observed an increased risk of breast cancer only in all women 50 years old or younger (including ER+ and ER– participants), and in ER+ participants aged 50 years or younger, along with a decrease in risk for ER– breast cancers in participants aged 50 years or younger. Significantly increased odds of breast cancer were also observed in an NHANES population across serum PFOA quartiles with a significant dose-response trend (Omoike et al., 2021). Two nested case-control studies did not report an association between breast cancer and PFOA concentrations measured in maternal serum throughout pregnancy and 1–3 days after delivery (Cohn et al., 2020) or in serum after case diagnosis and breast cancer (Hurley et al., 2018). One nested case-cohort study did not report an association between breast cancer and PFOA concentrations measured in a group of predominantly premenopausal women (Bonefeld-Jørgensen et al., 2014). In the C8 Health Project cohort, Barry et al. (2013) observed a significant inverse association with breast cancer for both unlagged (i.e., concurrent) and 10-year lagged (i.e., cumulative exposures occurring 10 years in the past) estimated cumulative PFOA serum concentrations. Similarly, a recent study in a Japanese population reported an inverse association across serum PFOA quartiles with a significant dose-response trend (Itoh et al., 2021). Overall, study design differences, lack of replication of the results, and a lack of mechanistic understanding of specific breast cancer subtypes or susceptibilities of specific populations limit firm conclusions regarding PFOA and breast cancer. However, there is suggestive evidence that PFOA exposure may be associated with an increased breast cancer risk based on studies in populations with specific genetic polymorphisms conferring increased susceptibility and for specific types of breast tumors.

3.5.4.1.2 Evidence From Animal Bioassays

In addition to the available epidemiological data, two multidose bioassays and one single-dose chronic cancer bioassay are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001). Increased incidences of neoplastic lesions were primarily observed in male rats, though results in females are supportive of potential carcinogenicity of PFOA. Testicular Leydig cell tumors (LCTs) were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. LCT incidence at similar dose levels was comparable between the two studies (11% and 14%). Pancreatic acinar cell tumors (PACTs) were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas and adenocarcinomas in males in all treatment groups compared with their respective controls (Table 3-17). These pancreatic tumor types were also observed in female rats in the highest dose group, a rare occurrence compared with historical controls (0/340), though these increases did not reach statistical significance. Biegel et al. (2001) similarly reported increases in the incidence of PACTs in male rats treated with PFOA, with zero incidences observed in control animals. In addition, NTP (2020) reported dose-dependent increases in the incidence of liver

adenomas and carcinomas in male rats (Table 3-16) and Biegel et al. (2001) also observed increased incidence of adenomas in male rats. Overall, NTP concluded that in their 2-year feeding studies, there was *clear evidence* of carcinogenic activity of PFOA in male Sprague-Dawley rats and *some evidence* of carcinogenic activity of PFOA in female Sprague-Dawley rats based on the observed tumor types (NTP, 2020).

The report from NTP (2020) provides evidence that chronic oral exposure accompanied by perinatal exposure (i.e., exposure beginning at gestation day 5 through lactation) to PFOA does not increase cancer risk when compared with chronic exposure scenarios beginning during the postnatal (i.e., exposure initiated after weaning) stage. The incidences of all tumor types examined did not differ significantly between the treatment groups administered PFOA during both perinatal and postweaning periods compared with the postweaning-only treatment groups (see further study design details in Section 3.4.4.2.1.2). Lifestage-dependent sensitivity to the carcinogenic effects of PFOA exposure was previously assessed in the study by Filgo et al. (2015) which exposed two mouse strains during gestation only (i.e., prenatal exposure with no comparisons to mice exposed through adulthood). Filgo et al. (2015) observed a nonmonotonic increase in hepatocellular adenomas in the female offspring of one strain (CD-1) and hepatocellular adenoma incidence in approximately 13% of all PFOA-exposed peroxisome proliferator-activated receptor (PPAR) α-knockout mice. However, these results are not conclusive due to the study's limited sample size and study design.

3.5.4.2 Mode of Action Analysis

In the 2016 PFOA HESD (U.S. EPA, 2016c), the EPA concluded that the induction of tumors was likely due to multiple MOAs, specifically noting interactions with nuclear receptors, perturbations in the endocrine system, interruption of intercellular communication, mitochondrial effects, and/or perturbations in the DNA replication and cell division processes. Since that time, the available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models. The few available mechanistic studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish which mechanism(s) are operative for PFOA-induced kidney cancer. Similarly for testicular cancer, the available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans.

As described in the following subsections, the available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models.

3.5.4.2.1 Mechanistic Evidence for Renal Tumors

As discussed in Section 3.5.13.4.5, there is convincing evidence for an association between renal carcinogenesis and serum PFOA concentrations in epidemiological studies from both the general population and residents of high-exposure communities (Shearer et al., 2021; Barry et al., 2013). However, there is limited mechanistic information from epidemiological studies explaining the observed renal carcinogenicity. Additionally, many animal models are limited in their ability to

replicate kidney damage due to PFOA exposure that is observed in humans (Li et al., 2017a). One factor that may be driving this inconsistency between humans and animals is the difference in renal clearance rates between human and animal models. Regardless of elimination differences, both animal toxicological studies and the limited available human biomonitoring data suggest that the kidneys may be a site of enrichment upon PFOA exposure and subsequent distribution (Shearer et al., 2021).

The few available studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish what mechanism(s) are the most relevant for PFOA-induced kidney cancer. The renal-specific evidence supporting multiple mechanisms involved in tumorigenesis is described in the subsections below, which are all key characteristics of carcinogens and may be related to PFOA-induced renal cell carcinoma.

3.5.4.2.1.1 Altered Cell Death, Cell Proliferation, or Nutrient Supply

There is evidence that relative kidney weight, particularly in male rats, is increased after PFOA treatment (see Appendix C, (U.S. EPA, 2024a)) (NTP, 2020, 2019; Butenhoff et al., 2004a). However, these increases in kidney weight and presumably increases in cell proliferation may be due to increased need for renal transporters and not necessarily an indicator of the initial stages of carcinogenesis (U.S. EPA, 2016a). Though there is conflicting evidence of alterations in relative kidney weight in female rats, NTP (2020) reported increased hyperplasia of urothelium that lines the renal papilla in female rats from the 0/1,000 and 300/1,000 ppm (63.4 and 63.5 mg/kg/day, respectively) dose groups at the interim sacrifice timepoint (16 weeks) and in female rats from the 0/300 (18.2 mg/kg/day), 0/1,000, and 300/1,000 ppm dose groups at the terminal sacrifice (107 weeks). These changes were accompanied by increased incidence of renal papilla necrosis at terminal sacrifice in both 1,000 ppm postweaning groups. Though NTP (2020) did not explore the mechanisms of toxicity underlying the observed renal effects, they note that prolonged exposure and relatively high dose levels along with the enhanced efficiency of excretion and increased urinary concentrations of PFOA in female rats (compared with males) may have resulted in cytotoxicity and hyperplasia of the papilla.

Evidence of cytotoxicity and cell cycle disruption was also provided by a single *in vitro* study in Vero cells (cell line derived from monkey kidney epithelial cells) (Fernández Freire et al., 2008). Fernández Freire et al. (2008) assessed potential cytotoxic effects and alterations in cell cycle progression in Vero cells treated with PFOA at concentrations of 50–500 μM for 24 hours. Cells treated with PFOA exhibited decreases in viability and proliferation, as indicated by alterations in mitochondrial metabolism (MTT assay) and the total number of cells (Bradford/TPC assay), though both assays exhibited a plateau in cytotoxicity at PFOA concentrations of approximately 200 μM and higher. The study also reported dose-dependent increases in the percentage of apoptotic cells with increasing PFOA concentrations. Flow cytometric analysis demonstrated G0/G1 cell cycle arrest in Vero cells treated with the maximum concentration of 500 μM PFOA. The percentage of cells in the G0–G1 stage were increased whereas the percentages of cells in the S and G2-M stages were decreased. The authors hypothesized that the observed cell cycle arrest may be linked to increased ROS and oxidative stress, further described below.

3.5.4.2.1.2 Oxidative Stress

The increases in cytotoxicity and apoptosis in Vero cells treated with up to 500 μM PFOA for 24 hours observed by Fernández Freire et al. (2008) were accompanied by a dose-dependent increase in ROS which was statistically significant in the cells treated with 500 μM. The authors noted that severe oxidative stress could induce cell cycle arrest and apoptosis, as described previously (Fernández Freire et al., 2008). However, in the only available animal toxicological study assessing oxidative damage in the kidney, levels of 8-hydroxydeoxyguanosine (8-OH-dG) DNA damage in the kidney were unchanged in male Fischer 344 rats administered PFOA via the diet (0.02% for 2 weeks) or by IP injection (100 mg/kg single injection) (Takagi et al., 1991). Though the renal-specific evidence of PFOA-induced oxidative stress is limited, further discussion on oxidative stress in other organ systems is discussed below, as well as in Section 3.5.3.

3.5.4.2.1.3 Epigenetics

Rashid et al. (2020) investigated epigenetic markers that could contribute to the kidney dysfunction associated with PFOA exposure. CD-1 mice were orally exposed to 1–20 mg/kg/day PFOA for 10 days and kidney tissues were evaluated for epigenetic alterations (DNA methylation and histone acetylation). Though no PFOA-induced changes in global methylation were noted (by measurements of 5-methyl cytosine and 5-hydroxy methylation levels), the study reported specific methylation changes with reduced representation bisulfite sequencing (RRBS). Overall, 879 genes were differentially methylated in in the 20 mg/kg/day dose group versus control. PFOA exposure also altered mRNA expression of several proteins that regulate DNA methylation, including DNA methyl transferases and translocation enzymes, as well as mRNA expression of several histone deacetylases. Combined, these results suggest that PFOA exposure triggered epigenetic alterations, including DNA methylation changes and potentially histone modifications, in the kidney (Rashid et al., 2020). However, further study is needed to explore connections between the observed epigenetic changes and subsequent regulation of genes associated with kidney tumorigenesis.

3.5.4.2.2 Mode of Action for Testicular Tumors

There is both epidemiological evidence and evidence from animal bioassays of an association between increased PFOA serum concentrations or doses and testicular carcinogenesis. Testicular cancer was observed in epidemiological studies from the C8 Health Project (Barry et al., 2013; Vieira et al., 2013). In addition, a recent meta-analysis concluded that there is a 3% increase in risk for testicular cancer with every 10 ng/mL increase in serum PFOA concentrations (Bartell and Vieira, 2021). In animal models, testicular tumors (Leydig cell tumors (LCTs)) were reported in two chronic studies in male Sprague-Dawley rats (Butenhoff et al., 2012; Biegel et al., 2001). Combined, these results indicate that the testes are a common site of PFOA-induced tumorigenesis.

The available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans. In a review of LCTs published by Clegg et al. (1997), a workgroup identified seven nongenotoxic hormonal MOAs, (i.e., androgen receptor antagonism; testosterone biosynthesis inhibition; 5α-reductase inhibition; aromatase inhibition; estrogen agonism; GnRH agonism; and dopamine agonism), five of which were considered relevant to humans, and the majority of which involved downstream increases

in luteinizing hormone (LH) levels and subsequent Leydig cell hyperplasia/tumorigenesis. The working group noted that sensitivity for the initiating events in these MOAs varies across species, with rodents being more sensitive relative to humans. It has also been proposed that PPARα agonism potentially mediates these effects, though the evidence supporting this claim is not as strong as for other tumor types (i.e., hepatic tumors) (Klaunig et al., 2012; Klaunig et al., 2003). However, CalEPA noted that "PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported" (CalEPA, 2021).

The testes-specific evidence for the six human-relevant MOAs are described in the subsections below, though, as described in Section 3.5.3, PFOA generally exhibits evidence of multiple key characteristics of carcinogens that may also be relevant to the MOA for testicular cancers associated with increased serum PFOA concentrations in humans.

3.5.4.2.2.1 Hormone-Mediated MOAs

Clegg et al. (1997) identified five human-relevant MOAs for LCTs that involve alterations in hormone balances, steroid receptor activity, or enzymes involved in steroid metabolism (5α-reductase inhibition, androgen receptor antagonism, aromatase inhibition, estrogen agonism, testosterone biosynthesis inhibition). In addition, some compounds have been shown to influence Leydig cell function, including steroidogenesis, via hormone-mediated MOAs that are initiated upon PPARα activation (Klaunig et al., 2003; Gazouli et al., 2002). Klaunig et al. (2003) described two proposed hormone-mediated MOAs and key events by which PPARα agonists could induce LCTs in rats: one MOA which is secondary to liver PPARα induction and one MOA which involves direct inhibition of testosterone biosynthesis in the testes. These two MOAs involve associative key events such as increased aromatase activity, increased serum estradiol (E2) levels, increased TGFα levels, decreased testosterone levels, increased LH levels, and/or Leydig cell proliferation. Evidence for the key events involved in the human-relevant MOAs for testicular tumors in rodents exposed to PFOA is summarized in the paragraphs below and in Table 3-23, Table 3-24, Table 3-25, and Table 3-26. There was no evidence of PFOA treatment resulting in 5α-reductase inhibition in the identified literature, and the majority of the limited available *in vitro* studies for PFOA report that PFOA does not act as an androgen receptor antagonist (McComb et al., 2019; Kang et al., 2016b; Du et al., 2013; Rosenmai et al., 2013). Thus, these two MOAs are not summarized herein.

3.5.4.2.2.1.1 Aromatase Inhibition MOA

In vivo studies in male rats and mice generally found no effect of oral PFOA exposure on testicular aromatase activity or mRNA expression, though there was some evidence for increased hepatic microsomal aromatase activity or mRNA expression (Li et al., 2011; Liu et al., 1996; Biegel et al., 1995). A reduction in serum testosterone is also opposite of the expected key event following aromatase inhibition (increased serum testosterone), further supporting that PFOA does not operate through this MOA. The hepatic aromatase activity provides some support for the MOA that is secondary to liver PPARα induction (Klaunig et al., 2003). Evidence demonstrating the lack of activity for the key events involved in the aromatase inhibition MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-23.

Table 3-23. Evidence of Key Events Associated with the Aromatase Inhibition Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: CYP19A1 Inhibition	Key Event 2: Increased Serum T	Key Event 3: Decreased Serum E2	Key Event 4: Increased Serum LH	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**CYP19A1 Activity in Liver**	**Serum T**	**Serum E2**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
0.2	– (14 d)	NR	– (14 d)	NR	NR	NR
0.31	NR	– (28 d)	NR	NR	NR	NR
0.64	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
1	– (6 wk)	↓ (6 wk) – (GD1–17) – (14 d)	– (14 d)	– (14 d)	– (6 wk)	– (6 wk)
1.1[b]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.25	NR	↓ (28 d)	NR	NR	NR	NR
1.3	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
2	↑ (14 d)	NR	↑ (14 d)	NR	NR	NR
2.2[b]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
2.5	NR	↓ (GD1–17)	NR	NR	NR	NR
4.6[b]	↑ (16 wk)	↓ (28 d) ↓ (GD1–17)	NR	NR	NR	– (16 wk)
5	– (6 wk)	↓ (6 wk)	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
10	NR	– (14 d)	↑ (14 d)	– (14 d)	NR	NR
13.6	NR	↑ (26 wk) – (4, 12, 39, 52, 65, 78, 91 wk)[c]	↑ (4, 12, 26, 39, 52 wk) – (65, 78, 91 wk)[c]	↓ (78 wk) – (4, 12, 26, 39, 52, 65, 91 wk)[c]	↑ (104 wk)	↑ (104 wk)
14.2	NR	NR	NR	NR	NR	↑ (105 wk)
20	↑ (14 d)	↓ (28 d) ↓ (1, 3, 5 d)	↑ (14 d)	NR	NR	NR
25	↑ (14 d)	– (14 d)	↑ (14 d)	– (14 d)	NR	NR
40	↑ (14 d)	NR	↑ (14 d)	NR	NR	NR
50	NR	– (14 d)	↑ (14 d)	– (14 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; CYP19A1 = cytochrome P-450 19A1 (aromatase); T = testosterone; E2 = β-estradiol; LH = luteinizing hormone; NR = not reported; wk = week(s); d = day(s); GD = gestational day.

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); Perkins et al. (2004); Song et al. (2018); and Zhang et al. (2014b). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[c] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.2 Estrogen Agonism MOA

Although increased aromatase activity was observed, indicating potential increases in the conversion of androgens to estrogens, evidence of estrogen agonism in rodents was not robust. Biegel et al. (2001) reported consistent increases in serum E2 in male rats treated with the same concentration of PFOA that induced LCTs (300 ppm; approximately 13.6 mg/kg/day); however, the estrogen levels were too low to be accurately measured with the radioimmunoassay methods utilized in the study. Cook et al. (1992) observed similar increases in serum E2 concentrations in male rats gavaged with 10, 25, or 50 mg/kg/day PFOA for 14 days, though the authors also used a radioimmunoassay and reported similarly low E2 concentrations. Perkins et al. (2004) additionally reported suggestive increases in serum E2 concentrations in male rats treated with up to 6.5 mg/kg/day PFOA for 13 weeks, though this response was not statistically significant. Overall, there is not sufficient evidence to support estrogen agonism as the MOA for PFOA-induced LCTs. Evidence for the key events involved in the estrogen agonism MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-24.

Table 3-24. Evidence of Key Events Associated with the Estrogen Agonism Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Increased Serum LH	Key Event 6: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**CYP19A1 Activity in Liver**	**Serum E2**	**TGFα in Testis**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
0.2	NR	– (14 d)	– (14 d)	NR	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
1	NR	– (6 wk)	– (14 d)	NR	– (14 d)	– (6 wk)	– (6 wk)
1.1[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.3	NR	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
2	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
2.2[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
4.6[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
5	NR	– (6 wk)	NR	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
10	NR	NR	↑ (14 d)	NR	– (14 d)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[d]	NR	↑ (4, 12, 26, 39, 52 wk)	NR	↓ (78 wk)	↑ (104 wk)	↑ (104 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Increased Serum LH	Key Event 6: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
			– (65, 78, 91 wk)[d]		– (4, 12, 26, 39, 52, 65, 91 wk)[d]		
14.2	NR	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1 ,7, 28 d)	NR	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR	NR
25	NR	↑ (14 d)	↑ (14 d)	↑ (14 d)	– (14 d)	NR	NR
40	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
50	NR	NR	↑ (14 d)	NR	– (14 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; CYP19A1 = cytochrome P-450 19A1 (aromatase); E2 = β-estradiol; TGFα = transforming growth factor α; LH = luteinizing hormone; NR = not reported; w = week(s); d = day(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); and Perkins et al. (2004). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.3 Testosterone Biosynthesis Inhibition MOA

Several of the available studies support an impact of PFOA on testosterone production in male rodents (Eggert et al., 2019; Lu et al., 2019; Song et al., 2018; Zhang et al., 2014b; Li et al., 2011; Martin et al., 2007; Biegel et al., 1995; Cook et al., 1992), as well as in men from the general population or high-exposure communities from epidemiological studies (Cui et al., 2020; Petersen et al., 2018; Lopez-Espinosa et al., 2016). However, neither the subchronic nor the chronic study in male rats that measured serum testosterone reported decreases across multiple time points ranging from 1 to 21 months (Perkins et al., 2004; Biegel et al., 2001) (Table 3-25). Though there is evidence of PFOA-induced inhibition of testosterone biosynthesis, this lack of response in the only study that both observed LCTs and measured testosterone serum levels limits potential conclusions about whether decreased testosterone plays a role in the MOA for LCTs (Biegel et al., 2001). Evidence for the key events involved in the testosterone biosynthesis inhibition MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-25.

Table 3-25. Evidence of Key Events Associated with the Testosterone Biosynthesis Inhibition Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Decreased Testosterone Biosynthesis	Key Event 3: Decreased Serum T	Key Event 4: Increased Serum LH	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**Testosterone Biosynthesis[c]**	**Serum T**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
0.31	NR	– (28 d)	– (28 d)	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
1	NR	↓ (6 wk)	↓ (6 wk) – (14 d) – (GD1–17)	– (14 d)	– (6 wk)	– (6 wk)
1.1[d]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.25	NR	↓ (28 d)	↓ (28 d)	NR	NR	NR
1.3	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
2.2[d]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
2.5	NR	NR	↓ (GD1–17)	NR	NR	NR
4.6[d]	↑ (16 wk)	↓ (28 d)	↓ (28 d) ↓ (GD1–17)	NR	NR	– (16 wk)
5	NR	↓ (6 wk)	↓ (6 wk)	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
10	NR	NR	– (14 d)	– (14 d)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	↑ (26 wk) – (4, 12, 39, 52, 65, 78, 91 wk)[e]	↓ (78 wk) – (4, 12, 26, 39, 52, 65, 91 wk)[e]	↑ (104 wk)	↑ (104 wk)
14.2	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↓ (28 d)	↓ (28 d) ↓ (1, 3, 5 d)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
25	NR	NR	– (14 d)	– (14 d)	NR	NR
50	NR	NR	– (14 d)	– (14 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; T = testosterone; LH = luteinizing hormone; wk = week(s); d = day(s); GD = gestational day.

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); Perkins et al. (2004); Song et al.

(2018); and Zhang et al. (2014b). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.
[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).
[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).
[c] Testosterone biosynthesis provided as gene expression of 3β-HSD, 17-β-HSD, and/or CYP17A1 in Zhang et al. (2014b) and as gene expression of 3β-HSD, 17-β-HSD, and/or CYP17A1 in Li et al. (2011).
[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.
[e] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.4 PPARα activation MOA

Support for at least partial PPARα mediation of testosterone production inhibition due to PFOA administration is available from one study in mice (Li et al., 2011). Significantly reduced plasma testosterone concentrations were observed in male wild-type PPARα mice and humanized PPARα transgenic mice. These decreases were evident but not statistically significant in PPARα-null mice. In addition, reduced reproductive organ weights and increased sperm abnormalities were also observed in PFOA-treated male PPARα wild-type and humanized PPARα mice but not in PPARα-null mice (Li et al., 2011). However, data are not currently sufficient to demonstrate that the other key steps in the postulated PPARα-mediated MOAs are present in PFOA-treated animals following exposures that lead to tumor formation. Additional studies are needed to demonstrate the increase of GnRH and LH in concert with the changes in aromatase and further study is needed to confirm the potential downstream increases in serum E2. There was also no indication of increased Leydig cell proliferation at the doses that caused adenomas in the Biegel et al. (2001) study. Thus, additional research is needed to determine if the hormone testosterone-E2 imbalance is a key factor in development of LCTs as a result of PFOA exposure. Evidence for the key events involved in the PPARα agonist-induced MOA for testicular tumors in rodents exposed to PFOA is summarized in Table 3-26.

Table 3-26. Evidence of Key Events Associated with PPARα Agonist-Induced Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**CYP19A1 Activity in Liver**	**Serum E2**	**TGFα in Testis**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
0.2	NR	– (14 d)	– (14 d)	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
1	NR	– (6 wk)	– (14 d)	NR	– (6 wk)	– (6 wk)
1.1[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
1.3	NR	NR	NR	NR	NR	– (105 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
1.94	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
2	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR
2.2[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
4.6[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
5	NR	– (6 wk)	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
10	NR	NR	↑ (14 d)	NR	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[d]	NR	↑ (4, 12, 26, 39, 52 wk) – (65, 78, 91 wk)[d]	NR	↑ (104 wk)	↑ (104 wk)
14.2	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↑ (14d)	↑ (14 d)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
25	NR	↑ (14 d)	↑ (14 d)	↑ (14 d)	NR	NR
40	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR
50	NR	NR	↑ (14 d)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; CYP19A1 = cytochrome P-450 19A1 (aromatase); E2 = β-estradiol; TGFα = transforming growth factor α; NR = not reported; wk = week(s); d = day(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in the table were extracted from Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); and Perkins et al. (2004). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.3 Mode of Action for Pancreatic Tumors

As discussed in Section 3.5.2, pancreatic acinar cell tumors (PACTs) were identified in male rats in two 2-year chronic cancer bioassays (NTP, 2020; Biegel et al., 2001). In fact, NTP (2020) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups, as well as increased incidence, though nonsignificant, in female rats from the highest

dose group. A subchronic drinking water exposure study in the LSL-KRas^{G12D}; Pdx-1 Cre (KC) mouse model for pancreatic cancer also provides evidence that PFOA exposure promotes the growth of pancreatic lesions (Kamendulis et al., 2022).

Two proposed MOAs for PFOA-induced pancreatic tumors in animal models were identified in the literature, including one study that utilizes a transgenic mouse model to mimic the histologic progression of pancreatic cancer in humans (Kamendulis et al., 2022; Klaunig et al., 2012; Klaunig et al., 2003). The proposed MOAs are: 1) changes in bile acids, potentially linked to activation of hepatic PPARα, leading to cholestasis, a positive cholecystokinin (CCK) feedback loop, and acinar cell proliferation; and 2) oxidative stress. However, the existing database is limited in its ability to determine the relationship between PFOA exposure and these MOAs, particularly for the PACTs observed in chronic rat studies. Evidence for the key events involved in the relevant MOAs for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-27 and Table 3-28.

3.5.4.2.3.1 Gastric Bile Alterations

Gastric bile compositional changes or flow alterations can lead to cholestasis, which is the reduction or stoppage of bile flow. Cholestasis may cause an increase in CCK, a peptide hormone that: stimulates digestion of fat and protein, causes increased production of hepatic bile, and stimulates contraction of the gall bladder. There is some evidence suggesting that pancreatic acinar cell adenomas may result from increased CCK levels resulting from blocked bile flow (Obourn et al., 1997), which may result in a CCK-activated feedback loop that leads to increased proliferation of secretory pancreatic acinar cells.

PFOA may change bile composition by competing with bile acids for biliary transport. Upregulation of MRP3 and MRP4 transporters (Maher et al., 2008) and downregulation of OATPs (Cheng and Klaassen, 2008a) linked to PPARα activation in mice may favor excretion of PFOA from the liver via bile. Minata et al. (2010) found that PFOA levels in bile were much higher in wild-type male mice versus PPARα-null mice, suggesting a link to PPARα. In this study, male mice were dosed with 0, 5.4, 10.8, and 21.6 mg/kg/day PFOA for 4 weeks, resulting in increased total bile acid in PPARα-null mice at the highest dose, which indicated that PFOA-induced activation of PPARα may result in increased PFOA excretion. This may, in turn, result in decreased flow of bile acids that compete for the same transporters. Notably, however, these alterations in male mice occurred at relatively high dose levels compared with those that resulted in PACTs in male rats following 2 years of PFOA exposure (NTP, 2020). In the NTP study, bile acid concentrations were increased greater than twofold in male rats exposed to PFOA in the diet at doses of 15.6 and 31.7 mg/kg/day for 4 weeks compared with the control group. In the same study, serum ALP levels were mildly increased (less than twofold). While these increases may be due to cholestasis, mild increases in ALP (and ALT) activity are also associated with the administration of hepatic microsomal enzyme inducer compounds, including PPARα agonists (NTP, 2020). There was no further evidence of cholestasis reported in the literature. Additionally, CalEPA noted that "PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported" (CalEPA, 2021).

Additionally, there is no evidence of alterations in CCK associated with PFOA exposure in animal models or human studies. In fact, medical surveillance data from male workers at 3M's

Cottage Grove plant demonstrated a significant negative association between CCK levels and serum PFOA (Olsen et al., 2000; Olsen et al., 1998). Further, cholestasis was not observed in the workers (Olsen et al., 2000). It has been suggested that the lack of a positive association may be due to PFOA levels being too low to increase CCK in humans, although it has been demonstrated that PFOA is not an agonist for the CCKA receptor that activates CCK release (Obourn et al., 1997). Overall, due to limited evidence for altered bile flow in animals that developed tumors and an overall lack of evidence for alterations in CCK levels in PFOA-exposed animals, there is not sufficient evidence to determine whether bile acid alterations contribute to the MOA for PACTs observed in rodents chronically exposed to PFOA. Evidence for the key events involved in the gastric bile acid alteration MOA for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-27.

Table 3-27. Evidence of Key Events Associated with the Gastric Bile Alterations Mode of Action for Pancreatic Tumors[a] in Male and Female Rats and Mice

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Altered Bile Flow and/or Bile Acid Composition	Key Event 3: Cholestasis	Key Event 4: Increase in CCK Levels	Key Event 5: Acinar Cell Proliferation or Hyperplasia	Outcome: Pancreatic Tumors
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**Altered Bile Flow and/or Bile Acid Composition**	**Cholestasis[c]**	**CCK Levels**	**Acinar Cell Proliferation or Hyperplasia**	**Pancreatic Tumors[d]**
1.1[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
1.3 (males)/ 1.6 (females)[f]	NR	NR	NR	NR	– (105 wk)	– (105 wk)
2.2[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
4.6[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
5.4	NR	– (4w)	↑ (4 wk) for ALT ↓ (4 wk) for bilirubin – (4 wk) for AST, bile acid	NR	NR	NR
10.8	NR	– (4w)	↑ (4 wk) for AST, ALT – (4 wk) for bile acid, bilirubin	NR	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[g]	NR	NR	NR	↑ (104 wk)	↑ (104 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Altered Bile Flow and/or Bile Acid Composition	Key Event 3: Cholestasis	Key Event 4: Increase in CCK Levels	Key Event 5: Acinar Cell Proliferation or Hyperplasia	Outcome: Pancreatic Tumors
14.2 (males)/ 16.1 (females)	NR	NR	NR	NR	– (105 wk)	– (105 wk)
15.6	↑ (16 wk)	NR	↑ (16 wk) for ALP, ALT, SDH, bile acid	NR	NR	NR
18.2 (females)[e]	↑ (16 wk)	NR	– (16 wk) for ALP, ALP, SDH	NR	– (104 wk)	– (104 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR	NR
21.6	NR	– (4 wk)	↑ (4 wk) for AST, ALT, bilirubin – (4 wk) for bile acid	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
31.7	↑ (16 wk)	NR	↑ (16 wk) for bile acid, ALP, ALT, SDH	NR	NR	NR
40	NR	↑ (2 d)	NR	NR	NR	NR
63.4 (females)[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP	NR	– (104 wk)	– (104 wk)
80	NR	↑ (2 d)	NR	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; CCK = cholecystokinin; wk = week(s); NR = not reported; ALT = alanine transaminase; ALP = alkaline phosphatase; SDH = sorbitol dehydrogenase; AST = aspartate transferase; d = day(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in the table were extracted from: Biegel et al. (2001); Butenhoff et al. (2012); Cheng et al. (2008a); Elcombe et al. (2010); Kamendulis et al. (2022); Martin et al. (2007); NTP (2020); and from wild-type animals in Minata et al. (2010). Doses in mg/kg/day for Minata et al. (2010) were converted from 12.5, 25, and 50 μmol/kg/d as reported in the primary study. Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls.

[a] Reviewed in Klaunig, 2003, 5772415 and Klaunig, 2012, 1289837.

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity (NTP, 2020), as hepatic β-oxidation activity (Biegel et al., 2001), and as CYP4A1 protein expression and hepatic β-oxidation activity (Elcombe et al., 2010).

[c] Observations consistent with cholestasis include significant increases in serum bile acid concentrations and increased serum liver enzyme activities (e.g., ALP, ALT) in NTP (2020), and increased total bilirubin and ALT in Minata et al. (2010).

[d] Pancreatic tumors reflect increased incidence of acinar cell adenoma and/or adenocarcinoma (combined) in male rats (NTP, 2020; Biegel et al., 2001).

[e] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures in male (20, 40, and 80 ppm, or 1.1, 2.2, and 4.6 mg/kg/day) and female (300 and 1,000 ppm, or 18.2 and 63.4 mg/kg/day) rats in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[f] All data are from male rats with the exception of Butenhoff et al. (2012) and NTP (2020), which include both males and females, as indicated.

[g] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.3.2 Oxidative Stress

More recent literature has suggested a potential role for oxidative stress in pancreatic carcinogenesis associated with PFOA exposure. Evidence for the key events involved in the proposed oxidative stress MOA for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-28. Hocevar et al. (2020) and Kamendulis et al. (2022) suggest that pancreatic cancer is induced through the activation of the UPR pathway, which leads to the activation of nuclear factor erythroid 2–related factor 2 (Nrf2), a regulator of the oxidative stress response, and protein kinase-like endoplasmic reticulum kinase (PERK), a signaler of endoplasmic reticulum (ER) stress, and subsequent upregulation of antioxidant responses (e.g., SOD gene expression). Activation of the UPR pathway can also stimulate ROS production. Activation of Sod1 in the mouse by the Nrf2 or PERK signaling pathways can stimulate cell proliferation through increased production of hydrogen peroxide which can then, in turn, act as a second messenger in mitogen signaling or through its elimination of ROS, leading to prevention of ROS-stimulated apoptosis (Kamendulis et al., 2022). Activation of PERK through the UPR pathway may also result in increased cytosolic calcium levels through activation of the inositol 1,4,5-trisphosphate receptor (IP3R), leading to ER stress and generation of ROS (Hocevar et al., 2020).

Induction of tumors by PFOA through oxidative stress is supported by two studies. Hocevar et al. (2020) evaluated PFOA-induced oxidative stress in mouse pancreatic acinar cells (266-6 cells) treated with 50 µg/mL PFOA for various durations. PFOA-exposed cells exhibited increased ER stress as well as activation of PERK, inositol-requiring kinase/endonuclease 1α (IRE1α), and activating transcription factor 6 (ATF6) signaling cascades of the UPR pathway. Exposure to PFOA at concentrations of 20, 50, or 100 µg/mL was also shown to result in time- and dose-dependent increases in cytosolic calcium levels, an effect that occurred predominantly through activation of IP_3R. Altogether, results in Hocevar et al. (2020) demonstrated that PFOA increased intracellular calcium levels through activation of the IP_3R, leading to ER stress, the generation of ROS and oxidative stress and subsequent PERK-dependent induction of antioxidant genes. The oxidative stress and ROS generated in response to PFOA may serve as a mechanism through which PFOA may induce pancreatic tumors.

Kamendulis et al. (2022) evaluated the ability for PFOA to promote pancreatic cancer using the LSL-KRasG12D;Pdx-1 Cre (KC) mouse model of pancreatic cancer, which has a mutation in the KRas gene, a mutations that is present in over 90% of human pancreatic cancers. This gene mutation in mice results in a histologic progression of pancreatic cancer that mirrors human pancreatic cancer progression, including formation of pancreatic intraepithelial neoplasia (PanIN). KC mice were exposed to 5 ppm PFOA in drinking water for up to 7 months, and increased PanIN was observed after 4 and 7 months of treatment compared with untreated KC mice.

Oxidative stress was also apparent in the PFOA-treated KC mice (Kamendulis et al., 2022). The authors reported increases in Sod enzyme activity at 4 and 7 months, along with threefold increases in Sod1 protein and mRNA levels and increased pancreatic catalase and thioredoxin reductase activities at 4 months relative to control. Pancreatic malondialdehyde, a product of oxidized lipids, was significantly increased at 7 months of exposure relative to untreated mice, but not at 4 months, indicating a potential accumulation of oxidative damage over time. Altogether, the results of Kamendulis et al. (2022) demonstrated that PFOA increased PanIN

area and number at 4 months, indicating early lesion formation. The increased desmoplasia and inflammation (MDA levels) after 7 months of exposure suggest PFOA increased disease severity over time, potentially through prolonged oxidative stress, resulting in pancreatic cancer progression.

Overall, although plausible, there is not sufficient evidence for key events related to an oxidative stress MOA to conclude that the pancreatic tumors in rodents chronically exposed to PFOA are the result of oxidative stress and related molecular events.

Table 3-28. Evidence of Key Events Associated with a Proposed Oxidative Stress Mode of Action Involving the UPR Pathway for Pancreatic Tumors[a] in Male and Female Rats and Mice.

Canonical MOA	Key Event 1: Activation of UPR Pathway	Key Event 2a: Activation of Nrf2 and PERK	Key Event 2b: ROS Production	Key Event 3: Upregulation of Antioxidant Responses	Key Event 4: Increased Production of Hydrogen Peroxide	Key Event 5a: Increased Cell Proliferation	Key Event 5b: Decreased Apoptosis	Outcome: Pancreatic Tumors
Dose (mg/kg/day)	**UPR Pathway**	**Nrf2 and PERK**	**ROS Production**	**Antioxidant Response**	**Hydrogen Peroxide Production**	**Cell Proliferation**	**Apoptosis**	**Pancreatic Tumors[b]**
1.1[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
1.28[d]	NR	NR	↑ (28 wk)	↑ (16 wk)	NR	NR	NR	↑ (16 wk)
1.3 (males)/ 1.6 (females)[e]	NR	NR	NR	NR	NR	– (105 wk)	NR	– (105 wk)
2.2[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
4.6[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
13.6	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
14.2 (males)/ 16.1 (females)	NR	NR	NR	NR	NR	– (105 wk)	NR	– (105 wk)
18.2[c] (females)	NR	NR	NR	NR	NR	– (104 wk)	NR	– (104 wk)
63.4[c] (females)	NR	NR	NR	NR	NR	– (104 wk)	NR	– (104 wk)
50 µg/mL[f]	↑ (in vitro)	↑ (in vitro)	NR	NR	NR	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; UPR = unfolded protein response; MOA = mode of action; ROS = reactive oxygen species; Nrf2 = nuclear factor erythroid 2–related factor 2; PERK = protein kinase-like endoplasmic reticulum kinase; NR = not reported; wk = week(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in the table were extracted from: Biegel et al. (2001); Butenhoff et al. (2012); Kamendulis et al. (2022); and NTP (2020). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls.

[a] Reviewed in Hocevar et al. (2020) and Kamendulis et al. (2022).

[b] Pancreatic tumors reflect increased incidence of acinar cell adenoma and/or adenocarcinoma (combined) in male rats (NTP, 2020; Biegel et al., 2001).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures in male (20, 40, and 80 ppm, or 1.1, 2.2, and 4.6 mg/kg/day) and female (300 and 1,000 ppm, or 18.2 and 63.4 mg/kg/day) rats in order to provide a representative set of the available mechanistic data involved in this MOA from

bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Dose from Kamendulis et al. (2022) converted from 5 ppm by summary authors using default assumptions for food consumption, water consumption, and body weight, in the absence of such data in the primary study, which used a Kras mutation model of mouse pancreatic cancer.

[e] All data are from male rats with the exception of Butenhoff et al. (2012) and NTP (2020), which include both males and females, as indicated.

[f] Indicates in vitro evidence from Hocevar et al. (2020), which used mouse pancreatic acinar cells (266-6 cells); data are included here owing to the only available demonstration of two of the key events in the proposed MOA.

3.5.4.2.4 Mode of Action for Hepatic Tumors

Two *high* confidence chronic studies on PFOA reported an increased incidence of hepatocellular adenomas in male rats (NTP, 2020; Biegel et al., 2001), one of which also demonstrated increased incidence of hepatocellular carcinomas specific to male rats exposed to PFOA perinatally. As described in the subsections below, the available mechanistic evidence across different *in vivo* and *in vitro* models establishes that multiple modes of action (MOA) are plausible for PFOA-induced liver cancer, including PPARα activation, activation of other nuclear receptors such as CAR, cytotoxicity, and an oxidative stress-mediated MOA. Evidence for the key events involved in the relevant MOAs for hepatic tumors in rodents exposed to PFOA is summarized in Table 3-29,Table 3-30,Table 3-31,Table 3-32, Table 3-33, and Table 3-34. Evidence related to genotoxicity and other plausible modes of action are also detailed in subsequent sections.

EPA previously concluded that liver tumor development in rats exposed to PFOA was not relevant to human health because it was determined to be mediated through PPARα activation. Evidence exists suggesting that although PPARα activators cause liver tumors in rodents, they may be unlikely to result in liver tumors in humans due to comparatively low hepatic PPARα expression, as well as biological differences between rodents and humans in the responses of events that are downstream of PPARα activation (Corton et al., 2018; U.S. EPA, 2016c). Specifically, some have argued that the MOA for liver tumor induction by PPARα activators in rodents has limited-to-no relevance to humans, due to differences in cellular expression patterns of PPARα and related proteins (e.g., cofactors and chromatin remodelers), as well as differences in binding site affinity and availability (Corton et al., 2018; Klaunig et al., 2003). However, there is also evidence that other MOAs are operative in PFOA-induced hepatic tumorigenesis (e.g., cytotoxicity (Felter et al., 2018) and liver necrosis in PFOA-exposed mice and rats; see Section 3.5.2). Recently published data suggest that oxidative stress and other mechanistic key characteristics associated with carcinogens may play a role in liver tumor development, as described further below. The existence of multiple plausible MOAs in addition to PPARα activation suggests that PFOA-induced liver cancer in rats may be more relevant to humans than previously thought.

The available literature on mechanisms related to PFOA-induced hepatic tumor development also supports EPA's prior conclusion that PFOA-induced tumors are likely due to nongenotoxic mechanisms involving nuclear receptor activation, perturbations of the endocrine system, and/or DNA replication and cell division (U.S. EPA, 2016a).

3.5.4.2.4.1 PPARα Activation

Exposure to several PFAS has been shown to activate PPARα, which is characterized by downstream cellular or tissue alterations in peroxisome proliferation, cell cycle control (e.g., apoptosis and cell proliferation), and lipid metabolism (U.S. EPA, 2016c). Notably, human

expression of PPARα mRNA and protein is only a fraction of what is expressed in rodent models, though there are functional variant forms of PPARα that are expressed in human liver to a greater extent than rodent models (Corton et al., 2018; Klaunig et al., 2003). Therefore, for PPARα activators that act solely or primarily through PPARα-dependent mechanisms (e.g., Wyeth-14,643 or di-2-ethyl hexyl phthalate), the hepatic tumorigenesis observed in rodents is expected to be infrequent and/or less severe in humans, or not observed at all (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

The MOA for PPARα activator-induced rodent hepatocarcinogenesis consists of the following sequence of key events: 1) PPARα activation in hepatic cells; 2) alterations in cell growth signaling pathways (e.g., increases in Kupffer cell activation leading to increases in TNFα); 3) perturbations of hepatocyte growth and survival (i.e., increased cell proliferation and inhibition of apoptosis); and 4) selective clonal expansion of preneoplastic foci cells leading to increases in hepatocellular adenomas and carcinomas (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003). Modulating factors in this MOA include increased oxidative stress and activation of NF-κB (Corton et al., 2018), both of which have been demonstrated for PFOA. This MOA is associated with, but not necessarily causally related to, nonneoplastic effects including peroxisome proliferation, hepatocellular hypertrophy, Kupffer cell-mediated events, and increased liver weight. There is also some overlap between signaling pathways and adverse outcomes, including tumorigenesis, associated with PPARα activation and the activation or degradation of other nuclear receptors, such as CAR, PXR, HNF4α, and PPARγ (Corton et al., 2018; Huck et al., 2018; Rosen et al., 2017; Beggs et al., 2016).

The key events underlying PFOA-induced hepatic tumor development through the PPARα MOA have been demonstrated in both in vivo and in vitro studies and have been discussed in detail previously (U.S. EPA, 2016a), as well as in Sections 3.5.2 and 3.5.3 of this document. A number of studies illustrate the potential of PFOA to activate human and rodent PPARα. For example, Buhrke et al. (2013) demonstrated PPARα activation in human Hep2G cells after 24-hour exposure to PFOA at a concentration of 25 μM. PFOA also activated mouse (Li et al., 2019b; Yan et al., 2015b; Takacs and Abbott, 2007; Maloney and Waxman, 1999) and human PPARα (Takacs and Abbott, 2007) in cell transfection studies. Gene expression analyses showed that PPARα activation was required for most transcriptional changes observed in livers of mice exposed to either PFOA or the known PPARα agonist Wyeth-14,643, demonstrating PFOA's ability to act as a PPARα agonist (Rosen et al., 2008a; Rosen et al., 2008b). Nonneoplastic (or pre-neoplastic) events that are associated with PPARα activation include peroxisome proliferation, hepatocellular hypertrophy, and increases in liver weight. Studies of PFOA exposure in rodents have reported one or more of these nonneoplastic effects (Section 3.5.2). For example, hepatocellular hypertrophy was observed in one of the two available chronic carcinogenicity studies of PFOA in rats (NTP, 2020), and both chronic carcinogenicity studies observed increases in liver weights (NTP, 2020; Biegel et al., 2001).

There is evidence from *in vivo* animal bioassays and in vitro studies of Kupffer cell activation, an indicator of alterations in cell growth, in response to PFOA treatment. Though this mechanism is itself PPARα-independent, factors secreted upon Kupffer cell activation may be required for increased cell proliferation by PPARα activators (Corton et al., 2018). Minata et al. (2010) observed a correlation between PFOA exposure and increased tumor necrosis factor alpha (*TNF-α*) mRNA levels in the livers of *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice treated with

PFOA (≤50 μmol/kg/day) for four weeks, while there was no effect of PFOA on wild-type (129S4/SvlmJ) mice in the same study. TNFα is a pro-inflammatory cytokine that can be released upon activation of Kupffer cells (Corton et al., 2018). Further study is needed to understand the potential role of other mediators of Kupffer cell activation since, unlike PPARα, PPARγ is expressed in Kupffer cells and can also be activated by PFOA.

Studies in both rats and mice have demonstrated (either directly or indirectly) that PFOA induces peroxisome proliferation in the liver, an indication of PPARα activation (Elcombe et al., 2010; Minata et al., 2010; Wolf et al., 2008; Martin et al., 2007; Yang et al., 2001; Pastoor et al., 1987). Gene expression profiling of HepG2 cells exposed to low PFOA concentrations (0.1 and 1 μM) revealed increased expression of cell cycle regulators (e.g., Cyclin D1, Cyclin E1). Higher PFOA concentrations generally had no effect on these genes, but were associated with increased expression of p53, p16, and p21 cell cycle regulators (Buhrke et al., 2013). Evidence for cell proliferation in the form of increased mitotic figures and/or bile duct hyperplasia as observed in PFOA-exposed male mice (Loveless et al., 2008), pregnant mice (Yahia et al., 2010), male rats (Elcombe et al., 2010), and female rats (NTP, 2020). Buhrke et al. (2013) also reported increased proliferation in HepG2 cells exposed to PFOA, in addition to PPARα activation. With respect to inhibition of apoptosis, there are conflicting reports, with some studies reported decreases in apoptosis following PFOA exposure (Son et al., 2008), while others report no effect or an increase in apoptosis (Blake et al., 2020; Elcombe et al., 2010; Minata et al., 2010). There is also evidence to support the clonal expansion key event. In an initiation-promotion study of liver tumors in rats, Abdellatif et al. (1990) reported that PFOA had promoting activity and increased the incidence of hepatocellular carcinomas following tumor initiation with diethylnitrosamine (DEN). Jacquet et al. (2012) exposed SHE cells to PFOA at concentrations ranging from 3.7×10^{-4} to 37.2 μM for 6 days with or without pre-treatment with the tumor initiator benzo-α-pyrene (BaP). PFOA exposure alone did not induce cell transformation, but PFOA did significantly induce transformation in BaP-sensitized cells, indicating that PFOA does not alone initiate cell transformation, but may have tumor promoter-like activity.

Two modulating factors have been proposed as part of the PPARα activation MOA that are relevant to PFOA: increased ROS and activation of NF-κB. Although there is not enough evidence to designate these effects as key events in the MOA, they have the potential to alter the ability of PPARα activators to increase liver cancer and are thus defined as modulating factors. PFOA exposure has been demonstrated to cause oxidative stress (detailed below in Section 3.5.4.2.4.5.2). Evidence for the key events involved in the PPARα activation MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-29 and Table 3-30, respectively.

Table 3-29. Evidence of Key Events Associated with the PPARα Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**PPARα Activation[b]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[c]**
1	NR	NR	– (7 d)	NR	NR	NR
1.1[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	– (104 wk)
1.3	NR	NR	– (104 wk)	NR	NR	– (104 wk)
2.2[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	↑ (104 wk)
3	NR	NR	– (7 d)	NR	NR	NR
4.6[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	↑ (104 wk)
5.4	NR	– (4 wk)	NR	– (4 wk)	NR	NR
10	NR	NR	↑ (7 d)	NR	NR	NR
10.8	NR	– (4 wk)	NR	↑ (4 wk)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	– (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	NR	↑ (104 wk)
14.2	NR	NR	– (104 wk)	NR	NR	– (104 wk)
19	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR	NR
21.6	NR	– (4 wk)	NR	↑ (4 wk)	NR	NR
23	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	– (1, 7, 28 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; d = day(s); wk = week(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from: Biegel et al. (2001); NTP (2020); Elcombe et al. (2010); Minata et al. (2010) (wild-type); Wolf et al. (2008) (sex of mice not stated); Martin et al. (2007); and Butenhoff et al. (2012).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Indirect measurement of PPARα induction provided as CYP4A1 protein expression and hepatic β-oxidation activity (Elcombe et al., 2010), as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] Hepatic tumors reflect increased incidence of adenoma in Biegel (2001), and carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[e] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

Table 3-30. Evidence of Key Events Associated with the PPARα Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**PPARα Activation[b]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation[c]**	**Apoptosis[d]**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[e]**
1	NR	NR	↓ (P_0 GD 1.5–17.5)[f] – (P_0 GD 1.5–11.5)	↑ (P_0 GD 1.5–17.5)[f] – (P_0 GD 1.5–11.5)	NR	NR
1.6	NR	NR	– (104 wk)	NR	NR	– (104 wk)
5	NR	NR	↑ (P_0 GD 1.5–11.5)[f] ↓ (P_0 GD 1.5–17.5)	↑ (P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)[f]	NR	NR
16.1	NR	NR	– (104 wk)	NR	NR	– (104 wk)
18.2 g	↑ (16 wk)	NR	– (104 wk)	NR	NR	– (104 wk)
63.4 g	↑ (16 wk)	NR	– (104 wk)	NR	NR	– (104 wk)

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls unless otherwise noted; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; P_0 = parental generation; GD = gestational day; wk = week(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from: NTP (2020); Blake et al. (2020) (dams); and Butenhoff et al. (2012).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020).

[c] Increased hepatic cell proliferation as provided by number of increased mitoses in NTP (2020).

[d] Apoptosis as both apoptosis and single-cell necrosis in Blake et al. (2020).

[e] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[f] No statistics were reported for hepatic cell proliferation or for apoptosis in Blake et al. (2020); thus, the arrows indicate direction of increased incidence relative to the control group per the authors' results narrative.

[g] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.2 Other Nuclear Receptors

In addition to PPARα, there is some evidence that other nuclear receptors, such as CAR, PXR, PPARγ, and ER, can be activated by PFOA. CAR, which has an established adverse outcome pathway of key events similar to that of PPARα, has been implicated in hepatic tumorigenesis in rodents. The key events of CAR-mediated hepatic tumors are: 1) CAR activation; 2) altered gene expression specific to CAR activation; 3) increased cell proliferation; and 4) clonal expansion leading to altered hepatic foci, leading to 5) liver tumors (Felter et al., 2018). Nonneoplastic events associated with this pathway include hypertrophy, induction of CAR-specific CYP enzymes (e.g., CYP2B), and inhibition of apoptosis. There is evidence that PFOA can activate CAR and initiate altered gene expression and associative events (Rosen et al., 2017; Elcombe et al., 2010; Rosen et al., 2008a; Rosen et al., 2008b; Martin et al., 2007). For example, Martin et al. (2007) and Elcombe et al. (2010) observed evidence of activation of CAR-related genes, many of which are also altered by PPARα activation, in rats following PFOA exposure, and Wen et al. (2019c) observed increased CAR activation in PFOA-exposed PPARα knockout mice

compared with PFOA-exposed wild-type mice. Other studies have shown altered gene expression of transcriptional targets of CAR in both wild-type and PPARα knockout mice exposed to PFOA (Rosen et al., 2017; Rosen et al., 2008a; Rosen et al., 2008b). As with PPARα-mediated tumorigenesis, there are claims that CAR-mediated tumorigenesis in animals is not relevant to human risk assessment due to differences in CAR-mediated alterations between species. For example, CAR activators (e.g., phenobarbital) induce cell proliferation and tumors in rodents but not in human cell lines (Elcombe et al., 2014). Hall et al. (Hall et al., 2012) noted that there is evidence that CAR in humans is more resistant to mitogenic effects (e.g., studies showing that human hepatocytes are resistant to induction of replicative DNA synthesis).

There is also evidence that PFOA can activate other nuclear receptors, such as PXR, PPARγ, and ERα. Martin et al. (2007) and Elcombe et al. (2010) observed evidence of PPARγ agonism and/or activation of PXR-related genes in rats following PFOA exposure, and Wen et al. (2019c) reported evidence suggesting increased ERα and PXR activation in PFOA-exposed PPARα knockout mice compared with wild-type. PFOA has also been shown to activate PXR in human HepG2 cells (Zhang et al., 2017). Buhrke et al. (2013) demonstrated PPARγ and PPARδ activation at PFOA concentrations of ≥100 μM in transfected HEK293 cells, and activation of PPARγ by PFOA in HepG2 cells (Buhrke et al., 2015).

There is also evidence that PFOA can suppress hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator of hepatic differentiation. Beggs et al. (2016) observed a decrease in HNF4α in the livers of ten-week-old CD-1 mice exposed to 3 mg/kg/day PFOA once daily by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcriptional regulation of liver-specific genes, and regulation of lipid metabolism. Beggs et al. (2016) also exposed human primary hepatocytes to 0.01–10 μM PFOA for 48 or 96 hours to determine pathways affected by PFOA exposure; after 96 hours of 10 μM PFOA, HNF4α protein expression was significantly decreased. In primary human hepatocytes exposed to 1, 25, or 100 μM PFOA for 24 hours, the number of differentially regulated genes was measured using a human genome gene chip; these microarray data demonstrated that PFOA exposure at 25 and 100 μM inhibited HNF4α function, as evidenced by changes in gene targets of HNF4α using upstream regulator analysis (Buhrke et al., 2015).

An evaluation of high-throughput screening (HTS) assay data from the ToxCast/Tox21 program provides further evidence that PFOA activates other nuclear receptors in addition to PPARα. Chiu et al. (2018) evaluated HTS data for PFOA in the context of the 10 key characteristics of carcinogens as described in Smith et al. (2016b). The assay results demonstrated PFOA activity in four ER assays (ERa, ERE, ERA_LUC, ERa_BLA), seven PPAR and PXR assays (PPARα, PPARγ, PPRE, hRRAg, PXR, PXRE, hPXR), two androgen receptor assays (rAR, AR_LUC), five enzyme assays (hBACE, hTie2, gLTB4, hORL1, hPY2), and six other assays (Nrf2, RXRb, hCYP2C9, AhR, ELG1, and TR LUC Via.) The results suggest a broad range of PFOA-induced receptor-mediated effects that were not exclusively receptor effects.

Many of the above-described nuclear receptors are known to play a role in liver homeostasis and disease and may be driving factors in the hepatotoxicity observed after PFOA exposure; however, their role in hepatic tumorigenesis is less clear. Evidence for the key events involved in the CAR activation MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-31 and Table 3-32.

Table 3-31. Evidence of Key Events Associated with the CAR Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**CAR Activation[b]**	**Altered Gene Expression[c]**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[d]**
1	– (7 d)	↑ (7 d)	NR	NR	NR
1.1[e]	NR	NR	↑ (16, 104 wk)	NR	– (104 wk)
1.3	NR	NR	– (104 wk)	NR	– (104 wk)
2.2[e]	NR	NR	↑ (16, 104 wk)	NR	↑ (104 wk)
3	↑ (7 d)	↑ (7 d)	NR	NR	NR
4.6[e]	NR	NR	↑ (16, 104 wk)	NR	↑ (104 wk)
5.4	NR	– (4 wk)	NR	NR	NR
10	↑ (7 d)	↑ (7 d)	NR	NR	NR
10.8	NR	– (4 wk)	NR	NR	NR
13.6	NR	NR	– (4, 12, 26, 39, 52, 65, 78, 91 wk)[f]	NR	↑ (104 wk)
14.2	NR	NR	– (104 wk)	NR	– (104 wk)
19	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR
21.6	NR	– (4 wk)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; CAR = constitutive androstane receptor; d = day(s); NR = not reported; wk = week(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from: Biegel et al. (2001); NTP (2020); Elcombe et al. (2010); Martin et al. (2007); Minata et al. (2010); Wen et al. (2019c) (wild-type); Rosen et al. (2008a); Rosen et al. (2008b); Rosen et al. (2017); and Butenhoff et al. (2012).

[a] Reviewed in Felter, et al. (2018).

[b] Direct and indirect measurement of CAR induction provided CAR gene expression in Wen et al. (2019c), as *Cyp3a1*, *Cyp3a3*, and *Cyp3a9* gene expression in Martin et al. (2007), as *Cyp2b1/2, Cyp3a1*, and *Cyp4a1 g*ene expression in Elcombe et al. (2010), and as CAR gene biomarker set expression in Rosen et al. (2017).

[c] Gene expression as measured by differential expression of CAR target genes by microarray analysis (Rosen et al., 2017) or RT-PCR (Wen et al., 2019c; Rosen et al., 2008b).

[d] Hepatic tumors reflect increased incidence of adenoma (Biegel et al., 2001), and carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[e] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[f] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

Table 3-32. Evidence of Key Events Associated with the CAR Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation[b]**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
1	NR	NR	↓ (P_0 GD 1.5–17.5)[c] – (P_0 GD 1.5–11.5)	NR	NR
1.6	NR	NR	– (104 wk)	NR	– (104 wk)
5	NR	NR	↑ (P_0 GD 1.5–11.5)[c] ↓ (P_0 GD 1.5–17.5)[c]	NR	NR
16.1	NR	NR	– (104 wk)	NR	– (104 wk)
18.2[d]	NR	NR	– (104 wk)	NR	– (104 wk)
63.4[d]	NR	NR	– (104 wk)	NR	– (104 wk)

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls unless otherwise noted; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; P_0 = parental generation; GD = gestational day; wk = week(s).
Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2020); Blake et al. (2020) (dams); and Butenhoff et al. (2012).
[a] Reviewed in Felter, et al. (2018).
[b] Proliferation as provided by number of increased mitoses in Blake et al. (2020), and liver cell proliferation or hyperplasia (no change) in NTP (2020).
[c] No statistics were reported for hepatic cell proliferation for Blake et al. (2020); thus, the arrows indicate direction of increased incidence relative to the control group per the authors' results narrative.
[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.3 Cytotoxicity

There is suggestive evidence that PFOA may act through a cytotoxic MOA. Felter et al. (2018) identified the following key events for establishing a cytotoxicity MOA: 1) the chemical is not DNA reactive; 2) clear evidence of cytotoxicity by histopathology such as the presence of necrosis and/or increased apoptosis; 3) evidence of toxicity by increased serum enzymes indicative of cellular damage that are relevant to humans; 4) presence of increased cell proliferation as evidenced by increased labeling index and/or increased number of hepatocytes; 5) demonstration of a parallel dose response for cytotoxicity and formation of tumors; and 6) reversibility upon cessation of exposure. As discussed above in the genotoxicity section (Section 3.5.3.1), there is little experimental evidence that PFOA can induce DNA damage, supporting the first key event of the cytotoxicity MOA. Quantitative liver histopathology is available in two studies (NTP, 2020; Butenhoff et al., 2012). Significantly increased single-cell (hepatocyte) death and necrosis in male and female was reported in Sprague-Dawley rats, with a significant dose-response trend. Evidence for the key events involved in the cytotoxicity MOA for hepatic

tumors in male and female rodents exposed to PFOA is summarized in Table 3-33 and Table 3-34.

In vitro results regarding apoptosis are variable. Wielsøe et al. (2015) observed no change in LDH release, a marker for cytotoxicity, in HepG2 cells after 24-hour exposure to PFOA doses as high as $2E^{-5}$M, while Panaretakis et al. (2001) demonstrated that PFOA exposure increased ROS generation, which led to activation of caspase-9 and induction of the apoptotic pathway in HepG2 cells.

Increased cell proliferation or markers of cell proliferation has been reported in vitro. Buhrke et al. (2013) determined that PFOA exposures of 10 μM and 25 μM for 24 hours resulted in increased proliferation of HepG2 cells. Increases in metabolic activity were also detected at 10, 25, and 50 μM exposures. Low PFOA concentrations (0.1 and 1 μM) were associated with increased expression of cell cycle regulators Cyclin D1, Cyclin E1, and Cyclin B1 whereas higher concentrations generally had no effect on these genes (except for increased expression of Cyclin E1 at 100 μM). The higher PFOA concentration of 100 μM was associated with increased expression of p53, p16, and p21 regulators (a nonsignificant increase was observed at 25 μM).

Although Wen et al. (2020) observed decreasing cell viability with increasing PFOA exposure in HepG2 cells after 48 hours of exposure (20 to 600 μM), no change in metabolic activity was observed. Wen et al. (2020) evaluated the impact of PFOA on several genes involved in cell cycle regulation, proliferation, and apoptosis and found that the expression of the *BAX* gene, a regulator of apoptosis, increased at 20, 50, and 150 μM, and decreased at 100 and 200 μM. The expression of cell cycle genes *CCNA2, CCNE1*, and *CCNB1* was altered, as was that of several genes related to cell proliferation (*CDKN1A* and *CDK4*): at lower concentrations (50 μM) of PFOA exposure, a minor increase in expression was observed, while significant decreases in expression was observed in a dose-dependent manner at concentrations >50 μM. Lipid metabolism and transport genes were also altered in the study: increased expression of lipid anabolism gene *ACSL1*, decreased expression of cholesterol synthesis enzyme gene *HMGCR*, decreased expression of fatty acid binding protein gene (*FABP1*), decreased expression *ACOX2*. There was no change in expression in the beta-oxidation acyl-CoA dehydrogenase enzyme encoding genes *ACAD11* and *ACADM.* In addition to the in vitro evidence for the key events in the cytotoxicity MOA for hepatic tumors, data from rodent studies are also available for PFOA. Histopathological and flow cytometric analyses are available for rodent studies, demonstrating hepatocyte cell death (Cope et al., 2021; NTP, 2020; Crebelli et al., 2019; NTP, 2019), increased proliferation in the presence of cell death (NTP, 2020; Loveless et al., 2008), and hyperplasia (NTP, 2020, 2019). Data are also available for increased serum enzymes related to hepatotoxicity in rodents exposed to PFOA (Cope et al., 2021; NTP, 2020; Guo et al., 2019; NTP, 2019; Yan et al., 2014; Butenhoff et al., 2012; Elcombe et al., 2010; Minata et al., 2010; Loveless et al., 2008). Evidence for the key events involved in the cytotoxicity MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-33 and Table 3-34.

Table 3-33. Evidence of Key Events Associated with the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**Cytotoxicity[b]**	**Serum Enzymes[c]**	**Regenerative Proliferation[d]**	**Hyperplasia and/or Preneoplastic Lesions[e]**	**Hepatic Tumors[f]**
0.08	NR	– (4 wk)	NR	NR	NR
0.10	– (F_1 GD 1.5–17.5) – (5 wk)	– (F_1 GD 1.5–17.5) – (5 wk)	NR	NR	NR
0.30	– (29 d)[g]	NR	– (29 d)[g]	– (29 d)[g]	NR
0.31	NR	– (4 wk)	NR	NR	NR
0.40	NR	– (4 wk)	NR	NR	NR
0.625	NR	↑ (4 wk)	NR	NR	NR
1.0	↑ (29 d)[g] – (F_1 GD 1.5–17.5) – (5 wk)	– (F_1 GD 1.5–17.5) – (5 wk)	– (29 d)[g]	↑ (29 d)[g]	NR
1.1[h]	↑ (16 wk) – (104 wk)	↑ (16 wk)	NR	↓ (104 wk)	– (104 wk)
1.25	NR	↑ (4 wk)	NR	NR	NR
1.3	– (104 wk)	↑ (12, 24, 52, 78 wk) – (104 wk)	NR	– (104 wk)	– (104 wk)
2.0	NR	↑ (4 wk)	NR	NR	NR
2.2[h]	↑ (16, 104 wk)	– (16 wk)	NR	↓ (104 wk)	↑ (104 wk)
2.5	NR	↑ (4 wk)	NR	NR	NR
4.6[h]	↑ (16, 104 wk)	– (16 wk)	NR	↓ (104 wk)	↑ (104 wk)
5.0	↑ (5 wk)	↑ (4 wk) ↑ (5 wk)	NR	NR	NR
5.4	NR	↑ (4 wk)	NR	NR	NR
10	↑ (29 d)[g]	↑ (4 wk)	↑ (29 d)[g]	↑ (29 d)[g]	NR
10.8	NR	↑ (4 wk)	NR	NR	NR
14.2	– (104 wk)	↑ (12, 24, 52, 78, 104 wk)	NR	– (104 wk)	– (104 wk)
15.6[h]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR
19	NR	– (1, 7, 28 d)	↑ (1, 7 d)	↑ (28 d) – (1, 7 d)	NR
20	NR	↑ (4 wk)	NR	NR	NR
21.6	NR	↑ (4 wk)	NR	NR	NR
23	NR	NR	↑ (1, 7, 28 d)	↑ (28 d) – (1, 7 d)	NR
30	↑ (29 d)[g]	NR	↑ (29 d)[g]	↑ (29 d)[g]	NR
31.7[h]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls unless otherwise noted; MOA = mode of action; NR = not reported; wk = week(s); F_1 = first generation of offspring; GD = gestational day; d = day(s).

Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from: NTP (2019); NTP (2020); Elcombe et al. (2010); Minata et al. (2010) (wild-type); Yan et al. (2014); Loveless et al. (2008); Crebelli et al. (2019); Guo et al. (2019); Butenhoff et al. (2012); and Cope et al. (2021) (low-fat diet only; F_1 pups exposed from GD 1.5 to 17.5, and evaluated at postnatal day (PND) 126).

[a] Reviewed in Felter et al. (2018).

[b] Cytotoxicity provided as increased incidence of late apoptosis/necrosis in Crebelli et al. (2019), necrosis in Butenhoff et al. (2012), and as necrosis and/or single-cell necrosis in NTP (2020) and Cope et al. (2021).

[c] Serum enzyme changes provided as changes in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate transaminase (AST) in Butenhoff et al. (2012), NTP (2020), NTP (2019), and Cope et al. (2021), and as changes in ALT and/or AST in Elcombe et al. (2010), Minata et al. (2010), Guo et al. (2019), and Yan et al. (2014).

[d] Regenerative proliferation provided as increased hepatic S-phase labeling indices (%) and/or increased number of hepatocytes in Elcombe et al. (2010) and as liver proliferation in NTP (2020).

[e] Hyperplasia and/or preneoplastic lesions provided as hepatocellular hyperplasia (qualitative results) in Elcombe et al. (2010); as bile duct hyperplasia in NTP (2020); as hyperplastic nodules in Butenhoff et al. (2012); and as bile duct hyperplasia in rats and mice in Loveless et al. (2008).

[f] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[g] No statistics were reported for histopathology results for Loveless et al. (2008); thus, the arrows indicate direction of increased incidence of individual cell necrosis for Key Event (KE)1, mitotic figures for KE3, and bile duct hyperplasia for KE4 relative to the control group.

[h] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, 80, 150, and 300 ppm in male rats, or 1.1, 2.2., 4.6, 15.6, and 31.7 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

Table 3-34. Evidence of Key Events Associated with the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)	**Cytotoxicity[b]**	**Serum Enzymes[c]**	**Regenerative Proliferation[d]**	**Hyperplasia and/or Preneoplastic Lesions[e]**	**Hepatic Tumors[f]**
0.1	– (F_1 GD 1.5–17.5)	– (F_1 GD 1.5–17.5)	NR	NR	NR
1.0	– (F_1 GD 1.5–17.5, P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	– (F_1 GD 1.5–17.5, P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	NR	NR	NR
1.6	– (104 wk)	↓ (78 wk) – (12, 24, 52, 104 wk)	NR	– (104 wk)	– (104 wk)
5.0	– (P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	↑ (P_0 GD 1.5–17.5) – (P_0 GD 1.5–11.5)	NR	NR	NR
6.25	NR	↑ (4 wk)	NR	NR	NR
12.5	NR	↑ (4 wk)	NR	NR	NR
16.1	– (104 wk)	– (12, 24, 52, 78, 104 wk)	NR	– (104 wk)	– (104 wk)
18.2 g	– (16 wk, 104 wk)	– (16 wk)	– (104 wk)	– (16, 104 wk)	– (104 wk)
25	NR	↑ (4 wk)	NR	NR	NR
50	NR	↑ (4 wk)	NR	NR	NR
63.4 g	↑ (104 wk) – (16 wk)	↑ (16 wk)	– (104 wk)	– (104 wk) – (16 wk)	– (104 wk)
100	NR	↑ (4 wk)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; F_1 = first generation of offspring; GD = gestational day; NR = not reported; P_0 = parental generation; wk = week(s).
Cells in bolded text and blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2019); NTP (2020); Butenhoff et al. (2012); Blake et al. (2020) (dams); and Cope et al. (2021) (low-fat diet only; F_1 pups exposed from GD 1.5 to 17.5 and evaluated at postnatal day (PND) 126).
[a] Reviewed in Felter et al. (2018).
[b] Cytotoxicity provided as increased incidence of hepatic necrosis in Butenhoff et al. (2012), focal necrosis in Blake et al. (2020), and as single-cell necrosis in NTP (2020) and Cope et al. (2021).
[c] Serum enzyme changes provided as changes in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate transaminase (AST) in Butenhoff et al. (2012), Blake et al. (2020), Cope et al. (2021), NTP (2020), and NTP (2019). For Butenhoff et al. (2012), only ALP was significantly decreased at 18 months (78 weeks).
[d] Regenerative proliferation provided as liver proliferation in NTP (2020).
[e] Hyperplasia and/or preneoplastic lesions provided as bile duct hyperplasia NTP (2020) and as hyperplastic nodules in Butenhoff et al. (2012).
[f] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).
[g] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.4 Genotoxicity

Evidence of PFOA genotoxicity (e.g., chromosomal aberrations, DNA breakage, micronuclei formation) is mixed, whereas most of the evidence for mutagenicity is consistently negative (Table 3-22). In an in vivo study in humans, Franken et al. (2017) observed an increase in DNA damage with increasing PFOA exposure, but the effect did not achieve statistical significance. The authors suggest that the DNA damage may have resulted from induction of oxidative stress. Additionally, Governini et al. (2015) reported that incidence of aneuploidy and diploidy was increased in PFAS-positive semen samples from nonsmokers (PFOA detected in 75% of the samples) compared with PFAS-negative samples. Of the five available animal toxicological studies that evaluated PFOA genotoxicity in vivo, only one yielded a positive result (micronuclei formation in peripheral blood cells from PFOA-exposed rats (NTP, 2019). A number of studies assessing genotoxicity of PFOA in vitro in both animal and human cell lines were reviewed. Results for chromosomal aberrations were negative for PFOA in human lymphocytes both with and without metabolic activation; results in CHO cells were mostly positive, both with and without activation, but the authors reported that the positive results were not reproducible. PFOA exposure induced DNA breakage in all in vitro DNA strand break assays that were reviewed, across three different human cell types. As noted in U.S. EPA (2016c) and Fenton et al. (2021), the clastogenic effects observed in some PFOA studies may arise from an indirect mechanism related to the physical-chemical properties of PFOA (specifically, PFOA is not subject to metabolism, it binds to proteins, it carries a net-negative electrostatic surface charge) and/or as a consequence of oxidative stress.

PFOA is non-mutagenic both with and without activation in several bacterial assays. Although three positive or equivocal results have been reported, these positive results were either exclusively at cytotoxic concentrations or were not reproducible (Table 3-22).

The available evidence suggests that PFOA is not mutagenic, but that PFOA exposure may cause DNA damage, although there is currently no known mechanistic explanation for the direct interaction between PFOA and genetic material. The available in vivo evidence suggests that

exposure to PFOA at levels resulting in cytotoxicity (e.g., hepatotoxicity, bone marrow toxicity) may lead to secondary genotoxicity in target tissues. Although unlikely, genotoxicity cannot be ruled out as a potential key event for PFOA-inducted hepatic tumor formation.

3.5.4.2.4.5 Consideration of Other Plausible Modes of Action

In addition to the evidence supporting modulation of receptor-mediated effects, and potential genotoxicity, PFOA also exhibits several other key characteristics (KCs) of carcinogens (Section 3.5.3), some of which are similarly directly evident in hepatic tissues.

For example, PFOA appears to induce oxidative stress, another KC of carcinogens, particularly in hepatic tissues (Section 3.4.1.3.7). Several studies in rats and mice showed evidence of increased oxidative stress and reduced capacity for defense against oxidants and oxidative damage in hepatic tissues.

3.5.4.2.4.5.1 Epigenetics

There is limited in vivo and in vitro evidence that PFOA induces epigenetic changes, (e.g., DNA methylation; Section 3.5.3.2) with very little liver-specific data. Two studies conducted with human cord blood reported associations between PFOA concentration and changes in DNA methylation (Miura et al., 2018; Kingsley et al., 2017), whereas an additional three studies reported no association between maternal PFOA exposure and global DNA methylation changes in the blood of the children or placenta (Ouidir et al., 2020; Leung et al., 2018; Liu et al., 2018a). Leung et al. (2018), however, did report some evidence of changes in methylation at CpG sites associated with PFOA exposure in a subset of a Faroese birth cohort with a mean cord blood PFOA concentration of 2.57 µg/L. Watkins et al. (2014) found no association between DNA methylation and PFOA in adults from the C8 Health Project.

Li et al. (2019b) observed PFOA-associated epigenetic alterations in the liver of female mouse pups following maternal exposure to PFOA. Histone acetyltransferase (HAT) levels were decreased, while histone deacetylase (HDAC) levels were increased at all dose levels. These results suggest that PFOA inhibits HAT and enhances HDAC activity, which was further demonstrated by a dose-dependent decrease in acetylation of histones H3 and H4 in the livers of PFOA-treated mice. The authors proposed that increased HDAC may activate PPARα, based upon known interactions between specific HDACs and PPARα (specifically, the class III HDAC SIRT1 deacetylates PPARα resulting in its activation), representing a regulatory role of an event included in the PPARα MOA.

In vitro studies have yielded mixed results with evidence of both hyper- and hypo-methylation of DNA in response to PFOA exposure (Section 3.5.3.2). For example, Pierozan et al. (2020) observed increased global methylation in the first daughter cell subculture of breast epithelial MCF-10A cells exposed to PFOA, although levels returned to baseline after the second passage. Two other studies found inverse relationships between global methylation and PFOA concentration in HepG2 and MCF7 cell lines (Liu and Irudayaraj, 2020 respectively; Wen et al., 2020).

3.5.4.2.4.5.2 Oxidative Stress

Results vary regarding the effect of PFOA exposure on markers of oxidative stress in in vitro and in vivo studies, both with and without a demonstrated relationship to PPARα activation.

Li et al. (2019b) observed a dose-dependent increase in 8-OHdG, as well as increases in the antioxidants catalase (CAT) and superoxide dismutase (SOD) (also indicative of oxidative stress) in the liver of female offspring of Kunming mice exposed to 1, 2.5, 5, or 10 mg/kg/day PFOA from GD 0 to GD 17, with pups sacrificed at PND 21. Serum AST and ALT levels were significantly increased in the PFOA-treated groups, indicating liver damage. Liver CAT content significantly increased in the 5 and 10 mg/kg/day dose groups. The authors propose that oxidative stress occurred through PPARα activation pathways and demonstrated changes in the mRNA level of PPARα-target genes in the same study. One such target gene is *Acox1*, which was significantly increased in livers of offspring of dams exposed to ≥2.5 mg/kg/day PFOA. Overexpression of *Acox1* has been reported to generate excess ROS, as ACOX1 is involved in fatty acid β-oxidation and produces hydrogen peroxide as a byproduct (Kim et al., 2014). This aligns with oxidative stress being proposed as a modulating factor in the PPARα-activation MOA for rodent hepatic tumors (Corton et al., 2018), as discussed above. Another study observed an increase in hydrogen peroxide in the liver of PFOA-exposed NMRI mice exposed to PFOA in utero (GD 5–9) (Salimi et al., 2019). Although they did not measure PPARα targets or PPARα itself, the type of oxidative stress observed aligns with the modulating factor in the MOA.

In contrast, Minata et al. (2010) did not observe an increase in a biomarker of oxidative stress in wild-type mice exposed to PFOA. The authors treated wild-type (129S4/SvlmJ) and *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice with PFOA (≤50 μmol/kg/day) for four weeks, after which no changes in 8-OHdG were observed in the wild-type mice. In contrast, a dose-dependent increase in 8-OHdG levels was observed in the *Pparα*-null mice, with a significant increase at 50 μmol/kg/day when compared with controls. The correlation between PFOA exposure and 8-OHdG was associated with increased tumor necrosis factor alpha (*TNF-α*) mRNA levels.

Takagi et al. (1991) performed a two-week subchronic (0.02% powdered PFOA in the diet) in male Fischer 344 rats and evaluated the levels of 8-OHdG in the liver and kidneys after exposure. The 8-OHdG level was significantly higher in the liver of exposed rats relative to controls, while there was no change in the kidneys, despite increased weights of both organs. Another group of rats were administered a single IP injection of PFOA (100 mg/kg) and sacrificed at days 1, 3, 5, and 8. Results were comparable to that of the dietary exposure study, with a significant increase in 8-OHdG levels in the liver (by day 1 following injection) as well as increased liver weight (by day 3).

PFOA exposure caused increases in 8-OHdG, a biomarker of oxidative stress, in human lymphoblast cells (TK6) and HepG2 hepatocytes (Yahia et al., 2016; Yao and Zhong, 2005). Peropadre et al. (2018) observed a slight elevation in 8-OHdG levels in PFOA-exposed human p53-deficient keratinocytes (HaCaT), and significantly elevated levels eight days following cessation of PFOA exposure. Several other in vitro studies reported increases in ROS in PFOA-exposed cells, including HepG2, nonhuman primate kidney, and human-hamster hybrid (AL) cells (Wielsøe et al., 2015; Zhao et al., 2011; Fernández Freire et al., 2008; Panaretakis et al., 2001). In contrast, Florentinet et al. (2011) did not observe increased ROS in HepG2 cells exposed to 5–400 μM PFOA for 24-hours, despite increased cytotoxicity at 200 μM PFOA and higher.

Some of the in vitro studies reported oxidative stress in relation to cell death and/or DNA damage. For example, Panaretakis et al. (2001) investigated ROS, mitochondrial damage, and

caspase-9 following PFOA exposure and determined that PFOA-induced apoptosis involved a ROS- and mitochondria-mediated pathway. ROS generation (H_2O_2 and superoxide anions) was detected in HepG2 cells following exposure to 200 and 400 μM PFOA. PFOA treatment also resulted in depolarization of the mitochondria and loss of mitochondrial transmembrane potential. A population of sub-G0/G2 phase of cell cycle was also observed. PFOA treatment was also associated with an increase in cells undergoing apoptotic DNA degradation. Caspase-9 activation was evident in cells exposed to 200 μM PFOA. The results of this study suggested that PFOA exposure increased ROS generation, which led to activation of caspase-9 and induction of the apoptotic pathway in HepG2 cells.

Wielsøe et al. (2015) observed a significant increase in ROS production in HepG2 cells exposed to 2.0E-7, 2.0E-6, and 2.0E-5M PFOA for 24 hours, along with a dose-dependent increase in DNA damage. Total antioxidant concentration was significantly decreased after 24 hours of exposure to all PFOA concentrations tested. This study demonstrated that genotoxic effects in vitro are the result of oxidative DNA damage following excess ROS production.

3.5.4.2.4.6 Conclusions

PFOA exposure is associated with several mechanisms that can contribute to carcinogenicity. There is robust evidence that PFOA activates PPARα and initiates downstream events that lead to hepatic tumorigenesis, including key events and modulating factors of the PPARα activator-induced MOA for rodent hepatocarcinogenesis (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

Additionally, PFOA exposure is associated with several mechanisms that can contribute to carcinogenicity, including epigenetic changes and oxidative stress, which may occur in conjunction with or independently of PPARα activation. It is plausible that these mechanisms may occur independently of PPARα-dependent mechanisms. These observations are consistent with literature reviews recently published by state health agencies which concluded that the hepatotoxic effects of PFOA may not entirely depend on PPARα activation (CalEPA, 2021; Gleason et al., 2017). For example, CalEPA concluded that PFOA "can induce biological activity and hepatotoxicity that is independent of PPARα activation. This indicates that the toxicity observed in rodent studies may not act entirely through the PPARα activation pathway. As such, OEHHA cannot conclude that all hepatotoxic endpoints of PFOA and PFOS in rodents are the result of PPARα activation" (CalEPA, 2021). Similarly, NJDWQI agreed that "effects of PFOA clearly occur through both PPAR-alpha independent and PPAR-alpha dependent processes" (Gleason et al., 2017). The existence of multiple MOAs in addition to PPARα activation suggest that PFOA-induced liver cancer in rats may be more relevant to humans than previously thought. Additional research is warranted to better characterize the MOAs for PFOA-induced hepatic tumorigenesis.

As described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "[i]n the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data; animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low-dose linearity." For the available data regarding the MOA of PFOA-induced hepatic carcinogenesis, there is an absence of definitive information supporting a single, scientifically justified MOA; in fact, there is evidence supporting the potential for

multiple plausible MOAs. Therefore, EPA takes the health-protective approach and concludes that the hepatic tumors observed by Biegel et al. (2001) and NTP (2020) can be relevant to human health.

3.5.4.3 Conclusions

The available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models. The few available mechanistic studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish which mechanism(s) are operative for PFOA-induced kidney cancer. Similarly for testicular cancer, the available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans. Combined, the epidemiological and animal toxicological literature indicate that the testes are a common site of PFOA-induced tumorigenesis. Overall, the EPA concluded that the available mechanistic data suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in studies of human populations and animal models. IARC (2016) and Zahm (2023), CalEPA (CalEPA, 2021) and NJDWQI (Gleason et al., 2017) similarly concluded that there is evidence for many potential mechanisms for PFOA-induced carcinogenicity. For example, IARC concluded there is strong mechanistic evidence of carcinogenicity in exposed humans and that PFOA is immunosuppressive, induces epigenetic alterations, induces oxidative stress, modulates receptor-mediated effects (via (PPAR) α, constitutive androstane receptor/pregnane X receptor [CAR/PXR], and PPARγ), and alters cell proliferation, cell death, and nutrient and energy supply (Zahm et al., 2023).

3.5.5 Cancer Classification

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the EPA reviewed the weight of the evidence and determined that PFOA is *Likely to Be Carcinogenic to Humans*, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor *Carcinogenic to Humans.*" This determination is based on the evidence of kidney and testicular cancer in humans and LCTs, PACTs, and hepatocellular adenomas and carcinomas in rats.

The *Guidelines* (U.S. EPA, 2005a) provide examples of data that may support the *Likely to Be Carcinogenic to Humans* descriptor; the available PFOA data are consistent with the following factors:

- "an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments";
- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans";
- "a rare animal tumor response in a single experiment that is assumed to be relevant to humans";

- "a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case" (U.S. EPA, 2005a).

The available evidence indicates that PFOA has carcinogenic potential in humans and at least one animal model. A plausible, though not definitively causal, association exists between human exposure to PFOA and kidney and testicular cancers in the general population and highly exposed populations. As stated in the *Guidelines for Carcinogen Risk Assessment,* "an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies." Two *medium* confidence independent studies provide evidence of an association between kidney cancer and elevated PFOA serum concentrations (Shearer et al., 2021; Vieira et al., 2013), while two studies in the same cohort provide evidence of an association between testicular cancer and elevated PFOA serum concentrations (Barry et al., 2013; Vieira et al., 2013). The PFOA cancer database would benefit from additional large *high* confidence cohort studies in independent populations.

The evidence of carcinogenicity in animals is based on three studies that used the same strain of rat. Taken together, these results provide evidence of increased incidence of three different tumor types (LCTs, PACTs, and hepatocellular tumors) in males administered diets contaminated with PFOA. Additionally, pancreatic acinar cell adenocarcinomas are a rare tumor type (NTP, 2020), and their occurrence in PFOA-treated animals in this study increases the confidence that this incidence is treatment-related since these tumors are unlikely to be observed in the absence of a carcinogenic agent (U.S. EPA, 2005a). The historical control incidence for pancreatic acinar cell adenocarcinomas in the female rats is 0/340 and in the male rats is 2/340, highlighting the rarity of this particular tumor type (NTP, 2020). Importantly, site concordance is not always assumed between humans and animal models; agents observed to produce tumors may do so at the same or different sites in humans and animals (U.S. EPA, 2005a). While site concordance was present between human studies of testicular cancer and animal studies reporting increased incidence of LCTs, evidence of carcinogenicity of PFOA from other cancer sites where concordance between humans and animals is not present is still relevant to the carcinogenicity determination for PFOA. See Table 3-35 below for specific rationale on how PFOA aligns with examples supporting the *Likely to Be Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 3-35. Comparison of the PFOA Carcinogenicity Database with the *Likely* Cancer Descriptor as Described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

Likely to Be Carcinogenic to Humans	
"An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** Epidemiological evidence supports a plausible association between exposure and cancer, though there are uncertainties regarding the MOAs for tumor types observed in humans. There is supporting experimental evidence, including carcinogenicity data from animal experiments.

Likely to Be Carcinogenic to Humans	
"An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** PFOA has tested positive in one species (rat), both sexes, and multiple sites (liver, pancreas, testes, uterus). There is also evidence of carcinogenicity in humans.
"A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset" (U.S. EPA, 2005a).	**This description is not applicable to PFOA.** The report by NTP (2020) does not indicate that perinatal exposure exacerbates the carcinogenic potential of PFOA.
"A rare animal tumor response in a single experiment that is assumed to be relevant to humans" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** The pancreatic adenocarcinomas observed in multiple male dose groups are a rare tumor type in this strain (NTP, 2020).
"A positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** Multiple positive tumor studies in the same strain of rat are supported by plausible associations between human exposure and kidney and testicular cancer.

Notes: DNA = deoxyribonucleic acid; MOA = mode of action.

EPA recognizes that other state and international health agencies have recently classified PFOA as carcinogenic to humans (IARC as reported in Zahm et al., 2023; CalEPA, 2021). As the SAB PFAS Review Panel (U.S. EPA, 2022e) noted, "the criteria used by California EPA, for determination that a chemical is a carcinogen, are not identical to the criteria in the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)" and, similarly, IARC's classification criteria are not identical to the EPA's guidelines (IARC, 2019). Rationale for why PFOA does not meet the Carcinogenic to Humans descriptor according to the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) is detailed in Section 5.4.

4 Dose-Response Assessment

Considerations in Selecting Studies and Endpoints for Dose-Response Analysis

There is evidence from both human epidemiological and animal toxicological studies that oral perfluorooctanoic acid (PFOA) exposure can result in adverse health effects across a range of health outcomes. In response to recommendations made by the SAB and the conclusions from EPA's systematic review of the available health effects evidence, presented in the EPA's preliminary analysis, the 2021 SAB review draft *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2021c), EPA focused its final toxicity value derivation efforts herein "on those health outcomes that have been concluded to have the strongest evidence" (U.S. EPA, 2022e). Therefore, EPA prioritized health outcomes and endpoints with the strongest overall weight of evidence, which were the outcomes with evidence *demonstrates* or evidence *indicates* integration judgments, based on the synthesis of the available human, animal, and mechanistic evidence (Section 3.4 and 3.5) for points of departure (POD) derivation using the systematic review methods described in Section 2 and Appendix A (U.S. EPA, 2024a). For PFOA, the health outcomes with the strongest weight of evidence are cancer (described in Section 4.2) and the noncancer health outcomes of immunological, developmental, cardiovascular (serum lipids), and hepatic effects (described in Section 4.1). For all other health outcomes (e.g., reproductive, endocrine, nervous, hematological, musculoskeletal), the evidence integration summary judgment for the human epidemiological and animal toxicological evidence was *suggestive* or *inadequate* and these outcomes were not assessed quantitatively. Health outcomes for which the results were *suggestive* are discussed in the evidence profile tables provided in Appendix C (U.S. EPA, 2024a), as well as Section 5.5.

In the previous section describing the hazard judgment decisions (Section 3.4 and 3.5), EPA qualitatively considered *high, medium,* and sometimes *low* confidence studies of PFOA exposure to characterize the weight of evidence for each health outcome. For the quantitative analyses described in the following subsections, EPA focused exclusively on *high* or *medium* confidence human epidemiological and animal toxicological studies for POD derivation, as recommended in Chapter 7.2 of the IRIS Handbook (U.S. EPA, 2022d). While the IRIS Handbook also includes consideration of *low* confidence studies for dose-response analysis under certain circumstances, this EPA assessment did not consider *low* confidence studies because of the relatively large and robust database for PFOA. At this stage, EPA considered additional study attributes to enable extrapolation to relevant exposure levels in humans. These attributes are described in Table 7-2 of the IRIS Handbook and include relevance of the test species, relevance of the studied exposure to human environmental exposures, quality of measurements of exposure and outcomes, and other aspects of study design including specific reconsideration of the potential for bias in the reported association between exposure and outcomes (U.S. EPA, 2022d).

Consideration of these attributes facilitates the transparent selection of studies and data for dose-response modeling and potential RfD or CSF derivation. Studies exhibiting these attributes are expected to provide more accurate human equivalent toxicity values and are therefore preferred in the selection process. Consideration of these attributes in the study selection process are described below for noncancer and cancer endpoints.

4.1 Noncancer

4.1.1 Study and Endpoint Selection

For study and endpoint selection for noncancer health outcomes, the human studies that provided all necessary analytical information (e.g., exposure distribution or variance, dose-response data, etc.) for POD derivation, analyzed the outcome of interest in the general population or susceptible population, and demonstrated a larger number of the study attributes outlined above were preferred. If available, *high* and *medium* confidence studies with exposures levels within or near the range of typical environmental human exposures, especially exposure levels comparable to human exposure levels in the general United States population, were preferred over studies reporting considerably higher exposure levels (e.g., occupational exposure levels). Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to the lower dose range of exposure levels that are relevant to the overall population. When available for a given health outcome, studies with analyses that addressed potential confounding factors affecting exposure concentrations (e.g., addressing temporal variations of PFOA concentrations during pregnancy due to hemodynamics) were also preferred. Additionally, when studies presented overlapping data on the same cohort or study population, various factors were considered to facilitate selection of one study for POD derivation. These factors included the duration of exposure, the length of observation of the study cohort, and the comprehensiveness of the analysis of the cohort in order to capture the most relevant results for dose-response analysis.

The preferred animal toxicological studies consisted of *medium* and *high* confidence studies with exposure durations appropriate for the endpoint of interest (e.g., chronic or subchronic studies vs. short-term studies for chronic effects) or with exposure during sensitive windows of development and with exposure levels near the lower dose range of doses tested across the evidence base. These types of animal toxicological studies increase the confidence in the RfD relative to other animal toxicological studies because they are based on data with relatively low risk of bias and are associated with less uncertainty related to low-dose and exposure duration extrapolations. See Section 5.3 for a discussion of animal toxicological studies and endpoints selected for POD derivation for this updated assessment compared with those selected for the 2016 PFOA HESD (U.S. EPA, 2016c).

4.1.1.1 Hepatic Effects

As reviewed in Section 3.4.1.4, *evidence indicates* that elevated exposures to PFOA are associated with hepatic effects in humans. As described in Table 3-4, the majority of epidemiological studies assessed endpoints related to serum biomarkers of hepatic injury (18 *medium* confidence studies), while fewer studies reported on liver disease or injury (5 *medium* confidence studies) and other serum markers of liver function (4 *medium* confidence studies). EPA prioritized studies that evaluated endpoints related to serum biomarkers of injury for quantitative analyses because the reported effects on these endpoints were well-represented within the database and were generally consistent across the available *medium* confidence studies. Additionally, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Specifically, all five *medium* confidence studies of general population adults from the updated literature searches reported

positive associations between PFOA serum concentrations and ALT, three of which reported statistically significant responses (Jain, 2019; Nian et al., 2019; Salihovic et al., 2018; Darrow et al., 2016; Gleason et al., 2015). These more recently published studies provided additional evidence for increased ALT in adults beyond the three *medium* confidence studies reporting positive associations for ALT from the 2016 PFOA HESD (Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010). Findings from studies of other liver enzymes, AST and GGT, in adults generally reported a positive association, though less consistently than studies of ALT; therefore, studies of AST and GGT are supportive of the selection of ALT as an endpoint for POD derivation because these results demonstrate coherence across the different liver serum enzyme endpoints.

As mentioned above, serum ALT measures are considered a reliable indicator of impaired liver function because increased serum ALT is indicative of leakage of ALT from damaged hepatocytes (Liu et al., 2014; Boone et al., 2005; U.S. EPA, 2002a). Additionally, evidence from both human epidemiological and animal toxicological studies indicates that increased serum ALT is associated with liver disease (Roth et al., 2021; Kwo et al., 2017; Ioannou et al., 2006b; Ioannou et al., 2006a). Human epidemiological studies have demonstrated that even low magnitude increases in serum ALT can be clinically significant when extrapolated to the overall population (Gilbert and Weiss, 2006). For example, a Scandinavian study in people without any symptoms of liver disease but with relatively small increased serum ALT levels were later diagnosed with liver diseases such as steatosis and chronic hepatitis C (Mathiesen et al., 1999). Additionally, a study in Korea found that the use of lowered thresholds for "normal" serum ALT values showed good prediction power for liver-related adverse outcomes such as mortality and hepatocellular carcinoma (Park et al., 2019a).

Numerous studies have also demonstrated an association between elevated ALT and liver-related mortality (reviewed by Kwo et al. (2017)). Furthermore, the American Association for the Study of Liver Diseases (AASLD) recognizes serum ALT as an indicator of overall human health and mortality (Kim et al., 2008). For example, as reported by Kwo et al. (2017), Kim et al. (2004) observed that higher serum ALT concentrations corresponded to an increased risk of liver-related death in Korean men and women; similarly, Ruhl and Everhart (2013, 2009) analyzed NHANES data and observed an association between elevated serum ALT and increased mortality, liver-related mortality, coronary heart disease in Americans, and Lee et al. (2008) found that higher serum ALT was associated with higher mortality in men and women in Olmstead County, Minnesota. Furthermore, the American College of Gastroenterology (ACG) recommends that people with ALT levels greater than 33 (men) or 25 IU/L (women) undergo screenings and assessments for liver diseases, alcohol use, and hepatotoxic medication use (Kwo et al., 2017). Taken together, results of human epidemiological and animal toxicological studies and the positions of the AASLD and the ACG demonstrate the clinical significance of increased serum ALT. It is also important to note that while evaluation of direct liver damage is possible in animal toxicological studies, it is difficult to obtain biopsy-confirmed histological data in humans. Therefore, liver injury in humans is typically assessed using serum biomarkers of hepatotoxicity (Costello et al., 2022).

Among the available *medium* confidence epidemiological studies reporting alterations in serum ALT in humans, studies of adults in the general population were prioritized over studies in other populations (e.g., occupational) or life stages (e.g. children), as the adult study findings provided

the most consistent evidence of increases in ALT (see Section 3.4.1.1). Several of these *medium* confidence studies reporting increases in ALT in adults were excluded from POD derivation for reasons such as combined adolescent and adult populations (Gleason et al., 2015), populations consisting of only elderly adults (Salihovic et al., 2018), use of correlation analyses only (Yamaguchi et al., 2013), and reporting analyses stratified by glomerular filtration status without stratifying by exposure level, which were not amenable to modeling (Jain, 2019).

Exclusions of these studies resulted in the consideration of four *medium* confidence studies for POD derivation (Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012; Lin et al., 2010) (Table 4-1). These studies exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). For example, the two largest studies of PFOA and ALT are Gallo et al. (2012) and Darrow et al. (2016), both conducted in over 30,000 individuals from the general population, aged 18-years and older, as part of the C8 Health Project in the United States. Further, Gallo et al. (2012) demonstrated a statistically significant trend of increased ALT across deciles of PFOA exposure and Darrow et al. (2016) provided an exposure-response gradient for PFOA. Two additional studies (Nian et al., 2019; Lin et al., 2010) were considered for POD derivation because they reported associations in general populations in the United States and a Chinese population located near a PFAS manufacturing facility, respectively. Nian et al. (2019) examined a large population of adults (1,605) in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project and reported significantly increased level of ALT associated with PFOA. Lin et al. (2010) was also considered for POD derivation since it is a large (2,216 men and 1,063 women) nationally representative study in an NHANES adult population and observed increased ALT levels per log-unit increase in PFOA and these associations remained after accounting for other PFAS in the regression models. However, several methodological limitations precluded its use for POD derivation. Limitations include lack of clarity about the base of logarithmic transformation applied to PFOA concentrations in regression models, and the choice to model ALT as an untransformed variable, which is a departure from the lognormality assumed in most of the ALT literature. Therefore, three *medium* confidence epidemiological studies were prioritized for POD derivation(Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012) (Table 4-1).

Liver toxicity results reported in animal toxicological studies after PFOA exposure are concordant with the observed increased ALT indicative of hepatic damage observed in epidemiological studies. Specifically, studies in rodents found that oral PFOA treatment resulted in increased relative liver weight (17/20 *high* and *medium* confidence studies), biologically significant alterations in levels of at least one serum biomarker of liver injury (i.e., ALT, AST, and ALP) (6/9 *high* and *medium* confidence studies), and evidence of histopathological alterations including hepatocyte degenerative or necrotic changes (12/12 *high* and *medium* confidence studies). These hepatic effects, particularly the increases in serum enzymes and histopathological evidence of liver damage, are supportive of increased ALT observed in human populations. Mechanistic studies in mammals and evidence from in vitro studies and nonmammalian animal models provide additional support for the biological plausibility and human relevance of the PFOA-induced hepatic effects observed in animals. These studies suggest multiple potential MOAs for the observed liver toxicity, including PPARα-dependent and -independent MOAs. The observed increases in liver enzymes (e.g., ALT) in rodents are supportive of the hepatic damage confirmed during histopathological examinations in several

studies. Taken together, the study results suggest that at least some mechanisms for PFOA-induced hepatic effects are relevant to humans.

For animal toxicological hepatic endpoints, EPA preferred studies reporting quantitative biologically or statistically significant measures of severe toxicity (i.e., histopathological lesions related to cell or tissue death or necrosis) with study designs best suited for quantitative analysis (e.g., large sample size, reported effects in the lower dose range). Of the seven studies that quantitatively reported incidences of hepatic cell or tissue death or necrosis, five were excluded; two studies were excluded because they did not report statistically or biologically significant responses (Butenhoff et al., 2012; Perkins et al., 2004) and three were excluded because they had relatively small sample sizes (i.e., $n \leq 10$) (Cope et al., 2021; Blake et al., 2020; NTP, 2019). After these exclusions, EPA identified two studies reporting adverse liver effects in male rodents due to PFOA exposure, NTP (2020), a chronic dietary study in Sprague-Dawley rats (see study design details in Section 3.4.4.2.1.2), and Loveless et al. (2008), a 29-day gavage dosing study in CD-1 mice, for POD derivation (Table 4-1). NTP (2020) conducted histopathological examinations of liver tissue in male rats and reported dose-dependent increases in the incidence of hepatocellular single cell death and hepatocellular necrosis. As this is one of the few available chronic PFOA toxicity studies that presented a large sample size ($n = 50$), numerous and relatively low dose levels, and assessment of a suite of hepatic endpoints, both the single cell death and necrosis endpoints in males from the 107-week time point were considered for derivation of PODs. Similar to the NTP study (2020), Loveless et al. (2008) reported a number of hepatotoxic effects, a low dose range, relatively large sample sizes ($n = 19–20$), and dose-dependent increases in histopathological outcomes indicating adverse effects in male mice gavaged with PFOA for 29 days. In addition, Loveless et al., (2008, 988599) was the only study in mice to report quantitative histopathological examinations of liver tissue in non-pregnant adults and had the longest exposure duration of the available mouse studies. Therefore, the incidences of two endpoints, focal cell necrosis and individual cell necrosis, in male mice from Loveless et al. (2008) were also considered for the derivation of PODs.

4.1.1.2 Immunological Effects

As reviewed in Section 3.4.2.4, *evidence indicates* that elevated exposures to PFOA are associated with immunological effects in humans. As described in Table 3-9, the majority of epidemiological studies assessed endpoints related to immunosuppression (1 *high* and 20 *medium* confidence studies) and immune hypersensitivity (1 *high* and 20 *medium* confidence studies), while 2 *medium* confidence studies also reported on endpoints related to autoimmune disease. Studies that reported on specific autoimmune diseases were excluded from POD derivation because there were a limited number of studies that assessed the same diseases (e.g., rheumatoid arthritis, celiac disease). Studies that evaluated endpoints related to immune hypersensitivity (e.g., asthma) were also not considered for POD derivation because there were inconsistencies in the direction and precision of effects across gender or age subgroups in the available studies. These inconsistencies limited the confidence needed to select particular studies and populations for dose-response modeling. Other immune hypersensitivity endpoints, such as odds of allergies and rhinoconjunctivitis, reported differing results across *medium* and *high* confidence studies and were therefore excluded from further consideration, though they provide qualitative support of an association between PFOA exposure and altered immune function.

Evidence of immunosuppression in children associated with exposure to PFOA reported by epidemiological studies was consistent across studies and endpoints. Specifically, epidemiological studies reported associations between PFOA exposure and reduced humoral immune response to routine childhood immunizations, including lower levels of tetanus and diphtheria (Timmermann et al., 2021; Abraham et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012), HiB (Abraham et al., 2020), and rubella (Zhang et al., 2023; Stein et al., 2016b; Granum et al., 2013) antibody titers. Reductions in antibody response were observed at multiple timepoints during childhood (specifically ages between 3-19 years in these studies), for either prenatal or postnatal childhood PFOA exposure levels, and were consistent across studies in children populations from *medium* confidence studies. Therefore, reduced antibody response in children was selected as an endpoint for POD derivation.

Measurement of antigen-specific antibodies following vaccination(s) is a measure of the overall ability of the immune system to respond to a challenge. The antigen-specific antibody response is extremely useful for evaluating the entire cycle of adaptive immunity, which is a type of immunity that develops when a person's immune system responds to a foreign substance or microorganism, and it has been used as a comprehensive approach to detect immunosuppression across a range of cells and signals (Myers, 2018). The SAB's PFAS review panel noted that reduction in the level of antibodies produced in response to a vaccine represents a "failure of the immune system to respond to a specific challenge and is considered an adverse immunological health outcome" (U.S. EPA, 2022e). This is consistent with a review article by Selgrade (2007) who suggested that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards—which has the potential to be a more adverse effect that just a single immunotoxic effect. Thus, decrements in the ability to maintain effective levels of antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFOA.

As noted by Dewitt et al. (2019; 2017; 2016a) and in comments from other subject matter experts on the SAB's PFAS review panel (U.S. EPA, 2022e), the clinical manifestation of a disease after chemical exposure is not required for a chemical to be classified as an immunotoxic agent and the ability to measure clinical outcomes as a result of mild to moderate immunosuppression in response to chemical exposure in traditional epidemiological studies can be challenging. Specifically, the SAB noted that "[d]ecreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease" (U.S. EPA, 2022e). The WHO *Guidance for immunotoxicity risk assessment for chemicals* similarly recommends measures of vaccine response as a measure of immune effects as "childhood vaccine failures represent a significant public health concern" (WHO, 2012). Decreases in antibody response, even at smaller magnitudes in individuals, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This response also translates across multiple species, including rodents, and extensive historical data indicate that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity.

Studies of developmental exposure to environmental toxicants demonstrate an association with immune suppression (Selgrade, 2007). When immunosuppression occurs during immune system development, the risks of developing infectious diseases and other immunosuppression-linked diseases may increase (Dietert et al., 2010). The immune system continues developing

postnatally; because of this, exposures to PFAS and other immunotoxic agents during development may have serious, long-lasting, and irreversible health consequences (Dewitt et al., 2019; Macgillivray and Kollmann, 2014; Selgrade, 2007). Indeed, Hessel et al. (2015) reviewed the effect of exposure to nine toxicants on the developing immune system and found that the developing immune system was at least as sensitive or more sensitive than the general (developmental) toxicity parameters that were assessed. Developmental immunotoxicity as a result of chemical exposure is generally observed at doses lower than required to elicit immunotoxicity in adults (vonderEmbse and DeWitt, 2018). Therefore, developmental immunotoxicity is generally a highly sensitive health outcome, both when considering other types of developmental toxicity and when comparing it to immunotoxicity observed in exposed adults. Luster et al. (2005) similarly noted that the specific immunotoxic endpoint of responses to childhood vaccines may be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent.

One *high* and 10 *medium* confidence studies (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Pilkerton et al., 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Stein et al., 2016b; Mogensen et al., 2015a; Granum et al., 2013; Grandjean et al., 2012) reported findings on antibody response to tetanus, diphtheria, or rubella in children or adolescents. Only one *low* confidence study reported findings on antibody response to Hib (Abraham et al., 2020), which was excluded from POD derivation because of the limited evidence and the *low* confidence rating. Three studies (Zhang et al., 2023; Pilkerton et al., 2018; Stein et al., 2016b) reported on antibody response to rubella in adolescents and one study reported on antibody response in young children (Granum et al., 2013). From the adolescent studies, one study observed decreased rubella antibody response in a specific subpopulation of only seropositive adolescents (Stein et al., 2016b) and the other two studies did not report statistically significant associations between PFOA and rubella antibody response in the overall population (Zhang et al., 2023; Pilkerton et al., 2018). Granum et al. (2013) reported a statistically significant association between PFOA exposure and rubella antibody response in young children. Because studies reporting rubella antibody response were mixed (2/4 demonstrating associations), rubella studies were not further considered for POD derivation. Overall, EPA prioritized studies reporting responses to tetanus and diphtheria because the responses were consistently observed across a large number of studies (*medium* and *low* confidence) in children from multiple populations for these two vaccine types.

Five separate studies (Shih et al., 2021; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a; Grandjean et al., 2012) reported on diphtheria and tetanus antibody responses and PFOA exposure in the same cohort (i.e., same individuals) of Faroese children. One study reported on the same Faroese children cohort in a more recent *medium* confidence publication (Budtz-Jørgensen and Grandjean, 2018). Because this most recent *medium* confidence study is the only one of the five studies that provided dose-response data with untransformed PFOA concentrations which are more amenable to BMD modeling, only results from Budtz-Jørgensen and Grandjean (2018) were prioritized for POD derivation and the four other studies conducted in the Faroe Island population were excluded. One *medium* confidence cross-sectional study (Timmermann et al., 2021) reported on tetanus and diphtheria antibody response and PFOA exposure in Greenlandic children. This study was also prioritized for POD derivation. The results from the Faroe Island and Greenlandic populations are qualitatively

supported by a *low* confidence cross-sectional study of associations between diphtheria and tetanus antibody responses and PFOA in German children (Abraham et al., 2020).

In total, two *medium* confidence epidemiologic studies that reported decreased antibody responses in children exposed to PFOA (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018) were considered for POD derivation (Table 4-1). These two epidemiological studies report data characterizing antibody responses to vaccinations in children using a variety of PFOA exposure measures across various populations and vaccinations. Budtz-Jørgensen and Grandjean (2018) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and PFOA exposure measured at age 5 or prenatally; Timmerman et al. (2021) investigated anti-tetanus and anti-diphtheria responses and PFOA exposure in Greenlandic children aged 7–12. Both studies examined antibody responses associated with PFOA exposure in well-characterized cohorts, and in the case of Budtz-Jørgensen and Grandjean (2018), multiple prior publications supported the finding of an inverse relationship between PFOA exposure concentrations and antibody response in the same study cohorts.

Immunotoxicity results reported in animal toxicological studies in adult rodents are concordant with the immunosuppression observed in epidemiological studies. Specifically, studies in rodents found that oral PFOA treatment resulted in reduced immune response (i.e., reduced globulin and immunoglobulin levels upon immune challenges) (four *medium* confidence studies) and altered immune cell populations (e.g., altered white blood cell counts, altered splenic and thymic cellularity) (one *high* and four *medium* confidence studies). Immunosuppression evidenced by functional assessments of the immune responses, such as analyses of globulin and immunoglobulin levels after challenges, are comparable and thus, supportive of the immunosuppression reported as decreased antibody responses seen in human populations and were therefore prioritized for quantitative assessment. Additionally, EPA identified immunosuppressive effects in multiple species and both sexes of animal toxicological studies, further supporting the selection of these endpoints for dose-response analyses. Animal toxicological studies assessing alterations in immune cell populations were not considered further as there were a limited number of studies assessing specific endpoints of interest. Although the other reported immune effects, such as altered organ weights and histopathology, are consistent with evidence indicating alterations in immune function and response from animal toxicological studies, they were not considered for POD derivation as these effects may be confounded by changes in body weight, effects were not consistent across studies, and/or a limited number of studies assessed specific outcomes. Of the four *medium* confidence studies reporting impaired IgM response in mice, EPA selected Dewitt et al. (2008), a 15-day drinking water exposure study in female mice, and Loveless et al. (2008), a 29-day study in male mice, for POD derivation as these two studies presented data for a larger number of dose groups spanning a broader dose range than either Dewitt et al. (2016b) or De Guise et al. (2021).

4.1.1.3 Cardiovascular Effects

As reviewed in Section 3.4.3.4, *evidence indicates* that exposure to PFOA are associated with cardiovascular effects in humans. As described in Table 3-12, the majority of epidemiological studies assessed endpoints related to serum lipids (2 *high*, 27 *medium*, and 19 *mixed*[18] confidence

[18] *Mixed* confidence studies on serum lipids were primarily of *medium* confidence for total cholesterol and HDL cholesterol, and *Low* confidence for LDL cholesterol and triglycerides.

studies) and blood pressure and hypertension (2 *high* and 18 *medium* confidence studies), while some studies also reported on cardiovascular disease (1 *high* and 6 *medium* confidence studies) and atherosclerosis (1 *high* and 3 *medium* confidence studies). Endpoints related to cardiovascular disease and atherosclerosis were excluded from consideration for POD derivation because there were limited high and medium confidence studies and they reported mixed (i.e., positive and inverse associations) or mostly null results. Endpoints related to blood pressure and hypertension were also excluded from quantitative analyses because there was higher confidence in analytically determined serum lipid levels compared with blood pressure measurements and there was a larger evidence base for serum lipids as compared to blood pressure. However, there was consistent evidence of associations between PFOA exposure and continuous measures of blood pressure and risk of hypertension in adults from the general population, including adults living in high-exposure communities located near PFAS manufacturing plants, which qualitatively support an association between PFOA and cardiovascular effects in humans.

The majority of studies in adults in the general population, including high-exposure communities, reported positive associations between PFOA serum concentrations and serum lipids. Studies in adults were prioritized based on reported age-dependent fluctuations in serum lipids as a result of puberty (Daniels et al., 2008), which may impact the consistency of results from studies in children. Specifically, *medium* confidence epidemiological studies in adults reported positive associations between PFOA exposure and total cholesterol (TC) (15/18 studies) and low-density lipoprotein (LDL) (12/17 studies). Of these two endpoints, EPA selected TC for quantitative assessments because the association was the most consistently observed in adults and the studies for TC were of higher confidence for outcome measurements compared with LDL. Additionally, the positive associations with TC in these studies were further supported by a recent meta-analysis that included 14 general population studies in adults (U.S. EPA, 2024b) and reported an association between increased cholesterol and increased PFOA exposure.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), ischemic stroke (IS), and cardiovascular mortality occurring in populations without prior CVD events (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Additionally, disturbances in cholesterol homeostasis contribute to the pathology of nonalcoholic fatty liver disease (NAFLD) and to accumulation of lipids in hepatocytes (Malhotra et al., 2020). Cholesterol is made and metabolized in the liver, and thus the evidence indicating that PFOA exposure disrupts lipid metabolism, suggests that toxic disruptions of lipid metabolism by PFOA are indications of hepatoxicity. Increases in serum cholesterol, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude increases in serum cholesterol could shift the distribution of serum cholesterol in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for cardiovascular disease. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern" (U.S. EPA, 2022e).

A total of 15 *medium* confidence studies (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020e; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Winquist and Steenland, 2014; Eriksen et al., 2013; Fitz-Simon et al., 2013; Nelson et al., 2010; Costa et al., 2009; Steenland et al., 2009; Sakr et al., 2007a; Olsen et al., 2003) reported positive associations between exposure to PFOA and total cholesterol in adults from the general population. One study (Winquist and Steenland, 2014) was excluded from POD derivation because the study estimated the risk of levels above clinical thresholds for TC and these data were not amenable to modeling continuous changes in TC. Three studies were excluded from POD derivation because they were in occupationally exposed adult populations only and would not represent typical exposure scenarios for human environmental exposure (Costa et al., 2009; Sakr et al., 2007a; Olsen et al., 2003). Three studies (Canova et al., 2020; Lin et al., 2020e; Eriksen et al., 2013) were excluded from POD derivation due to narrow age ranges (i.e., 50–65 years of age, 55–75 years of age, 40–60 years of age, and 20–39 years of age, respectively) of the study populations that were less comprehensive than the age groups included by other studies and therefore, may not apply across the general adult population. One study (Jain and Ducatman, 2019b) was excluded from POD derivation because the study reported findings stratified by BMI status without stratification by exposure.

Although the positive associations between PFOA and TC were supported by the findings of a recent meta-analysis that included 14 general population studies of adults (U.S. EPA, 2024b), EPA did not use the pooled effect from this meta-analysis for POD derivation. This meta-analysis was not comprehensive of the entire database of studies on PFOA and TC because it was performed specifically with the purpose of informing aspects of the Pooled Cohort Atherosclerotic Cardiovascular Disease (ASCVD) model which relies on CVD risk reduction analysis for those ages 40–89 (U.S. EPA, 2024b). The results of another recent meta-analysis on PFOA and serum lipids (Abdullah Soheimi et al., 2021) was excluded from POD derivation because the pooled effects reported combined 11 studies with TC, triglycerides and LDL in multiple populations (i.e., children, adolescents, pregnant women, and adults). As previously noted, serum lipids rise in early childhood and fluctuate in puberty (Daniels et al., 2008), and combining study populations at different lifestages would likely result in unaddressed confounding by age.

Four studies presented overlapping data from NHANES (Fan et al., 2020; Dong et al., 2019; Liu et al., 2018d; Nelson et al., 2010). Of these four, Dong et al. (2019) was selected for POD derivation because this larger study included data from all NHANES cycles between 2003 and 2014, while the other three studies reported results for only one or two cycles within the 2003-2014 range and were therefore not further considered. Similarly, two studies (Fitz-Simon et al., 2013; Steenland et al., 2009) presented data on the C8 Health Project population. Fitz-Simon et al. (2013) was not selected for POD derivation because it was a part of a short-term follow-up and was not as comprehensive as the population examined by Steenland et al. (2009). Therefore, Steenland et al. (2009) was also selected for POD derivation. Finally, Lin et al. (2019) was also selected for POD derivation because it provided data for a large number of adults (n = 940) in the general U.S. population from the Diabetes Prevention Program (DPP) population, with PFOA levels at baseline comparable to those from NHANES 1999–2000.

In summary, three *medium* confidence epidemiologic studies were considered for POD derivation (Table 4-1) (Dong et al., 2019; Lin et al., 2019; Steenland et al., 2009). These

candidate studies describe a variety of PFOA exposure measures across various adult populations and exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). Dong et al. (2019) investigated the NHANES population (2003–2014), Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population), and Lin et al. (2019) collected data from prediabetic adults from the DPP and DPPOS study (1996–1999).

Though results reported in animal toxicological studies support the alterations in lipid metabolism associated with PFOA exposure observed in epidemiological studies, there are species differences in the direction of effect with increasing dose. As a result of these differences, there is some uncertainty about the human relevance of these observed responses in rodents. Additionally, the available mechanistic data do not provide an increased understanding of the observed non-monotonicity of serum lipid levels and decreased serum lipid levels at higher dose levels in rodents (Section 3.4.3.2). Due to the uncertainties regarding human relevance of the animal toxicology studies, EPA did not derive PODs for animal toxicological studies reporting cardiovascular effects, such as altered serum lipid levels.

4.1.1.4 Developmental Effects

As reviewed in Section 3.4.4.4, *evidence indicates* that elevated exposures to PFOA are associated with developmental effects in humans. As described in Table 3-15, the majority of epidemiological studies assessed endpoints related to fetal growth restriction (26 *high* and 25 *medium* confidence studies) and gestational duration (13 *high* and 13 *medium* confidence studies), while fewer studies reported on endpoints related to fetal loss (2 *high* and 6 *medium* confidence studies) and birth defects (4 *medium* confidence studies). Evidence for birth defects was limited in that there are only 4 *medium* confidence studies and those studies provided mixed findings. Therefore, birth defects not prioritized for POD derivation. Although half of the available *high* and *medium* confidence studies reported increased incidence of fetal loss (2/4), EPA did not prioritize this endpoint for POD derivation as there were a relatively limited number of studies compared with endpoints related to gestational duration and fetal growth restriction and results from the *high* confidence studies were mixed. The impacts observed on fetal loss are supportive of an association between PFOA exposure and adverse developmental effects.

Approximately half of the available studies reporting metrics of gestational duration observed increased risk associated with PFOA exposure, including among *high* confidence studies. Six of the 14 *medium* or *high* confidence studies reported inverse associations for gestational age at birth and 5 of the 11 *medium* or *high* confidence studies reported an increased risk of preterm birth. Gestational age was not prioritized for quantitative analyses because the majority of studies did not report inverse associations and this endpoint is more prone to measurement error (see Section 3.4.4.1.2). There were generally more consistent findings showing positive associations between PFOA exposure and preterm birth, particularly from the *high* confidence studies. However, there were some concerns with sample timing and potential influence of pregnancy hemodynamics on the observed outcomes, as the majority of studies reporting increased odds of preterm birth sampled PFOA concentrations later in pregnancy. While overall there appears to be some associations between PFOA exposure and gestational duration, the inconsistencies in the database and lack of studies sampling in the first trimester of pregnancy resulted in this effect not being considered for POD derivation. Additionally, the database for fetal growth restriction was

both larger and consisted of more *medium* and *high* confidence studies. Therefore, studies demonstrating fetal growth restriction were prioritized for POD derivation.

The majority of *high* and *medium* confidence epidemiological studies (17/25) reported associations between PFOA and decreased mean birth weight in infants. Studies on changes in standardized birth weight measures (i.e., z-scores) also reported some inverse associations in *high* and *medium* confidence studies. Endpoints characterizing fetal growth restriction were included for POD derivation because multiple studies reported effects on these endpoints, particularly decreased birth weight, and reported generally consistent findings across *high* and *medium* confidence studies. As noted in the Developmental Human Evidence Study Evaluation Considerations (Section 3.4.4.1.2), measures of birth weight were considered higher confidence outcomes compared with other measures of fetal growth restriction such as birth length, head circumference, or ponderal index because birth weight measures are less prone to measurement error (Shinwell and Shlomo, 2003). Studies reporting changes in mean birth weight were more amenable to modeling compared with those reporting changes in standardized (e.g., z-score) birth weight measurements. Standardized measurements depend on sources of standardization and are harder to interpret and compare across studies. As a result, measures of mean changes in birth weight were considered for quantitative analysis.

Low birth weight (LBW) is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999). LBW is widely considered a useful population level public health measure (Vilanova et al., 2019; Cutland et al., 2017; WHO and UNICEF, 2004; Lira et al., 1996) and is on the World Health Organization's (WHO's) global reference list of core health indicators (WHO, 2018a, 2014). Decreases in birthweight, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude decreases in birthweight could shift the distribution of birthweight in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for decreased birthweight and subsequent effects related to decreased birthweight. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern" (U.S. EPA, 2022e).

Substantial evidence links LBW to a variety of irreversible adverse health outcomes at various later life stages. It has been shown to predict prenatal mortality and morbidity (Cutland et al., 2017; WHO, 2014; U.S. EPA, 2013) and is a leading cause of infant mortality in the United States (CDC, 2021). Low-birth-weight infants are also more likely to have underdeveloped and/or improperly-functioning organ systems (e.g., respiratory, hepatic, cardiovascular), clinical manifestations of which can include breathing problems, red blood cell disorders (e.g., anemia), and heart failure (U.S. EPA, 2013; Zeleke et al., 2012; Guyatt and Snow, 2004; WHO and UNICEF, 2004; JAMA, 2002). Additionally, low-birth-weight infants evaluated at 18 to 22 months of age demonstrated impaired mental development (Laptook et al., 2005).

LBW is also associated with increased risk for diseases in adulthood, including obesity, diabetes, and cardiovascular disease (Smith et al., 2016a; Risnes et al., 2011; Gluckman et al., 2008; Ong and Dunger, 2002 as reported in Yang 2022, 10176603; Osmond and Barker, 2000). Poor academic performance, cognitive difficulties (Hack et al., 2002; Larroque et al., 2001), and depression (Loret de Mola et al., 2014) in adulthood have also been linked to LBW. These associations between LBW and infant mortality, childhood disease, and adult disease establish LBW as an adverse effect. Considering the established consequences of LBW, as well as the consistency of the database and large number of *medium* and *high* confidence studies reporting mean birth weight and other binary birth weight-related measures, the endpoint of decreased birth weight in infants was selected for POD derivation.

Given the abundance of *high* confidence epidemiological studies that evaluated decreases in birth weight, *low* and *medium* confidence studies were excluded from POD derivation. Thus, 15 *high* confidence studies reporting inverse associations between exposure to PFOA and mean birth weight (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Bell et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Govarts et al., 2016; Whitworth et al., 2012) were considered for POD derivation. Three studies (Gardener et al., 2021; Ashley-Martin et al., 2017; Whitworth et al., 2012) were excluded because they reported sex-stratified results rather than results in both sexes or results for the overall population in terms of standardized measurements (e.g., z-score) only. Analyses utilizing standardized measurements as the dependent variable are internally valid, but this type of analysis estimates a change in birthweight relative to the study population, which would not be generalizable to other populations. Two studies (Luo et al., 2021; Bell et al., 2018) were not considered because they used non-preferred exposure measures such as infant whole blood samples from a heel stick and postpartum maternal exposure samples, which are prone to exposure misclassification. Four studies (Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017) were not considered for POD derivation because of inconsistencies in associations by sex or study location with no clear biological explanation for the inconsistency.

As a result of these exclusions, the six remaining *high* confidence epidemiologic studies (Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Govarts et al., 2016) were considered for POD derivation (Table 4-1). The candidate epidemiological studies described a variety of PFOA exposure measures across both fetal and neonatal developmental windows. All six studies reported their exposure metric in units of ng/mL and reported the β coefficients per ng/mL or ln(ng/mL), along with 95% confidence intervals, estimated from linear regression models. Yao et al. (2021) was not further considered because the PFOA exposure concentrations in this cohort were considerably higher than typical human environmental exposure levels and the exposure median in this study was at least 10 times higher than the other studies considered. Two of the six studies examined PFOA levels primarily during trimester one (Sagiv et al., 2018 Wikström, 2020, 6311677) and one during trimesters two and three (Starling et al., 2017). Two studies examined PFOA collected within days of birth (Chu et al., 2020; Govarts et al., 2016). Wikström et al. (2020) reported associations between PFOA levels and decreased birth weight in the large Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study cohort with samples collected between 2007 and 2010. Sagiv et al. (2018) reported on first trimester PFOA samples

collected between 1999–2002 in a Project Viva birth cohort in the U.S. Chu et al. (2020) reported inverse associations between maternal PFOA collected within three days of delivery and birth weight in the Chinese Guangzhou Birth Cohort Study (2013). Starling et al. (2017) reported associations between PFOA collected in later pregnancy (range: 20 to 34 weeks gestational age) and decreased birth weight in the Healthy Start prospective cohort in Colorado (2009–2014). Govarts et al. (2016) reported a modest inverse association between PFOA in cord blood and birth weight in the Flemish Human Environment Health Survey II (FLEHS II) cohort (2008–2009).

Developmental toxicity results reported in animal toxicological studies are concordant with the observed developmental effects in epidemiological studies. Specifically, studies in rodents found that gestational PFOA exposure resulted in reduced offspring weight (8/11 studies; 2 *high* and 6 *medium* confidence), decreased offspring survival (6/9 studies; 1 *high* and 5 *medium* confidence), developmental delays (2/2 studies; both *medium* confidence), physical abnormalities (2/2 studies; both *medium* confidence) and altered placental parameters (2/2 studies; both *medium* confidence). Some of the developmental effects seen in the offspring of rodents treated with PFOA (e.g., reduced offspring weight) are consistent with the decreases in birth weight and adverse effects associated with LBW observed in human populations.

Given the large number of adverse effects identified in the animal toxicological database for the developmental health outcome, EPA prioritized only the most sensitive effects (i.e., those observed at lower dose levels and/or higher magnitude) in offspring that were supported by multiple studies for derivation of PODs. EPA focused on the animal toxicological studies with effects in offspring, as opposed to placental or maternal effects, because these effects provide concordance with the approximate timing of decreased birth weight observed in human infants. Though several studies measured pregnant dam weight or dam weight at birth, there were inconsistencies in results across the database, with some studies reporting decreased maternal weight, some reporting no effect, and some reporting increased maternal weight as a result of PFOA exposure. These inconsistencies may stem from the potential confounding effect of reduced offspring weight observed in those same studies. EPA also focused on endpoints for which results from multiple animal toxicological studies corroborated the observed effect, thereby increasing the confidence in that effect. EPA additionally focused on studies with exposure durations lasting through the majority of gestation and/or lactation (i.e., from GD 1 through early postnatal development) rather than those that targeted a specific period of gestation or postnatal development as they were more likely to detect developmental effects. Multiple animal toxicological studies observed effects at low dose levels and demonstrated a dose-related response in decreased offspring weight, decreased pup survival, and developmental delays. Therefore, these endpoints were prioritized for dose-response analysis.

Numerous studies in both rats and mice reported decreased offspring body weight after gestational PFOA exposure. Reduced fetal body weight was consistently observed, with 5/5 studies in mice reporting this effect (Blake et al., 2020; Li et al., 2018a; Suh et al., 2011; Yahia et al., 2010; Lau et al., 2006). Reduced pup body weight was also consistently observed; the majority of the available studies (10/13) reported this effect, two of which were *high* confidence studies in rats (NTP, 2020; Butenhoff et al., 2004a), indicating consistency across species. EPA selected both reduced pup and fetal weights because the timing is concordant with the endpoint of decreased infant birth weight prioritized for POD derivation from the human epidemiological

studies and also represents two different developmental stages (i.e., fetus and pup) across the sensitive perinatal period of development.

Of the five studies reporting decreased fetal body weight in mice, results from Li et al. (2018a) were selected for POD derivation because the exposure duration encompassed the majority of gestation, the study used a relatively large number of dose groups, and the effect was observed in multiple dose groups. The two *high* confidence rat studies reporting reduced pup weight were not selected for POD derivation due to study design limitations, including the use of relatively high dose levels, and non-monotonic responses, although they provide qualitative support for this effect in mice. Of the eight studies reporting reduced pup body weight in mice (Song et al., 2018; Hu et al., 2012; White et al., 2011; Hu et al., 2010; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006), decreased pup weight relative to litter at PND 22 as reported by Lau et al. (2006) was ultimately selected for POD derivation because this study reported results as pup weight averaged by litter rather than individual pups, used an exposure duration that spanned the majority of gestation, used a larger number of dose groups than the other studies, and reported the effect in multiple dose groups.

In addition to effects on offspring weight, six studies in mice (Song et al., 2018; White et al., 2011; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) reported alterations in pup survival after gestational exposure to PFOA. Pup survival was selected over fetal survival because the metrics used to determine fetal mortality varied (e.g., reported as prenatal loss, litter loss, resorption, reduced fetal survival) and difficult to directly compare, Additionally, pup survival provides concordance with the timing of the effect of decreased infant birth weight in humans. Among the six available studies examining pup survival, Abbott et al. (2007) was determined to be *low* confidence for this endpoint and was therefore excluded for quantitative assessment. EPA selected results from Song et al. (2018) (PND 21) and Lau et al. (2006) (PND 0 and 23) for POD derivation because this study presented data for a larger number of treatment groups spanning broader or lower dose ranges as compared with Wolf et al. (2007), White et al. (2011), and Yahia et al. (2010).

Three studies in mice (Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) reported developmental delays, specifically delayed eye opening, as a result of gestational PFOA exposure. Abbott et al. (2007) was not further considered for POD derivation due to the extensive litter loss in dose groups greater than 1 mg/kg/day and the effect was only observed in that dose group, limiting the available dose-response range as compared to Lau et al. (2006) and Wolf et al. (2007). EPA selected results from Lau et al. (2006) over Wolf et al. (2007) for POD derivation because Lau et al. (2006) presented data for a larger number of dose groups spanning a greater dose range. Additionally, Lau et al. (2006) reported the effect in multiple dose groups.

Table 4-1 summarizes the studies and endpoints considered for POD derivation.

Table 4-1. Summary of Observed Endpoints in Humans and Rodent Studies Considered for Dose-Response Modeling and Derivation of Points of Departure

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
Immune Effects				
Reduced Antibody Concentrations for Diphtheria and Tetanus	Budtz-Jørgensen and Grandjean (2018)[a] *Medium* Timmerman et al. (2021) *Medium*	Human (male and female children)	Yes	Decreases in antibody responses to pathogens such as diphtheria and tetanus were observed at multiple ages during childhood, associated with both prenatal and childhood PFOA exposure levels. Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects. Effects were observed in multiple populations and are supported by studies of other vaccine types (e.g., rubella (Granum et al., 2013)).
Reduced immunoglobulin M (IgM) Response	Loveless et al. (2008) *Medium* DeWitt et al. (2008) *Medium*	C57BL/6N mice (adult females), Crl:CD-1(ICR)BR mice (adult males)	Yes	Functional assessment indicative of immunosuppression. Immune effects were consistently observed across multiple studies including reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. Reduced IgM response is coherent with epidemiological evidence of reduced immune response to vaccinations.
Developmental Effects				
Decreased Birth Weight	Chu et al. (2020) *High* Govarts et al. (2016) *High* Sagiv et al. (2018) *High* Starling et al. (2017) *High* Wikström et al. (2020) *High*	Human (male and female infants)	Yes	Evidence for developmental effects is based on consistent inverse effects for FGR including birth weight measures, which are the most accurate endpoint examined. Some deficits were consistently reported for birth weight and standardized birth weight in many *high* and *medium* confidence cohort studies. Effect was generally large in magnitude and coherent with epidemiological evidence for other biologically related effects.
Decreased Birth Weight	Yao et al. (2021) *High*	Human (male and female infants)	No	Effect was supportive of epidemiological evidence for this effect, but the exposure median in this study was at least 10 times higher than the other studies considered (see Appendix D, (U.S. EPA, 2024a)).
Decreased Pup Survival	Song et al. (2018) *Medium* Lau et al. (2006) *Medium*	Kunming mice (F_1 males and females, PND 21)	Yes	Effects on pre- and postnatal offspring survival were consistently observed across multiple studies and species. Decreased pup survival was reported in six studies and three strains of mice (Song et al., 2018; White et al., 2011; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) and is

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
		CD-1 mice (F_1 males and females, PND 0 and PND 23)		coherent timing of the critical effect selected in humans (i.e., decreased birth weight in infants). This critical effect is supported by observations of prenatal loss, litter loss/resorption, reduced fetal survival, and increased postweaning mortality observed in mice and rats. Song et al. (2018) and Lau et al. (2006) were selected for POD derivation because they reported data for a larger number of dose groups and tested lower or broader dose ranges than the other four studies reporting this effect.
Decreased Fetal Body Weight	Li et al. (2018a) *Medium*	Kunming mice (F_1 males and females, GD 18)	Yes	Effects on pre- and postnatal offspring weight were consistently observed across multiple studies and species. Decreased fetal weight was observed in 5/5 studies in mice and is supported by reduced pup weight observed in studies of mice and rats. Li et al. (2018a) was selected for POD derivation because the study tested a relatively large number of dose groups and had decreased variability compared with the other four studies. Note that decreases in maternal body weight were also considered for POD derivation but was not a selected endpoint because the decreased fetal body weight could be a potential confounder and was found to be a more sensitive effect.
Decreased Pup Body Weight (relative to litter)	Lau et al. (2006) *Medium*	CD-1 mice (F_1 males and females, PND 22)	Yes	Effects on pre- and postnatal offspring weight were consistently observed across multiple studies and species. Decreased pup weight was observed in nine studies across two species and is supported by reduced fetal weight reported by five studies in mice. Reduced pup weight at PND 22 reported by Lau et al. (2006) was selected for POD derivation because the study reported pup weight relative to litter, tested a relatively large number of dose groups compared with the other six studies in mice, and reported the effect in multiple dose groups.
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 mice (F_1 males and females, PND 14 – PND 18)	Yes	Effect also observed in Wolf et al. (2007) and Abbott et al. (2007). Lau et al. (2006) was selected for dose-response because this study reported dose response information across a larger number of dose groups (5) and a relatively low dose range (1, 3, 5, 10 and 20 mg/kg/day).
		Serum Lipid Effects		
Increased Total Cholesterol	Dong et al. (2019) *Medium* Lin et al. (2019) *Medium* Steenland et al. (2009)[b] *Medium*	Human (male and female adults)	Yes	Effect was consistent and observed across multiple adult populations including general population adults in NHANES (Dong et al., 2019); from prediabetic adults from the DPP and DPPOS cohort (Lin et al., 2019) and the C8 Health project high-exposure community (Steenland et al., 2009), as well as when study designs excluded individuals prescribed cholesterol medication, minimizing concerns of bias due to medical intervention (Dong et al., 2019; Steenland et al., 2009). Endpoint is supported by associations between PFOA and blood pressure.

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
Hepatic Effects				
Increased ALT	Gallo et al. (2012) *Medium* Darrow et al. (2016)[b] *Medium* Nian et al. (2019) *Medium*	Human (male and female adults)	Yes	Effect was consistent and observed across multiple populations including general population adults (Lin et al., 2010) (NHANES) and high-exposure communities including the C8 Health Project (Darrow et al., 2016; Gallo et al., 2012) and Isomers of C8 Health Project in China (Nian et al., 2019).
Increased ALT	Lin et al. (2010) *Medium*	Human (male and female adults)	No	While this is a large nationally representative population, several methodological limitations preclude its use for POD derivation. Limitations include lack of clarity about base of logarithmic transformation applied to PFOA concentrations in regression models, and the choice to model ALT as an untransformed variable, a departure from the typically lognormality assumed in most of the ALT literature.
Necrosis (focal, individual cell, both) in the Liver	Loveless et al. (2008) *Medium* NTP (2020) *High*	Crl:CD-1(ICR)BR mice (adult males), Sprague-Dawley rats (adult males)	Yes	Effect was accompanied in both studies by other liver lesions including cytoplasmic alteration and apoptosis. Necrotic liver cells were also observed in male mice in Crebelli et al. (2019) and pregnant dams in Blake et al. (2020). Effect is further supported by changes in serum ALT levels in animals and humans. Data from females were not considered for POD derivation as they appear to be less sensitive, potentially due to toxicokinetic differences between the sexes in rats.

Notes: ALT = alanine transaminase; BMD = benchmark dose; F1 = first generation; NHANES = National Health and Nutrition Examination Survey; POD = point of departure.
[a] Supported by Grandjean et al. (2012), Grandjean et al. (2017a), and Grandjean et al. (2017b).
[b] See Section 5.6.3 for discussion on the approach to estimating BMDs from regression coefficients.

4.1.2 Estimation or Selection of Points of Departure (PODs) for RfD Derivation

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR intended to represent a minimal, biologically significant level of change. The *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) describes a hierarchy by which BMRs are selected, with the first and preferred approach being the use of a biological or toxicological basis to define what minimal level of response or change is biologically significant. If biological or toxicological information is lacking, the guidance document recommends BMRs that could be used in the absence of information about a minimal clinical or biological level of change considered to be adverse—specifically, a BMR of 1 standard deviation (SD) change from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data. When severe or frank effects are modeled, a lower BMR can be adopted. For example, developmental effects are serious effects that can result in irreversible structural or functional changes to the organism, and the *Benchmark Dose Technical Guidance* suggests that studies of developmental effects can support lower BMRs. BMDs for these effects may employ a BMR of 0.5 SD change from the control mean for continuous data or a BMR of 5% for dichotomous data (U.S. EPA, 2012a). A lower BMR can also be used if it can be justified on a biological and/or statistical basis. The *Benchmark Dose Technical Guidance* (page 23; (U.S. EPA, 2012a)) shows that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of 1 SD results in a ~10% extra risk of being at risk of having an adverse effect. A BMR smaller than 0.5 SD change from the control mean is generally used for severe effects (e.g., 1% extra risk of cancer mortality).

Based on rationales described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the IRIS Handbook (U.S. EPA, 2022d) and past IRIS assessment precedent, BMRs were selected for dose-response modeling of PFOA-induced health effects for individual study endpoints as described below and summarized in Table 4-2 along with the rationales for their selection. For this assessment, EPA took statistical and biological considerations into account to select the BMR. For dichotomous responses, the general approach was to use 10% extra risk as the BMR for borderline or minimally adverse effects and either 5% or 1% extra risk for adverse effects, with 1% reserved for the most severe effects (e.g., mortality, infertility). For continuous responses, the preferred approach for defining the BMR was to use a preestablished cutoff for the minimal level of change in the endpoint at which the effect is generally considered to become biologically significant (e.g., greater than or equal to 42 IU/L serum ALT in human males (Valenti et al., 2021)). In the absence of an established cutoff, a BMR of 1 SD change from the control mean, or 0.5 SD for effects considered to be severe, was generally selected. Specific considerations for BMR selection for endpoints under each of the priority noncancer health outcomes are described in the subsections below and alongside the modeling methods and results provided in Appendix E (U.S. EPA, 2024a). Considerations for BMR selection for cancer endpoints are described in Section 4.2 and Appendix E (U.S. EPA, 2024a).

4.1.2.1 Hepatic Effects

For the hepatic endpoint of increased serum ALT in adults associated with PFOA exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically

significant adverse serum ALT level (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for the endpoint of elevated ALT. Specifically, EPA modeled elevated human ALT using cutoff levels of 42 IU/L for males and 30 IU/L for females (Valenti et al., 2021). These are the most updated clinical consensus cutoffs which post-date the American Association for the Study of Liver Diseases (AASLD) journal of Clinical Liver Disease recommended values of 30 IU/L for males, and 19 IU/L for females (Ducatman et al., 2023; Kasarala and Tillmann, 2016). Valenti et al. (2021, 1036989) determined the values using the same approach at the same center, but using an updated standardized method, a large cohort of apparently healthy blood donors (ages 18-65 years) and showed that the updated cutoffs were able to better predict liver disease.

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of U.S. EPA (2012a)) to estimate the probability of an individual with an adverse serum ALT level as a function of PFOA exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the endpoint of elevated serum ALT.

EPA selected a BMR of 5% extra risk because studies have demonstrated that ALT levels at or slightly above the selected cutoff levels can be associated with more severe liver diseases (Wedemeyer et al., 2010; Mathiesen et al., 1999), increased risk of liver-related mortality (Park et al., 2019a; Ruhl and Everhart, 2009; Kim et al., 2004), and mortality (Lee et al., 2008). Based on the severity of the health effects associated with increased ALT, EPA determined that a BMR of 5% extra risk is warranted (U.S. EPA, 2012a); a 10% extra risk would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing more severe liver diseases such as advanced fibrosis, chronic liver disease, and even liver-related death. Since there is currently a relatively high prevalence of elevated ALT in the general population (14% and 13% of U.S. male and female adults, respectively, aged 20 and older (Valenti et al., 2021)), a small increase in the prevalence of elevated ALT associated with PFOA exposure would likely increase the number of individuals with severe liver-related health effects. This also supports using a more health protective BMR of 5% extra risk (rather than 10%) for POD derivation. EPA presents PODs with a 10% extra risk BMR for comparison to the selected 5% BMR in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effects of single cell and focal liver necrosis observed in adult rats following PFOA exposure, there is currently inadequate available biological or toxicological information to permit determination of an effect-specific minimal biologically significant response level. Therefore, in accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a BMR of 10% extra risk was used because it is considered the standard reporting level for quantal (dichotomous) data and a minimally biologically significant response level (see Table 4-2).

4.1.2.2 Immune Effects

For the developmental immune endpoint of decreased diphtheria and tetanus antibody response in children associated with PFOA exposure, the BMD and the BMDL were estimated using a BMR of 0.5 SD change from the control mean (see Table 4-2). Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018) and Timmerman et al. (2021) measured antibody concentrations in childhood and PFOA exposure during gestation or childhood, these are considered developmental studies based on EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), which includes the following definition:

> "Developmental toxicology - The study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the lifespan of the organism."

EPA guidance recommends the use of a 1 or 0.5 SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect. A blood concentration for tetanus and diphtheria antibodies of 0.1 IU/mL has been cited in the literature as a "protective level" (Grandjean et al., 2017b; Galazka and Kardymowicz, 1989). However, in the *Immunological Basis for Immunization Series* of modules (WHO, 2018b), the WHO argued that assay-specific "protective levels" of tetanus antitoxin may not actually guarantee immunity. Galazka et al. (1993) similarly argued that several factors give rise to variability in the vulnerability of individuals to diphtheria and there is no consensus on what level offers full protection. As such, EPA determined that there is no clear definition of an adverse effect threshold for the endpoints of reduced tetanus or diphtheria antibody concentrations in children.

With these two factors in mind, a 0.5 SD was selected as the BMR because: 1) the health outcome is developmental, and 2) there is no accepted definition of an adverse level of change or clinical cutoff for reduced antibody concentrations in response to vaccination. Therefore, EPA performed the BMDL modeling using a BMR equivalent to a 0.5 SD change in $\log_2$-transformed antibody concentrations, as opposed to a fixed change in the antibody concentration distributions. EPA also presented BMDL modeling using a BMR equivalent to a 1 SD change, as recommended by agency guidance (U.S. EPA, 2012a).

For the effect of reduced IgM response observed in animal toxicological studies, there is currently inadequate available biological or toxicological information to permit determination of a minimal biologically significant response level. In accordance with recommendations in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) for continuous data in adult animal models with no known biologically significant response level, a BMR of 1 SD change from the control mean was employed (see Table 4-2).

4.1.2.3 Cardiovascular Effects

For the cardiovascular endpoint of increased serum TC in adults associated with PFOA exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant adverse serum TC concentration (Dong et al., 2019; Steenland et al., 2009) or a BMR of 0.5 SD (Lin et al., 2019), depending on the data provided by the study (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect and identified the definition of hypercholesterolemia from the American Heart Association (NCHS, 2019) as providing the most recent upper reference limit for clinically adverse serum TC. Specifically, when possible, EPA modeled human cholesterol using a cutoff level of 240 mg/dL for elevated serum total cholesterol (NCHS, 2019).

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of U.S. EPA (2012a)) to estimate the probability of an individual with an adverse TC level as a function of PFOA exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the effect of elevated serum TC. For Lin (2019), EPA relied on the mean serum TC concentrations reported across PFOA quartiles (i.e., continuous data) provided by the study, and therefore considered a BMR of a change in the mean equal to 0.5 SD or 1 SD from the control mean.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), IS, and cardiovascular mortality occurring in populations without prior CVD events (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Based on the severity of the cardiovascular-related health effects associated with increased cholesterol, EPA determined that selection of a BMR of 5% extra risk or 0.5 SD is warranted (U.S. EPA, 2012a); a 10% extra risk or 1SD would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing increased incidence of cardiovascular disease events. Since there is currently a relatively high prevalence of elevated TC in the general population (11.5% of U.S. adults aged 20 and older (NCHS, 2019)), a small increase in the prevalence of elevated TC associated with PFOA exposure would likely increase risk of severe health outcomes, such as cardiovascular-related events. Thus, this supports using a more conservative BMR of 5% extra risk or 0.5 SD for POD derivation. EPA presents PODs with a BMR of 10% extra risk (Dong et al., 2019; Steenland et al., 2009) or 1 SD (Lin et al., 2019) for comparison purposes in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

4.1.2.4 Developmental Effects

For the developmental endpoint of decreased birth weight associated with PFOA exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant birth weight deficit (see Table 4-2). As described in Appendix E (U.S. EPA, 2024a), LBW is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include but is not exclusive to babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999).

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Low birthweight is associated with increased risk for adverse health effects throughout life (Tian et al., 2019; Reyes and Mañalich, 2005; Hack et al., 1995) and therefore, supports a more stringent BMR below 10% (for dichotomous data) or 1 SD (for continuous data). Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of U.S. EPA (2012a)) to estimate the probability of an individual with a birth weight deficit as a function of PFOS exposure. This approach effectively dichotomized the data, resulting in a BMR defined as a 5% increase in the number of infants with birth weights below 2,500 g.

For delayed time to eye opening and decreased pup survival observed in animal studies, a BMR of 0.5 SD from the control was employed (see Table 4-2). For decreased fetal and pup weights observed in animal studies, a BMR of 5% relative deviation was employed. These BMR selections are consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) and the IRIS Handbook (U.S. EPA, 2022d), which note that studies of adverse developmental effects represent a susceptible lifestage and can support BMRs that are lower than 10% extra risk (dichotomous data) and 1 SD change from the control mean (continuous data). A 5% relative deviation in markers of growth in gestational exposure studies (i.e., fetal and pup weight) has generally been considered an appropriate biologically significant response level and has been used as the BMR in final IRIS assessments (e.g., U.S. EPA (2003), U.S. EPA (2004), and U.S. EPA (2012b)). Additionally, the 5% BMR selection is statistically supported by data which compared a BMR of 5% relative deviation for decreased fetal weight to NOAELs and other BMR measurements, including 0.5 SD, and found they were statistically similar (Kavlock et al., 1995). EPA presented modeling results using a BMR of 0.5 SD for decreased fetal or pup body weight, a BMR of 0.1 SD for the frank effects of decreased fetal or pup survival, and a BMR of 1 SD for delayed time to eye opening for comparison purposes, based on severity of the endpoints, as recommended by agency guidance (U.S. EPA, 2012a) (see Appendix E, (U.S. EPA, 2024a)).

Table 4-2. Benchmark Response Levels Selected for BMD Modeling of Health Outcomes

Endpoint	BMR	Rationale
Immune Effects		
Reduced antibody concentrations for diphtheria and tetanus in children	0.5 SD	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect and selects a 1 or 0.5 SD change in

Endpoint	BMR	Rationale
		cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a)
Reduced immunoglobulin M (IgM) response	1 SD	Insufficient information available to determine minimal biologically significant response level. The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 1 SD was used as per EPA guidance (U.S. EPA, 2012a)
Developmental Effects		
Decreased Birth Weight in Infants	5% extra risk of exceeding adversity cutoff (hybrid approach)	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a). The use of the hybrid approach results in dichotomization of the data and therefore a 5% BMR was selected (U.S. EPA, 2012a)
Decreased Fetal or Pup Weight	5%	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Decreased Pup Survival	0.5 SD	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Delayed Time to Eye Opening	0.5 SD	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Cardiovascular Effects		
Increased Cholesterol	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect.
	0.5 SD	Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects, a 0.5 SD was used as the BMR. A change from the mean of 0.5 SD limits further increases in the prevalence of this effect

Endpoint	BMR	Rationale
Hepatic Effects		
Increased ALT	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased ALT is not a frank effect but is associated with increased incidence of severe liver-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect
Single Cell and Focal Liver Necrosis	10%	Insufficient information available to determine minimal biologically significant response level. The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 10% was used as per EPA guidance (U.S. EPA, 2012a)

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMR = benchmark response; CDC = Centers for Disease Control; SD = standard deviation.

4.1.3 Pharmacokinetic Modeling Approaches to Convert Administered Dose to Internal Dose in Animals and Humans

4.1.3.1 Pharmacokinetic Model for Animal Internal Dosimetry

Following review of the available models in the literature (see Section 3.3.2), EPA chose the Wambaugh et al. (2013) model to describe PFOA dosimetry in experimental animals based on the following criteria:

- availability of model parameters across the species of interest,
- agreement with out-of-sample datasets (see Appendix F, (U.S. EPA, 2024a)), and
- flexibility to implement life-course modeling.

These criteria originated from the goal of accurately predicting internal dose metrics for toxicology studies that were selected for dose-response analysis. The species used in the toxicological studies (i.e., species of interest) were rats, mice, and nonhuman primates; model parameters for these species of interest were available. Good agreement with training and test (out-of-sample) datasets shows that the model performance is good compared with both the data used to identify model parameters and to external data. This was assessed using the mean square log error (MSLE) to compare model predicted concentration values to observed PFOA serum concentrations following single dose exposure to animals. Training set data demonstrated an MSLE of 0.40 for PFOA. For test set data, the MSLE was 1.4 for PFOA. As evidenced in the supplementary code, the discrepancy in model predictions for test set data is driven by higher animal PFOA doses that were outside the scope of the original model calibration. The general agreement between test and training datasets increases confidence that the model can be used to make accurate predictions of internal dose metrics for the dose magnitudes used in the available toxicology studies. The ability to implement life-course modeling was necessary to properly

predict internal dose metrics for developmental studies and endpoints as the animal transitioned through numerous lifestages.

In this case, an oral dosing version of the original model structure introduced by Andersen et al. (2006) and summarized in Section 3.3.2 was selected for having the fewest number of parameters that would need estimation. In addition, the Wambaugh et al. (2013) approach allowed for a single model structure to be used for all species in the toxicological studies allowing for model consistency for the predicted dose metrics associated with LOAELs and NOAELs from 13 animal toxicological studies of PFOA.

The Wambaugh et al. (2013) model was selected for pharmacokinetic modeling for animal internal dosimetry for several important reasons: 1) it allowed for sex-dependent concentration-time predictions for PFOA across all three species of interest, 2) it adequately predicted dosimetry of newer datasets published after model development, and 3) it was amendable to addition of a lifestage component for predicting developmental study designs. These analyses are further described in the subsections below. Uncertainties and limitations of the selected modeling approach are described in Section 5.6.1.

4.1.3.1.1 Animal Model Parameters

Pharmacokinetic parameters for different species and strains represented in the Wambaugh et al. (2013) model are presented in Table 4-3.

Table 4-3. PK Parameters From Wambaugh et al. (2013) Meta-Analysis of Literature Data for PFOA

Parameter	Units	CD1 Mouse (F)[a]	C57BL/6 Mouse (F)[a]	Sprague-Dawley Rat (F)[a]	Sprague-Dawley Rat (M)[a]	Cynomolgus Monkey (M/F)[a]
Body Weight[b] (BW)	kg	0.02	0.02	0.20 (0.16–0.23)	0.24 (0.21–0.28)	7 (M), 4.5 (F)
Cardiac Output[c] (Q_{cc})	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
Absorption Rate (k_a)	1/h	290 (0.6–73,000)	340 (0.53–69,000)	1.7 (1.1–3.1)	1.1 (0.83–1.3)	230 (0.27–73,000)
Central Compartment Volume (V_{cc})	L/kg	0.18 (0.16–2.0)	0.17 (0.13–2.3)	0.14 (0.11–0.17)	0.15 (0.13–0.16)	0.4 (0.29–0.55)
Intercompartment Transfer Rate (k_{12})	1/h	0.012 ($3.1 \times e^{-10}$ – 38,000)	0.35 (0.058–52)	0.098 (0.039–0.27)	0.028 (0.0096–0.08)	0.0011 ($2.4 \times e^{-10}$ – 35,000)
Intercompartment Ratio ($R_{V2:V21}$)	Unitless	1.07 (0.26–5.84)	53 (11–97)	9.2 (3.4–28)	8.4 (3.1–23)	0.98 (0.25–3.8)
Maximum Resorption Rate (T_{maxc})	µmol/h	4.91 (1.75–2.96)	2.7 (0.95–22)	1.1 (0.25–9.6)	190 (5.5–50,000)	3.9 (0.65–9,700)
Renal Resorption Affinity (K_T)	µmol	0.037 (0.0057–0.17)	0.12 (0.033–0.24)	1.1 (0.27–4.5)	0.092 ($3.4 \times e^{-4}$ – 1.6)	0.043 ($4.3 \times e^{-5}$ – 0.29)
Free Fraction	Unitless	0.011 (0.0026–0.051)	0.034 (0.014–0.17)	0.086 (0.031–0.23)	0.08 (0.03–0.22)	0.01 (0.0026–0.038)
Filtrate Flow Rate (Q_{filc})	Unitless	0.077 (0.015–0.58)	0.017 (0.01–0.081)	0.039 (0.014–0.13)	0.22 (0.011–58)	0.15 (0.02–24)
Filtrate Volume (V_{filc})	L/kg	0.00097 ($3.34 \times e^{-9}$ – 7.21)	$7.6 \times e^{-5}$ ($2.7 \times e^{-10}$ – 6.4)	$2.6 \times e^{-5}$ ($2.9 \times e^{-10}$ – 28)	0.0082 ($1.3 \times e^{-8}$ – 7.6)	0.0021 ($3.3 \times e^{-9}$ – 6.9)

Notes: F = female; M = male.
Means and 95% credible intervals (in parentheses) from Bayesian analysis are reported. For some parameters, the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).
[a] Datasets modeled for the CD1 mouse were from Lou et al. (2009), for the C57BL/6 mouse were from DeWitt et al. (2008), for the rat were from Kemper (2003), and for the monkey from Butenhoff et al. (2004b).
[b] Estimated average body weight for species used except with Kemper (2003) where individual rat weights were available and assumed to be constant.
[c] Cardiac outputs obtained from Davies and Morris (1993).

4.1.3.1.2 Out-of-Sample Comparisons

To evaluate the model's ability to predict PFOA concentration-time data in the species of interest, EPA compared model fits to in vivo datasets either not considered in or published after the 2016 PFOA HESD (Table 4-4). For rats, this included Kudo et al. (2002), Kim et al. (2016), and Dzierlenga et al. (2019a). Model simulations demonstrated good agreement with available data for adult time-course PFOA PK predictions in the rat. For mice however, only one adult PFOA study was available for comparison (Fujii et al., 2015) and that study only tracked PFOA concentrations through 24 hours. As mentioned in Section 3.3.2.1, a 24 hour observation window is insufficient to accurately estimate the terminal excretion half-life of PFOA. Therefore, only the original study used for parameter determination, Lou et al. (2009), was compared with model simulations. This comparison approach demonstrated agreement with the in vivo data.

Using the Wambaugh et al. (2013) model, EPA predicted the half-life, V_d, and clearance and compared these species-specific predictions to values obtained from in vivo studies when data were available.

Because male mouse parameters are not available for PFOA, only female parameters are used for all PFOA modeling in mice. This assumption is addressed in Wambaugh et al. (2013) and is based on a lack of evidence for sex-dependent PK differences in the mouse.

Table 4-4. Model Predicted and Literature PK Parameter Comparisons for PFOA

	Male					Female				
	t1/2,α (days)	**t1/2,β (days)**	**Vd,α (L/kg)**	**Vd,β (L/kg)**	**CL (L/d/kg)**	**t1/2,α (days)**	**t1/2,β (days)**	**Vd,α (L/kg)**	**Vd,β (L/kg)**	**CL (L/d/kg)**
					Rat					
Model	5.8	16.5	0.12	0.35	0.0147	0.16	2.84	0.16	2.81	0.686
Literature	1.64[a], 2.8[b]	10.25[b]	0.11[a,c],	0.15[b,c]	0.047[a], 0.013[b]	0.19[a], 0.028[b]	0.22[b]	0.17[a,c],	0.12[b,c]	0.613[a], 0.81[b]
					Mouse					
Model	–	–	–	–	–	17.8	18.9	0.18	0.19	0.007
Literature	–	–	–	–	–	–	–	–	–	–

Notes: CL = clearance; PK = pharmacokinetic; $t_{1/2,\alpha}$ = initial-phase elimination half-life; $t_{1/2,\beta}$ = terminal-phase elimination half-life; $V_{d,\alpha}$ = volume of distribution during the initial phase; $V_{d,\beta}$ = volume of distribution during the terminal phase.
[a] Information obtained from Kim et al. (2016).
[b] Information obtained from Dzierlenga et al. (2019a).
[c] Literature volumes of distribution represent central compartment volumes from a one-compartment or two-compartment model.

4.1.3.1.3 Life-Course Modeling

The Wambaugh et al. (2013) model was modified to account for gestation, lactation, and postweaning phases (Figure 4-1). Using the original model structure and published parameters, simulations assumed that dams were dosed prior to conception and up to the date of parturition. Following parturition, a lactational phase involved PFOA transfer from the breastmilk to the suckling pup where the pup was modeled with a simple one-compartment PK model. Finally, a postweaning phase utilized the body weight-scaled Wambaugh model to simulate dosing to the growing pup and accounted for filtrate rate as a constant fraction of cardiac output.

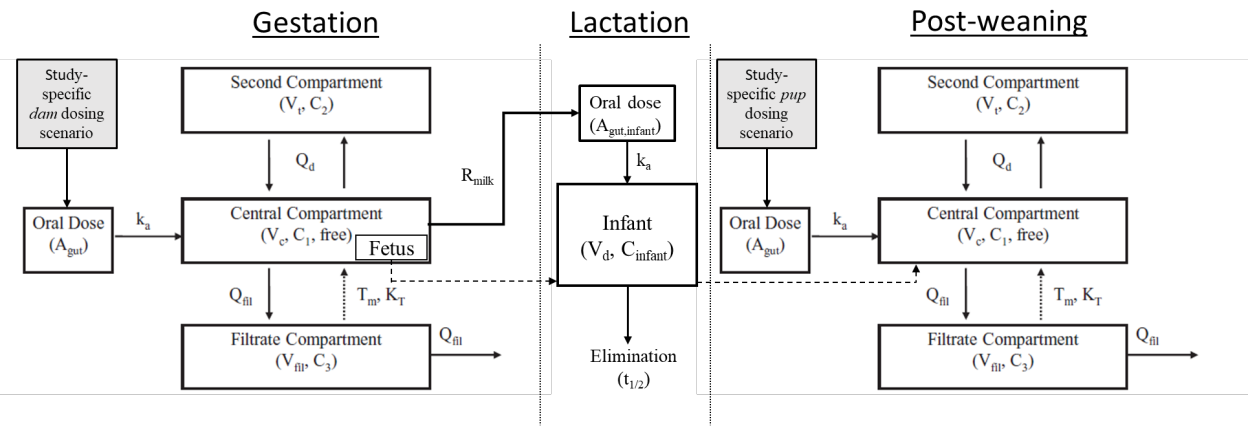


Figure 4-1. Model Structure for Lifestage Modeling

Model parameters for three-compartment model are the same as those described earlier. Pup-specific parameters include milk consumption in kg_{milk}/day (R_{milk}), infant-specific volume of distribution (V_d), and infant-specific half-life ($t_{1/2}$).

This methodology was adapted from Kapraun et al. (2022) and relies on the following assumptions for gestation/lactation modeling:

- During gestation and up through the instant birth occurs, the ratio of the fetal concentration (mg of substance per mL of tissue) to the maternal concentration is constant.
- Infant animal growth during the lactational period is governed by the infant growth curves outlined in Kapraun et al. (2022).
- Rapid equilibrium between maternal serum PFOA and milk PFOA is assumed and modeled using a serum:milk partition coefficient.
- All (100%) of the substance in the breast milk ingested by the offspring is absorbed by the offspring.
- The elimination rate of the substance in offspring is proportional to the amount of substance in the body and is characterized by an infant-specific half-life that is a fixed constant for any given animal species as described in Table 4-5 below.
- Following the lactation period, infant time-course concentrations are tracked using the more physiologically based Wambaugh model to model postweaning exposure and infant growth.

A simple one-compartment model for infant lactational exposure was chosen because of differences between PFOA Vd reported in the literature and Wambaugh et al. (2013) model-predicted Vd following extrapolation to a relatively low infant body weight. Because Vd is assumed to be extracellular water in human, Goeden et al. (2019) adjusts for lifestage-specific changes in extracellular water using an adjustment factor where infants have 2.1 times more extracellular water than adults resulting in a larger V_d. However, this large difference in extracellular water is not observed in rats (Johanson, 1979). Johanson (1979) demonstrated a 5% decrease in blood water content from early postnatal life (~0.5 weeks) to adulthood (> 7 weeks) in the rat. Therefore, EPA used the literature reported V_d (Dzierlenga et al., 2019a; Lou et al., 2009) for the one-compartment model to describe infant toxicokinetics. Finally, the Wambaugh

et al. (2013) model was not parameterized on a postpartum infant, and it was not possible to evaluate the mechanistic assumptions for renal elimination with postnatal toxicokinetic data. While there is one study that doses PFOA in young, postweaning, juvenile animals (Hinderliter et al., 2006b), concentrations at only two time points are reported for each age group making it not possible to estimate infant/juvenile pharmacokinetic parameters such as half-life. Therefore, the parameters listed in Table 4-5 in a one-compartment gestation/lactation model were used in conjunction with the parameters published in Wambaugh et al. (2013) to predict developmental dose metrics for PFOA.

Table 4-5. Additional PK Parameters for Gestation/Lactation for PFOA

Parameter	Units	Rat	Mouse
Maternal Milk:Blood Partition Coefficient (P_{milk})	Unitless	0.11[a,b]	0.32[e]
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.42[b]	0.25[f]
Elimination Half-Life ($t_{1/2}$)	Days	2.23[c]	18.5 g
Volume of Distribution (V_d)	L/kg	0.18[d]	0.2 g
Starting Milk Consumption Rate (r^0_{milk})	kg_{milk}/day	0.001[h]	0.0001[i]
Week 1 Milk Consumption Rate (r^1_{milk})	kg_{milk}/day	0.003[h]	0.0003[i]
Week 2 Milk Consumption Rate (r^2_{milk})	kg_{milk}/day	0.0054[h]	0.00054[i]
Week 3 Milk Consumption Rate (r^3_{milk})	kg_{milk}/day	0.0059[h]	0.00059[i]

Notes: PK = pharmacokinetic.
[a] Information obtained from Loccisano et al. (2013) (derived from Hinderliter et al. (2005)).
[b] Information obtained from Hinderliter et al. (2005).
[c] Average of male/female half-lives reported in Dzierlenga et al. (2019a), Kim et al. (2016), and Kemper et al. (2003).
[d] Information obtained from Kim et al. (2016) and Dzierlenga et al. (2019a).
[e] Information obtained from Fujii et al. (2020).
[f] Information obtained from Blake et al. (2020).
[g] Information obtained from Lou et al. (2009).
[h] Information obtained from Kapraun et al. (2022) (adapted from Lehmann et al. (2014)).
[i] Information obtained from Kapraun et al. (2022) (mouse value is 10% of rat based on assumption that milk ingestion rate is proportional to body mass).

These developmental-specific parameters include the maternal milk:blood PFOA partition coefficient (P_{milk}), the ratio of the concentrations in the fetus(es) and the mother during pregnancy (R_{fm}), the species-specific in vivo determined half-life ($t_{1/2}$) and V_d for PFOA, and the species-specific milk consumption rate during lactation (r^i_{milk}) for the i^{th} week of lactation. Milk rate consumptions are defined as:

- r^0_{milk}, the starting milk consumption rate in kg milk per day (kg/d);
- r^1_{milk}, the (average) milk consumption rate (kg/d) during the first week of lactation (and nursing);
- r^2_{milk}, the (average) milk consumption rate (kg/d) during the second week of lactation; and
- r^3_{milk}, the (average) milk consumption rate (kg/d) during the third week of lactation.

where R_{milk} used in the model is a piecewise linear function comprising each r^i_{milk} depending on the week of lactation.

Using this gestation/lactation model, EPA simulated two studies for PFOA exposure (one in mice and one in rats) to ensure the model predicted the time-course concentration curves for both

the dam and the pup. For all gestation/lactation studies, time zero represents conception followed by a gestational window (21 days for the rat, 17 days for the mouse). Dosing prior to day zero represents pre-mating exposure to PFOA.

Figure 4-2 demonstrates the model's ability to predict gestation and lactation study design in rat dams exposed to 30 mg/kg/day PFOA from GD 1-LD 22 that gave birth to pups who are exposed through gestation and lactation until weaning (Hinderliter et al., 2005). Comparatively, Figure 4-3 demonstrates model fits for PFOA exposure in mice from a cross-fostering study (White et al., 2009). In each case, the original Wambaugh et al. (2013) model with increasing maternal weight predicts dam concentrations in female rats and mice while the one-compartmental lactational transfer model predicts infant concentrations for pups exposed either *in utero* or during lactation only.

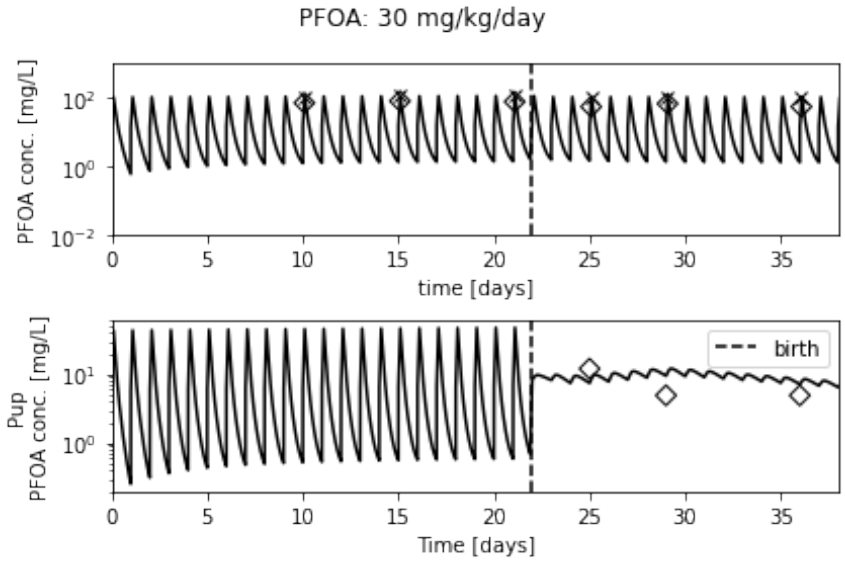


Figure 4-2. Gestation and Lactation Predictions of PFOA in the Rat

Top panel represents time-course model predicted dam concentrations (solid line) where open diamonds (◊) represent the in vivo dam concentrations reported in Hinderliter et al. (2005) and x's represent the model-predicted value at the reported time. Bottom panel demonstrates the model predicted pup concentrations (solid line) where open diamonds (◊) represent the reported pup concentrations in Hinderliter et al. (2005) with PFOA exposure is from the breast milk. Vertical dashed line represents birth.

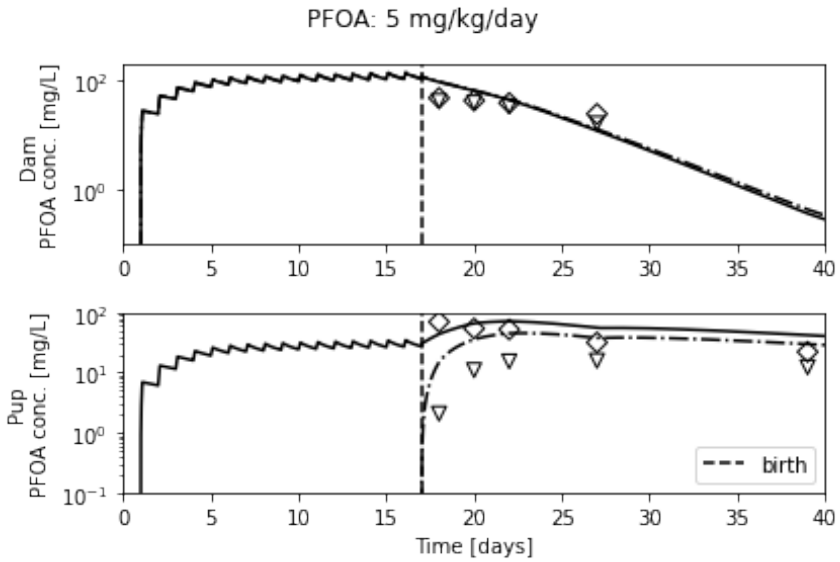


Figure 4-3. Gestation and Lactation Predictions of PFOA in the Mouse in a Cross-Fostering Study

Top panel represents predicted dam concentrations while bottom panel represents the predicted pup concentrations from White et al. (2009). Solid lines (–) represent a 5 mg/kg/day maternal dose paired with nursing pups that were exposed to PFOA in utero and open diamonds (◊) represent the reported dam and infant concentrations for this exposure scenario. Comparatively, dot-dashed lines (• –) represent the simulations from the cross-fostering study where dams were exposed to 5 mg/kg/day PFOA and pups born to the control dam were exposed through lactation. Open triangles (▽) represent the reported dam and infant concentrations for this cross-foster study.

The purpose of the animal PBPK model is to make predictions of internal dose in laboratory animal species used in toxicity studies and extrapolate these internal dose points of departure to humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose metrics across lifestages were selected for simulation in a mouse, rat, monkey, or human. Concentrations of PFOA in blood were considered for all the dose metrics. For studies in adult animals the dose-metric options were generally a maximum blood concentration (C_{max}, mg/L) and a time averaged blood concentration i.e., the area under the curve over the duration of the study (AUC, mg * day/L) or the blood concentration over the last 7 days (C_{last7}, mg/L). In developmental studies, dose metrics were developed for the dam, the fetus (during gestation), and the pup (during lactation) for both time C_{max} and averaged blood concentrations (C_{avg}). In the dam, the C_{max} and C_{avg}, were calculated over a range of lifestages: during gestation ($C_{avg\_dam\_gest}$), during lactation ($C_{avg\_dam\_lact}$), or combined gestation and lactation ($C_{avg\_dam\_gest\_lact}$). In pups for C_{max}, two different lifestages were calculated either during gestation or lactation ($C_{max\_pup\_gest}$, $C_{max\_pup\_lact}$). In pups for time averaged metrics, a C_{avg} was calculated during gestation, lactation, or combined gestation and lactation ($C_{avg\_pup\_gest}$, $C_{avg\_pup\_lact}$, and $C_{avg\_pup\_gest\_lact}$).

EPA selected the metric of C_{last7} for studies examining noncancer effects using nondevelopmental exposure paradigms. This metric provides a consistent internal dose for use across disparate chronic and subchronic study designs where steady state may or may not have been reached in the animal following continuous dosing. When the animal has reached steady state, C_{last7} is equal to the steady-state concentration and for non-steady-state study designs, this metric averages the concentration variability over a week's worth of dosing rather than using a

single, maximal concentration. This allows for extrapolation to the human model where a steady-state assumption is implemented for adult dose metric calculations.

For developmental endpoints, the metric of C_{max} is typically used when there is a known mechanism of action (MOA) related to a threshold effect during a specific window of susceptibility. From the *Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation* (U.S. EPA, 2014), the choice of this metric "depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue." Furthermore, the guidance clarifies that "for chronic effects, in the absence of MOA information to the contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to the active toxicant is the most appropriate dose metric (e.g., AUC)" (U.S. EPA, 2014). Repeat dosing coupled with a lack of a defined MOA for the apical endpoints used for dose-response modeling resulted in EPA excluding C_{max} as an internal dose metric for animal toxicological endpoints. However, EPA provides modeling results using C_{max} for comparison purposes in Appendix E (U.S. EPA, 2024a).

EPA selected the metric of C_{avg} for studies with reproductive or developmental exposure designs encompassing gestation and/or lactation. One factor considered for this selection pertains to the long half-life of PFOA and the degree of accumulation throughout pregnancy and lactation. Because PFOA is not cleared within 24 hours, daily dosing throughout pregnancy/lactation will result in a C_{max} that falls on the final day of pregnancy or lactation or a C_{last7} only representative of the final days of gestation or lactation, even if dosing ceases after birth, due to ongoing lactational exposure. The endpoints in this assessment (decreased fetal or pup weight, decreased pup survival, delayed time to eye opening) do not have established MOAs or known windows of susceptibility and instead are expected to result from sustained internal dose from repeated exposures. If, as anticipated, this window of susceptibly for a given endpoint is not on the final day or the last week of exposure, the C_{max} or C_{last7} will not capture the exposure at the time associated with the adverse effect. A C_{avg} metric is more representative of the exposure throughout the potential window of susceptibility. This selection is also supported by the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), which state that when pharmacokinetic data are available, as is the case for PFOA, "adjustments may be made to provide an estimate of equal average concentration at the site of action for the human exposure scenario of concern." The selection of C_{avg} for developmental animal studies is therefore consistent with the guidance for humans.

Finally, for NTP (2020), an additional dose metric was derived which averages out the concentration in the pup from conception to the end of the 2 years ($C_{avg\_pup\_total}$). Specifically, it adds the area under the curve in gestation/lactation to the area under the curve from diet (postweaning) and then divides by 2 years.

4.1.3.2 Pharmacokinetic Model for Human Dosimetry

The key factors considered in model determination were to implement a human model from the literature that was able to model gestational and lactational exposure to infants, that was able to describe time-course changes in serum concentration due to changes in body weight during growth, and that required minimal new development. Previous modeling efforts suggest that limiting model complexity helps to prevent errors and facilitates rapid implementation (Bernstein

et al., 2021). For the human epidemiological and animal toxicological endpoints of interests, serum concentration was identified as a suitable internal dosimetry target, which provides support for using a simpler model that did not have individual tissue dosimetry. For these reasons, EPA selected the one-compartment human developmental model published by Verner et al. (2016). Several alternative models to EPA's updated version of the Verner et al. (2016) model for the calculation of POD_{HED} from an internal POD were considered. This included consideration of full PBPK models (i.e., the Loccisano family of models (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011)), as well as other one-compartment PK models (e.g., Goeden et al. (2019)). Discussion on the justification for selection of the Verner et al. (2016) model as the basis for the pharmacokinetic modeling approach used for PFOA is available in Sections 5.6.2 and 5.7.

Several adjustments were undertaken to facilitate the application of the model for this use. First, the model was converted from acslX language to an R/MCSim framework. This allows the code to be more accessible to others by updating it to a contemporary modeling language, as acslX software is no longer available or supported. The starting point for the conversion to R/MCSim was another model with a similar structure that was in development by EPA at that time (Kapraun et al., 2022). Second, the modeling language conversion body weight curves for nonpregnant adults were revised based on CDC growth data for juveniles and values from EPA's *Exposure Factors Handbook* in adults (U.S. EPA, 2011b; Kuczmarski et al., 2002). Linear interpolation was used to connect individual timepoints from these two sources to produce a continuous function over time. Body weight during pregnancy was defined based on selected studies of maternal body weight changes during pregnancy (U.S. EPA, 2011b; Portier et al., 2007; Thorsdottir and Birgisdottir, 1998; Carmichael et al., 1997; Dewey et al., 1993). Age-dependent breastmilk intake rates were based on the 95th percentile estimates from EPA's *Exposure Factors Handbook* and was defined relative to the infant's body weight (U.S. EPA, 2011b).

A third modification was the update of parameters: the half-life, the volume of distribution (V_d), the ratio of PFOA concentration in cord blood to maternal serum, and the ratio of PFOA concentration in breastmilk and maternal serum. Details for how these parameters were updated are given in the following paragraphs. In the model, half-life and V_d are used to calculate the clearance, which is used in the model directly and is also used for calculation of steady-state concentrations in adults. Other than half-life and, because of that, clearance, the updated parameters were similar to the original parameters (Table 4-6). The results of the new R model and updated acslX model with the original parameters were essentially identical (see Appendix, (U.S. EPA, 2024a)). With the updated parameters, the predicted PFOA serum concentrations are approximately 70% of the original values during pregnancy, and the child's serum concentration is approximately 60% of the original values during the first year of life.

The use of the Verner model in humans presents a substantial advancement in approach for endpoints in children compared with the previous EPA assessment of PFOA (U.S. EPA, 2016c). The 2016 PFOA HESD did not explicitly model children, but instead applied an uncertainty factor to an RfD based on long-term adult exposure to account for the potential for increased susceptibility in children. The current approach explicitly models PFOA exposure to infants during nursing who are undergoing rapid development, including growth, through childhood and who do not reach steady state until near adulthood. This allows for a more accurate estimation of

exposures associated with either serum levels in children or dose metric from developmental animal toxicological studies. The Verner model also explicitly models the mother from her birth through the end of breastfeeding which allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy. Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Application of the updated Verner model to three cohorts with paired maternal measurements and subsequent samples in children between ages of 6 months and 6 years showed good agreement between reported and predicted serum levels in the children (see Appendix, (U.S. EPA, 2024a)). This suggests that the assumptions made governing lactational transfer and the selected half-life value are reasonable. A local sensitivity analysis was also performed to better understand the influence of each parameter on model output (see Appendix, (U.S. EPA, 2024a)).

Table 4-6. Updated and Original Chemical-Specific Parameters for PFOA in Humans

Parameter	Updated Value	Original Value[a]
Volume of Distribution (mL/kg)	170[b]	170
Half-life (yr)	2.7[c]	3.8
Clearance (mL/kg/d)	0.120[d]	0.085
Cord Serum:Maternal Serum Ratio	0.83[e]	0.79
Milk:Serum Partition Coefficient	0.049[f]	0.058

Notes:
[a] Verner et al. (2016).
[b] Thompson et al. (2010a).
[c] Li et al. (2017d).
[d] Calculated from half-life and volume of distribution. $Cl = Vd * \ln(2)/t_{1/2}$.
[e] Average values for total PFOA Cord Serum:Maternal Serum ratios (see Appendix, (U.S. EPA, 2024a)). This is a similar approach to that used by Verner et al. (2016), but also includes studies made available after the publication of that model.
[f] Average value of studies as reported in Table 4-7. This is a similar approach to that used by Verner et al. (2016), but also includes studies made available after the publication of that model.

EPA selected a reported half-life value from an exposure to a study population that is demographically representative of the general population, with a clear decrease in exposure at a known time, with a high number of participants and a long follow-up time. Based on these criteria, a half-life of 2.7 years was determined for PFOA as reported in Li et al. (2018c; 2017d). This value comes from a large population (n = 455) who originally had contaminated drinking water for which the study documents the decrease in exposure levels after the installation of filtration with an average final serum sample taken 3.9 years after the beginning of water filtration. Li et al. (2018c) also reported a similar half-life of 2.7 years for PFOA in a separate community with a similar study design. In that study, serial blood samples were collected from participants after the beginning of drinking water filtration after a long period of exposure to drinking water contaminated with PFOA. The second study involved 106 participants with a median number of six samples per person but with only a 2-year observation period Li et al. (2017d). The good agreement between the second study and the previous, larger study in diverse populations support the use of this value as a good estimate of the PFOA elimination half-life.

A summary of PFOA half-life values is presented in the Appendix (U.S. EPA, 2024a). Uncertainties related to EPA's selected half-life are discussed in Section 5.6.2.

The updated value for human V_d of PFOA, 170 mL/kg, was sourced from Thompson et al. (2010a) who used a one-compartment PK model. This calculation involves several assumptions: that the participants' serum concentrations are at steady-state, their exposure can be estimated from the drinking water concentration in their community, there is 91% bioavailability for exposure from drinking water, and the half-life of PFAS is 2.3 years, which comes from the report of Bartell et al. (2010). EPA considered updating this parameter to 200 mL/kg, which is the value that would be calculated using the EPA chosen half-life value of 2.7 years. However, the value of 2.3 years was calculated under very similar conditions as the other data in the Thompson et al. (2010a) population and 2.3 years may better reflect the clearance rate in that specific population at that time. This calculation was performed in a population with PFOA contamination. V_d is a parameter that is relatively easily obtained from an analysis of PK data from controlled experimental studies, as it is related to the peak concentration observed after dosing and is expected to be similar between human and nonhuman primates (Mordenti et al., 1991). For comparison, the optimized V_d for PFOA from oral dosing in monkeys was 140 mL/kg (Andersen et al., 2006).

Another group has approached the calculation of V_d by taking the average of reported animal and human values and reported values of 200 mL/kg for PFOA (Gomis et al., 2017). This calculation included the V_d value from Thompson et al. (2010a) and did not include additional values derived from human data. The resulting average value shows that the value from Thompson et al. (2010a) is reasonable; EPA selected the Thompson et al. (2010a) result based on the fact that it is the only value derived from human data that EPA considers to be reliable for risk estimation in the general population.

A summary of PFOA V_d values is presented in the Appendix (U.S. EPA, 2024a). Uncertainties related to EPA's selected V_d are discussed in Section 5.6.2.

In the original model, the ratio of PFOA concentration in cord blood to maternal serum, and the ratio of PFOA concentration in breastmilk and maternal serum were based on an average of values available in the literature; here, EPA identified literature made available since the original model was published and updated those parameters with the averages of all identified values (Table 4-7). The values for cord blood to maternal serum ratio are presented in the Appendix (U.S. EPA, 2024a). One restriction implemented on the measurements of the cord blood to maternal serum ratio was to only include reports where the ratio was reported, and not to calculate the ratio from reported mean cord and maternal serum values.

Table 4-7. Summary of Studies Reporting the Ratio of PFOA Levels in Breastmilk and Maternal Serum or Plasma

Source	HERO ID	Milk:Maternal Plasma Ratio	Included in Verner et al. (2016) Analysis
Haug et al. (2011)	2577501	0.038	No
Seung-Kyu Kim et al. (2011b)	2919258	0.025	No
Liu et al. (2011)	2919240	0.11	No
Cariou et al. (2015)[a]	3859840	0.034	Yes
Sunmi Kim et al. (2011a)[b]	1424975	0.04	Yes
Verner et al. (2016)	3299692	0.058[c]	–

Source	HERO ID	Milk:Maternal Plasma Ratio	Included in Verner et al. (2016) Analysis
Additional Studies	–	0.049[d]	–

Notes: Whether studies were included in the analysis of Verner et al. (2016) is noted. The reported values were based on the mean of ratios in the study populations except when noted otherwise.
[a] Median result based on the report of Pizzurro et al. (2019).
[b] Median result as reported by the authors.
[c] Average value of milk:maternal plasma ratio used by Verner et al. (2016).
[d] Average value of milk:maternal plasma ratio with the inclusion of additional studies not in the original analysis. This value was used in the human PK model.

This updated model was used to simulate the HED from the animal PODs that were obtained from BMD modeling of the animal toxicological studies (see Appendix, (U.S. EPA, 2024a)). It was also used to simulate selected epidemiological studies (Section 4.1.1.2) to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling (see Appendix, (U.S. EPA, 2024a)). For PODs resulting from chronic exposure, such as a long-term animal toxicological study or an epidemiological study on an adult cohort, the steady-state approximation was used to calculate a POD_{HED} that would result in the same dose metric after chronic exposure. For PODs from exposure to animals in developmental scenarios, the updated Verner model was used to calculate a POD_{HED} that results in the same dose metric during the developmental window selected. The updated Verner model was also used to calculate a POD_{HED} for PODs based on epidemiological observations of maternal serum concentration during pregnancy, cord blood concentration, and serum concentrations in children.

The pharmacokinetic modeling code for both the updated Wambaugh et al. (2013) and Verner et al. (2016) models that was used to calculate human equivalence doses is available in an online repository (https://github.com/U.S. EPA/OW-PFOS-PFOA-MCLG-support-PK-models). The model code was thoroughly QA'd through the established EPA Quality Assurance Project Plan (QAPP) for PBPK models (U.S. EPA, 2018).

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOA Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Different approaches were taken to estimate POD_{HED}s depending on the species (i.e., human versus animal model) and lifestage (e.g., developmental, adult). The PODs from epidemiological studies (immune, developmental, hepatic, and serum lipid endpoints) were derived using hybrid or benchmark dose modeling (see Appendix E.1, (U.S. EPA, 2024a)) which provided an internal serum concentration in ng/L. The internal dose PODs were converted to a POD_{HED} using the modified Verner model described in Section 4.1.3.1.3 to calculate the dose that results in the same serum concentrations. Specifically, reverse dosimetry was performed by multiplying an internal dose POD by a model predicted ratio of a standard exposure and the internal dose for that standard exposure. This expedited procedure can be performed because the human model is linear, that is, the ratio of external and internal dose is constant with dose. Additional details are provided below and in Table 4-8.

The PODs from the animal toxicological studies were derived by first converting the administered dose to an internal dose as described in Section 4.1.3.1.1. The rationale for the

internal dosimetric selected for each endpoint is described in the Appendix E.2 (U.S. EPA, 2024a). Because a toxicological endpoint of interest results from the presence of chemical at the organ-specific site of action, dose-response modeling is preferentially performed on internal doses rather than administered doses and assumes the internal dose metric is proportional to the target tissue dose In addition, the nonlinear elimination described in Wambaugh et al. (2013) requires conversion to an internal dose as the relationship between internal and external dose will not scale linearly. The internal doses were then modeled using the Benchmark Dose Software (BMDS) (see Appendix E, (U.S. EPA, 2024a)). If BMD modeling did not produce a viable model, a NOAEL or LOAEL approach was used consistent with EPA guidance (U.S. EPA, 2012a). The internal dose animal PODs were converted to a POD_{HED} using the model described in Section 4.1.3.1.3. Reverse dosimetry for the animal PODs used the ratio of standard exposure and internal dose as was applied to PODs from epidemiological data. For animal toxicological studies using the average concentration over the final week of the study ($C_{last7,avg}$), the POD_{HED} is the human dose that would result in the same steady-state concentration in adults. When a concentration internal dose metric in the pup during lactation and/or gestation was selected, the POD_{HED} is the dose to the mother that results in the same average concentration in the fetus/infant over that period.

This approach for interspecies extrapolation follows EPA's guidance to prefer the use of a PK or PBPK model over the use of a data-derived extrapolation factor (DDEF) (U.S. EPA, 2014). A PK model allows for predictions of dosimetry for specific exposure scenarios in animals and humans and can incorporate PK details such as maternal accumulation and subsequent gestation/lactational transfer to a fetus/infant. Using a hierarchical decision-making framework, a DDEF approach is only considered when a validated PK or PBPK model is not available. Furthermore, EPA considers DDEF values based on the ratio of maximum blood concentration from acute, high-dose exposures to likely not be protective for typical exposure scenarios to humans, chronic low-dose exposure or lactational exposure to a nursing infant (Dourson et al., 2019). While a repeat dose DDEF has been presented (Dourson et al., 2019), this factor relied on maximum concentrations from Elcombe et al. (2013), for which the results are not considered relevant to the general population as discussed in Section 4.1.3.2.

Table 4-8 displays the POD and estimated internal and POD_{HED}s for immune, developmental, cardiovascular (serum lipids), and hepatic endpoints from animal and/or human studies selected for the derivation of candidate RfDs.

Table 4-8. POD_{HED}s Considered for the Derivation of Candidate RfD Values

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Immunological Effects						
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOA concentrations at age 5 and anti-tetanus antibody serum concentrations at age 7	$BMDL_{0.5SD}$	3.47 ng/mL	3.05×10^{-7}	BMR of 0.5 SD provided reasonably good estimate of 5% extra risk; single- and multi-PFAS models resulted in same BMDL; selected BMDL was based on significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOA concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	3.31 ng/mL	5.21×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in similar BMDLs; selected BMDL was based on significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOA concentrations and anti-tetanus antibody concentrations at ages 7–12	$BMDL_{0.5SD}$	2.26 ng/mL	3.34×10^{-7}	BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk; BMDL was based on nonsignificant regression parameter; no multi-PFAS modeling was conducted
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOA concentrations at age five and anti-diphtheria antibody serum concentrations at age 7	$BMDL_{0.5SD}$	3.32 ng/mL	2.92×10^{-7}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 30% change in the effect size when controlling for PFOS; selected BMDL was based on significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b]	Human, male and female; PFOA concentrations in the mother[c] and anti-	$BMDL_{0.5SD}$	1.24 ng/mL	1.95×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; single- and

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Medium	diphtheria antibody serum concentrations at age 5				multi-PFAS models resulted in similar BMDLs though there was a 30% change in the effect size when controlling for PFOS
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOA concentrations and anti-diphtheria antibody concentrations at ages 7–12	$BMDL_{0.5SD}$	1.49 ng/mL	2.20×10^{-7}	BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk; BMDL was based on nonsignificant regression parameter
Decreased IgM response to SRBC	Dewitt et al. (2008) *Medium*	C57BL/6N Mice, females, adults, Study 1	$BMDL_{1SD}$, Polynomial Degree 4	18.2 mg/L $C_{last7,avg}$	2.18×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Dewitt et al. (2008) *Medium*	C57BL/6N Mice, females, adults, Study 2	NOAEL[d] (1.88 mg/kg/day)	45.3 mg/L $C_{last7,avg}$	5.43×10^{-3}	Test for constant variance and test for nonconstant variance failed therefore a NOAEL approach was taken
	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, males, adults	$BMDL_{1SD}$, Exponential 3	57.6 mg/L $C_{last7,avg}$	6.91×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Developmental Effects						
Decreased Birth Weight	Chu et al. (2020) *High*	Human, male and female; PFOA serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	2.0 ng/mL	3.15×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on significant regression parameter
	Govarts et al. (2016) *High*	Human, male and female; PFOA concentrations in umbilical cord	$BMDL_{5RD}$, Hybrid	1.2 ng/mL	2.28×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on nonsignificant regression parameter

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Sagiv et al. (2018) *High*	Human, male and female; PFOA serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	9.1 ng/mL	1.21×10^{-6}	Selected BMDL was based on nonsignificant regression parameter
	Starling et al. (2017) *High*	Human, male and female; PFOA serum concentrations in second and third trimesters	$BMDL_{5RD}$, Hybrid	1.8 ng/mL	2.65×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on significant regression parameter
	Wikström et al. (2020) *High*	Human, male and female; PFOA serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	2.2 ng/mL	2.92×10^{-7}	Selected BMDL was based on significant regression parameter
Decreased Pup Survival	Song et al. (2018) *Medium*	Kunming Mice, F_1 males and females (PND 21)	$BMDL_{0.5SD}$, Polynomial Degree 3	12.3 mg/L $C_{avg\_pup\_gest\_lact}$	6.40×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 0)	NOAEL[d] (3 mg/kg/day)	19.1 mg/L $C_{avg\_pup\_gest}$	3.23×10^{-3}	No models had adequate fit. Test for constant variance failed, and test for nonconstant variance failed. NOAEL approach taken
	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 23)	NOAEL[d] (3 mg/kg/day)	26.6 mg/L $C_{avg\_pup\_gest\_lact}$	1.38×10^{-3}	Test for constant variance failed. For nonconstant variance models, goodness of fit for nonconstant models was poor. NOAEL approach taken
Decreased Fetal Body Weight	Li et al. (2018a) *Medium*	Kunming Mice, F_1 males and females (GD 18)	NOAEL[d] (1 mg/kg/day)	8.5 mg/L $C_{avg\_pup\_gest}$	1.44×10^{-3}	No models had adequate fit. Test for constant variance failed, and test for nonconstant variance failed. NOAEL approach taken
Decreased Pup Body Weight	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 23)	NOAEL[d] (1 mg/kg/day)	15.8 mg/L $C_{avg\_pup\_gest\_lact}$	8.2×10^{-4}	No models had adequate fit. Test for constant variance failed. For nonconstant

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
						variance models, goodness of fit for nonconstant models was poor. NOAEL approach taken
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 14 – PND 18)	$BMDL_{0.5SD}$, Polynomial Degree 2	8.0 mg/L $C_{avg\_pup\_gest\_lact}$	4.17×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Cardiovascular Effects (Serum Lipids)						
Increased Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	$BMDL_{5RD}$, Hybrid	2.29 ng/mL	2.75×10^{-7}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	$BMDL_{5RD}$, Hybrid	4.25 ng/mL	5.10×10^{-7}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Lin et al. (2019) *Medium*	Human, male and female, age 25 and older	$BMDL_{0.5SD}$, Linear	5.28 ng/mL	6.34×10^{-7}	Analyses include individuals prescribed cholesterol medication and significant regression parameter
Hepatic Effects						
Increased ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	17.9 ng/mL	2.15×10^{-6}	BMDL based on significant regression parameter
	Darrow et al. (2016) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	66.0 ng/mL	7.92×10^{-6}	BMDL based on modeled serum PFOA concentrations and significant regression parameter
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	$BMDL_{5RD}$, Hybrid	3.76 ng/mL	4.51×10^{-7}	BMDL based on significant regression parameter

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Increased Focal Necrosis	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, adult male	$BMDL_{10RD}$, Dichotomous Hill	10.0 mg/L $C_{last7,avg}$	1.20×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and presented most protective BMDL in consideration of the adversity of effect
Increased Individual Cell Necrosis	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, adult male	$BMDL_{10RD}$, Probit	36.0 mg/L $C_{last7,avg}$	4.32×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Increased Hepatocyte Single Cell Death	NTP (2020) *High*	Sprague-Dawley Rats, males; perinatal and postweaning	$BMDL_{10RD}$, Gamma	100 mg/L $C_{avg\_pup\_total}$	1.20×10^{-2}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Increased Necrosis	NTP (2020) *High*	Sprague-Dawley Rats, males; perinatal and postweaning	$BMDL_{10RD}$, Multistage Degree 1	26.9 mg/L $C_{avg\_pup\_total}$	3.23×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC

Notes: AIC = Akaike information criterion; ALT = alanine aminotransferase; AUC = area under the curve; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 0.5 SD from the control mean; $BMDL_{5RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response; $BMDL_{10RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change in response; $C_{avg\_pup\_gest}$ = average blood concentration normalized per day during gestation; $C_{avg\_pup\_total}$ = average blood concentration in pup; $C_{last7,avg}$ = average blood concentration over the last 7 days; F_1 = first generation; IgM = immunoglobulin M; NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program; POD_{HED} = point-of-departure human equivalence dose; RfD = reference dose; SRBC = sheep red blood cell.

[a] See Appendix (U.S. EPA, 2024a) for additional details on BMD modeling.

[b] Supported by Grandjean et al. (2012), Grandjean et al. (2017a), and Grandjean et al. (2017b).

[c] Maternal serum concentrations were taken either in the third trimester (32 weeks) or about two weeks after the expected term date.

[d] No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.

4.1.4.1 Hepatic Effects

Increased ALT in individuals aged 18 and older (Darrow et al., 2016; Gallo et al., 2012) or 22 and older (Nian et al., 2019)

The POD for increased ALT in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E.1, (U.S. EPA, 2024a)) on the measured (Nian et al., 2019; Gallo et al., 2012) or modeled (Darrow et al., 2016) PFOA serum concentrations collected from adults aged 18 years and older, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2).The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (see Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Focal Necrosis, Crl:CD-1(ICR)BR mice, male, $C_{last7,avg}$ (Loveless et al., 2008)

Increased incidence of focal necrosis of the liver was observed in male ICR mice. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Individual Cell Necrosis, Crl:CD-1(ICR)BR mice, male, $C_{last7,avg}$ (Loveless et al., 2008)

Increased incidence of individual cell necrosis of the liver was observed in male ICR mice. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Necrosis, Sprague-Dawley rats, males, perinatal and postweaning, $C_{avg\_pup\_total}$ (NTP, 2020)

Increased incidence of necrosis of the liver was observed in adult male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). For

endpoints derived from NTP (2020), an additional dose metric was developed which averages the concentration in the offspring from conception to the end of the 2-year postnatal exposure period ($C_{avg\_pup\_total}$; see Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Hepatocyte Single Cell Death, Sprague-Dawley rats, males, perinatal and postweaning, $C_{avg\_pup\_total}$ (NTP, 2020)

Increased incidence of single cell death of the liver was observed in adult male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). For endpoints derived from NTP (2020), an additional dose metric was developed which averages the concentration in the offspring from conception to the end of the 2-year postnatal exposure period ($C_{avg\_pup\_total}$; see Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

4.1.4.2 Immune Effects

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 7 (Budtz-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 7 was derived by quantifying a benchmark dose (see Appendix E.1, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations at age 5, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period, where the exposure to the child was only through lactation, which was much greater than the exposure to the mother. After 1 year, the exposure to the child, relative to body weight, was set to the same value as the mother. The model provided predictions for a child aged 5 years, when the serum concentrations used to determine the POD were collected, and reverse dosimetry was used to determine the POD_{HED} that results in the POD serum concentration. Because different growth curves specific to male and female children were used in the model, the model predicted slightly different (less than 5%) serum concentrations for each. The slightly lower HED in males was then selected as it was the most health protective.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 5 (Budtz-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 5 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from the mother either in the third trimester (32 weeks) or about two weeks after the expected term date, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was selected was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7, except that the model was only run until the maternal age of 25 years, when delivery occurs in the model. As the POD was based on maternal serum concentrations taken before and after birth, the time of delivery was chosen as an average of the two. Reverse dosimetry was performed on model predicted maternal serum concentration at that time to calculate the POD_{HED}. This metric was independent of the sex of the child in the model.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at ages 7–12 (Timmermann et al., 2021)

The POD for decreased antibody production in children aged 7–12 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations at ages 7–12, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7 (Budtz-Jørgensen and Grandjean, 2018), but the model was run until the median age of this cohort at blood collection, 9.9 years. Reverse dosimetry was used to calculate the POD_{HED} that resulted in a serum level equal to the POD at that age. Because different growth curves specific to male and female children were used in the model, the model predicted slightly different (less than 5%) serum concentrations for each sex. The lower HED was then selected as it was the most health protective.

Decreased IgM response to SRBC, C57BL/6N mice, Female, Studies 1 and 2, $C_{last7,avg}$ (Dewitt et al., 2008)

Decreased mean response of SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Studies 1 and 2). Using the Wambaugh et al. (2013) model, daily exposure to PFOA in the drinking water was simulated for 15 days using female C57BL/6 mice parameters (Section 4.1.3.1). Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 1 SD from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). For Study 1, the BMDS produced a BMDL in mg/L. For Study 2, the tests for constant and nonconstant variance failed therefore a NOAEL approach was taken. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Decreased IgM response to SRBC, Crl:CD-1(ICR)BR mice, Male, $C_{last7,avg}$ (Loveless et al., 2008)

Decreased mean response of IgM serum titer was observed in male Crl:CD-1(ICR)BR mice. Using the Wambaugh et al. (2013) model, daily oral gavage exposure to PFOA was simulated for 29 days using male CD1 mice parameters. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 1 SD from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

4.1.4.3 Cardiovascular Effects

Increased total cholesterol in adults aged 20–80, excluding individuals prescribed cholesterol medication (Dong et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from adults aged 20–80 years not prescribed cholesterol medication through the NHANES, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Increased total cholesterol in individuals aged 18 and older, excluding individuals prescribed cholesterol medication (Steenland et al., 2009)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from adults aged 18 years and older not prescribed cholesterol medication from the C8 study population, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Increased total cholesterol in individuals aged 25 and older (Lin et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using BMDS (see Appendix E, (U.S. EPA, 2024a)) from the measured PFOA serum concentrations collected in adults 25 years and older who were at high risk of developing type 2 diabetes and hyperlipidemia from the DPP and Outcomes Study (DPPOS), which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was selected per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

4.1.4.4 Developmental Effects

Decreased birthweight using the mother's serum PFOA concentration collected in third trimester (Chu et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from the mother in the third trimester (blood was collected within 3 days after delivery), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This calculation was performed similarly for each of the birthweight endpoints. The model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age. The model was stopped at a time to match the median gestational age of the cohort at sample time for samples taken during pregnancy, or at delivery (25 years maternal age) in the case of maternal samples at delivery or samples of cord blood. Reverse dosimetry was performed to calculate the POD_{HED} resulting in serum levels matching the POD at the model end time. For this study, maternal blood was drawn within a few days of the birth of the child, so delivery was chosen as the model end time. This metric was independent of the sex of the child in the model.

Decreased birthweight using the serum PFOA concentrations collected from umbilical cord samples (Govarts et al., 2016)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from an umbilical cord sample, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at delivery and reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD serum level in cord serum. This metric was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA concentration collected in the first and second trimesters (Sagiv et al., 2018)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from the mother primarily in the first trimester (median gestational age: 9 weeks; range: 5–19 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 9 weeks. The time after conception was calculated as the fraction of pregnancy competed after 9 weeks (9/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA concentration collected in second and third trimesters (Starling et al., 2017)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from the mother in trimesters 2 and 3 (median gestational age of 27 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 27 weeks. The time after conception was calculated as the fraction of pregnancy competed after 27 weeks (27/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA concentration collected in first and second trimesters (Wikström et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOA serum concentrations collected from the mother in the trimesters 1 and 2 (median gestational age of 10 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 10 weeks. The time after conception was calculated as the fraction of pregnancy completed at 10 weeks (10/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased Pup Survival, Kunming Mice, F_1 males and females (PND 21), $C_{avg\_pup\_gest\_lact}$ (Song et al., 2018)

Decreased mean response of number of offspring survival at weaning on PND 21 was observed in F1 male and female Kunming mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was selected for POD derivation was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) and a BMR of a change in the mean equal to 0.1 standard deviations from the control mean was provided for comparison purposes because decreased pup survival is a severe, frank effect (U.S. EPA, 2012a)(see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest,lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

Decreased Pup Survival, CD-1 Mice, F_1 males and females (PND 0), $C_{avg\_pup\_gest}$ (Lau et al., 2006)

Decreased mean response of pup survival was observed in F1 male and female CD-1 mice at PND 0. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) and a BMR of a change in the mean equal to 0.1 standard deviations from the control mean was provided for comparison purposes because decreased pup survival is a severe, frank effect (U.S. EPA, 2012a) (see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). The tests for constant and nonconstant variance failed therefore a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. This metric was independent of the sex of the child in the model.

Decreased Pup Survival, CD-1 Mice, F_1 males and females (PND 23), $C_{avg\_pup\_gest\ lact}$ (Lau et al., 2006)

Decreased mean response of pup survival was observed in F1 male and female CD-1 mice at PND 23. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) and a BMR of a change in the mean equal to 0.1 standard deviations from the control mean was provided for comparison purposes because decreased pup survival is a severe, frank effect (U.S. EPA, 2012a) (see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The tests for constant and nonconstant variance failed therefore a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

Decreased Fetal Body Weight, Kunming Mice, F_1 males and females (GD 18), $C_{avg\_pup\_gest}$ (Li et al., 2018a)

Decreased mean response of fetal body weight was observed in F_1 male and female Kunming mice. Continuous models were used to fit dose-response data. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). The tests for constant and nonconstant variance failed therefore a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. This metric was independent of the sex of the child in the model.

Decreased Pup Body Weight (relative to litter), CD-1 Mice, F_1 males and females (PND 23), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2006)

Decreased mean response of pup body weight was observed in F_1 male and female CD-1 mice at PND 23. Continuous models were used to fit dose-response data. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The BMDS did not produce a model with adequate fit, so a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

Delayed Time to Eye Opening, CD-1 Mice, F_1 males and females (PND 14 – PND 18), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2006)

Decreased mean response of time to eye opening was observed in F1 male and female CD-1 mice. Continuous models were used to fit dose-response data. BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a BMR of a change in the mean equal to 1 standard deviations from the control mean was provided for comparison purposes (see Appendix E.2, (U.S. EPA, 2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation and lactation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the entire 1-year breastfeeding period because the lactational duration in humans that equates to time to eye opening in rodents is unknown. Additionally, there is currently no mechanistic information to identify a specific window of susceptibility in lactation for this endpoint. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because different growth curves specific to male and female children were used in the model, the model predicted slightly (less than 5%) different serum concentrations for each sex. The lower HED was selected to be more health protective.

4.1.5 Derivation of Candidate Chronic Oral Noncancer Reference Doses (RfDs)

Though multiple candidate POD_{HED}s were derived for multiple health systems from both epidemiological and animal toxicological studies, EPA selected the POD_{HED}s with the greatest strength of evidence and the lowest risk of bias represented by *high* or *medium* confidence studies for candidate RfD derivation, as described below. For epidemiological studies, similar to the discussion of study selection factors in Sections 4 and 4.1.1, EPA critically considered attributes for each POD_{HED} including timing of endpoint collection or measurement, uncertainties associated with modeling (see Appendix E (U.S. EPA, 2024a) and Table 4-8), and consideration of confounding. For animal toxicological studies, attributes considered included study confidence (i.e., *high* confidence studies were prioritized over *medium* confidence studies), amenability to benchmark dose modeling, study design, sensitive lifestages, and health effects observed after exposure in the lower dose range among the animal toxicological studies. As described in the subsections below, this examination of epidemiological and toxicological studies led to the exclusion of a number of studies from consideration for candidate RfD derivation. Health outcome- and study-specific considerations are discussed in Sections 4.1.5.1 (Hepatic), 4.1.5.2 (Immune), 4.1.5.3 (Cardiovascular), and 4.1.5.4 (Developmental).

Once studies and their corresponding POD_{HED}s were prioritized for candidate RfD derivation, EPA applied uncertainty factors (UFs) according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). Considerations for individual UFs differed between epidemiological and animal toxicological studies and are further described in Section 4.1.5.5. Presentation of the candidate RfDs for each health outcome is provided in Section 4.1.5.6.

4.1.5.1 Hepatic Effects

Three *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012). EPA considered all three studies as they represented the low-dose range of effects across hepatic endpoints and provided data from relatively large populations, including U.S. populations. Additionally, these studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. The three studies reported analyses examining different forms of confounding factors and consideration of cumulative PFOA exposure (Darrow et al., 2016), sensitivity analyses excluding participants with lifestyle characteristics (e.g., excluding smokers, drinkers, medicine takers) impacting outcome assessment (Nian et al., 2019), and nonlinear exposure-response relationships (Gallo et al., 2012). All three of these studies provided the necessary data for modeling.

One *high* confidence animal toxicological study was carried forward for candidate RfD determination (NTP, 2020). NTP (2020) was prioritized for candidate RfD development because it was determined to be a *high* confidence study and it used a chronic exposure duration that encompassed sensitive periods of development, whereas Loveless et al. (2008) was a *medium* confidence study that used a short-term (28-day) exposure duration and predated current criteria for hepatic histopathological assessment of cell death (Elmore et al., 2016). Increased liver necrosis from NTP (2020) was selected for candidate RfD derivation over the effect of increased

hepatocyte single cell death due to the increased biological severity of the former endpoint. Increased liver necrosis additionally resulted in a more protective POD_{HED}.

4.1.5.2 Immune Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). EPA considered both studies as they both represented the low-dose range of effects across immunological endpoints and provided data regarding sensitive populations (i.e., children). Although EPA derived POD_{HED}s for two time points reported by Budtz-Jørgensen and Grandjean (2018) (i.e., PFOA serum concentrations at age 5 and antibody concentrations at age 7; PFOA serum concentrations in the mother during the third trimester or approximately 2 weeks after the expected term date and antibody concentrations at age 5), EPA did not carry forward POD_{HED}s based on serum PFOA concentrations measured in the mother for candidate RfD derivation because of concerns surrounding potential increased risk of bias due to pregnancy-related hemodynamic effects. EPA also derived candidate RfDs for both tetanus and diphtheria vaccine responses from Timmerman et al. (2021) for comparison to a second population of children. In total, four immunological POD_{HED}s from two epidemiological studies were carried forward for candidate RfD derivation.

One *medium* confidence animal toxicological study was carried forward for candidate RfD determination (Dewitt et al., 2008). The POD_{HED} from Study 1 was selected over Study 2 because the former was amenable to benchmark dose modeling and had a POD_{HED} based on a BMDL, the preferred POD for animal toxicological studies (U.S. EPA, 2022d, 2012a). Study quality evaluations and further consideration did not identify notable characteristics distinguishing the two candidate studies (Dewitt et al., 2008; Loveless et al., 2008), but because the POD_{HED}s of reduced IgM response in rodents represented effects at the highest dose range of responses and because the observed effects were from *medium* confidence less-than-chronic studies, EPA selected the most health protective POD_{HED} based on Dewitt et al. (2008) for candidate RfD derivation. The candidate RfD derived from Dewitt et al. (2008) is expected to be protective of the immune effects observed in Loveless et al. (2008).

4.1.5.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Dong et al., 2019; Steenland et al., 2009). Of the three studies for which POD_{HED}s were derived, Dong et al. (2019) and Steenland et al. (2009) excluded individuals who were prescribed cholesterol medication, minimizing concerns surrounding confounding due to the medical intervention altering serum total cholesterol levels. This is in contrast to Lin et al. (2019) which did not control for individuals prescribed cholesterol medication and was therefore excluded from further consideration. Modeling of both Dong et al. (2019) and Steenland et al. (2009) resulted in POD_{HED}s with minimal risk of bias, representing both the general population and a high-exposure community, respectively and thus, were both considered further for candidate RfD derivation.

4.1.5.4 Developmental Effects

Two *high* confidence epidemiological studies were carried forward for candidate RfD determination for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al.,

2018). Of the five epidemiological studies for which POD_{HED}s were derived, Sagiv et al. (2018) and Wikström et al. (2020) assessed maternal PFOA serum concentrations primarily in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Although Wikström et al. (2020) collected approximately 4% of samples during early weeks of the second trimester, sensitivity analyses showed no differences when trimester two samples were excluded. Additionally, these two studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias and reflected two different study populations. Therefore, both studies were considered further for candidate RfD derivation. The other three studies assessed PFOA concentrations in either umbilical cord blood or primarily during the second or third trimesters, increasing the uncertainty associated with the derived POD_{HED}s due to potential pregnancy-related hemodynamic effects, and as a result, were excluded from consideration for candidate RfD derivation (Chu et al., 2020; Starling et al., 2017; Govarts et al., 2016).

Two *medium* confidence animal toxicological studies representing two endpoints, decreased pup survival and delayed time to eye opening, were carried forward for candidate RfD determination (Song et al., 2018; Lau et al., 2006). These two datasets were amenable to benchmark dose modeling and had POD_{HED}s based on BMDLs, the preferred POD for animal toxicological studies (U.S. EPA, 2022d, 2012a). In contrast, the endpoints of decreased fetal body weight derived from data published by Li et al. (2018a) and decreased pup survival and decreased pup weight derived from data published by Lau et al. (2006) were not amenable to BMD modeling and had NOAELs as the basis of the POD_{HED}s. Therefore, these POD_{HED}s were excluded from further consideration for candidate RfD derivation. As the delayed time to eye opening and decreased pup survival endpoints reported by Lau et al. (2006) and Song et al. (2018), respectively, encompassed sensitive populations (i.e., fetuses and pups) and different effects in two different strains of mice, these two POD_{HED}s were considered further for candidate RfD derivation. These two endpoints appear to be more sensitive (i.e., have lower POD_{HED}s) than the effects reported by Li (2018a) and Lau (2006).

4.1.5.5 Application of Uncertainty Factors

To calculate the candidate RfD values, EPA applied UFs to the POD_{HED}s derived from selected epidemiological and animal toxicological studies (Table 4-9 and Table 4-10). UFs were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b).

Table 4-9. Uncertainty Factors for the Development of the Candidate Chronic RfD Values From Epidemiological Studies (U.S. EPA, 2002b)

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to effects observed in epidemiological studies as the study population is humans.
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1	A UF_S of 1 is applied when effects are observed in adult human populations that are assumed to have been exposed to a contaminant over the course of many years. A UF_S of 1 is applied for developmental effects because the developmental period is recognized as a susceptible lifestage when exposure during a time window of

UF	Value	Justification
		development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF_D	1	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterization of the contaminant's toxicity.
UF_C	10	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: BMDL = benchmark dose level; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = composite UF.

An interspecies UF (UF_A) of 1 was applied to POD_{HED}s derived from epidemiological studies because the dose-response information from these studies is directly relevant to humans. There is no need to account for uncertainty in extrapolating from laboratory animals to humans.

An intraspecies UF (UF_H) of 10 was applied to POD_{HED}s derived from epidemiological studies to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, lifestage, and health status) and extrinsic (lifestyle) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to quantitatively characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation UF (UF_L) of 1 was applied to POD_{HED}s derived from epidemiological studies because a BMDL is used as the basis for the POD_{HED} derivation. This was the case for all epidemiological endpoints and studies advanced for candidate RfD derivation.

A UF for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 1 was applied to POD_{HED}s derived from epidemiological studies. A UF_s of 1 was applied to the hepatic and cardiovascular endpoints because the effects were observed in adult populations that were assumed to have been exposed to PFOA over the course of many years. A UF_s of 1 was applied to the developmental endpoints because the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). A UF_S of 1 was also applied to the immune endpoints observed in children and adolescents because exposure is assumed to occur from gestation through childhood, when the response variable was measured. There is uncertainty regarding the critical window of exposure that results in these immune effects in children and adolescents. Therefore, EPA expects that any exposure during this period of development has the potential to impact this response (U.S. EPA, 1991). According to the WHO/International Programme on Chemical Safety (IPCS) *Immunotoxicity Guidance for Risk Assessment*, developmental immunotoxicity is assessed during the prenatal, neonatal, juvenile and adolescent life stages because immune system development occurs throughout these life stages and should be viewed differently in part due to increased susceptibility compared with the immune system of adults from a risk assessment perspective (IPCS, 2012).

A database UF (UF_D) of 1 was applied to account for deficiencies in the database for PFOA. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a large number of *medium* and *high* confidence epidemiological studies which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. Effects identified in developmental and multigenerational reproductive toxicity studies have been quantitatively considered in this assessment.

The composite UF that was applied to candidate RfDs derived from all of the epidemiological studies were the same value (UF_C = 10) (Table 4-9).

Increased uncertainty is associated with the use of animal toxicological studies as the basis of candidate RfDs. The composite UF applied to animal toxicological studies considered for candidate RfD derivation were either one of two values, depending on the duration of exposure (i.e., chronic vs. subchronic) or exposure window (e.g., gestational) (Table 4-10).

Table 4-10. Uncertainty Factors for the Development of the Candidate Chronic RfD Values From Animal Toxicological Studies (U.S. EPA, 2002b)

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied for the extrapolation from animal models to humans due to the implementation of a PK model for animal POD_{HED} derivation.
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1 or 10	A UF_S of 10 is applied for the extrapolation of subchronic-to-chronic exposure durations. A UF_S of 1 is applied to studies with chronic exposure durations or that encompass a developmental period (i.e., gestation). The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF_D	1	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterization of the contaminant's toxicity.
UF_C	30 or 300	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: BMDL = benchmark dose level; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = total uncertainty factors.

A UF_A of 3 was applied to POD_{HED}s derived from animal toxicological studies to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). The threefold factor is applied to account for toxicodynamic differences between the animals and

humans. The HEDs were derived using a model that accounted for PK differences between animals and humans.

A UF_H of 10 was applied to POD_{HED}s derived from animal toxicological studies to account for variability in the responses within human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, lifestage, and health status) and extrinsic (lifestyle) factors can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A UF_L of 1 was applied to POD_{HED}s derived from animal toxicological studies because a BMDL was used as the basis for the POD_{HED} derivation. BMDLs were available for all animal toxicological endpoints and studies advanced for candidate RfD derivation.

A UF_S of 1 was applied to POD_{HED}s derived from chronic animal toxicological studies as well as animal toxicological studies that encompass a developmental period (i.e., gestation). A UF_s of 1 was applied to developmental endpoints because the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). A UF_s of 10 was applied to POD_{HED}s derived from studies that implemented a less-than-chronic exposure duration because extrapolation is required to translate from a subchronic POD_{HED} to a chronic RfD.

A database UF (UF_D) of 1 was applied to account for deficiencies in the database for PFOA. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a large number of *medium* and *high* confidence epidemiological studies which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. Effects identified in developmental and multigenerational reproductive toxicity studies have been quantitatively considered in this assessment.

In summary, the composite UF that was applied to candidate RfDs derived from all of the epidemiological studies were the same value (UF_C = 10) (Table 4-9). The composite UF that was applied to candidate RfDs derived from animal toxicological studies was either UF_C = 30 or 300 (Table 4-10). In all of these cases, the total uncertainty is well below the maximum recommended UF_C = 3,000 (U.S. EPA, 2002b).

4.1.5.6 Candidate RfDs

Table 4-11 shows the UFs applied to each candidate study to subsequently derive the candidate RfDs.

Table 4-11. Candidate Reference Doses (RfDs)

Endpoint	Study, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate RfD[a] (mg/kg/day)
Immune Effects										
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female, PFOA concentrations at age 5 and antibody concentrations at age 7	3.05×10^{-7}	1	10	1	1	1	10	$3.05 \times 10^{-8} = 3 \times 10^{-8}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOA and antibody concentrations at ages 7–12	3.34×10^{-7}	1	10	1	1	1	10	$3.34 \times 10^{-8} = 3 \times 10^{-8}$
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female, PFOA concentrations at age 5 and antibody concentrations at age 7	2.92×10^{-7}	1	10	1	1	1	10	$2.92 \times 10^{-8} = 3 \times 10^{-8}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOA and antibody concentrations at ages 7–12	2.20×10^{-7}	1	10	1	1	1	10	$2.20 \times 10^{-8} = 2 \times 10^{-8}$
Decreased IgM response to SRBC	Dewitt et al. (2008) *Medium*	Mouse, female, adults, study 1	2.18×10^{-3}	3	10	10	1	1	300	$7.27 \times 10^{-6} = 7 \times 10^{-6}$
Developmental Effects										
Decreased Birth Weight	Sagiv et al. (2018) *High*	Human, male and female, PFOA concentrations in first and second trimesters	1.21×10^{-6}	1	10	1	1	1	10	$1.21 \times 10^{-7} = 1 \times 10^{-7}$
	Wikström et al. (2020) *High*	Human, male and female, PFOA concentrations in first and second trimesters	2.92×10^{-7}	1	10	1	1	1	10	$2.92 \times 10^{-8} = 3 \times 10^{-8}$
Decreased Offspring Survival	Song et al. (2018) *Medium*	Kunming Mice, F_1 males and females	6.40×10^{-4}	3	10	1	1	1	30	$2.13 \times 10^{-5} = 2 \times 10^{-5}$

Endpoint	Study, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate RfD[a] (mg/kg/day)
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 14 – PND 18)	4.17×10^{-4}	3	10	1	1	1	30	$1.39 \times 10^{-5} = 1 \times 10^{-5}$
Cardiovascular Effects										
Increased Serum Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	2.75×10^{-7}	1	10	1	1	1	10	$2.75 \times 10^{-8} = 3 \times 10^{-8}$
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	5.10×10^{-7}	1	10	1	1	1	10	$5.10 \times 10^{-8} = 5 \times 10^{-8}$
Hepatic Effects										
Increased Serum ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	2.15×10^{-6}	1	10	1	1	1	10	$2.15 \times 10^{-7} = 2 \times 10^{-7}$
	Darrow et al. (2016) *Medium*	Human, female, age 18 and older	7.92×10^{-6}	1	10	1	1	1	10	$7.92 \times 10^{-7} = 8 \times 10^{-7}$
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	4.51×10^{-7}	1	10	1	1	1	10	$4.51 \times 10^{-8} = 5 \times 10^{-8}$
Necrosis	NTP (2020) *High*	Sprague-Dawley rats, perinatal and postweaning (2-year), male	3.23×10^{-3}	3	10	1	1	1	30	$1.08 \times 10^{-4} = 1 \times 10^{-4}$

Notes: ALT = alanine aminotransferase; NTP = National Toxicology Program; POD_{HED} = point-of-departure human equivalence dose; RfD = reference dose; SRBC = sheep red blood cells; UF_A = interspecies uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic extrapolation uncertainty factor; UF_L = extrapolation from a LOAEL-to-NOAEL uncertainty factor; UF_D = database uncertainty factor; UF_C = composite uncertainty factor.

[a] RfDs were rounded to one significant figure.

[b] Supported by Grandjean et al. (2012), Grandjean et al. (2017a), and Grandjean et al. (2017b).

4.1.6 RfD Selection

As presented in Section 4.1.5 (Table 4-11), EPA derived and considered multiple candidate RfDs across the four noncancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental). EPA derived candidate RfDs based on both epidemiological and animal toxicological studies. As depicted in Figure 4-4, the candidate RfDs derived from epidemiological studies were all within 1 order of magnitude of each other (10^{-7} to 10^{-8} mg/kg/day), regardless of endpoint, health outcome, or study population.

Candidate RfDs derived from animal toxicological studies were generally 2–3 orders of magnitude higher than candidate RfDs derived from epidemiological studies. However, EPA does not necessarily expect concordance between animal and epidemiological studies in terms of either the adverse effect(s) observed or the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" (U.S. EPA, 1991). Additionally, for developmental effects, the guidance says that "the experimental animal data were generally predictive of adverse developmental effects in humans, but in some cases, the administered dose or exposure level required to achieve these adverse effects was much higher than the effective dose in humans" (U.S. EPA, 1991).

As shown in Table 4-11 and Figure 4-4, there is greater uncertainty associated with the use of animal toxicological studies as the basis of RfDs than human epidemiological studies. Though there are some uncertainties in the use of epidemiological studies for quantitative dose-response analyses (see Sections 5.1, 5.6, and 5.7), human data eliminate the uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species which are major uncertainties associated with the PFOA animal toxicological studies due to the half-life differences and sex-specific toxicokinetic differences in rodent species. These uncertainties may explain, in part, the higher magnitude of candidate RfDs derived from animal toxicological studies compared to the candidate RfDs derived from epidemiological studies. Moreover, the human epidemiological studies also have greater relevance to human exposure than animal toxicological studies because they directly measure environmental or serum concentrations of PFOA. In accordance with EPA's current best practices for systematic review, "animal studies provide supporting evidence when adequate human studies are available, and they are considered to be the studies of primary interest when adequate human studies are not available" (U.S. EPA, 2022d). For these reasons, EPA determined that candidate RfDs based on animal toxicological studies would not be further considered for health outcome-specific RfD selection or overall RfD selection. See Section 5.2 for further comparisons between toxicity values derived from epidemiological and animal toxicological studies.

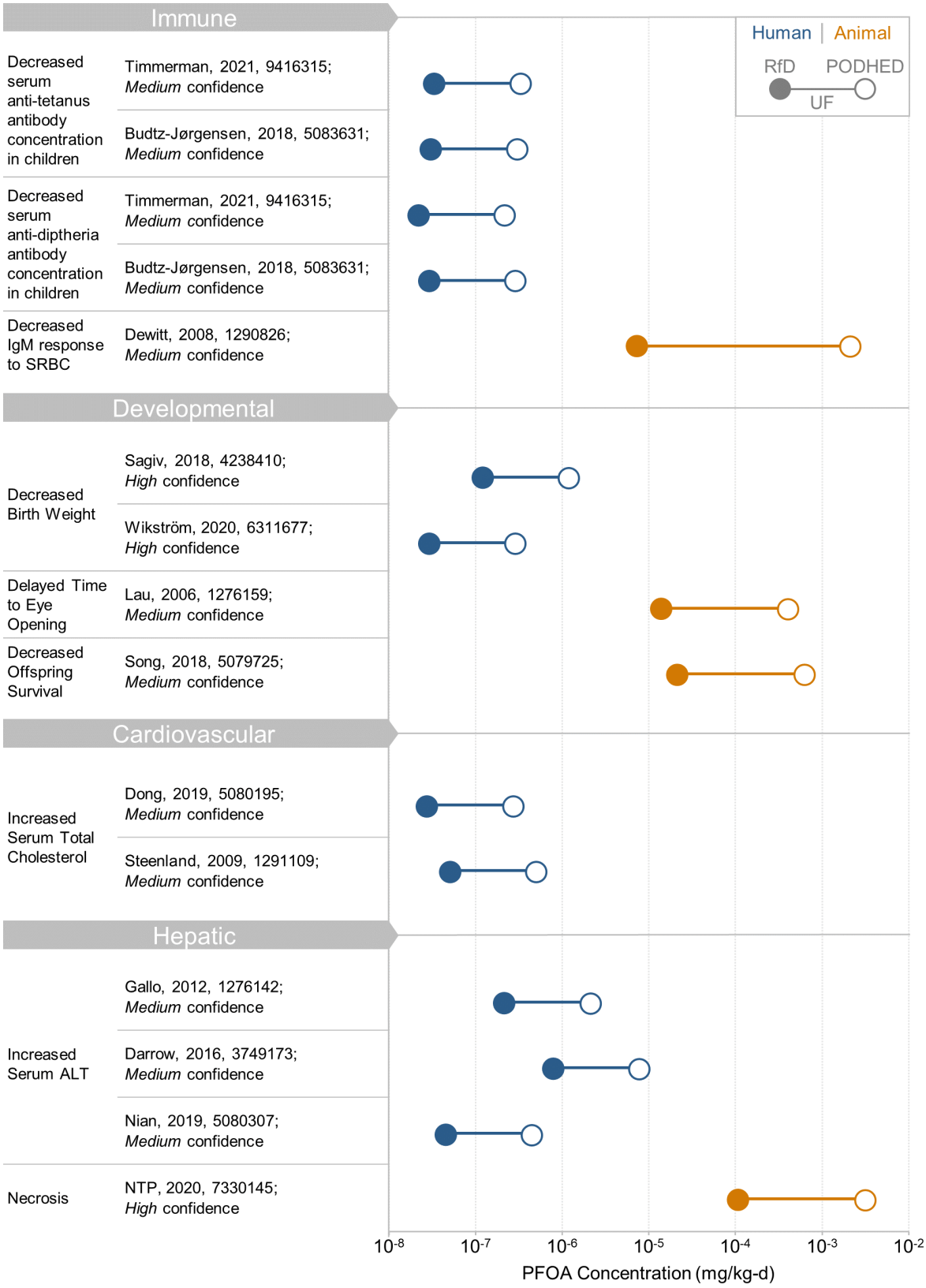


Figure 4-4. Comparison of Candidate RfDs Resulting from the Application of Uncertainty Factors to POD_{HED}s Derived from Epidemiological and Animal Toxicological Studies

As described in the subsections below, EPA selected amongst the candidate RfDs to identify an RfD representative of each of the four priority health outcomes (i.e., health outcome-specific RfDs), as well as an overall RfD that is protective of the effects of PFOA on all health outcomes and endpoints (Figure 4-5).

4.1.6.1 Health Outcome-Specific RfDs

At least two candidate RfDs were derived from epidemiological studies for each of the four prioritized noncancer health outcomes. EPA considered several factors when selecting health outcome-specific RfDs, including relevance of exposure or population characteristics to the general population, potential confounding factors, and characteristics of the modeled data. Health outcome- and study-specific considerations are discussed in Sections 4.1.6.1.1 (Hepatic), 4.1.6.1.2 (Immune), 4.1.6.1.3 (Cardiovascular), and 4.1.6.1.4 (Developmental), below.

4.1.6.1.1 Hepatic Effects

Three *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased ALT (Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012). The two largest studies of PFOA and ALT in adults, Gallo et al. (2012) and Darrow et al. (2016), were both conducted in over 30,000 adults from the C8 Study. Gallo et al. (2012) reported measured serum ALT levels, unlike Darrow et al. (2016) which reported a modeled regression coefficient as the response variable. Another difference between the two studies is reflected in exposure assessment: Gallo et al. (2012) includes measured PFOA serum concentrations, while Darrow et al. (2016) based PFOA exposure on modeled PFOA serum levels. Due to these factors, the candidate RfD derived from Darrow et al. (2016) was excluded from further consideration as the health outcome-specific RfD for hepatic effects.

The third study by Nian et al. (2019) examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project and observed significant increases in lognormal ALT per each ln-unit increase in PFOA, as well significant increases in ORs of elevated ALT. Both Nian et al. (2019) and Gallo et al. (2012) provided measured PFOA serum concentrations and a measure of serum ALT levels. However, the Gallo et al. (2012) study was conducted in a community exposed predominately to PFOA, whereas Nian et al. (2019) was conducted in a community exposed predominately to PFOS. The candidate RfD derived from Gallo et al. (2012) was ultimately selected as the health outcome-specific RfD due to reduced risk of bias related to potential confounding from other PFAS in this population. The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-5).

4.1.6.1.2 Immune Effects

Candidate RfDs were derived from two *medium* confidence epidemiological studies for the endpoint of decreased antibody production in response to various vaccinations in children (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). Candidate RfDs were derived from Timmerman et al. (2021) were considered lower confidence candidate RfDs than those derived from Budtz-Jørgensen and Grandjean (2018). PODHEDs derived from Timmerman et al. (2021) were considered to have increased uncertainty compared with Budtz-Jørgensen and Grandjean (2018) due to two features of the latter study that strengthen the confidence in the PODHEDs: 1) the analyses considered co-exposures of other PFAS (i.e.,

PFOS); and 2) the response reported by this study was more precise in that it reached statistical significance. Therefore, the candidate RfDs from Timmerman et al. (2021) were not considered for selection as the health outcome-specific RfD.

The RfDs for anti-tetanus response in 7-year-old Faroese children and anti-diphtheria response in 7-year-old Faroese children, both from Budtz-Jørgensen and Grandjean (2018) were ultimately selected for the immune outcome as they are the same value and have no distinguishing qualitative (e.g., strength of evidence) or quantitative (e.g., model fit) characteristics that would facilitate selection of one over the other. The resulting health outcome-specific RfD is 3×10^{-8} mg/kg/day (Figure 4-5). Note that all candidate RfDs based on epidemiological studies for the immune outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased TC (Dong et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOA exposure measures across various populations. Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Both of these studies excluded individuals prescribed cholesterol medication which minimizes concerns of confounding due to medical intervention. The candidate RfD for increased TC from Dong et al. (2019) was ultimately selected for the health outcome-specific RfD for cardiovascular effects as there is marginally increased confidence in the modeling from this study. Steenland et al. (2009) presented analyses using both PFOA and TC as categorical and continuous variables. The results using the natural log transformed TC and the natural log transformed PFOA were stated to fit the data slightly better than the ones using untransformed PFOA. However, the dramatically different changes in regression slopes between the two analyses by Steenland et al. (2009) resulting in extremely different PODs raise concerns about the appropriateness of using this data. Therefore, the resulting health outcome-specific RfD based on results from Dong et al. (2019) is 3×10^{-8} mg/kg/day (Figure 4-5). Note that both candidate RfDs for the cardiovascular outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.4 Developmental Effects

Two *high* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018). These candidate studies assessed maternal PFOA serum concentrations primarily in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Both were *high* confidence prospective cohort studies with many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. Between these two studies, PFOA exposure concentrations observed in Wikström et al. (2020) are more comparable to current exposure levels in the U.S. general population and therefore may be more relevant to the general population than the candidate RfD derived from Sagiv et al. (2018). Additionally, the BMDL derived from Wikström et al. (2020) was based on a statistically significant regression parameter. For these reasons, the RfD for decreased birth weight from Wikström et al. (2020) was selected as the basis for the health outcome-specific RfD for developmental effects. The resulting health outcome-specific RfD is 3×10^{-8} mg/kg/day (Figure 4-5). Note that both candidate RfDs based on epidemiological studies for the

developmental outcome were within one order of magnitude of the selected health outcome-specific RfD.

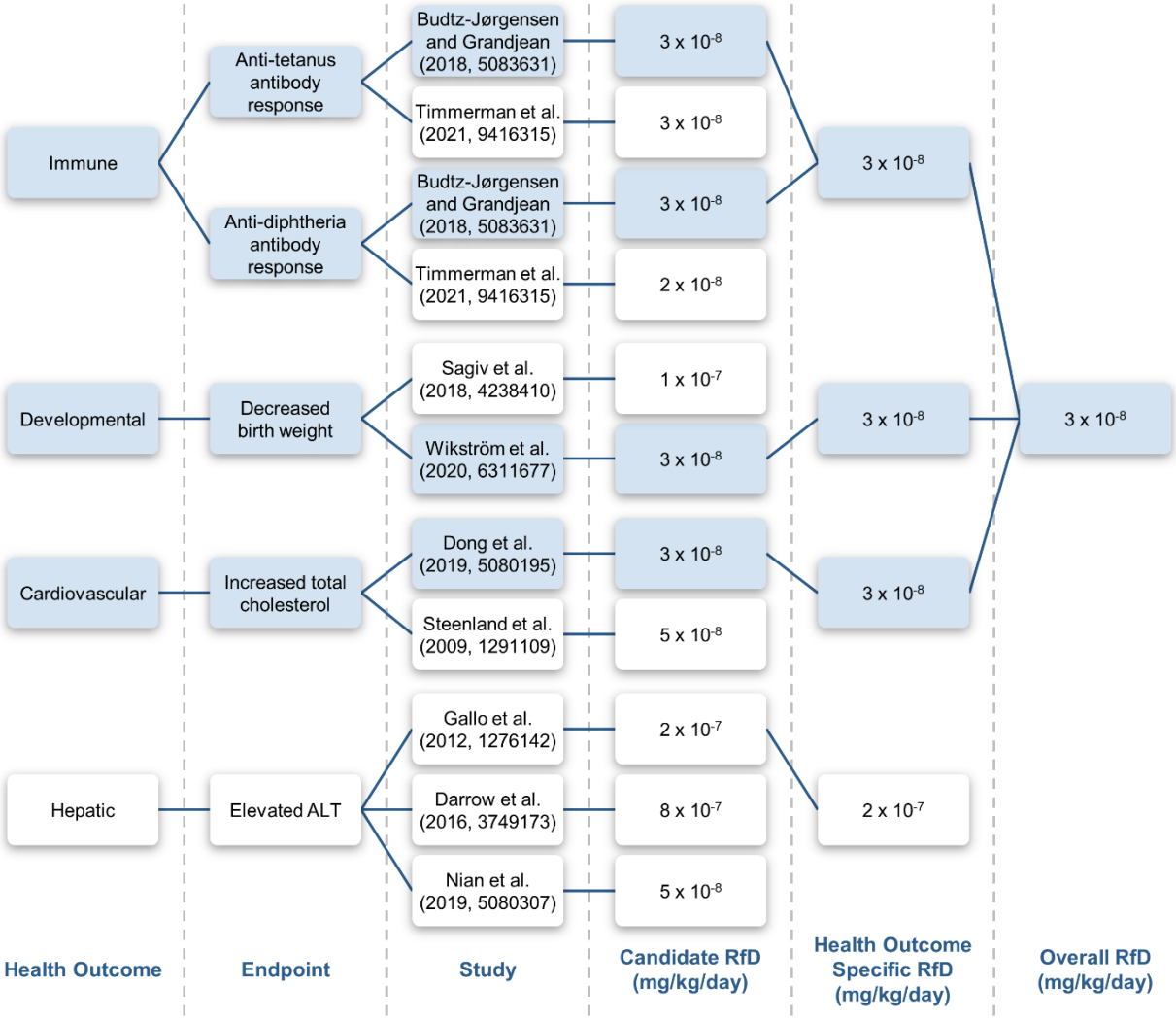


Figure 4-5. Schematic Depicting Selection of the Overall RfD for PFOA

RfD = reference dose.
Blue highlighted boxes indicate outcomes, endpoints, studies, candidate RfDs, and health outcome-specific RfDs that were selected as the basis of the overall RfD.

4.1.6.2 Overall Noncancer RfD

The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOA exposure. In fact, candidate RfDs within the immune, developmental, and cardiovascular outcomes are the same value (i.e., 3×10^{-8} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOA of 3×10^{-8} mg/kg/day. The immune, developmental, and cardiovascular RfDs based on endpoints of decreased anti-tetanus and anti-diphtheria antibody concentrations in children, decreased birth weight, and increased total cholesterol, respectively, serve as co-critical effects for this RfD.

Notably, the RfD is protective of effects that may occur in sensitive populations (e.g., infants, children; see Section 5.8), as well as hepatic effects in adults that may result from PFOA exposure. As two of the co-critical effects identified for PFOA are developmental endpoints and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOA is applicable to both short-term and chronic risk assessment scenarios.

The critical studies that serve as the basis of the RfD are all *medium* or *high* confidence epidemiological studies. The critical studies are supported by multiple other *medium* or *high* confidence studies in both humans and animal models and have health outcome databases for which EPA determined *evidence indicates* that oral PFOA exposure is associated with adverse effects. Additionally, the selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans.

4.2 Cancer

As described in the introduction of Section 4, there is evidence from both epidemiological and animal toxicological studies that oral PFOA exposure may result in adverse health effects across many health outcomes, including cancer (Section 3.5). In Section 3.5.5, EPA concluded that PFOA is *Likely to be Carcinogenic to Humans* in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Therefore, the quantification of cancer effects was prioritized along with the four noncancer health outcomes that are described in Section 4.1. EPA considered only *high* or *medium* confidence human and animal toxicological studies for CSF derivation.

4.2.1 Study and Endpoint Selection

Human studies selected for CSF derivation reported all necessary analytical information (e.g., exposure distribution or variance) for the outcome of interest (any cancer). If available, *high* and *medium* confidence studies with exposures levels near the range of typical environmental human exposures, especially exposure levels comparable to human exposure in the general population, were preferred over studies reporting considerably higher exposure levels. Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to exposure levels that may be more relevant to the U.S. general population. Additionally, the most recent and comprehensive publication on a single study population was preferred over prior publications on the same or portions of the same population (e.g., selection of Vieira et al. (2013) over other C8 Health Project studies (see Section 4.2.1.1)).

Preferred animal toxicological studies consisted of *medium* and *high* confidence studies with chronic exposure durations to capture potential latency of cancer effects. Studies with exposure durations during development (e.g., gestation) were also considered informative for assessing potential early lifestage susceptibility to cancer (see Section 4.2.4). Studies encompassing lower dose ranges were also preferred. These types of animal toxicological studies increase the confidence in the CSF relative to other animal toxicological studies because they are based on data with relatively low risk of bias, have sufficient study designs to observe the critical effects, and are associated with less uncertainty related to low-dose and exposure duration extrapolations.

4.2.1.1 Epidemiological Studies

The available evidence indicates that there is an increase in risk for kidney or Renal cell carcinoma (RCC) and testicular cancers with PFOA exposure (Bartell and Vieira, 2021; Shearer et al., 2021; Chang et al., 2014; Raleigh et al., 2014; Barry et al., 2013; Vieira et al., 2013; Steenland and Woskie, 2012). Results are most consistent for kidney cancer in adults based on a nested case-control study (Shearer et al., 2021), two C8 Health Project studies (Barry et al., 2013; Vieira et al., 2013), two occupational mortality studies (Raleigh et al., 2014; Steenland and Woskie, 2012), and a meta-analysis of epidemiological literature that concluded that there was an increased risk of kidney tumors correlated with increased PFOA serum concentrations (Bartell and Vieira, 2021). Therefore, the endpoint of kidney cancer was selected for CSF derivation.

Testicular cancer was identified as supporting evidence for carcinogenicity in humans in the 2016 PFOA HESD (U.S. EPA, 2016c). However, additional epidemiological studies examining risk of testicular cancer were not identified in the updated literature search and only two studies in the same high-exposure community (C8 Health Project) reported this association (Barry et al., 2013; Vieira et al., 2013). Therefore, the endpoint of testicular cancer in humans was not selected for dose-response modeling. Evidence was mixed or limited for other cancer sites (e.g., breast, liver cancers), which were not considered further.

Two studies reporting associations between kidney cancer and PFOA serum concentrations, Shearer et al. (2021) and Vieira et al. (2013), were selected for dose-response modeling. Shearer et al. (2021) was selected because it is a well-conducted, U.S.-based multicenter case-control study in the general population reporting a relatively large number of cases (N = 326). Median PFOA levels in controls was 5.0 ng/mL, comparable with 4.8 ng/mL in adults 60 and over from NHANES 1999–2000. Additionally, the analyses accounted for numerous confounders including BMI, smoking, history of hypertension, eGFR, previous freeze-thaw cycle, calendar and study year of blood draw, sex, race and ethnicity, study center. There was also a statistically significant increase in odds of RRC per doubling of PFOA (OR = 1.71, 95% CI: 1.23, 2.37) and in the highest versus lowest quartile (OR = 2.63, 95% CI: 1.33, 5.2) and a statistically significant increasing trend with increasing PFOA exposure across quartiles (p-trend = 0.007). Statistically significant increased odds of RCC were observed in participants ages 55–59 years, and in both men and women, separately.

EPA also selected the C8 Health Project study (Vieira et al., 2013) for dose-response modeling. The Vieira et al. (2013) study was a cancer registry-based case-control conducted in 13 counties in Ohio and West Virginia that surround the DuPont Washington Works PFOA facility (C8 study area). Analyses were adjusted for several factors including age, sex, diagnosis year, smoking status (current, past, unknown, or never), and insurance provider (government-insured Medicaid, uninsured, unknown, or privately insured). There was a statistically significant increase in the odds of kidney cancer when comparing both the high (OR = 2.0; 95% CI: 1.3, 3.2) and the very high (OR = 2.0; 95% CI: 1.0, 3.9) exposure categories to the unexposed reference population. Vieira et al. (2013) was selected for modeling over Barry et al. (2013), the populations of which likely overlapped, because Barry et al. (2013) did not report the necessary exposure measurements for CSF calculation. Specifically, exposure levels were reported separately for the community participants and workers, but not for the overall study population and therefore, CSF calculations were not feasible. Vieira (2013, 2919154) included the most complete and up-to-date data from this population, including all information needed for CSF derivation.

The high-exposure occupational study by Steenland and Woskie (2012) was not selected for dose-response analysis because it was limited by the small number of observed cancer cases (six kidney cancer deaths) and the exposure levels reported in the study population (average annual serum concentration of 350 ng/mL) are less comparable to the U.S. general population than the levels reported by Shearer et al. (2021) and Vieira et al. (2013). The study by Raleigh et al. (2014) was also not selected prioritized because of the concerns of exposure assessment methods (i.e., estimated air PFOA concentrations rather than biomonitoring data) and study quality (i.e., relatively small numbers of cases and lack of information regarding adjustment of risk factors for kidney cancer such as smoking status and BMI).

4.2.1.2 Animal Toxicological Studies

Three chronic studies are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001). Combined, at least two of the three studies report increased incidences each of hepatic, testicular, and pancreatic neoplastic lesions. Increased incidences of neoplastic lesions were primarily observed in male rats, though results in females, particularly the reports of rare tumor types (i.e., pancreatic acinar cell adenomas and adenocarcinomas), are supportive of potential carcinogenicity of PFOA. Additionally, NTP (2020) observed marginally increased incidences of uterine adenocarcinomas in female Sprague-Dawley rats during the extended evaluation (i.e., uterine tissue which included cervical, vaginal, and uterine tissue remnants). Uterine adenocarcinomas were not selected for CSF derivation because "the strength of the response was marginal and there was a low concurrent control incidence that lowered confidence in the response" (NTP, 2020). Butenhoff et al. (2012) identified mammary fibroadenomas and ovarian tubular adenomas in female rats, though there were no statistical differences in incidence rates between PFOA-treated groups and controls. These tumor types were also not selected for CSF derivation because the incidences were not observed by NTP (2020). As these results are inconclusive and there was increased magnitude of hepatic and pancreatic tumor incidences in males, likely due to the increased sensitivity of male rats resulting from toxicokinetic differences between the sexes (see Section 3.3.1), quantitative analyses were focused on males rather than females.

Butenhoff et al. (2012) and Biegel et al. (2001) reported dose-dependent increases in testicular LCTs. Additionally, LCT incidence at similar dose levels was comparable between the two studies (11 and 14%, respectively). PACTs were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas and adenocarcinomas in males in all treatment groups compared with their respective controls. These rare tumor types were also observed in female rats in the highest dose group, though the increased incidence did not reach statistical significance. Biegel et al. (2001) reported increases in the incidence of PACTs in male rats treated with PFOA, with zero incidences observed in control animals. In addition, both NTP (2020) and Biegel et al. (2001) reported dose-dependent increases in the incidence of liver adenomas in male rats. NTP (2020) also reported several male rats with hepatocellular carcinomas in the highest dose group (300/80 ppm). Butenhoff et al. (2012) additionally reported incidences of hepatocellular carcinomas in male rats from every treatment group, including controls, and female rats in the highest dose group. Given the consistency across the three available studies, the observation of malignant pancreatic and

hepatic tumors, and the site concordance between the testicular tumors in rats and humans, tumors from all three sites (i.e., liver, pancreas, testes) were selected for CSF derivation.

In further evaluation of the studies, Biegel et al. (2001) was not considered for dose-response modeling because it is a single-dose study. Therefore, NTP (2020) was selected for candidate CSF derivation for the PACTs and hepatocellular tumors and Butenhoff et al. (2012) was selected for candidate CSF derivation for LCTs.

4.2.2 Candidate CSF Derivation

4.2.2.1 Epidemiological Studies

EPA calculated CSFs for RCC from Shearer et al. (2021) and for kidney cancer from Vieira et al. (2013) based on the method used in CalEPA (2021) and for its *Public Health Goals for Arsenic in Drinking Water* (OEHHA, 2004). Details are provided in the Appendix (U.S. EPA, 2024a). The underlying model involves a linear regression between PFOA exposure and cancer relative risk used to estimate the dose-response between PFOA and RCC or kidney cancer risk. This was calculated using a weighted linear regression of the quartile specific RRs, with the weights defined as the inverse of the variance of each RR. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age (or models were adjusted for age in Vieira et al. (2013)), the ORs represent a good approximation of the underlying RRs. The CSF is then calculated as the excess cancer risk associated with each ng/mL increase in serum PFOA (internal CSF). The internal CSF was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done assuming a baseline risk (R_0) of RCC or kidney cancer in an unexposed or lower exposure reference group. Since this is not available in a case-control study, the lifetime risk of RCC in U.S. males is used. For Shearer et al. (2021), the lifetime RCC risk was estimated by multiplying the lifetime risk of kidney cancer in U.S. males (American Cancer Society, 2020) by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R_0 of $0.0202 \times 90\% = 0.0182$. For Vieira et al. (2013), the lifetime kidney cancer of R_0 of 0.0202 was used, and the model fit was better when the highest exposure level was excluded. The internal CSF was then calculated as either the product of the upper 95% CI or the central tendency of the dose-response slope and R_0 and represents the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/(kg-day) increase in dose. This was done by dividing the internal serum CSF by the selected clearance value, which is equivalent to dividing by the change in external exposure that results in a 1 ng/mL increase in serum concentration at steady-state. The clearance value used was the same as that used in the updated Verner model for endpoints related to developmental exposure (Table 4-6).

The results of the modeling and the candidate CSFs derived are presented in Table 4-12.

Table 4-12. Candidate Cancer Slope Factors Based on Epidemiological Data

Tumor Type	Reference, Confidence	Strain/ Species/Sex/ Age	POD Type, Model	Internal CSF – Increase in Cancer Risk per 1 ng/mL Serum Increase	CSF – Increase in Cancer Risk per 1 ng/(kg*d) Increase in Dose
Renal cell carcinoma (RCC)	Shearer et al. (2021) *Medium*	Human, male and female 55–74 yr	CSF serum in adults (per ng/mL of serum PFOA); upper limit of the 95% CI	3.52×10^{-3} $(ng/mL)^{-1}$ (see Appendix (U.S. EPA, 2024a)for additional detail)	0.0293 $(ng/kg/d)^{-1}$
Kidney cancer	Vieira et al. (2013) *Medium*	Human, male and female, median age 67 years	CSF serum in adults (per ng/mL of serum PFOA); upper limit of the 95% CI, highest exposure group excluded	4.81×10^{-4} $(ng/mL)^{-1}$ (see Appendix (U.S. EPA, 2024a)for additional detail)	0.00401 $(ng/kg/d)^{-1}$

Notes: CI = Confidence Interval; CSF = cancer slope factor; POD = point of departure.

EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) notes that approaches for combining datasets in dose-response modeling may be used when datasets are statistically and biologically compatible. This type of approach was utilized in the CalEPA analysis of kidney cancer (CalEPA, 2021). EPA therefore considered this approach for candidate CSF derivation and performed a sensitivity analysis to derive a CSF_{serum} based on the pooled data from Shearer et al. (2021) and Vieira et al. (2013). These analyses are presented in Appendix E (U.S. EPA, 2024a). However, EPA identified several considerable differences between the two studies, including the outcome measured (RCC versus any kidney cancer) and the exposure metric (measured vs. modeled serum PFOA), among others. Additionally, the slope of the dose-response relationship was very different between the two studies (0.0981, 95% CI: 0.0025, 0.1937 *vs.* 0.0122, 95% CI: 0.0006, 0.0238 from Shearer et al. (2021) and Vieira et al. (2013), respectively). Given these differences, EPA determined that these two studies are not statistically or biologically comparable and therefore, they were not pooled for dose-response modeling (U.S. EPA, 2012a).

4.2.2.2 Animal Toxicological Studies

In the 2016 PFOA HESD (U.S. EPA, 2016c), EPA derived a CSF based on LCTs reported by Butenhoff et al. (2012). At that time, the dose-response relationship for the LCTs observed by Butenhoff et al. (2012) was modeled using EPA's Benchmark Dose Software (BMDS) Version 2.3.1. The multistage cancer model predicted the dose at which a 4% increase in tumor incidence would occur. The 4% increase was chosen as the low end of the observed response range within the Butenhoff et al. (2012) results. EPA has reanalyzed the LCTs reported by Butenhoff et al. (2012) in the current effort using the updated animal and human PK models described in Section 4.1.3 and an updated version of BMDS (Version 3.2). These modeling results are described in Appendix E (U.S. EPA, 2024a). A BMR of 10% was modeled because it is the recommended standard level for comparison across chemicals (U.S. EPA, 2012a). However, for this dataset, a

BMR of 10% resulted in a BMDL value higher than the lowest dose tested (see Appendix E (U.S. EPA, 2024a)). Therefore, a BMR of 4% was ultimately selected because it was representative of the low end of the observed response range within the study results (U.S. EPA, 2012a).

EPA also derived candidate CSFs for the tumor types observed in the NTP study that provide further evidence of carcinogenic activity of PFOA in male Hsd:Sprague-Dawley rats: hepatocellular neoplasms (hepatocellular adenomas and carcinomas) and acinar cell neoplasms (adenomas and adenocarcinomas) of the pancreas (NTP, 2020) (Table 4-13). A BMR of 10% was selected for these tumor types, consistent with the BMD Technical Guidance (U.S. EPA, 2012a). For all tumor types, dichotomous models were used to fit dose-response data.

For LCTs reported by Butenhoff et al. (2012), EPA selected the AUC averaged over the study duration (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, as the dose metric for modeling cancer endpoints. This is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the IRIS Handbook (U.S. EPA, 2022d), which recommend the cumulative dose received over a lifetime as the measure of exposure to a carcinogen when modeling chronic cancer effects. For tumor types reported by NTP (2020), the $C_{avg\_pup\_total}$ was selected for this model to account for the perinatal window of exposure. As discussed previously in Section 4.1.3.1.3, the $C_{avg\_pup\_total}$ metric averages out the concentration in the pup from conception to the end of the 2 years by adding the area under the curve in gestation/lactation to the area under the curve from diet (postweaning) and dividing by 2 years. The BMDS produced BMDLs in mg/L for all tumor types. The animal PODs were converted to POD_{HED}s by multiplying the POD by the human clearance value (Table 4-6). This POD_{HED} is equivalent to the constant exposure, per body weight, which would result in serum concentration equal to the POD at steady state. The candidate CSF is then calculated by dividing the BMR by the POD_{HED}. These modeling results are described further in the Appendix (U.S. EPA, 2024a).

Table 4-13. Candidate Cancer Slope Factors Based on Animal Toxicological Data from 2-year Cancer Bioassays

Tumor Type	Reference, Confidence	Strain/ Species/Sex	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED}	CSF (BMR/POD_{HED})	Notes on Model Selection
Leydig Cell Adenomas in the Testes	Butenhoff et al. (2012) *Medium*	Male Sprague-Dawley Rats	$BMDL_{4RD}$, Multistage Degree 1	27,089.3 AUC_{avg} (mg × d/L)	4.75×10^{-3} mg/kg/day	8.42 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Hepatocellular Adenomas or Carcinoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 2	88.7 ($C_{avg\_pup\_total}$ in mg/L)	1.06×10^{-2} mg/kg/day	9.4 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Hepatocellular Adenomas	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 2	93.0 ($C_{avg\_pup\_total}$ in mg/L)	1.12×10^{-2} mg/kg/day	9.0 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Pancreatic Acinar Cell Adenoma or Adenocarcinoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 3	15.2 ($C_{avg\_pup\_total}$ in mg/L)	1.83×10^{-3}	54.7 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Pancreatic Acinar Cell Adenoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 1	15.7 ($C_{avg\_pup\_total}$ in mg/L)	1.88×10^{-3}	53.2 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.

Notes: AUC = area under the curve; $BMDL_{4RD}$ = benchmark dose level corresponding to the 95% lower confidence limit of a 4% change; $BMDL_{10RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change; BMR = benchmark response; CSF = cancer slope factor; NTP = National Toxicology Program.
[a] See Appendix (U.S. EPA, 2024a) for additional details on benchmark dose modeling.

4.2.3 Overall CSF Selection

Overall, recently published studies and the candidate CSFs indicate that PFOA is a more potent carcinogen than previously understood and described in the 2016 PFOA HESD (U.S. EPA, 2016c). To select an overall CSF, EPA focused on the CSFs derived from the epidemiological data consistent with the IRIS Handbook which states "when both laboratory animal data and human data with sufficient information to perform exposure-response modeling are available, human data are generally preferred for the derivation of toxicity values" (U.S. EPA, 2022d). As with data underlying noncancer RfDs, the use of human data eliminates the uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species which are major uncertainties associated with the PFOA animal toxicological studies due to the half-life differences and sex-specific toxicokinetic differences in rodent species. The use of human data also ensures that the values are based on human-relevant exposure conditions and human-relevant tumor types/sites.

Therefore, EPA selected the critical effect of renal cell carcinomas in human males reported by Shearer et al. (2021) as the basis of the overall CSF for PFOA. Shearer et al. (2021) is a well-conducted, multicenter case-control epidemiological study nested within NCI's PLCO with median PFOA levels relevant to the general U.S. population. The CSF derived from Shearer et al. (2021) was selected as the overall CSF over the CSF derived from Vieira et al. (2013) due to multiple study design considerations. Specifically, Shearer et al. (2021) exhibited several preferred study attributes compared with the Vieira et al. (2013) include specificity in the health outcome considered (RCC vs. any kidney cancer), the type of exposure assessment (serum biomarker vs. modeled exposure), the source population (multicenter vs. Ohio and West Virginia regions), and study size (324 cases and 324 matched controls vs. 59 cases and 7,585 registry-based controls).

The resulting overall CSF for PFOA based on RCC reported by Shearer et al. (2021) is 0.0293 $(ng/kg/day)^{-1}$ (29,300 $(mg/kg/day)^{-1}$).

4.2.4 Application of Age-Dependent Adjustment Factors

EPA's *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* require the consideration of applying age-dependent adjustment factors (ADAFs) to CSFs to address the potential for increased risk for cancer due to early lifestage susceptibility to chemical exposure (U.S. EPA, 2005a, b). Per EPA guidelines, ADAFs are only to be used for carcinogenic chemicals with a mutagenic MOA when chemical-specific data about early-life susceptibility are lacking. For carcinogens with any MOA, including mutagens and non-mutagens, but with available chemical-specific data for early-life exposure, those data should be used.

As described in Section 3.5.3.1.1, most of the studies assessing mutagenicity following PFOA exposure were negative and therefore, PFOA is unlikely to cause tumorigenesis via a mutagenic MOA. Given the lack of evidence of a mutagenic MOA, EPA does not recommend applying ADAFs when quantitatively determining the cancer risk for PFOA (U.S. EPA, 2011a).

EPA additionally evaluated whether there are chemical-specific data for early-life exposure to PFOA and determined that there is insufficient information available from epidemiological and animal toxicological studies to adequately determine whether exposure during early-life periods,

per EPA's above-referenced supplemental guidance, may increase incidence or reduce latency for cancer compared with adult-only exposure. No current studies allow for comparisons of cancer incidence after early-life versus adult-only PFOA exposure. However, there are two studies that assessed cancer risk after PFOA exposure during various developmental stages.

An NTP 2-year cancer bioassay in rats chronically exposed to PFOA both perinatally and postweaning did not report an increased cancer risk compared with chronic postweaning-only exposure (see further study design details in Section 3.4.4.2.1.2 and study results in Section 3.5.2), which suggests no increased cancer risk as a result of lifetime exposure compared with postweaning-only exposure. The NTP cancer bioassay does not include dose groups that were only exposed during early-lifestages (i.e., only during development) and therefore, the findings of the NTP cancer bioassay do not provide a basis for quantitatively estimating the difference in susceptibility between early-life and adult exposures. The other study, by Filgo et al. (2015), reported equivocal evidence of hepatic tumors in three strains of F_1 female mice perinatally treated with PFOA from GD 1–17, with potential residual exposure through lactation, and necropsy at 18 months of age. This study is also limited in that there was no adult-only exposure comparison group, the authors only assessed female mice, and the authors only histopathologically examined the liver (Filgo et al., 2015). In summary, the available studies do not provide information on whether early-life PFOA exposures result in increased cancer incidence compared with adult-only exposure. Due to the lack of evidence supporting postnatal early-life susceptibility to PFOA exposure, EPA did not adjust the risk value using chemical-specific data.

5 Effects Characterization

5.1 Addressing Uncertainties in the Use of Epidemiological Studies for Quantitative Dose-Response Analyses

In the 2016 *Health Effects Support Document for Perfluorooctanoic Acid (PFOA*) and Drinking Water Health Advisory (U.S. EPA, 2016a, c), EPA qualitatively considered epidemiological studies as a supporting line of evidence but did not quantitatively consider them for POD derivation, citing the following as reasons to exclude the epidemiological data that were available at that time from quantitative analyses:

- Unexplained inconsistencies in the epidemiological database,
- The use of mean serum PFOA concentrations rather than estimates of exposure,
- Declining serum PFOA values in the U.S. general population over time (CDC, 2017),
- Uncertainties related to potential exposure to additional PFAS, telomer alcohols that metabolically break down into PFOA, and other bio-persistent contaminants, and
- Uncertainties related to the clinical significance of effects observed in epidemiological studies.

Since 2016, EPA has identified many additional epidemiology studies that have increased the database of information for PFOA (see Sections 3.1.1, 3.4, and 3.5). Further, new tools that have facilitated the use of study quality evaluation as part of systematic review have enabled EPA to systematically assess studies in a way that includes consideration of confounding. As a result, EPA is now in a position to be able to quantitatively consider epidemiological studies of PFOA for POD derivation in this assessment.

In this assessment EPA has assessed the strength of epidemiological and animal evidence following current agency best practices for systematic review (U.S. EPA, 2022d), a process that was not followed in 2016. By performing an updated assessment using systematic review methods, EPA determined that five health outcomes and five epidemiological endpoints within these outcomes (i.e., decreased antibody response to vaccination in children, decreased birthweight, increased total cholesterol, increased ALT, and increased risk of kidney cancer) have sufficient weight of evidence to consider quantitatively. Each endpoint quantified in this assessment has consistent evidence from multiple *medium* and/or *high* confidence epidemiological and animal toxicological studies supporting an association between PFOA exposure and the adverse effect. Each of the endpoints were also specifically supported by multiple *high* and/or *medium* confidence epidemiological studies with low risk of bias in different populations, including general and highly exposed populations. Many of these supporting studies have been published since 2016 and have strengthened the weight of evidence for this assessment.

As described in Section 4.1.3, EPA has improved upon the pharmacokinetic modeling approach used in 2016. Though there are challenges in estimations of human dosimetry from measured or modeled serum concentrations (see Section 5.6.2), EPA has evaluated the available literature and

developed a pharmacokinetic model that estimates PFOA exposure concentrations from the serum PFOA concentrations provided in epidemiological studies, which reduces uncertainties related to exposure estimations in humans. This new approach is supplemented with the uncertainty factor (UF) accounting for intraspecies variation of 10× applied to each POD_{HED}, which accounts for the sensitivities of specific populations, including those that may have increased susceptibility to PFOA toxicity due to differential toxicokinetics.

An additional source of uncertainty in using epidemiological data for POD derivation for chronic, non-developmental effects, is the documented decline in human serum PFOA levels over time, which raises concerns about whether one-time serum PFOA measurements are a good representation of lifetime peak exposure. Because of PFOA's long half-life in serum, however, one-time measurements likely reflect several years of exposure (Lorber and Egeghy, 2011). Importantly, EPA considered multiple time periods when estimating PFOA exposure, ranging from the longest period with available data on PFOA serum levels within the U.S. population (1999–2018) to the shortest and most recent period (2017–2018) (see Appendix E, (U.S. EPA, 2024a)), when performing dose-response modeling of the ALT and TC endpoints in the epidemiological data. EPA selected PODs for these two endpoints using PFOA exposure estimates based on the serum PFOA data for 1999–2018, which is likely to capture the peak PFOA exposures in the United States, which occurred in the 1990's (Dong et al., 2019). The modeling results show that the BMDL estimates for increased TC derived using the longest range of exposure data (1999-2018) are consistently lower than those based on the 2017–2018 PFOA exposure data whereas for ALT, the BMDL estimates using data from the longest exposure period are consistently higher than those based on the 2017–2018 PFOA exposure data. Given these analyses, it appears that selection of one exposure time period over another does not predictably impact the modeling results. Therefore, for this assessment, EPA consistently selected the time periods more likely to capture peak PFOA exposures (e.g., 1999–2018) as the basis of BMDL estimates for all endpoints of interest (see Appendix E, (U.S. EPA, 2024a)).

It is plausible that observed associations between adverse health effects and PFOA exposure could be explained in part by confounding from other PFAS exposures, including the metabolism of precursor compounds to PFOA in the human body. However, mixture analysis remains an area of emerging research (Taylor et al., 2016), and there is no scientific consensus yet for the best approach to account for exposure by co-occurring PFAS. Additionally, multipollutant analyses from studies included in this assessment did not provide direct evidence that associations between exposure to PFOA and health effects are confounded by or are fully attributable to confounding by co-occurring PFAS. A detailed discussion of statical approaches for accounting for co-occurring PFAS and results from studies performing multipollutant analysis is provided in Section 5.1.1. For an extended review of the uncertainties associated with PFAS co-exposures, see the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

Additionally, there is uncertainty about the magnitude of the contribution of PFAS precursors to PFOA serum concentrations, especially as biotransformation efficiency appears to vary depending on the precursor of interest (Mcdonough et al., 2022; D'eon and Mabury, 2011; Lorber and Egeghy, 2011; Vestergren et al., 2008). The contributions of PFAS precursors to serum concentrations also varies between populations with differing PFAS exposure histories

(i.e., individuals living at or near sites with aqueous film-forming foam use may have different precursor PFOA contributions than the general population).

In addition, some populations may be disproportionately exposed to other contaminants, such as polychlorobiphenyls and methylmercury. To address this, EPA quantified associations between PFOA serum concentrations and endpoints of interest in populations with varying exposure histories, including the general population and high-exposure communities. EPA observed associations for several endpoints in populations known to have been predominantly exposed to PFOA (e.g., C8 Health Project participants), reducing the uncertainty related to potential confounding of other contaminants, including PFAS precursor compounds. These sensitivity analyses are supportive of EPA's conclusions regarding the effects of PFOA reported across many epidemiological studies.

In this assessment, studies were not excluded from consideration based primarily on lack of or incomplete adjustments for potential confounders including socioeconomic status (SES) or race/ethnicity. A small number of studies examining PFAS serum levels across SES and racial/ethnic groups were identified, many of which reported on a national scale (e.g., using NHANES data). The identified studies (most from the early-mid 2000's) reported that serum concentrations of PFOA were lower among people of color on average nationwide (Buekers et al., 2018; Kato et al., 2014; Nelson et al., 2012; Calafat et al., 2007). However, certain races/ethnicities may have relatively higher serum concentrations than others depending on the geographic location and the specific PFAS sampled (Park et al., 2019c). EPA acknowledges that in observational epidemiological studies, potential residual confounding may result from complexities related to SES and racial/ethnic disparities. Additional racially and ethnically diverse studies in multiple U.S. communities are needed to fill this important data gap. The Appendix (U.S. EPA, 2024a) provides detailed information on the available epidemiological studies and identifies the study-specific confounding variables that were considered, such as SES.

Lastly, the potential uncertainty related to the clinical significance of effects observed in the PFOA epidemiological studies is sometimes cited for dismissing the epidemiological data quantitatively. However, as described in Section 4.1.1, the four selected critical effects (i.e., decreased antibody response to vaccination, increased serum ALT, increased TC, and decreased birthweight) are biologically significant effects and/or precursors to disease (e.g., CVD), which, according to agency guidance and methods, both warrant consideration as the basis of RfDs for PFOA (U.S. EPA, 2022d, 2005a, 2002b). EPA's *A Review of the Reference Dose and Reference Concentration Processes*, states that a reference dose (RfD) should be based on an adverse effect or a precursor to an adverse effect (e.g., increased risk of an adverse effect occurring) (U.S. EPA, 2002b). Also, at the individual level, the interpretation and impact of small magnitude changes in endpoints such as increased TC, increased ALT, decreased birth weight, and decreased antibody response to vaccination may be less clear. However, at the population level, even small magnitude changes in these effects will shift the distribution in the overall population and increase the number of individuals at risk for diseases, such as cardiovascular disease and liver disease(Gilbert and Weiss, 2006).

There are challenges associated with quantitative use of epidemiological data for risk assessment (Deener et al., 2018) as described above; however, improvements such as methodological advancements that minimize bias and confounding, strengthened methods to estimate and

measure exposure, and updated systematic review practices facilitate the use of epidemiological studies to quantitatively inform risk.

5.1.1 Uncertainty due to Potential Confounding by Co-Occurring PFAS

5.1.1.1 PFAS Co-exposure Statistical Approaches and Confounding Analysis

A potential source of uncertainty in epidemiologic studies examining associations between a particular PFAS and health outcomes is confounding by other co-occurring PFAS. In studies of PFOA, such confounding may occur if there are other PFAS that are moderately or highly correlated with PFOA, associated with the outcome of interest, and not on the causal pathway between PFOA and the outcome. If the association between co-occurring PFAS and the outcome is in the same direction as the association between PFOA and that outcome, the anticipated direction of bias resulting from not accounting for other PFAS would be away from the null. For an extended review of the uncertainties associated with PFAS co-exposures, see the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

Several statistical methods are used to estimate associations while accounting for potential confounding by co-occurring PFAS and other pollutants. One common approach is to include co-occurring PFAS as covariates in regression models. This approach allows for an estimation of the association between PFOA and the outcome of interest, adjusted for other covariates and the co-pollutants. Another approach is to screen large groups of exposures to identify which ones are most strongly related to the outcome, using principal components analysis, elastic net regression, and Bayesian kernel machine regression (BKMR). Each of these approaches has strengths and limitations. For example, when PFOA and the co-pollutants are highly correlated, then multipollutant models could be affected by multicollinearity or result in amplification bias, rather than reduce confounding bias compared with single pollutant models (Weisskopf et al., 2018). Additionally, accounting for a variable in a multivariable regression model that is not a significant predictor of the response variable reduces the degrees of freedom and effectively dilutes the significance of the other exposure variables that are predictors of the response. The use of screening approaches, while effective at accounting for co-pollutants, can result in estimates that are not easily interpretable and make it difficult to differentiate the impact and contribution of individual PFAS (Meng et al., 2018). Mixture analysis is an emerging research area (Liu et al., 2022; Taylor et al., 2016), and there is no scientific consensus yet on the best approach for estimating independent effects of PFOA within complex PFAS mixtures.

In this assessment, the risk of bias due to confounding by co-occurring PFAS was considered as part of the study quality evaluation process. To further support the assessment, Section 5.1.1.2 below summarizes evidence from *high* and *medium* confidence studies that included at least one of the approaches described above (hereafter referred to collectively as "multipollutant models") to account for co-pollutants, in order to assesses the extent to which there may be confounding by other PFAS in studies reporting the associations between PFOA and birth weight.

5.1.1.2 Multipollutant Models of PFOA and Birth Weight

When assessing the associations between PFOA and a health effect of interest (e.g., decreased birth weight), there is concern for potential confounding by other PFAS when there is a strong correlation between the occurrence of PFOA and another PFAS and when the magnitude of the association between the co-exposure and the health effect is large.

In order to identify the most co-occurring PFAS, Table 5-1 shows correlations between PFOA and other PFAS exposures in the nine studies on PFOA and birth weight that included mutually adjusted models. Four of these studies are *medium* confidence studies (Meng et al., 2018; Woods et al., 2017; Lenters et al., 2016; Robledo et al., 2015) and five are *high* confidence studies (Luo et al., 2021; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017). Moderately positive correlations (around 0.6) between PFOA and PFOS were consistently observed in six of the seven studies that reported such information. Correlations between PFOA with other commonly examined PFAS, including PFNA (four studies), PFDA (four studies), and PFHxS (five studies), were less consistent but generally weaker than correlations with PFOS, suggesting that other PFAS may not consistently co-occur with PFOA.

Table 5-1. Correlation Coefficients Between PFOA and Other PFAS in Mutually Adjusted Studies

Reference	Study Setting	Correlations with PFOA			
		PFOS	PFNA	PFDA	PFHxS
Ashley-Martin et al. (2017)[a] *High*	Canada (10 cities)	0.59	–[b]	–	0.47
Luo et al. (2021)[a] *High*	Guangzhou, China	0.11	0.28	0.19	0.02
Manzano-Salgado et al. (2017a)[c] *High*	Gipuzkoa, Sabadell, and Valencia, Spain	NR	NR	NR	NR
Shoaff et al. (2018)[d] *High*	Cincinnati, Ohio, USA	0.60	–	–	–
Starling et al. (2017)[e] *High*	Colorado, USA	0.68	0.76	0.56	0.61
Lenters et al. (2016)[e] *Medium*	Greenland; Kharkiv, Ukraine; Warsaw, Poland	0.61	0.30	0.50	0.34
Meng et al. (2018)[d] *Medium*	Denmark	0.66	0.47	0.28	0.33
Robledo et al. (2015)[c] *Medium*	Michigan and Texas, USA	NR	NR	NR	NR
Woods et al. (2017)[f] *Medium*	Cincinnati, Ohio, USA	+[g]	+	+	+

Notes: NR = not reported.
Shaded cells indicate analytes for which a correlation with PFOA was not measured or reported.
[a] Pearson correlation of log_{10}-transformed (Ashley-Martin et al., 2017) and ln-transformed (Luo et al., 2021) PFAS values.
[b] Analyte not measured.
[c] Correlation coefficients not reported.
[d] Pearson correlation of PFAS values, unclear if transformed prior to correlation analysis.
[e] Spearman rank correlation of PFAS values.
[f] Correlation type not specified.

[g] Correlations not reported numerically. Heat map of correlation coefficients (Figure S2, in Woods et al. (Woods et al., 2017)) shows positive correlations between PFOA and PFOS, PFNA, PFHxS, and PFDA, ranging from about 0.6 to about 0.1, respectively.

Results from mutually adjusted models are summarized and compared in Table 5-2. The statistical approaches for accounting for PFAS co-exposures varied across the studies. Six studies included at least one additional PFAS as a predictor in ordinary least squares (OLS) regression models (Meng et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017; Robledo et al., 2015). Woods et al. (Woods et al., 2017) included multiple PFAS as predictors in a Bayesian hierarchical linear model. Three studies (Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016) used elastic net regression and one study used BKMR (Luo et al., 2021). The impact of other PFAS adjustment on the association between PFOA and birth weight is evaluated by comparing the magnitude and direction of the effects from the single-PFOA model (when available) to those from mutually adjusted models.

Six studies provided results from both single and multipollutant models (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016). Multipollutant models in these studies included PFOS but varied with respect to other PFAS considered (Table 5-2). Lenters et al. (Lenters et al., 2016) also adjusted for other types of chemicals (such as phthalates and organochlorides) in addition to several PFAS. Generally, the direction of effect estimates remained the same following adjustment for other PFAS, but precision was reduced. None of the studies that showed birth weight deficits in single-pollutant models reported greater or more precise results following statistical adjustment for other PFAS.

Starling et al. (Starling et al., 2017) observed a statistically significant association between PFOA and birth weight reductions in the single pollutant model. This association increased in magnitude but precision was decreased in the multipollutant OLS model with four other PFAS. PFOA was also retained in the elastic net regression model, showing an inverse relationship with birth weight, but the association was attenuated. Lenters et al. (Lenters et al., 2016) reported associations between PFOA and reduced birth weight in single pollutant OLS and in elastic net regression models with PFOA retained but the association attenuated. Luo et al. (Luo et al., 2021) observed non-significant inverse associations between PFOA and birth weight in single pollutant and in BKMR models. Manzano-Salgado et al. (Manzano-Salgado et al., 2017a) and Shoaff et al. (Shoaff et al., 2018) reported null results in single and in multi-PFAS regression models. Meng et al. (Meng et al., 2018) observed an association between PFOA and reduced birth weight in the single pollutant model; this association was attenuated in a multipollutant model with PFOS.

Three studies provided results only from multipollutant models (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015), thus making assessment of impact of co-pollutants difficult. Ashley-Martin et al. (Ashley-Martin et al., 2017) and Robledo et al. (Robledo et al., 2015) reported non-significant inverse associations between PFOA and birth weight in girls in multipollutant models. Woods et al. (Woods et al., 2017) reported on an overlapping population from the same HOME cohort as Shoaff et al. (Shoaff et al., 2018) and observed non-significant inverse associations from a multipollutant Bayesian hierarchical linear model. PFOA was not selected for inclusion in an elastic net regression model that included other endocrine-disrupting chemicals in addition to PFAS.

In summary, in the six studies that included both single and multipollutant models, associations were often attenuated following adjustment for other PFAS (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016). Three additional studies presented results from multipollutant models only, making it difficult to determine the extent to which confounding by other PFAS may have impacted the PFOA birth weight associations (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015). Considering all nine studies together, it is challenging to draw definitive conclusions about the extent of confounding by co-occurring PFAS, particularly given differences in modeling approaches, PFAS adjustment sets, and exposure contrasts used across studies. Additionally, these studies represented only a small fraction of the total number of studies examining associations between PFOA and birth weight and it is unclear whether their results are generalizable to the broader evidence base. Although it is an important source of uncertainty, there is no evidence in the entirety of the large evidence base that the observed associations between PFOA and birth weight deficits are fully attributable to confounding by co-occurring PFAS.

Similar conclusions can be drawn for other health outcomes. Budtz-Jorgensen (Budtz-Jørgensen and Grandjean, 2018) evaluated the possibility of confounding across PFAS in analyses of decreased antibody response. The study reported significant decreases in the antibody response with elevated PFOA exposure, and there was no notable attenuation of the observed effects after estimates were adjusted for PFOS (see Section 3.4.2.1.2.1) (Budtz-Jørgensen and Grandjean, 2018). A limited number of studies performed co-exposure analyses for increased ALT and increased TC in adults. Lin et al. (Lin et al., 2010) performed multipollutant modeling for the effects on serum ALT and observed that when PFOS, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, the significant positive association between PFOA exposure and ALT remained and was slightly larger. Fan et al. (Fan et al., 2020) examined cross-sectional associations between exposure to PFOA and increased TC in single- and multipollutant models in a sample of adults from NHANES (2012–2014). Exposure to PFOA was associated with statistically significantly elevated TC in the single-pollutant model, but the association was no longer significant in multipollutant analyses. A statistically significant positive association was also observed for PFAS mixture and TC in WQS regression analyses (Fan et al., 2020).

Overall, there is no evidence that the consistently observed associations between exposures to PFOA and the four priority noncancer health outcomes are confounded by or are fully attributable to confounding by co-occurring PFAS.

Table 5-2. Impact of Co-Exposure Adjustment on Estimated Change in Mean Birth Weight (grams) per Unit Change (ng/mL) in PFOA Levels.

Reference	Single PFAS Model Result (95% CI)[a,b]	Multi-PFAS Model Result (95% CI)[a,b]	Elastic Net Regression Result[b]	Exposure Comparison	Effect of Other PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Ashley-Martin et al. (Ashley-Martin et al., 2017) *High*	NR	Girls: −89.51 (−263.40, 84.38) Boys: −35.51 (−198.99, 127.97)	–[c]	log_{10}-unit (ng/mL) increase	–	PFOS, PFHxS
Luo et al. (Luo et al., 2021) *High*	−62.37 (−149.08, 24.35)	−24 (−84, 36)[d]	–	Single PFAS model: ln-unit (ng/mL) increase Multi-PFAS model: 75th vs. 25th percentile	Attenuated	PFOS, PFBA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, 6:2 Cl-PFESA, 8:2 Cl-PFESA
Manzano-Salgado et al. (Manzano-Salgado et al., 2017a) *High*	−9.33 (−38.81, 20.16)	1.02 (−42.73, 44.77)	–	log_2-unit (ng/mL) increase	Slightly attenuated	PFOS, PFNA, PFHxS
Shoaff et al. (Shoaff et al., 2018) *High*	−0.03 (−0.17, 0.10)[e]	0.00 (−0.16, 0.18)[e]	–	log_2-unit (ng/mL) increase	Slightly attenuated	PFOS, PFNA, PFHxS
Starling et al. (Starling et al., 2017) *High*	−51.4 (−97.2, −5.7)	−69.66 (−148.19, 8.87)	−14.47	ln-unit (ng/mL) increase	Attenuated	PFOS, PFNA, PFDA, PFHxS
Lenters et al. (Lenters et al., 2016) *Medium*	−78.52 (−137.01, −20.03)	–	−38.82	2 SD ln-unit (ng/mL) increase	Attenuated	PFOS, PFNA, PFDA, PFHxS, PFHpA, PFUnDA, PFDoDA
Meng et al. (Meng et al., 2018)[f] *Medium*	−35.6 (−66.3, −5.0)	−9.94 (−52.63, 32.75)	–	log_2-unit (ng/mL) increase	Attenuated	PFOS

Reference	Single PFAS Model Result (95% CI)[a,b]	Multi-PFAS Model Result (95% CI)[a,b]	Elastic Net Regression Result[b]	Exposure Comparison	Effect of Other PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Robledo et al. (Robledo et al., 2015)[g] *Medium*	NR	Girls: −61.64 (−159.15, 35.87) Boys: 4.78 (−85.44, 95.01)	–	1 SD ln-unit (ng/mL) increase	–	PFOS, PFDA, PFNA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH
Woods et al. (Woods et al., 2017) *Medium*	NR	−13 (−54, 27)[h]	N/S	log_{10}-unit (ng/mL) increase	–	PFOS, PFNA, PFDA, PFHxS

Notes: N/S = PFOA not selected in elastic net regression model; SD = standard deviation.
[a] From ordinary least squares regression models unless otherwise specified.
[b] Outcome variable unit is grams unless otherwise specified.
[c] Not applicable.
[d] Results estimated from Luo et al. (Luo et al., 2021) Figure 3 using WebPlotDigitizer. Results are from a Bayesian kernel machine regression model comparing the PFOA at its 75th vs. 25th percentile, holding other PFAS constant at their 50th percentiles.
[e] Outcome variable unit in Shoaff et al. (Shoaff et al., 2018) models is birth weight *z*-score.
[f] Meng et al. (Meng et al., 2018) estimates associations between serum PFOA and birth weight in three samples of the Danish National Birth Cohort, two of which were analyzed by the same laboratory for PFOA, PFOS, PFDA, PFNA, PFHxS, and PFHpS and one of which was analyzed by a separate laboratory for PFOA and PFOS only.
[g] Robledo et al. (Robledo et al., 2015) estimated associations using both maternal and paternal PFAS concentrations; results shown here are from maternal PFAS models, also adjusted for "the individual and partner sum of remaining chemical concentrations in each chemical's respective class."
[h] Effect estimates and posterior 95% credible intervals based on a Bayesian hierarchical linear model. Results estimated from Woods et al. (Woods et al., 2017) Figure 1 using WebPlotDigitizer.

5.2 Comparisons Between Toxicity Values Derived from Animal Toxicological Studies and Epidemiological Studies

As recommended by the SAB (U.S. EPA, 2022e), EPA derived candidate RfDs and CSFs for multiple health outcomes using data from both epidemiological and animal toxicological studies. Candidate RfDs from epidemiological and animal toxicological studies within a health outcome differed by approximately two to three orders of magnitude (see Figure 4-, with epidemiological studies producing lower values. EPA does not necessarily expect concordance between animal and epidemiological studies in terms of the adverse effect(s) observed, as well as the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" (U.S. EPA, 1991). EPA further describes these factors in relation to this assessment below.

First, there are well-established differences in the toxicokinetics between humans and animal models such as rats and mice. As described in Section 3.3.1.4.5, PFOA half-life estimates vary considerably by species, being lowest in rodents (hours to days) and several orders of magnitude higher in humans (years). All candidate toxicity values based on animal toxicological studies were derived from studies conducted in rats or mice, adding a potential source of uncertainty related to toxicokinetic differences in these species compared with humans. For PFOA, sex-specific differences in the toxicokinetics of rats is an additional source of uncertainty. To address toxicokinetic differences between species and sexes, EPA utilized a pharmacokinetic (PK) model to estimate the internal dosimetry of each animal model and convert the values into predicted levels of human exposure that would result in the corresponding observed health effects. However, the outputs of these models are *estimates* and may not fully account for species-specific toxicokinetic differences, particularly differences in excretion. The application of uncertainty factors (i.e., UF_A) also may not precisely reflect animal-human toxicokinetic differences.

Second, candidate toxicity values derived from epidemiological studies are based on responses associated with actual environmental exposure levels, whereas animal toxicological studies are limited to the tested dose levels that are often several orders of magnitude higher than the ranges of exposure levels in humans. Extrapolation from relatively high experimental doses to environmental exposure levels introduces a potential source of uncertainty for toxicity values derived from animal toxicological studies; exposures at higher dose levels could result in different responses, perhaps due to differences in mechanisms activated, compared with responses to lower dose levels. One example of this is the difference between epidemiological and animal toxicological studies in the effect of PFOA exposure on serum lipid levels (i.e., potential non-monotonic dose-response relationships that are not easily assessed in animal studies due to low dose levels needed to elicit the same response observed in humans).

Third, there may be differences in mechanistic responses between humans and animal models. One example of this is the PPARα response. It is unclear to what extent PPARα influences the responses to PFOA exposure observed in humans, though it has been shown that the rodent PPARα response is greater than that observed in humans (see Section 3.4.1.3.1). Mechanistic differences could influence dose-response relationships and subsequently result in differences

between toxicity values derived from epidemiological and animal toxicological studies. There may be additional mechanisms that differ between humans and animal models that could contribute to the magnitude of responses and doses required to elicit responses across species.

The factors described above represent some but not all potential contributors that may explain the differences between toxicity values derived from epidemiological and animal toxicological studies. In this assessment, EPA prioritized epidemiological studies of *medium* or *high* confidence for the selection of health outcome-specific and overall RfDs and CSFs (see Section 4.1.6). The use of human data to derive toxicity values removes uncertainties and assumptions about human relevance inherent in extrapolating from and interpreting animal toxicological data in quantitative risk assessment.

5.3 Updated Approach to Animal Toxicological RfD Derivation Compared with the 2016 PFOA HESD

For POD derivation in this assessment, EPA considered the studies identified in the recent literature searches and also re-examined the candidate RfDs derived in the 2016 PFOA HESD (U.S. EPA, 2016c) and the animal toxicological studies and endpoints on which they were based. The updated approach used for hazard identification and dose response in the current assessment as compared with the 2016 PFOA HESD led to some differences between animal toxicological studies and endpoints used as the basis of candidate RfDs for each assessment. These updates and the resulting differences are further described below.

For the 2016 PFOA HESD, EPA did not use BMD modeling to derive PODs, and instead relied on the NOAEL/LOAEL approach for all candidate studies and endpoints (U.S. EPA, 2016c). The NOAEL/LOAEL approach allows for the incorporation of multiple endpoints from a single study to derive a single POD, if the endpoints have the same NOAEL and/or LOAEL. For example, in the 2016 PFOA HESD, EPA derived a candidate RfD based on the endpoints of decreased parental body weight and increased parental absolute and relative kidney weight reported by Butenhoff et al. (Butenhoff et al., 2004a), all of which shared a common POD (LOAEL). For the current assessment, EPA preferentially used BMD modeling to derive PODs because it allows for greater precision than the NOAEL/LOAEL approach and considers the entire dose-response curve. This approach requires the consideration of endpoints on an individual basis and further examination of the weight of evidence for particular endpoints, as well as the dose-response relationship reported for each endpoint, in order to derive a BMDL. When considering an effect on a standalone basis rather than together with other effects occurring at the same exposure level, EPA sometimes determined the weight of evidence was not sufficient to consider an individual endpoint for POD derivation. For the current assessment, EPA used a systematic review approach consistent with the IRIS Handbook (U.S. EPA, 2022d) to consider the weight of evidence for both the health outcomes as well as for individual endpoints of interest when selecting endpoints and studies for dose-response modeling. In the case of the endpoints selected in 2016 from the Butenhoff et al. (Butenhoff et al., 2004a) study, systemic effects such as body weight and renal effects such as kidney weight were reevaluated and determined to have *evidence suggestive* of an association with PFOA exposure. As described in Section 4.1.1 of this assessment, EPA derived PODs only for endpoints from health outcomes with *evidence indicating* or *evidence demonstrating* an association with PFOA exposure.

Additionally, for the current assessment, EPA preferentially selected endpoints for which there were a greater number of studies supporting the observed effect. For example, for the 2016 PFOA HESD, EPA derived a candidate RfD based on the co-critical effect of accelerated male puberty reported by Lau et al. (Lau et al., 2006). Results of the current assessment's literature search showed that no *high* or *medium* confidence studies supporting that observed effect have been published since 2016. As Lau et al. (Lau et al., 2006) was also the only study identified in 2016 that reported an acceleration of male puberty (a second study reported a delay in male puberty (Butenhoff et al., 2004a) and there were several other developmental endpoints (e.g., reduced offspring weight and survival, delayed eye opening) that were supported by multiple studies, EPA did not further consider this endpoint from Lau et al. (Lau et al., 2006) for POD derivation in the present assessment. Similarly, upon further evaluation during the current assessment of the co-critical effects of reduced forelimb and hindlimb ossification in pups reported by Lau et al. (Lau et al., 2006), it was determined that an unexplained non-linear dose-response trend adds uncertainty to selection of the LOAEL as the POD. As reduced ossification was only observed at the highest dose tested (10 mg/kg/day) by the one other study (Yahia et al., 2010) that tested dose levels close to the LOAEL from Lau et al. (Lau et al., 2006) (1 mg/kg/day) and because no studies identified during literature searches for the current assessment reported this effect, EPA relied on other endpoints from Lau et al. (Lau et al., 2006) that were amenable to BMD modeling, exhibited dose-dependent response trends, and were supported by at least one other study in the available literature.

For some health effects that served as the basis for candidate RfDs in the 2016 PFOA HESD, new studies published since 2016 provide more information about these same endpoints. For example, in 2016, EPA derived a candidate RfD based on increased liver weight and necrosis in rats reported by Perkins et al. (Perkins et al., 2004). Since that time, NTP (NTP, 2020) published an animal bioassay that has additional or improved study attributes compared to the older study. Specifically, the NTP (NTP, 2020) study was identified as a *high* confidence study (rather than *medium* confidence) that used a chronic (rather than 14-week) exposure duration, larger sample sizes (n = 50 rather than n = 15), and a dose range that was more sensitive to the histopathological effects in both male and female rats. Therefore, EPA considered liver necrosis data as reported by NTP (NTP, 2020) for POD derivation rather than data from the *medium* confidence study by Perkins et al. (Perkins et al., 2004).

For transparency, EPA has provided a comparison of studies and endpoints used to derive candidate RfDs for both the 2016 PFOA HESD and the present assessment (Table 5-3).

Table 5-3. Comparison of Candidate RfDs Derived from Animal Toxicological Studies for Priority Health Outcomes[a]

Studies and Effects Used in 2016 for Candidate RfD Derivation[b]	Studies and Effects Used in 2024 for Candidate RfD Derivation
Immune	
Dewitt et al. (Dewitt et al., 2008), *medium* confidence – reduced immunoglobulin M (IgM) response	Dewitt et al. (Dewitt et al., 2008), *medium* confidence – reduced IgM response
Developmental	

Studies and Effects Used in 2016 for Candidate RfD Derivation[b]	Studies and Effects Used in 2024 for Candidate RfD Derivation
Lau et al. (Lau et al., 2006), *medium* confidence – reduced pup ossification (forelimb and hindlimb) and accelerated male puberty (preputial separation)	Lau et al. (Lau et al., 2006), *medium* confidence – delayed time to eye opening
Wolf et al. (Wolf et al., 2007), *medium* confidence – decreased pup body weight	Song et al. (Song et al., 2018), *medium* confidence – decreased pup survival
Hepatic	
Perkins et al. (Perkins et al., 2004), *medium* confidence – increased liver weight and necrosis	NTP (NTP, 2020), *high* confidence – liver necrosis

Notes: RfD = reference dose; IgM = immunoglobulin M; NTP = National Toxicology Program.
[a] Note that candidate RfDs for the fourth priority noncancer health outcome (i.e., cardiovascular) are not presented in this table because candidate RfDs based on animal toxicological studies representing this health outcome were not derived in the 2016 PFOA HESD or the current assessment.
[b] Candidate RfDs from the 2016 PFOA HESD that correspond to non-priority health outcomes (e.g., renal) are not presented here.

5.4 Consideration of Alternative Conclusions Regarding the Weight of Evidence of PFOA Carcinogenicity

While reviewing the weight of evidence for PFOA, EPA also evaluated consistencies of the carcinogenicity database with other cancer descriptors according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). In the 2016 PFOA HESD, EPA determined that the available carcinogenicity database for PFOA at that time was consistent with the descriptions for *Suggestive Evidence of Carcinogenic Potential* (U.S. EPA, 2016c). Upon reevaluation for this assessment, the agency identified several new studies reporting on cancer outcomes that strengthened the evidence. As a result of conducting a weight of evidence evaluation of the available carcinogenicity database, EPA determined that PFOA is consistent with the descriptions for *Likely to Be Carcinogenic to Humans* according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), as described above. More specifically, the available data for PFOA surpass many of the descriptions for *Suggestive Evidence of Carcinogenic Potential* provided in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The examples for which the PFOA database exceeds the *Suggestive* descriptions (outlined below) include:

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans.' The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system (see discussions of conflicting evidence and differing results, below);
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend." (U.S. EPA, 2005a).

There are multiple *medium* or *high* confidence human and animal toxicological studies that provide evidence of multiple tumor types resulting from exposure to PFOA. The observed tumor types are generally consistent across human subpopulations (i.e., kidney (Shearer et al., 2021; Vieira et al., 2013) and testicular (Barry et al., 2013; Vieira et al., 2013)) and studies of equal confidence did not provide conflicting evidence for these cancer types. Studies within the same species of rat consistently report multisite tumorigenesis (i.e., testicular, pancreatic, and hepatic (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001)) and there is no indication that a high background incidence or other intrinsic factors related to these tumor types are driving the observed responses. The SAB PFAS Review Panel agreed that: "a) the evidence for potential carcinogenicity of PFOA has been strengthened since the 2016 PFOA HESD; b) the results of human and animal studies of PFOA are consistent with the examples provided above and support a designation of 'likely to be carcinogenic to humans'; and c) the data exceed the descriptors for the three designations lower than 'likely to be carcinogenic'" (U.S. EPA, 2022e). See Table 5-4 below for specific details on how PFOA exceeds the examples supporting the *Suggestive Evidence of Carcinogenic Potential* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

While the SAB panel agreed that the data for PFOA exceed a *Suggestive* cancer descriptor, the final report also recommends "explicit description of how the available data for PFOA do not meet the criteria for the higher designation as 'carcinogenic'" (U.S. EPA, 2022e). After reviewing the descriptions of the descriptor *Carcinogenic to Humans,* EPA has determined that at this time, the evidence supporting the carcinogenicity of PFOA does not warrant a descriptor exceeding *Likely to Be Carcinogenic to Humans*. The *Guidelines* indicate that a chemical agent can be deemed *Carcinogenic to Humans* if it meets all of the following conditions:

- "there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and
- there is extensive evidence of carcinogenicity in animals, and
- the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and
- there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information" (U.S. EPA, 2005a).

As discussed in Section 3.5.5, convincing epidemiological evidence supporting a causal association between human exposure to PFOA and cancer is currently lacking. The SAB similarly concluded that "the available epidemiologic data do not provide convincing evidence of a causal association but rather provide evidence of a plausible association, and thus do not support a higher designation of 'carcinogenic to humans'" (U.S. EPA, 2022e).

Additionally, though the available evidence indicates that there are positive associations between PFOA and multiple cancer types, there is uncertainty regarding the identification of carcinogenic MOA(s) for PFOA, particularly for renal cell carcinomas and testicular cancer in humans. The evidence of carcinogenicity in animals is limited to a single strain of rat, although PFOA tested positive for multisite tumorigenesis. The animal and mechanistic databases do not provide clarity to discern the MOA(s) of PFOA in humans, though there is some animal toxicological study

evidence supporting hormone-mediated MOAs for testicular tumors and oxidative stress-mediated MOAs for pancreatic tumors. The full mode of action analysis, including in-depth discussions on the potential MOAs for kidney and testicular tumors, as well as discussions on the potential MOAs and human relevance for pancreatic and liver tumors observed in rats, is presented in Section 3.5.4.2. See Table 5-4 below for specific details on how PFOA does not align with the examples supporting the *Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 5-4. Comparison of the PFOA Carcinogenicity Database with Cancer Descriptors as Described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

Comparison of Evidence for *Carcinogenic* and *Suggestive* Cancer Descriptors	
Carcinogenic to Humans	
"This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** There is evidence of a plausible association between PFOA exposure and cancer in humans, however, the database is limited to only two independent populations, there is uncertainty regarding the potential confounding of other PFAS, and there is limited mechanistic information that could contribute to the determination of a causal relationship.
Or, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* of the following conditions are met:	
"There is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association," (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** There is evidence of an association between human exposure and cancer, however, there is limited mechanistic information that could contribute to the determination of a causal relationship.
"There is extensive evidence of carcinogenicity in animals," (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** While there are three chronic cancer bioassays available, each testing positive in at least one tumor type, they were all conducted in the same strain of rat. The database would benefit from additional *high* confidence chronic studies in other species and/or strains.
"The mode(s) of carcinogenic action and associated key precursor events have been identified in animals and" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** A definitive MOA has not been identified for each of the PFOA-induced tumor types identified in rats.
"There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** The animal database does not provide significant clarity on the MOA(s) of PFOA in humans, though there is some evidence supporting hormone-mediated MOAs for testicular tumors and oxidative stress-mediated MOAs for pancreatic tumors.
Suggestive Evidence of Carcinogenic Potential	
"A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system" (U.S. EPA, 2005a).	**PFOA data exceed this description.** Statistically significant increases in tumor incidence of multiple tumor types were observed across several human and animal toxicological studies.

Comparison of Evidence for *Carcinogenic* and *Suggestive* Cancer Descriptors	
"A small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed" (U.S. EPA, 2005a).	**This description is not applicable to the tumor types observed after PFOA exposure.**
"Evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships)" (U.S. EPA, 2005a).	**PFOA data exceed this description.** The studies from which carcinogenicity data are available were determined to be *high* or *medium* confidence during study quality evaluation.
"A statistically significant increase at one dose only, but no significant response at the other doses and no overall trend" (U.S. EPA, 2005a).	**PFOA data exceed this description.** Increases in kidney cancer in humans were statistically significant in two exposure groups in one study (Vieira et al., 2013), and there was a statistically significant increased odds for the highest exposure quartile and an increasing trend across exposure quartiles in a second study (Shearer et al., 2021). Statistically significant increases in hepatic and pancreatic tumors in male rats were observed in multiple dose groups with a statistically significant trend overall (NTP, 2020).

Notes: MOA = mode of action.

5.5 Health Outcomes with Evidence Integration Judgments of *Evidence Suggests* Bordering on *Evidence Indicates*

EPA evaluated 16 noncancer health outcomes as part of this assessment. In accordance with recommendations from the SAB (U.S. EPA, 2022e) and the IRIS Handbook (U.S. EPA, 2022d), for both quantitative and qualitative analyses in the final assessment, EPA prioritized health outcomes with either *evidence demonstrating* or *evidence indicating* associations between PFOA exposure and adverse health effects. Health outcomes reaching these tiers of judgment were the hepatic, immune, developmental, cardiovascular, and cancer outcomes. Some other health outcomes were determined to have *evidence suggestive* of associations between PFOA and adverse health effects as well as some characteristics associated with the *evidence indicates* tier, and EPA made judgments on these health outcomes as described below.

For PFOA, two health outcomes that had characteristics of both *evidence suggests* and *evidence indicates* were the reproductive and endocrine outcomes. Endpoints relevant to these two health outcomes had been previously considered for POD derivation in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). However, upon further examination using the protocols for evidence integration outlined in Appendix A (U.S. EPA, 2024a) and Section 2.1.5, EPA concluded that the available epidemiological and animal toxicological evidence did not meet the criteria recommended for subsequent quantitative dose-response analyses. Although these health outcomes were not prioritized in the current assessment, based on the available data, EPA concluded that PFOA exposure may cause adverse reproductive or endocrine effects.

Epidemiological studies published since the 2016 PFOA HESD considered for evidence integration for adverse endocrine effects included many *high* and *medium* confidence studies. There was *slight evidence* to suggest human endocrine toxicity, including associations between PFOA exposure and changes in serum thyroxine (T4) in children, though there was considerable uncertainty in the results due to inconsistencies across sexes and age groups and a limited number of studies. Animal toxicological studies considered for evidence integration included eight *high* or *medium* confidence studies. Collectively, the animal evidence for an association between PFOA exposure and effects on the endocrine system was considered *moderate*, based on observed alterations in thyroid and adrenocortical hormone levels, increased thyroid gland weight, and increased thyroid follicular cell hypertrophy. Overall, the available evidence was *suggestive* but not *indicative* of adverse endocrine effects due to PFOA exposure. Therefore, EPA did not prioritize this health outcome for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of endocrine evidence synthesis and integration.

Epidemiological studies of reproductive effects in males published since the 2016 PFOA HESD that were considered for evidence integration included three *medium* confidence studies (Cui et al., 2020; Petersen et al., 2018; Lopez-Espinosa et al., 2016) and one *low* confidence study (Di Nisio et al., 2019). Although there was *slight evidence* to suggest human male reproductive toxicity, including for effects on testosterone levels and sperm parameters, the associations were inconsistent across studies and populations, and it was difficult to assess the impacts of the alterations. Animal toxicological studies considered for evidence integration included three *high* confidence studies (NTP, 2020, 2019; Biegel et al., 2001) and five *medium* confidence studies (Song et al., 2018; Lu et al., 2016; Zhang et al., 2014b; Butenhoff et al., 2012; Li et al., 2011). The available animal data provided *slight evidence* that exposure to PFOA results in adverse effects to the male reproductive system, including changes to the testes and epididymis. However, the evidence from animal studies was inconsistent. Therefore, this health outcome was not prioritized for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of male reproductive evidence synthesis and integration.

Female reproductive epidemiological studies published since the 2016 PFOA HESD that were considered for evidence integration included 1 *high* confidence study (Ding et al., 2020) and 10 *medium* confidence studies (Kim et al., 2020; Donley et al., 2019; Ernst et al., 2019; Wang et al., 2019; Crawford et al., 2017; Lum et al., 2017; Timmermann et al., 2017b; Wang et al., 2017; Lopez-Espinosa et al., 2016; Romano et al., 2016). Although there was *slight evidence* to suggest human female reproductive toxicity, including preeclampsia and gestational hypertension, there was conflicting evidence on altered puberty onset and limited data suggesting reduced fertility and fecundity. The associations were inconsistent across reproductive hormone parameters, and it was difficult to assess the adversity of these alterations. Animal toxicological studies considered for evidence integration included one *high* confidence study (NTP, 2019) and three *medium* confidence studies (Zhang et al., 2020b; Chen et al., 2017c; Butenhoff et al., 2012). The available animal data provided *slight evidence* that exposure to PFOA can result in alterations in ovarian physiology and hormonal parameters in adult female rodents following exposure to doses as low as 1 mg/kg/day. However, as with the available epidemiological studies, the evidence from animal studies was inconsistent. Therefore, this health outcome was not prioritized for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of female reproductive evidence synthesis and integration.

Similar adverse reproductive and endocrine effects have been observed among the family of PFAS. For example, the developing fetus and thyroid were identified as targets following oral exposure to PFBS (U.S. EPA, 2021f), though the observed reproductive effects were considered equivocal. Additionally, EPA's 2021 assessment of GenX chemicals identified the reproductive system as a potential toxicological target (U.S. EPA, 2021e) and the final IRIS Toxicological Reviews for both PFBA (U.S. EPA, 2022c) and PFHxA (U.S. EPA, 2023b) concluded that the available *evidence indicates* that the observed thyroid effects were likely due to PFBA and PFHxA exposure, respectively. Given the similarities across PFAS, these findings support potential associations between PFOA and reproductive and endocrine effects.

As the databases for endocrine and reproductive outcomes were *suggestive* of human health effects resulting from PFOA exposure, they were not prioritized during the updated literature reviews conducted in February 2022 and 2023. However, EPA acknowledges that future studies of these currently "borderline" associations could impact the strength of the association and the weight of evidence for these health outcomes. The currently available studies suggest the potential for endocrine and reproductive effects after PFOA exposure. Studies on endocrine and reproductive health outcomes represent two important research needs.

5.6 Challenges and Uncertainty in Modeling

5.6.1 Modeling of Animal Internal Dosimetry

There are several limitations and uncertainties associated with using pharmacokinetic models in general and estimating animal internal dosimetry. In this assessment, EPA utilized the Wambaugh et al. (Wambaugh et al., 2013) animal internal dosimetry model because it had availability of model parameters across all species of interest, agreement with out-of-sample datasets (see Appendix F, (U.S. EPA, 2024a)), and flexibility to implement life-course modeling (see Section 4.1.3.1). However, there were some limitations to this approach.

First, posterior parameter distributions summarized in Table 4-3 for each sex/species combination were determined using a single study. Therefore, uncertainty in these parameters represents only uncertainty in fitting that single study; any variability between studies or differences in study design were not accounted for in the uncertainty of these parameters. Second, issues with parameter identifiability for some sex/species combinations resulted in substantial uncertainty for some parameters. For example, filtrate volume (Vfil) represents a parameter with poor identifiability when determined using only serum data, due to lack of sensitivity to serum concentrations (see Appendix F, (U.S. EPA, 2024a)). Measurements in additional matrices, such as urine, would help inform this parameter and reduce the uncertainty reflected in the wide confidence intervals of the posterior distribution. These parameters with wide posterior CIs represent parameters that are not sensitive to the concentration-time datasets on which the model was trained (see Appendix F, (U.S. EPA, 2024a)). However, these uncertain model parameters did not impact the median prediction used for BMD modeling and simply demonstrate that the available data are unable to identify all parameters across every species over the range of doses used for model calibration. Finally, the model is only parameterized using adult, single dose, PFOA study designs. Gestational and lactational PK modeling parameters were later identified from numerous sources (Table 4-5) to allow for the modeling of these lifestages, with a more detailed description of the life-course modeling in Section 4.1.3.1.3.

The Wambaugh et al. (Wambaugh et al., 2013) model fit the selected PFOA developmental study data well, though there are additional limitations to using this method to model developmental lifestages. First, perinatal fetal concentrations assume instantaneous equilibration across the placenta and do not account for the possibility of active transporters mediating distribution to the fetus. Second, clearance in the pup during lactation is assumed to be a first-order process governed by a single half-life. At low doses, this assumption is in line with adult clearance, but it is unclear how physiological changes during development impact the infant half-life. Finally, PFOA concentrations in breast milk are assumed to partition passively from the maternal blood. This assumption does not account for the presence of active transport in the mammary gland or time-course changes for PFOA uptake to the milk. Despite these limitations, the incorporation of model parameters related to developmental lifestages is a significant improvement over the model used in the 2016 PFOA HESD, which did not implement life-course modeling (U.S. EPA, 2016c).

5.6.2 Modeling of Human Dosimetry

Uncertainties may stem from efforts to model human dosimetry. One limitation is that the clearance parameter, which is a function of the measured half-life and V_d values, is difficult to estimate in the human general population. Specifically for PFOA, the measurement of half-life is hindered by slow excretion and ongoing exposure. Additionally, it is unclear whether some of the variability in measured half-life values reflects actual variability in the population as opposed to uncertainty in the measurement of the value.

In the Verner et al. (Verner et al., 2016) model, half-life, V_d, and hence clearance values are assumed to be constant across ages and sexes. The excretion of PFOA in children and infants is not well understood. The ontogeny of renal transporters, age-dependent changes in overall renal function, and the amount of protein binding (especially in serum) could all play a role in PFOA excretion and could vary between children and adults. It is even difficult to predict the overall direction of change in excretion in children (higher or lower than in adults) without a clear understanding of these age-dependent differences. V_d is also expected to be different in children. Children have a higher body water content, which results in a greater distribution of hydrophilic chemicals to tissues compared with blood in neonates and infants compared with adults (Fernandez et al., 2011). This is well known for pharmaceuticals, but PFOA is unlike most pharmaceuticals in that it undergoes extensive protein interaction, such that its distribution in the body is driven primarily by protein binding and active transport. Hence, it is difficult to infer the degree to which increased body water content might impact the distribution of PFOA.

The updated half-life value was developed based upon a review of recent literature (see Section 3.3.1.4.5). Many half-life values have been reported for the clearance of PFOA in humans (see Appendix B, (U.S. EPA, 2024a)). The slow excretion of PFOA requires measurement of a small change in serum concentration over a long time; the difficulties associated with making these measurements may represent one reason for the variance in reported values. Another challenge is the ubiquity of PFOA exposure. Ongoing exposure will result in a positive bias in observed half-life values if not considered (Russell et al., 2015). In studies that calculate the half-life in a population with greatly decreased PFOA exposures, typically due to the end of occupational exposure or the introduction of drinking water filtration, the amount of bias due to continuing exposure will depend on the ratio of the prior and ongoing exposures. That is, for a given ongoing exposure, a higher prior exposure may be less likely to overestimate half-life compared

with a lower prior exposure. However, a half-life value determined from a population with very high exposure may not be informative of the half-life in typical exposure scenarios because of non-linearities in PK that may occur due to the saturation of PFAS-protein interactions. This will likely take the form of an under-estimation of the half-life that is relevant to lower levels, which are more representative of the general population due to saturation of renal resorption and increased urinary clearance in the study population. One probable example of this is the elimination half-life of approximately 120 or 200 days reported by Dourson and Gadagbui (Dourson and Gadagbui, 2021), who analyzed a clinical trial with exposures to PFOA of between 50 and 1,200 mg weekly for a period of 6 weeks. In this study, the average plasma level after 6 weeks ranged from 34 µg/mL at 0.1 mg/kg/day to 492.7 µg/mL at 2.3 mg/kg/day (Dourson et al., 2019). This is orders of magnitude greater than the blood levels seen in the general population (the 95th percentile serum PFOA concentration in NHANES 2007–08 was 9.7 ng/mL (Kato et al., 2011)) and is in the range of the maximum values seen at the peak of PFOA manufacturing (Post et al., 2012). The high exposure and short follow-up time may be the source of the short half-life observed in this population. In addition, this study was also carried out in patients with advanced cancer, which may have an effect on the rate of PFOA excretion.

A recent review publication (Campbell et al., 2022) addressing the variation in reported half-life values for PFOA promoted a half-life value of 1.3 years, based on the authors' analysis of half-life values estimated from paired blood and urine samples (Zhang et al., 2013c). The rationale for this was the exclusion of studies that may be biased upward by ongoing exposure, and studies that did not analyze linear and branched isomers of PFOA separately. A commentary in response to the review disputed this conclusion and the approach used to make it (Post et al., 2022). The authors pointed out two citations that explore the effect of explicitly correcting for background exposure: Russell et al. (Russell et al., 2015) and Bartell (Bartell, 2012). Both estimated half-lives >2 years after accounting for ongoing exposure. They go on to list several high-quality studies that estimated half-lives much longer than the value calculated from Zhang et al. (Zhang et al., 2013c). They also pointed out methodological limitations of Zhang et al. (Zhang et al., 2013c) and noted that another estimate of renal clearance using the same approach resulted in a considerably different value (Gao et al., 2015b). EPA is aware of two other studies estimating renal clearance of PFOA from measurements in urine, and both estimated longer half-lives than the value calculated by Zhang et al. (Zhang et al., 2013c). Fu et al. (Fu et al., 2016) estimated a half-life of 4.1 years and Fujii et al. (Fujii et al., 2015) estimated a renal clearance value of 0.044 mL/kg/day, equivalent to a half-life of 7.3 years. These additional measurements of PFOA half-life using a similar study design show that Campbell et al. (Campbell et al., 2022) selected an outlier study, both from other urinary clearance studies and from direct-observation studies.

Another factor EPA considered when evaluating Zhang et al. (Zhang et al., 2013c) was that the estimated value for the half-life of PFOS, geometric mean of 5.8 years for young females and 18 years for males and older females, is higher than is typically estimated. This result for PFOS illustrates that there are uncertainties in any single estimate. Campbell et al. (Campbell et al., 2022) selected an outlier study for the half-life of PFOA, both from other urinary clearance studies and from direct-observation studies. The range of results from among various studies represents a range of uncertainty and EPA has chosen a half-life based on study quality (i.e., representative population, environmentally relevant exposure, and multiple sampling of each individual) that results in a value intermediate among the published estimates.

There are few reported V_d values for humans because this parameter requires knowledge of the total dose or exposure, and V_d values are difficult to determine from environmental exposures. In addition to the V_d reported by Thompson et al. (Thompson et al., 2010b), which was selected by EPA for model parameterization, Dourson and Gadagbui (Dourson and Gadagbui, 2021) reported a human V_d of 91 mL/kg from a clinical trial on PFOA. This value is much lower than other reported values across mammalian species and may reflect an earlier initial distribution step rather than the distribution observed after chronic exposure. Chronic exposure may result in a greater distribution to tissues relative to the plasma, and this process may be slowed by extensive binding of PFOA to plasma proteins. Additionally, the exposure levels used in the clinical trial are much higher than typically seen in the general population, which could result in a different distribution profile.

Lastly, the description of breastfeeding in the updated Verner et al. (Verner et al., 2016) model relied on a number of assumptions: that infants were exclusively breastfed for 1 year, that there was a constant relationship between maternal serum and breastmilk PFOA concentrations, and that weaning was an immediate process with the infant transitioning from a breastmilk-only diet to the background exposure at 1 year. This is a relatively long duration of breastfeeding; only 27% of children in the United States are being breastfed at 1 year of age (CDC, 2013). Along with using the 95th percentile of breastmilk consumption, this provides a scenario of high but realistic lactational exposure. Lactational exposure to the infant is much greater than background exposure, so the 1-year breastfeeding duration is a conservative approach and will result in a lower POD_{HED} than a scenario with earlier weaning. Children in the United States are very unlikely to be exclusively breastfed for up to 1 year, and this approach does not account for potential PFOA exposure via the introduction to solid foods. However, since lactational exposure is much greater than exposure after weaning, a breastfeeding scenario that does not account for potential PFOA exposure from introduction of infants to solid foods is not expected to introduce substantial error.

5.6.3 Approach of Estimating a Benchmark Dose from a Regression Coefficient

EPA identified epidemiological studies that reported associations between PFOA exposure and response variables as regression coefficients. Since such a regression coefficient is associated with a change in the biological response variable, it is biologically meaningful and can therefore be used for POD derivation. EPA modeled these regression coefficients using the same approach used to model studies that reported measured response variables. The SAB PFAS Review Panel agreed with this approach, stating, "it would seem straightforward to apply the same methodology to derive the beta-coefficients ("re-expressed," if necessary, in units of per ng/mL) for antibody responses to vaccines and other health-effect-specific endpoints. Such a coefficient could then be used for deriving PODs" (U.S. EPA, 2022e). When modeling regression coefficients that were reported per log-transformed units of exposure, EPA used the SAB's recommended approach and re-expressed the reported β coefficients in units of per ng/mL (see Appendix E, (U.S. EPA, 2024a)). Sensitivity analyses to evaluate the potential impact of re-expression in a hybrid approach when modeling hepatic and serum lipid studies for PFOA showed little impact on BMDLs (see Appendix E, (U.S. EPA, 2024a)).

To evaluate this potential uncertainty in BMDLs derived based on regression coefficients, EPA obtained the measured dose-response data across exposure deciles from Steenland et al. (Steenland et al., 2009) (kindly provided to EPA on June 30, 2022 via email communication with the corresponding study author) and conducted sensitivity analyses to compare BMDs produced by the reported regression coefficients with the measured response variable (i.e., mean total cholesterol and odds ratios of elevated total cholesterol). For PFOA, the analyses did not generate viable models and therefore the comparison could not be made. These analyses are presented in detail in Appendix E (U.S. EPA, 2024a).

For PFOS, however, $BMDL_5$ values estimated using the regression coefficient and using the measured response variable were 9.52 ng/L and 26.39 ng/L, respectively. The two BMDL estimates from the two approaches are within an order of magnitude, less than a threefold difference. The RfD allows for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. Therefore, EPA is confident in its use of regression coefficients, re-expressed or not as the basis of POD_{HED}s.

5.7 Human Dosimetry Models: Consideration of Alternate Modeling Approaches

Physiologically based pharmacokinetic (PBPK) models are typically preferred over a one-compartment approach because they can provide individual tissue information and have a one-to-one correspondence with the biological system that can be used to incorporate additional features of pharmacokinetics, including tissue-specific internal dosimetry and local metabolism. In addition, though PBPK models are more complex than one-compartment models, many of the additional parameters are chemical-independent and have widely accepted values. Even some of the chemical-dependent values can be extrapolated from animal toxicological studies when parameterizing a model for humans, for which data are typically scarcer.

The decision to select a non-physiologically based model as opposed to one of the PBPK models was influenced in part by past issues identified during evaluation of the application of PBPK models to other PFAS for the purpose of risk assessment. During the process of adapting a published PBPK model for EPA needs, models are subjected to an extensive EPA internal QA review. During initial review of the Loccisano family of models (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011), an unusual implementation of PFOA plasma binding appeared to introduce a mass balance error. Because of the stated goal of minimizing new model development (see Section 4.1.3.2), EPA did not pursue resolution of the discrepancies, which would have required modifications to one of these models for application in this assessment.

Given the previous issues that EPA encountered for other PFAS when implementing PBPK models and the known issue with the Loccisano model and the models based upon it, EPA selected a one-compartment model because it was the most robust available approach for this effort. Following the consideration and analysis of different models, EPA concluded that a one-compartment model is sufficient to predict blood (or serum/plasma) concentrations. Serum/plasma is a good biomarker for exposure, because a major proportion of the PFOA in the body is found in serum/plasma due to albumin binding (Forsthuber et al., 2020). There were no other specific tissues that were considered essential to describing the dosimetry of PFOA.

The two one-compartment approaches identified in the literature for PFOA was the model of Verner et al. (Verner et al., 2016) and the model developed by the Minnesota Department of Health (MDH model) (Goeden et al., 2019). These two models are structurally very similar, with a single compartment each for mother and child, first-order excretion from those compartments, and a similar methodology for describing lactational transfer from mother to child. The following paragraphs describe the slight differences in model implementations, but it is first worth emphasizing the similarity in the two approaches. The overall agreement in approach between the two models supports its validity for the task of human health risk assessment for PFOA.

One advantage of the Verner model is that it explicitly models the mother from birth through the end of breastfeeding. The MDH model, however, is limited to predictions for the time period after the birth of the child with maternal levels set to an initial steady-state level. An explicit description of maternal blood levels allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy, as has been observed for serum PFOA in serial samples from pregnant women (Glynn et al., 2012). This decrease occurs due to the relatively rapid increase in body weight during pregnancy (compared with the years preceding pregnancy) and the increase in blood volume that occurs to support fetal growth (Sibai and Frangieh, 1995). Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Another distinction of the Verner model is that it is written in terms of rates of change in mass rather than concentrations, as in the MDH model. This approach includes the effect of dilution of PFOA during childhood growth without the need for an explicit term in the equations. Not accounting for growth will result in the overprediction of serum concentrations in individuals exposed during growth. Despite this, PFOA concentration in infants at any specific time is driven more by recent lactational exposure than by earlier exposure (either during pregnancy or early breastfeeding), which tends to minimize the impact of growth dilution. Additionally, this structural consideration best matches the approach taken in our animal model, presenting a harmonized approach. These structural considerations favor the application of the updated Verner model over the MDH model.

EPA evaluated two other factors that were present in the MDH model: the application of a scaling factor to increase the V_d in children and the treatment of exposure as a drinking water intake rather than a constant exposure relative to body weight. After testing these features within the updated Verner model structure, EPA determined that neither of these features were appropriate for this assessment, primarily because they did not meaningfully improve the comparison of model predictions to validation data.

In the MDH model, V_d in children starts at 2.4 times the adult V_d and decreases relatively quickly to 1.5 times the adults V_d between 6 and 12 months, reaching the adult level at 10 years of age. These scaling values originated from measurements of body water content relative to weight compared with the adult value. There is no chemical-specific information to suggest that V_d is larger in children compared with adults for PFOA. However, it is generally accepted in pharmaceutical research that hydrophilic chemicals have greater V_d in children (Batchelor and Marriott, 2015), which is attributed to increased body water. Still, PFOA is amphiphilic, not simply hydrophilic, and its distribution is driven by interactions with binding proteins and

transporters, not by passive diffusion with body water. While it is plausible that V_d is larger in children, it is unknown to what degree.

Since increased V_d in children is plausible but neither supported nor contradicted by direct evidence, EPA evaluated the effect of variable V_d by implementing this change the updated Verner model and comparing the results with constant and variable V_d (see Appendix F, (U.S. EPA, 2024a)). This resulted in reduced predictions of serum concentrations, primarily during their peak in early childhood. The model with variable V_d did not decrease the root mean squared error compared with the model with constant V_d. Since the model with constant V_d had better performance and was an overall simpler solution, EPA did not implement variable V_d in the application of the model for POD_{HED} calculation.

The other key difference between the MDH model and the updated Verner model is that instead of constant exposure relative to body weight, exposure in the MDH model was based on drinking water consumption, which is greater relative to body weight in young children compared with adults. Drinking water consumption is also greater in lactating women. To evaluate the potential impact of calculating a drinking water concentration directly, bypassing the RfD step, EPA implemented drinking water consumption in the modified Verner model (see Appendix F, (U.S. EPA, 2024a)). EPA evaluated this decision for PFOA and PFOS together because the choice of units used for human exposure represents a substantial difference in risk assessment methodology. For reasons explained below, EPA ultimately decided to continue to calculate an RfD in terms of constant exposure, with a maximum contaminant level goal (MCLG) calculated thereafter using lifestage specific drinking water consumption values.

When comparing exposure based on drinking water consumption to the traditional RfD approach, the impact on the serum concentrations predicted by the updated Verner model differed between PFOA and PFOS. For PFOA, the predicted serum concentration in the child was qualitatively similar, with the main effect seen in overprediction of timepoints that occur later in childhood. These timepoints are more susceptible to changes in exposure, as early childhood exposure is dominated by lactational exposure. Lactational exposure is slightly increased in this scenario, because of increased drinking water consumption during lactation. However, the main source of PFOA or PFOS in breastmilk in the model with exposure based on drinking water consumption is that which accumulated over the mother's life prior to childbirth, not that which was consumed during lactation. For PFOS, the increased exposure predicted based on children's water intake results in much greater levels in later childhood compared with the model with constant exposure relative to body weight. Use of water ingestion rates to adjust for dose in the Verner model fails to match the decrease in PFOS concentration present in the reported data with multiple timepoints and overestimates the value for the Norwegian Mother, Father, and Child Cohort Study (MoBa) cohort with a single timepoint. There was a much greater effect on PFOS model results relative to PFOA, but in both cases model performance, as quantified by root mean squared error, was superior with constant exposure compared with exposure based on drinking water consumption. This comparison suggests that incorporating variations in drinking water exposure in this way is not appropriate for the updated Verner model.

In addition to the comparison with reported data, EPA's decision to use the Verner model was also considered in the context of the effect on the derivation of MCLGs under SDWA. The epidemiological endpoints can be placed into three categories based on the age of the individuals

at the time of exposure measurement: adults, children, and pregnant women. Because increased drinking water exposure is only applied to children and lactating women, the group of endpoints in children are the only ones that would be affected. While the RfD estimated using the updated Verner model assumed constant exposure, the MCLG based on noncancer effects or for nonlinear carcinogens is an algebraic calculation that incorporates the RfD, RSC, and drinking water intake. The drinking water intake used for this type of MCLG calculation would be chosen based on the exposure interval used in the critical study and/or the target population relevant to the timing of exposure measurement and the response variable that serves as the basis of the RfD. Therefore, even if the RfD does not incorporate increased drinking water intake in certain lifestages, the subsequent MCLG calculation does take this into account. Furthermore, the derivation of an RfD is useful for general assessment of risk and not limited to drinking water exposure.

For these reasons and based on EPA's analyses presented in Appendix F (U.S. EPA, 2024a), EPA determined that the updated Verner model was the most appropriate available model structure for POD_{HED} calculation for PFOA. Specifically, EPA concluded that the determination that assuming V_d in children equal to the adult values and calculating an RfD assuming a constant dose (mg/kg/day) were appropriate for this assessment.

5.8 Sensitive Populations

Some populations may be more susceptible to the potential adverse health effects of toxic substances such as PFOA. These potentially susceptible populations include populations exhibiting a more severe response than others despite similar PFOA exposure due to increased biological sensitivity, as well as populations exhibiting a more severe response due to higher PFOA exposure and/or exposure to other chemicals or nonchemical stressors. Populations with greater biological sensitivity may include pregnant women and their developing fetuses, lactating women, the elderly, children, adolescents, and people with certain underlying medical conditions (see Section 5.8.1). Additionally, some available data indicates that there may be sex-specific differences in sensitivity to potential effects of PFOA (see Section 5.8.2). Populations that could exhibit a greater response to PFOA exposure due to higher exposures to PFOA or other chemicals include communities overburdened by chemical exposures or nonchemical stressors such as communities with environmental justice concerns (see Section 5.8.3).

The potential health effects after PFOA exposure have been evaluated in some sensitive populations (e.g., pregnant women, children) and a small number of studies have assessed differences in exposure to PFOA across populations to assess whether racial/ethnic or socioeconomic differences are associated with greater PFOA exposure. However, the available research on PFOA's potential impacts on sensitive populations is limited and more research is needed. Health effects differences in sensitivity to PFOA exposure have not allowed for the identification or characterization of all potentially sensitive subpopulations. This lack of knowledge about susceptibility to PFOA represents a potential source of uncertainty in the assessment of PFOA.

5.8.1 Fetuses, Infants, Children

One of the more well-studied sensitive populations to PFOA exposure is developing fetuses, infants, and children. Both animal toxicological and epidemiological data suggest that the

developing fetus is particularly sensitive to PFOA-induced toxicity. As described in Sections 3.4.4.1 and 3.4.2.1, results of some epidemiological studies indicate an association between PFOA exposure during pregnancy and/or early childhood and adverse outcomes such as decreased birth weight and decreased antibody response to vaccination. The available animal toxicological data lend support to these findings; as described in Section 3.4.4.2, numerous studies in rodents report effects similar to those seen in humans (e.g., decreased body weights in offspring exposed to PFOA during gestation). Additionally, PFOA exposure to humans during certain lifestages or exposure windows (e.g., prenatal or early postnatal exposure windows) may be more consequential than others. These potentially different effects in different populations and/or exposure windows have not been fully characterized. More research is needed to fully understand the specific critical windows of exposure during development.

With respect to the decreased antibody production endpoint, children who have autoimmune diseases (e.g., juvenile arthritis) or are taking medications that weaken the immune system would be expected to mount a relatively low antibody response compared to other children and would therefore represent potentially susceptible populations for PFOA exposure. There are also concerns about declines in vaccination status (Bramer et al., 2020; Smith et al., 2011) for children overall, and the possibility that diseases that are considered eradicated (such as diphtheria or tetanus) could return to the United States (Hotez, 2019). As noted by Dietert et al. (Dietert et al., 2010), the risks of developing infectious diseases may increase if immunosuppression occurs in the developing immune system.

5.8.2 Sex Differences

In humans, potential sex differences in the disposition of PFOA in the body, as well as in the potential for adverse health effects in response to PFOA exposure, have not been fully elucidated. With respect to sex differences in the development of adverse health effects in response to PFOA exposure, the available epidemiological data are insufficient to draw conclusions, although some studies reported sex differences (e.g., an association between PFOA exposure and serum ALT in girls but not boys (Attanasio, 2019; Mora et al., 2018)). In some studies in rats, males appeared to be more sensitive to some effects than females, even when females received much higher PFOA doses (NTP, 2020; Butenhoff et al., 2004a).

With respect to ADME, research in humans indicates that PFOA half-lives in males are generally longer than those in females (Li et al., 2018c; Gomis et al., 2017; Fu et al., 2016). Some animal studies (in rats in particular) show the same sex difference, but additional research is needed to determine whether the underlying mechanisms identified in rats are relevant to humans. Female rats have been shown to absorb PFOA faster than male rats (Kim et al., 2016), and PFOA may distribute to some compartments (i.e., liver cytosol) to a greater extent in female rats compared with males (Han et al., 2005). Several studies have demonstrated that female rats and rabbits eliminate PFOA from the body faster than males (Dzierlenga et al., 2019a; NTP, 2019; Hinderliter et al., 2006b; Hundley et al., 2006). These studies and others are further described in Section 3.3.1 and Appendix B (U.S. EPA, 2024a).

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats (Cheng et al., 2006; Hinderliter et al., 2006b; Kudo et al., 2002). Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs and OATPs located in the proximal portion of the descending tubule (Yang et al., 2010;

Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). Generally, both *in vivo* and *in vitro* studies reported differences in renal transporters that are regulated by sex hormones and show consistent results indicating increased resorption of PFOA in male rats (see Section 3.3.1 and Appendix B, (U.S. EPA, 2024a)). Hinderliter et al. (Hinderliter et al., 2006b) found that a developmental change in renal transport occurs in rats between 3 and 5 weeks of age that allows for expedited excretion of PFOA in females and an inverse development in males. When considered together, the studies of the transporters suggest that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate, but male rats are not.

Although sex differences in rats have been relatively well studied, sex differences observed in mice were less pronounced (Lou et al., 2009; Lau et al., 2006) and were actually reversed in cynomolgus monkeys and hamsters (Hundley et al., 2006; Butenhoff et al., 2004b), indicating species-specific factors impacting elimination across sexes.

Although there is some evidence to suggest sex differences in humans exposed to PFOA, the mechanisms for these potential differences have not been fully explored. For example, postmenopausal females and adult males have longer PFOA elimination half-lives than premenopausal adult females (Zhang et al., 2013c). Partitioning to the placenta, amniotic fluid, fetus, menstruation, and breast milk represent important routes of elimination in humans and may account for some of the sex differences observed for blood and urinary levels of PFOA by sex and age. It is unclear whether hormone-dependent renal transporters play an additional role in the observed sex differences in PFOA half-life in humans. Additional research is needed to further elucidate these sex differences and their implications, and to ascertain whether the sex differences observed in some animal species are relevant to humans. This data gap represents a source of uncertainty in the elucidation of the risks of PFOA to humans.

5.8.3 Other Susceptible Populations

As noted in the SAB PFAS review panel's final report (U.S. EPA, 2022e), there is uncertainty about whether there are susceptible populations, such as certain racial/ethnic groups, that might be more sensitive to the health effects of PFOA exposure because of either greater biological sensitivity or higher exposure to PFOA and/or other environmental chemicals. Although some studies have evaluated differences in PFAS exposure levels across SES and racial/ethnic groups (see Section 5.1), studies of differential health effects incidence and PFOA exposure are limited. To fully address equity and environmental justice concerns about PFOA, these data gaps regarding differential exposure and health effects after PFOA exposure need to be addressed. In the development of the proposed PFAS NPDWR, EPA conducted an analysis to evaluate potential environmental justice impacts of the proposed regulation (See Chapter 8 of the *Economic Analysis for the Final PFAS National Primary Drinking Water Regulation* (U.S. EPA, 2024b)). EPA acknowledges that exposure to PFOA, and PFAS in general, may have a disproportionate impact on certain communities (e.g., low SES communities; Tribal communities; minority communities; communities in the vicinity of areas of historical PFOA manufacturing and/or contamination) and that studies of these communities are high priority research needs.

6 References

3M Company. (2000). Voluntary Use and Exposure Information Profile Perfluorooctanic Acid and Salts; U.S. EPA Administrative Record AR226-0595 [EPA Report]. Washington, D.C.: U. S. Environmental Protection Agency.

Abbott, BD; Wolf, CJ; Schmid, JE; Das, KP; Zehr, RD; Helfant, L, et al. (2007). Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. Toxicological Sciences 98: 571-581. http://dx.doi.org/10.1093/toxsci/kfm110

Abdallah, MA; Wemken, N; Drage, DS; Tlustos, C; Cellarius, C; Cleere, K, et al. (2020). Concentrations of perfluoroalkyl substances in human milk from Ireland: Implications for adult and nursing infant exposure. Chemosphere 246: 125724. http://dx.doi.org/10.1016/j.chemosphere.2019.125724

Abdellatif, AG; Préat, V; Vamecq, J; Nilsson, R; Roberfroid, M. (1990). Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. Carcinogenesis 11: 1899-1902. http://dx.doi.org/10.1093/carcin/11.11.1899

Abdullah Soheimi, SS; Abdul Rahman, A; Abd Latip, N; Ibrahim, E; Sheikh Abdul Kadir, SH. (2021). Understanding the impact of perfluorinated compounds on cardiovascular diseases and their risk factors: A meta-analysis study [Review]. International Journal of Environmental Research and Public Health 18: 8345. http://dx.doi.org/10.3390/ijerph18168345

Abe, T; Takahashi, M; Kano, M; Amaike, Y; Ishii, C; Maeda, K, et al. (2017). Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. Archives of Toxicology 91: 2365-2374. http://dx.doi.org/10.1007/s00204-016-1888-3

Abraham, K; Mielke, H; Fromme, H; Völkel, W; Menzel, J; Peiser, M, et al. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Archives of Toxicology 94: 2131-2147.

Ahrens, L. (2011). Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate [Review]. Journal of Environmental Monitoring 13: 20-31. http://dx.doi.org/10.1039/c0em00373e

Ait Bamai, Y; Goudarzi, H; Araki, A; Okada, E; Kashino, I; Miyashita, C; Kishi, R. (2020). Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: The Hokkaido study on environment and children's health. Environment International 143: 105979.

Alkhalawi, E; Kasper-Sonnenberg, M; Wilhelm, M; Völkel, W; Wittsiepe, J. (2016). Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the Duisburg Birth Cohort. Journal of Toxicology and Environmental Health, Part A: Current Issues 79: 1041-1049.

American Cancer Society. (2020). Cancer facts and figures 2019 key statistics about kidney cancer. https://www.cancer.org/cancer/kidney-cancer/about/key-statistics.html.

Ammitzbøll, C; Börnsen, L; Petersen, ER; Oturai, AB; Søndergaard, HB; Grandjean, P; Sellebjerg, F. (2019). Perfluorinated substances, risk factors for multiple sclerosis and cellular immune activation. Journal of Neuroimmunology 330: 90-95.

Andersen, CS; Fei, C; Gamborg, M; Nohr, EA; Sørensen, TI; Olsen, J. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. American Journal of Epidemiology 172: 1230-1237. http://dx.doi.org/10.1093/aje/kwq289

Andersen, ME; Butenhoff, JL; Chang, SC; Farrar, DG; Kennedy, GL; Lau, C, et al. (2008). Perfluoroalkyl acids and related chemistries--toxicokinetics and modes of action. Toxicological Sciences 102: 3-14. http://dx.doi.org/10.1093/toxsci/kfm270

Andersen, ME; Clewell, HJ; Tan, YM; Butenhoff, JL; Olsen, GW. (2006). Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys--probing the determinants of long plasma half-lives. Toxicology 227: 156-164. http://dx.doi.org/10.1016/j.tox.2006.08.004

Apelberg, BJ; Goldman, LR; Calafat, AM; Herbstman, JB; Kuklenyik, Z; Heidler, J, et al. (2007a). Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environmental Science and Technology 41: 3891-3897. http://dx.doi.org/10.1021/es0700911

Apelberg, BJ; Witter, FR; Herbstman, JB; Calafat, AM; Halden, RU; Needham, LL; Goldman, LR. (2007b). Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115: 1670-1676. http://dx.doi.org/10.1289/ehp.10334

Arbuckle, T; Kubwabo, C; Walker, M; Davis, K; Lalonde, K; Kosarac, I, et al. (2013). Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. International Journal of Hygiene and Environmental Health 216: 184-194.

Armstrong, LE; Guo, GL. (2019). Understanding environmental contaminants' direct effects on non-alcoholic fatty liver disease progression. Current Environmental Health Reports 6: 95–104. http://dx.doi.org/10.1007/s40572-019-00231-x

Ashley-Martin, J; Dodds, L; Arbuckle, TE; Bouchard, MF; Fisher, M; Morriset, AS, et al. (2017). Maternal concentrations of perfluoroalkyl substances and fetal markers of metabolic function and birth weight. American Journal of Epidemiology 185: 185-193.

ATSDR. (2021). Toxicological profile for perfluoroalkyls [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://dx.doi.org/10.15620/cdc:59198

ATSDR (Agency for Toxic Substances and Disease Registry). (2022). PFAS exposure assessments final report: Findings across ten exposure assessment sites. Atlanta, GA: U.S. Department of Health and Human Services. https://www.atsdr.cdc.gov/pfas/docs/PFAS-EA-Final-Report-508.pdf

Attanasio, R. (2019). Sex differences in the association between perfluoroalkyl acids and liver function in US adolescents: Analyses of NHANES 2013-2016. Environmental Pollution 254: 113061.

Avanasi, R; Shin, HM; Vieira, VM; Bartell, SM. (2016). Variability and epistemic uncertainty in water ingestion rates and pharmacokinetic parameters, and impact on the association between perfluorooctanoate and preeclampsia in the C8 Health Project population. Environmental Research 146: 299-307.

Averina, M; Brox, J; Huber, S; Furberg, AS. (2021). Exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Fit Futures study. Environmental Research 195: 110740. http://dx.doi.org/10.1016/j.envres.2021.110740

Averina, M; Brox, J; Huber, S; Furberg, AS; Sørensen, M. (2019). Serum perfluoroalkyl substances (PFAS) and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern Norway. Environmental Research 169: 114-121.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bonefeld-Jørgensen, EC, et al. (2016). Perfluoroalkyl acids in maternal serum and indices of fetal growth: The Aarhus Birth Cohort. Environmental Health Perspectives 124: 848-854.

Bae, J; Kim, S; Schisterman, EF; Boyd Barr, D; Buck Louis, GM. (2015). Maternal and paternal serum concentrations of perfluoroalkyl and polyfluoroalkyl substances and the secondary sex ratio. Chemosphere 133: 31-40. http://dx.doi.org/10.1016/j.chemosphere.2015.03.015

Balk, FGP; Winkens Pütz, K; Ribbenstedt, A; Gomis, MI; Filipovic, M; Cousins, IT. (2019). Children's exposure to perfluoroalkyl acids - a modelling approach. Environmental Science: Processes & Impacts 21: 1875-1886. http://dx.doi.org/10.1039/c9em00323a

Bao, WW; Qian, ZM; Geiger, SD; Liu, E; Liu, Y; Wang, SQ, et al. (2017). Gender-specific associations between serum isomers of perfluoroalkyl substances and blood pressure among Chinese: Isomers of C8 Health Project in China. Science of the Total Environment 607-608: 1304-1312.

Barry, V; Winquist, A; Steenland, K. (2013). Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives 121: 1313-1318. http://dx.doi.org/10.1289/ehp.1306615

Bartell, SM. (2012). Bias in half-life estimates using log concentration regression in the presence of background exposures, and potential solutions. Journal of Exposure Science and Environmental Epidemiology 22: 299-303. http://dx.doi.org/10.1038/jes.2012.2

Bartell, SM; Calafat, AM; Lyu, C; Kato, K; Ryan, PB; Steenland, K. (2010). Rate of Decline in Serum PFOA Concentrations after Granular Activated Carbon Filtration at Two Public Water Systems in Ohio and West Virginia. Environmental Health Perspectives 118: 222-228. http://dx.doi.org/10.1289/ehp.0901252

Bartell, SM; Vieira, VM. (2021). Critical Review on PFOA, Kidney Cancer, and Testicular Cancer. Journal of the Air and Waste Management Association. http://dx.doi.org/10.1080/10962247.2021.1909668

Bassler, J; Ducatman, A; Elliott, M; Wen, S; Wahlang, B; Barnett, J; Cave, MC. (2019). Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. Environmental Pollution 247: 1055-1063. http://dx.doi.org/10.1016/j.envpol.2019.01.064

Batchelor, HK; Marriott, JF. (2015). Paediatric pharmacokinetics: key considerations. British Journal of Clinical Pharmacology 79: 395-404. http://dx.doi.org/10.1111/bcp.12267

Beach, SA; Newsted, JL; Coady, K; Giesy, JP. (2006). Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS) [Review]. Reviews of Environmental Contamination and Toxicology 186: 133-174. http://dx.doi.org/10.1007/0-387-32883-1_5

Beck, IH; Timmermann, CAG; Nielsen, F; Schoeters, G; Jøhnk, C; Kyhl, HB, et al. (2019). Association between prenatal exposure to perfluoroalkyl substances and asthma in 5-year-old children in the Odense Child Cohort. Environmental Health: A Global Access Science Source 18: 97.

Beesoon, S; Martin, JW. (2015). Isomer-Specific Binding Affinity of Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) to Serum Proteins. Environmental Science and Technology 49: 5722-5731.

Beggs, KM; Mcgreal, S. R.; Mccarthy, A; Gunewardena, S; Lampe, JN; Lau, C; Apte, U. (2016). The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicology and Applied Pharmacology 304: 18-29. http://dx.doi.org/10.1016/j.taap.2016.05.001

Behr, AC; Kwiatkowski, A; Ståhlman, M; Schmidt, FF; Luckert, C; Braeuning, A; Buhrke, T. (2020a). Impairment of bile acid metabolism by perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in human HepaRG hepatoma cells. Archives of Toxicology 94: 1673-1686. http://dx.doi.org/10.1007/s00204-020-02732-3

Behr, AC; Plinsch, C; Braeuning, A; Buhrke, T. (2020b). Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). Toxicology In Vitro 62: 104700. http://dx.doi.org/10.1016/j.tiv.2019.104700

Bell, EM; Yeung, EH; Ma, W; Kannan, K; Sundaram, R; Smarr, MM; Buck Louis, GM. (2018). Concentrations of endocrine disrupting chemicals in newborn blood spots and infant outcomes in the upstate KIDS study. Environment International 121: 232-239.

Benskin, JP; De Silva, AO; Martin, LJ; Arsenault, G; McCrindle, R; Riddell, N, et al. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. Environmental Toxicology and Chemistry 28: 542-554. http://dx.doi.org/10.1897/08-239.1

Benskin, JP; Muir, DCG; Scott, BF; Spencer, C; De Silva, AO; Kylin, H, et al. (2012). Perfluoroalkyl Acids in the Atlantic and Canadian Arctic Oceans. Environmental Science and Technology 46: 5815-5823. http://dx.doi.org/10.1021/es300578x

Bernstein, AS; Kapraun, DF; Schlosser, PM. (2021). A Model Template Approach for Rapid Evaluation and Application of Physiologically Based Pharmacokinetic Models for Use in Human Health

Risk Assessments: A Case Study on Per- and Polyfluoroalkyl Substances. Toxicological Sciences 182: 215–228. http://dx.doi.org/10.1093/toxsci/kfab063

Biegel, LB; Hurtt, ME; Frame, S. R.; O'Connor, JC; Cook, JC. (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicological Sciences 60: 44-55. http://dx.doi.org/10.1093/toxsci/60.1.44

Biegel, LB; Liu, RC; Hurtt, ME; Cook, JC. (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicology and Applied Pharmacology 134: 18-25. http://dx.doi.org/10.1006/taap.1995.1164

Birnbaum, LS; Fenton, SE. (2003). Cancer and developmental exposure to endocrine disruptors [Review]. Environmental Health Perspectives 11: 389-394. http://dx.doi.org/10.1289/ehp.5686

Bjerregaard-Olesen, C; Bach, CC; Long, M; Wielsøe, M; Bech, BH; Henriksen, TB, et al. (2019). Associations of Fetal Growth Outcomes with Measures of the Combined Xenoestrogenic Activity of Maternal Serum Perfluorinated Alkyl Acids in Danish Pregnant Women. Environmental Health Perspectives 127: 17006.

Bjorke-Monsen, AL; Varsi, K; Averina, M; Brox, J; Huber, S. (2020). Perfluoroalkyl substances (PFASs) and mercury in never-pregnant women of fertile age: association with fish consumption and unfavorable lipid profile. 3: 277-284. http://dx.doi.org/10.1136/bmjnph-2020-000131

Blake, BE; Cope, HA; Hall, SM; Keys, RD; Mahler, BW; Mccord, J, et al. (2020). Evaluation of Maternal, Embryo, and Placental Effects in CD-1 Mice following Gestational Exposure to Perfluorooctanoic Acid (PFOA) or Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX). Environmental Health Perspectives 128: 27006.

Blomberg, AJ; Shih, YH; Messerlian, C; Jørgensen, LH; Weihe, P; Grandjean, P. (2021). Early-life associations between per- and polyfluoroalkyl substances and serum lipids in a longitudinal birth cohort. Environmental Research 200: 111400. http://dx.doi.org/10.1016/j.envres.2021.111400

Bogdanska, J; Borg, D; Bergström, U; Mellring, M; Bergman, Å; Depierre, J; Nobel, S. (2020). Tissue distribution of 14C-labelled perfluorooctanoic acid in adult mice after 1-5 days of dietary exposure to an experimental dose or a lower dose that resulted in blood levels similar to those detected in exposed humans. Chemosphere 239: 124755.

Bonefeld-Jørgensen, EC; Long, M; Fredslund, SO; Bossi, R; Olsen, J. (2014). Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort. Cancer Causes and Control 25: 1439-1448. http://dx.doi.org/10.1007/s10552-014-0446-7

Boone, L; Meyer, D; Cusick, P; Ennulat, D; Bolliger, AP; Everds, N, et al. (2005). Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies [Review]. Veterinary Clinical Pathology 34: 182-188. http://dx.doi.org/10.1111/j.1939-165X.2005.tb00041.x

Botelho, SC; Saghafian, M; Pavlova, S; Hassan, M; Depierre, JW; Abedi-Valugerdi, M. (2015). Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid. Chemosphere 129: 225-231. http://dx.doi.org/10.1016/j.chemosphere.2014.06.093

Boulanger, B; Vargo, J; Schnoor, JL; Hornbuckle, KC. (2004). Detection of perfluorooctane surfactants in Great Lakes water. Environmental Science and Technology 38: 4064-4070. http://dx.doi.org/10.1021/es0496975

Bouwmeester, MC; Ruiter, S; Lommelaars, T; Sippel, J; Hodemaekers, HM; van den Brandhof, EJ, et al. (2016). Zebrafish embryos as a screen for DNA methylation modifications after compound exposure. Toxicology and Applied Pharmacology 291: 84-96. http://dx.doi.org/10.1016/j.taap.2015.12.012

Braissant, O; Wahli, W. (1998). Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. Endocrinology 139: 2748-2754.

Bramer, CA; Kimmins, LM; Swanson, R; Kuo, J; Vranesich, P; Jacques-Carroll, LA; Shen, AK. (2020). Decline in Child Vaccination Coverage During the COVID-19 Pandemic - Michigan Care

Improvement Registry, May 2016-May 2020. MMWR Morbidity and Mortality Weekly Report 69: 630-631. http://dx.doi.org/10.15585/mmwr.mm6920e1

Brieger, A; Bienefeld, N; Hasan, R; Goerlich, R; Haase, H. (2011). Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. Toxicology In Vitro 25: 960-968. http://dx.doi.org/10.1016/j.tiv.2011.03.005

Brochot, C; Casas, M; Manzano-Salgado, C; Zeman, FA; Schettgen, T; Vrijheid, M; Bois, FY. (2019). Prediction of maternal and foetal exposures to perfluoroalkyl compounds in a Spanish birth cohort using toxicokinetic modelling. Toxicology and Applied Pharmacology 379: 114640.

Buck Louis, GM; Sapra, KJ; Barr, DB; Lu, Z; Sundaram, R. (2016). Preconception perfluoroalkyl and polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. Reproductive Toxicology 65: 11-17.

Buck Louis, GM; Zhai, S; Smarr, MM; Grewal, J; Zhang, C; Grantz, KL, et al. (2018). Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environment International 119: 515-526.

Buck, RC; Franklin, J; Berger, U; Conder, JM; Cousins, IT; de Voogt, P, et al. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins [Review]. Integrated Environmental Assessment and Management 7: 513-541. http://dx.doi.org/10.1002/ieam.258

Budtz-Jørgensen, E; Grandjean, P. (2018). Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS ONE 13: e0205388. http://dx.doi.org/10.1371/journal.pone.0205388

Buekers, J; Colles, A; Cornelis, C; Morrens, B; Govarts, E; Schoeters, G. (2018). Socio-economic status and health: evaluation of human biomonitored chemical exposure to per- and polyfluorinated substances across status [Review]. International Journal of Environmental Research and Public Health 15: 2818. http://dx.doi.org/10.3390/ijerph15122818

Buhrke, T; Kibellus, A; Lampen, A. (2013). In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. Toxicology Letters 218: 97-104. http://dx.doi.org/10.1016/j.toxlet.2013.01.025

Buhrke, T; Krüger, E; Pevny, S; Rößler, M; Bitter, K; Lampen, A. (2015). Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology 333: 53-62. http://dx.doi.org/10.1016/j.tox.2015.04.004

Bulka, CM; Avula, V; Fry, RC. (2021). Associations of exposure to perfluoroalkyl substances individually and in mixtures with persistent infections: Recent findings from NHANES 1999-2016. Environmental Pollution 275: 116619. http://dx.doi.org/10.1016/j.envpol.2021.116619

Burkemper, JL; Aweda, TA; Rosenberg, AJ; Lunderberg, DM; Peaslee, GF; Lapi, SE. (2017). Radiosynthesis and biological distribution of F-18-labeled perfluorinated alkyl substances. Environmental Science & Technology Letters 4: 211-215.

Buser, MC; Scinicariello, F. (2016). Perfluoroalkyl substances and food allergies in adolescents. Environment International 88: 74-79.

Butenhoff, J; Costa, G; Elcombe, C; Farrar, D; Hansen, K; Iwai, H, et al. (2002). Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicological Sciences 69: 244-257. http://dx.doi.org/10.1093/toxsci/69.1.244

Butenhoff, JL; Kennedy, GL; Chang, SC; Olsen, GW. (2012). Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. Toxicology 298: 1-13.

Butenhoff, JL; Kennedy, GL; Frame, S. R.; O'Connor, JC; York, RG. (2004a). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196: 95-116.

Butenhoff, JL; Kennedy, GL; Hinderliter, PM; Lieder, PH; Jung, R; Hansen, KJ, et al. (2004b). Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. Toxicological Sciences 82: 394-406. http://dx.doi.org/10.1093/toxsci/kfh302

Butenhoff, JL; Kennedy, GL; Jung, R; Chang, SC. (2014). Evaluation of perfluorooctanoate for potential genotoxicity. Toxicology Reports 1: 252-270. http://dx.doi.org/10.1016/j.toxrep.2014.05.012

C8 Science Panel. (2012a). C8 portable link reports. http://www.c8sciencepanel.org/index.html

C8 Science Panel. (2012b). C8 study results - Status reports. Available online at http://www.c8sciencepanel.org/study_results.html

Cai, D; Li, QQ; Chu, C; Wang, SZ; Tang, YT; Appleton, AA, et al. (2020). High trans-placental transfer of perfluoroalkyl substances alternatives in the matched maternal-cord blood serum: Evidence from a birth cohort study. Science of the Total Environment 705: 135885.

Calafat, AM; Kato, K; Hubbard, K; Jia, T; Botelho, JC; Wong, LY. (2019). Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013-2014 National Health and Nutrition Examination Survey. Environment International 131: 105048. http://dx.doi.org/10.1016/j.envint.2019.105048

Calafat, AM; Wong, LY; Kuklenyik, Z; Reidy, JA; Needham, LL. (2007). Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environmental Health Perspectives 115: 1596-1602. http://dx.doi.org/10.1289/ehp.10598

CalEPA. (2021). Public Health Goals: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water (First Public Review Draft ed.). California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Branch. https://oehha.ca.gov/sites/default/files/media/downloads/crnr/pfoapfosphgdraft061021.pdf

Callan, AC; Rotander, A; Thompson, K; Heyworth, J; Mueller, JF; Odland, JØ; Hinwood, AL. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Science of the Total Environment 569-570: 1107-1113.

Campbell, J; Clewell, H; Cox, T; Dourson, M; Ethridge, S; Forsberg, N, et al. (2022). The Conundrum of the PFOA human half-life, an international collaboration. Regulatory Toxicology and Pharmacology 132: 105185. http://dx.doi.org/10.1016/j.yrtph.2022.105185

Canova, C; Barbieri, G; Zare Jeddi, M; Gion, M; Fabricio, A; Daprà, F, et al. (2020). Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environment International 145: 106117. http://dx.doi.org/10.1016/j.envint.2020.106117

Canova, C; Di Nisio, A; Barbieri, G; Russo, F; Fletcher, T; Batzella, E, et al. (2021). PFAS Concentrations and Cardiometabolic Traits in Highly Exposed Children and Adolescents. International Journal of Environmental Research and Public Health 18. http://dx.doi.org/10.3390/ijerph182412881

Cao, L; Guo, Y; Chen, Y; Hong, J; Wu, J; Hangbiao, J. (2022). Per-/polyfluoroalkyl substance concentrations in human serum and their associations with liver cancer. Chemosphere 296: 134083. http://dx.doi.org/10.1016/j.chemosphere.2022.134083

Cao, W; Liu, X; Liu, X; Zhou, Y; Zhang, X; Tian, H, et al. (2018). Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a Chinese birth cohort. Environment International 116: 197-205.

Cao, Y; Ng, C. (2021). Absorption, distribution, and toxicity of per- and polyfluoroalkyl substances (PFAS) in the brain: a review [Review]. Environmental Science: Processes & Impacts 23: 1623-1640. http://dx.doi.org/10.1039/d1em00228g

Cardenas, A; Hivert, MF; Gold, DR; Hauser, R; Kleinman, KP; Lin, PD, et al. (2019). Associations of perfluoroalkyl and polyfluoroalkyl substances with incident diabetes and microvascular disease. Diabetes Care 42: 1824-1832.

Cariou, R; Veyrand, B; Yamada, A; Berrebi, A; Zalko, D; Durand, S, et al. (2015). Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environment International 84: 71-81.

Carmichael, S; Abrams, B; Selvin, S. (1997). The pattern of maternal weight gain in women with good pregnancy outcomes. American Journal of Public Health 87: 1984-1988. http://dx.doi.org/10.2105/AJPH.87.12.1984

Cavallini, G; Donati, A; Taddei, M; Bergamini, E. (2017). Peroxisomes proliferation and pharmacological stimulation of autophagy in rat liver: evidence to support that autophagy may remove the "older" peroxisomes. Molecular and Cellular Biochemistry 431: 97-102. http://dx.doi.org/10.1007/s11010-017-2979-z

Caverly Rae, JM; Frame, S. R.; Kennedy, GL; Butenhoff, JL; Chang, SC. (2014). Pathology review of proliferative lesions of the exocrine pancreas in two chronic feeding studies in rats with ammonium perfluorooctanoate. Toxicology Reports 1: 85-91. http://dx.doi.org/10.1016/j.toxrep.2014.04.005

CDC. (2013). Breastfeeding report card : United States 2013. Atlanta, GA.

CDC. (2017). Fourth national report on human exposure to environmental chemicals, updated tables, January 2017, volume one. Atlanta, GA. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf

CDC. (2021). Mortality in the United States, 2020. (Data Brief No. 427). Atlanta, GA: National Center for Health Statistics. https://www.cdc.gov/nchs/products/databriefs/db427.htm

Chang, CJ; Barr, DB; Ryan, PB; Panuwet, P; Smarr, MM; Liu, K, et al. (2022). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. Environment International 158: 106964. http://dx.doi.org/10.1016/j.envint.2021.106964

Chang, ET; Adami, HO; Boffetta, P; Cole, P; Starr, TB; Mandel, JS. (2014). A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans [Review]. Critical Reviews in Toxicology 44 Suppl 1: 1-81. http://dx.doi.org/10.3109/10408444.2014.905767

Chen, A; Jandarov, R; Zhou, L; Calafat, AM; Zhang, G; Urbina, EM, et al. (2019). Association of perfluoroalkyl substances exposure with cardiometabolic traits in an island population of the eastern Adriatic coast of Croatia. Science of the Total Environment 683: 29-36.

Chen, F; Yin, S; Kelly, BC; Liu, W. (2017a). Isomer-specific transplacental transfer of perfluoroalkyl acids: Results from a survey of paired maternal, cord sera, and placentas. Environmental Science and Technology 51: 5756-5763.

Chen, H; Wang, Q; Cai, Y; Yuan, R; Wang, F; Zhou, B. (2020). Investigation of the Interaction Mechanism of Perfluoroalkyl Carboxylic Acids with Human Serum Albumin by Spectroscopic Methods. International Journal of Environmental Research and Public Health 17.

Chen, L; Tong, C; Huo, X; Zhang, J; Tian, Y. (2021). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and birth outcomes: A longitudinal cohort with repeated measurements. Chemosphere 267: 128899. http://dx.doi.org/10.1016/j.chemosphere.2020.128899

Chen, MH; Ha, EH; Wen, TW; Su, YN; Lien, GW; Chen, CY, et al. (2012). Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS ONE 7: e42474. http://dx.doi.org/10.1371/journal.pone.0042474

Chen, MH; Ng, S; Hsieh, CJ; Lin, CC; Hsieh, WS; Chen, PC. (2017b). The impact of prenatal perfluoroalkyl substances exposure on neonatal and child growth. Science of the Total Environment 607-608: 669-675.

Chen, Q; Huang, R; Hua, L; Guo, Y; Huang, L; Zhao, Y, et al. (2018). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: A prospective birth cohort study. Environmental Health: A Global Access Science Source 17: 1-12.

Chen, Y; Zhou, L; Xu, J; Zhang, L; Li, M; Xie, X, et al. (2017c). Maternal exposure to perfluorooctanoic acid inhibits luteal function via oxidative stress and apoptosis in pregnant mice. Reproductive Toxicology 69: 159-166.

Cheng, W; Ng, CA. (2017). A permeability-limited physiologically based pharmacokinetic (PBPK) model for perfluorooctanoic acid (PFOA) in male rats. Environmental Science and Technology 51: 9930-9939. http://dx.doi.org/10.1021/acs.est.7b02602

Cheng, W; Ng, CA. (2018). Predicting relative protein affinity of novel per- and polyfluoroalkyl substances (PFASs) by an efficient molecular dynamics approach. Environmental Science and Technology 52: 7972-7980.

Cheng, X; Klaassen, CD. (2008a). Critical role of PPAR-alpha in perfluorooctanoic acid- and perfluorodecanoic acid-induced downregulation of Oatp uptake transporters in mouse livers. Toxicological Sciences 106: 37-45. http://dx.doi.org/10.1093/toxsci/kfn161

Cheng, X; Klaassen, CD. (2008b). Perfluorocarboxylic acids induce cytochrome P450 enzymes in mouse liver through activation of PPAR-alpha and CAR transcription factors. Toxicological Sciences 106: 29-36. http://dx.doi.org/10.1093/toxsci/kfn147

Cheng, X; Maher, J; Lu, H; Klaassen, CD. (2006). Endocrine regulation of gender-divergent mouse organic anion-transporting polypeptide (Oatp) expression. Molecular Pharmacology 70: 1291-1297. http://dx.doi.org/10.1124/mol.106.025122

Chiu, WA; Guyton, KZ; Martin, MT; Reif, DM; Rusyn, I. (2018). Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. ALTEX 35: 51-64. http://dx.doi.org/10.14573/altex.1703231

Christensen, KY; Raymond, M; Meiman, J. (2019). Perfluoroalkyl substances and metabolic syndrome. International Journal of Hygiene and Environmental Health 222: 147-153.

Christensen, KY; Raymond, M; Thompson, BA; Anderson, HA. (2016). Perfluoroalkyl substances in older male anglers in Wisconsin. Environment International 91: 312-318.

Chu, C; Zhou, Y; Li, QQ; Bloom, MS; Lin, S; Yu, YJ, et al. (2020). Are perfluorooctane sulfonate alternatives safer? New insights from a birth cohort study. Environment International 135: 105365.

Cichoz-Lach, H; Michalak, A. (2014). Oxidative stress as a crucial factor in liver diseases [Review]. World Journal of Gastroenterology 20: 8082-8091. http://dx.doi.org/10.3748/wjg.v20.i25.8082

Clegg, ED; Cook, JC; Chapin, RE; Foster, PMD; Daston, GP. (1997). Leydig cell hyperplasia and adenoma formation: Mechanisms and relevance to humans [Review]. Reproductive Toxicology 11: 107-121. http://dx.doi.org/10.1016/S0890-6238(96)00203-1

Cochran, RS. (2015) Evaluation of Perfluorinated Compounds in Sediment, Water, and Passive Samplers Collected from the Barksdale Air Force Base. (Master's Thesis). Texas Tech University, [n.l.].

Cohn, BA; La Merrill, MA; Krigbaum, NY; Wang, M; Park, JS; Petreas, M, et al. (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reproductive Toxicology 92: 112-119.

Cong, J; Chu, C; Li, QQ; Zhou, Y; Qian, ZM; Geiger, SD, et al. (2021). Associations of perfluorooctane sulfonate alternatives and serum lipids in Chinese adults. Environment International 155: 106596. http://dx.doi.org/10.1016/j.envint.2021.106596

Convertino, M; Church, TR; Olsen, GW; Liu, Y; Doyle, E; Elcombe, CR, et al. (2018). Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systemic health risk of perfluorooctanoate (pfoa). Toxicological Sciences 163: 293-306.

Cook, JC; Murray, SM; Frame, SR; Hurtt, ME. (1992). Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicology and Applied Pharmacology 113: 209-217. http://dx.doi.org/10.1016/0041-008X(92)90116-A

Cope, HA; Blake, BE; Love, C; McCord, J; Elmore, SA; Harvey, JB, et al. (2021). Latent, sex-specific metabolic health effects in CD-1 mouse offspring exposed to PFOA or HFPO-DA (GenX) during gestation. Emerging Contaminants 7: 219-235. http://dx.doi.org/10.1016/j.emcon.2021.10.004

Corley, RA; Mendrala, AL; Smith, FA; Staats, DA; Gargas, ML; Conolly, RB, et al. (1990). Development of a physiologically based pharmacokinetic model for chloroform. Toxicology and Applied Pharmacology 103: 512-527.

Corton, JC; Cunningham, ML; Hummer, BT; Lau, C; Meek, B; Peters, JM, et al. (2014). Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study [Review]. Critical Reviews in Toxicology 44: 1-49. http://dx.doi.org/10.3109/10408444.2013.835784

Corton, JC; Peters, JM; Klaunig, JE. (2018). The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions [Review]. Archives of Toxicology 92: 83-119. http://dx.doi.org/10.1007/s00204-017-2094-7

Costa, G; Sartori, S; Consonni, D. (2009). Thirty years of medical surveillance in perfluorooctanoic acid production workers. Journal of Occupational and Environmental Medicine 51: 364-372. http://dx.doi.org/10.1097/JOM.0b013e3181965d80

Costa, O; Iñiguez, C; Manzano-Salgado, CB; Amiano, P; Murcia, M; Casas, M, et al. (2019). First-trimester maternal concentrations of polyfluoroalkyl substances and fetal growth throughout pregnancy. Environment International 130: 104830. http://dx.doi.org/10.1016/j.envint.2019.05.024

Costello, E; Rock, S; Stratakis, N; Eckel, SP; Walker, DI; Valvi, D, et al. (2022). Exposure to per- and Polyfluoroalkyl Substances and Markers of Liver Injury: A Systematic Review and Meta-Analysis [Review]. Environmental Health Perspectives 130: 46001. http://dx.doi.org/10.1289/EHP10092

Crawford, NM; Fenton, SE; Strynar, M; Hines, EP; Pritchard, DA; Steiner, AZ. (2017). Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility. Reproductive Toxicology 69: 53-59.

Crebelli, R; Caiola, S; Conti, L; Cordelli, E; De Luca, G; Dellatte, E, et al. (2019). Can sustained exposure to PFAS trigger a genotoxic response? A comprehensive genotoxicity assessment in mice after subacute oral administration of PFOA and PFBA. Regulatory Toxicology and Pharmacology 106: 169-177.

Creton, S; Aardema, MJ; Carmichael, PL; Harvey, JS; Martin, FL; Newbold, RF, et al. (2012). Cell transformation assays for prediction of carcinogenic potential: State of the science and future research needs. Mutagenesis 27: 93-101. http://dx.doi.org/10.1093/mutage/ger053

Cui, L; Liao, CY; Zhou, QF; Xia, TM; Yun, ZJ; Jiang, GB. (2010). Excretion of PFOA and PFOS in male rats during a subchronic exposure. Archives of Environmental Contamination and Toxicology 58: 205-213. http://dx.doi.org/10.1007/s00244-009-9336-5

Cui, L; Zhou, QF; Liao, CY; Fu, JJ; Jiang, GB. (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Archives of Environmental Contamination and Toxicology 56: 338-349. http://dx.doi.org/10.1007/s00244-008-9194-6

Cui, Q; Pan, Y; Wang, J; Liu, H; Yao, B; Dai, J. (2020). Exposure to per- and polyfluoroalkyl substances (PFASs) in serum versus semen and their association with male reproductive hormones. Environmental Pollution 266 Pt. 2: 115330.

Cui, R; Li, C; Wang, J; Dai, J. (2019). Induction of hepatic miR-34a by perfluorooctanoic acid regulates metabolism-related genes in mice. Environmental Pollution 244: 270-278. http://dx.doi.org/10.1016/j.envpol.2018.10.061

Cui, Y; Liu, W; Xie, W; Yu, W; Wang, C; Chen, H. (2015). Investigation of the Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) on Apoptosis and Cell Cycle in a Zebrafish (Danio rerio) Liver Cell Line. International Journal of Environmental Research and Public Health 12: 15673-15682. http://dx.doi.org/10.3390/ijerph121215012

Cutland, CL; Lackritz, EM; Mallett-Moore, T; Bardají, A; Chandrasekaran, R; Lahariya, C, et al. (2017). Low birth weight: Case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. Vaccine 35: 6492-6500. http://dx.doi.org/10.1016/j.vaccine.2017.01.049

D'Agostino, RB; Vasan, RS; Pencina, MJ; Wolf, PA; Cobain, M; Massaro, JM; Kannel, WB. (2008). General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 117: 743-753. http://dx.doi.org/10.1161/CIRCULATIONAHA.107.699579

D'eon, JC; Mabury, SA. (2011). Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? [Review]. Environmental Science & Technology 45: 7974-7984. http://dx.doi.org/10.1021/es200171y

Dairkee, SH; Luciani-Torres, G; Moore, DH; Jaffee, IM; Goodson, WH. (2018). A ternary mixture of common chemicals perturbs benign human breast epithelial cells more than the same chemicals do individually. Toxicological Sciences 165: 131-144. http://dx.doi.org/10.1093/toxsci/kfy126

Dalla Zuanna, T; Savitz, DA; Barbieri, G; Pitter, G; Zare Jeddi, M; Daprà, F, et al. (2021). The association between perfluoroalkyl substances and lipid profile in exposed pregnant women in the Veneto region, Italy. Ecotoxicology and Environmental Safety 209: 111805. http://dx.doi.org/10.1016/j.ecoenv.2020.111805

Dalsager, L; Christensen, N; Halekoh, U; Timmermann, CAG; Nielsen, F; Kyhl, HB, et al. (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. Environment International 149: 106395. http://dx.doi.org/10.1016/j.envint.2021.106395

Dalsager, L; Christensen, N; Husby, S; Kyhl, H; Nielsen, F; Høst, A, et al. (2016). Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environment International 96: 58-64.

Daniels, SR; Greer, FR; Nutr, C. (2008). Lipid screening and cardiovascular health in childhood. Pediatrics 122: 198-208. http://dx.doi.org/10.1542/peds.2008-1349

Darrow, LA; Groth, AC; Winquist, A; Shin, HM; Bartell, SM; Steenland, K. (2016). Modeled perfluorooctanoic acid (PFOA) exposure and liver function in a mid-Ohio valley community. Environmental Health Perspectives 124: 1227-1233.

Darrow, LA; Howards, PP; Winquist, A; Steenland, K. (2014). PFOA and PFOS serum levels and miscarriage risk. Epidemiology 25: 505-512. http://dx.doi.org/10.1097/EDE.0000000000000103

Darrow, LA; Stein, CR; Steenland, K. (2013). Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environmental Health Perspectives 121: 1207-1213. http://dx.doi.org/10.1289/ehp.1206372

Das, KP; Wood, CR; Lin, MT; Starkov, AA; Lau, C; Wallace, KB, et al. (2017). Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378: 37-52. http://dx.doi.org/10.1016/j.tox.2016.12.007

Dassuncao, C; Hu, XC; Nielsen, F; Weihe, P; Grandjean, P; Sunderland, EM. (2018). Shifting Global Exposures to Poly- and Perfluoroalkyl Substances (PFASs) Evident in Longitudinal Birth Cohorts from a Seafood-Consuming Population. Environmental Science and Technology 52: 3738-3747.

Davies, B; Morris, T. (1993). Physiological parameters in laboratory animals and humans [Review]. Pharmaceutical Research 10: 1093-1095. http://dx.doi.org/10.1023/A:1018943613122

De Boo, HA; Harding, JE. (2006). The developmental origins of adult disease (Barker) hypothesis [Review]. Australian and New Zealand Journal of Obstetrics and Gynaecology 46: 4-14. http://dx.doi.org/10.1111/j.1479-828X.2006.00506.x

de Cock, M; de Boer, MR; Lamoree, M; Legler, J; van De Bor, M. (2014). First Year Growth in Relation to Prenatal Exposure to Endocrine Disruptors - A Dutch Prospective Cohort Study. International Journal of Environmental Research and Public Health 11: 7001-7021.

de Cock, M; De Boer, MR; Lamoree, M; Legler, J; Van De Bor, M. (2016). Prenatal exposure to endocrine disrupting chemicals and birth weight-A prospective cohort study. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 51: 178-185.

De Guise, S; Levin, M. (2021). Suppression of Th2 cytokines as a potential mechanism for reduced antibody response following PFOA exposure in female B6C3F1 mice. Toxicology Letters 351: 155-162. http://dx.doi.org/10.1016/j.toxlet.2021.09.002

De Toni, L; Radu, CM; Sabovic, I; Di Nisio, A; Dall'Acqua, S; Guidolin, D, et al. (2020). Increased cardiovascular risk associated with chemical sensitivity to perfluoro-octanoic acid: role of

impaired platelet aggregation. International Journal of Molecular Sciences 21: 399. http://dx.doi.org/10.3390/ijms21020399

Deener, KC; Sacks, JD; Kirrane, EF; Glenn, BS; Gwinn, MR; Bateson, TF; Burke, TA. (2018). Epidemiology: a foundation of environmental decision making [Review]. Journal of Exposure Science & Environmental Epidemiology 28: 515-521. http://dx.doi.org/10.1038/s41370-018-0059-4

Dewey, KG; Heinig, MJ; Nommsen, LA. (1993). Maternal weight-loss patterns during prolonged lactation. American Journal of Clinical Nutrition 58: 162-166.

Dewitt, JC; Blossom, SJ; Schaider, LA. (2019). Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence [Review]. Journal of Exposure Science and Environmental Epidemiology 29: 148-156. http://dx.doi.org/10.1038/s41370-018-0097-y

Dewitt, JC; Copeland, CB; Luebke, RW. (2009). Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. Toxicological Sciences 109: 106-112. http://dx.doi.org/10.1093/toxsci/kfp040

Dewitt, JC; Copeland, CB; Strynar, MJ; Luebke, RW. (2008). Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environmental Health Perspectives 116: 644-650.

DeWitt, JC; Germolec, DR; Luebke, RW; Johnson, VJ. (2016a). Associating changes in the immune system with clinical diseases for interpretation in risk assessment. Current Protocols in Toxicology 67: 1.1-18.11.22. http://dx.doi.org/10.1002/0471140856.tx1801s67

Dewitt, JC; Keil, DE. (2017). Current issues in developmental immunotoxicity. In G Parker (Ed.), Immunopathology in Toxicology and Drug Development: Volume 1, Immunobiology, Investigative Techniques, and Special Studies (pp. 601-618). Totowa, NJ: Humana Press. http://dx.doi.org/10.1007/978-3-319-47377-2_13

Dewitt, JC; Williams, WC; Creech, NJ; Luebke, RW. (2016b). Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARα and T- and B-cell targeting. Journal of Immunotoxicology 13: 38-45. http://dx.doi.org/10.3109/1547691X.2014.996682

Di Nisio, A; Sabovic, I; Valente, U; Tescari, S; Rocca, MS; Guidolin, D, et al. (2019). Endocrine disruption of androgenic activity by perfluoroalkyl substances: clinical and experimental evidence. Journal of Clinical Endocrinology and Metabolism 104: 1259-1271.

Dietert, RR; Dewitt, JC; Germolec, DR; Zelikoff, JT. (2010). Breaking patterns of environmentally influenced disease for health risk reduction: Immune perspectives [Review]. Environmental Health Perspectives 118: 1091-1099. http://dx.doi.org/10.1289/ehp.1001971

Ding, N; Harlow, SD; Randolph, JF; Calafat, AM; Mukherjee, B; Batterman, S, et al. (2020). Associations of perfluoroalkyl substances with incident natural menopause: The study of women's health across the nation. Journal of Clinical Endocrinology and Metabolism 105: E3169-E3182.

Dinglasan-Panlilio, MJ; Prakash, SS; Baker, JE. (2014). Perfluorinated compounds in the surface waters of Puget Sound, Washington and Clayoquot and Barkley Sounds, British Columbia. Marine Pollution Bulletin 78: 173-180. http://dx.doi.org/10.1016/j.marpolbul.2013.10.046

Dixon, LJ; Barnes, M; Tang, H; Pritchard, MT; Nagy, LE. (2013). Kupffer cells in the liver [Review]. Comprehensive Physiology 3: 785-797. http://dx.doi.org/10.1002/cphy.c120026

Domazet, SL; Grøntved, A; Timmermann, AG; Nielsen, F; Jensen, TK. (2016). Longitudinal associations of exposure to perfluoroalkylated substances in childhood and adolescence and indicators of adiposity and glucose metabolism 6 and 12 years later: The European Youth Heart Study. Diabetes Care 39: 1745-1751.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Koponen, J, et al. (2019). Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environment International 124: 58-65.

Dong, GH; Tung, KY; Tsai, CH; Liu, MM; Wang, D; Liu, W, et al. (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environmental Health Perspectives 121: 507-513, 513e501-508. http://dx.doi.org/10.1289/ehp.1205351

Dong, Z; Wang, H; Yu, YY; Li, YB; Naidu, R; Liu, Y. (2019). Using 2003-2014 U.S. NHANES data to determine the associations between per- and polyfluoroalkyl substances and cholesterol: Trend and implications. Ecotoxicology and Environmental Safety 173: 461-468.

Donley, GM; Taylor, E; Jeddy, Z; Namulanda, G; Hartman, TJ. (2019). Association between in utero perfluoroalkyl substance exposure and anti-Müllerian hormone levels in adolescent females in a British cohort. Environmental Research 177: 108585.

Dourson, M; Gadagbui, B. (2021). The Dilemma of perfluorooctanoate (PFOA) human half-life. 126: 105025. http://dx.doi.org/10.1016/j.yrtph.2021.105025

Dourson, ML; Gadagbui, B; Onyema, C; Mcginnis, PM; York, RG. (2019). Data derived Extrapolation Factors for developmental toxicity: A preliminary research case study with perfluorooctanoate (PFOA). Regulatory Toxicology and Pharmacology 108: 104446.

Du, G; Huang, H; Hu, J; Qin, Y; Wu, D; Song, L, et al. (2013). Endocrine-related effects of perfluorooctanoic acid (PFOA) in zebrafish, H295R steroidogenesis and receptor reporter gene assays. Chemosphere 91: 1099-1106. http://dx.doi.org/10.1016/j.chemosphere.2013.01.012

Ducatman, A; Tan, Y; Nadeau, B; Steenland, K. (2023). Perfluorooctanoic Acid (PFOA) Exposure and Abnormal Alanine Aminotransferase: Using Clinical Consensus Cutoffs Compared to Statistical Cutoffs for Abnormal Values. Toxics 11: 449. http://dx.doi.org/10.3390/toxics11050449

Ducatman, A; Zhang, J; Fan, H. (2015). Prostate-specific antigen and perfluoroalkyl acids in the C8 health study population. Journal of Occupational and Environmental Medicine 57: 111-114.

Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA; Gibbs, ST, et al. (2019a). Toxicokinetics of perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) in male and female Hsd:Sprague dawley SD rats following intravenous or gavage administration. Xenobiotica 50: 1-11.

Dzierlenga, M, .W.; Crawford, L, .; Longnecker, M, .P. (2020a). Birth weight and perfluorooctane sulfonic acid: a random-effects meta-regression analysis. Environmental Epidemiology 4: e095. http://dx.doi.org/10.1097/EE9.0000000000000095

Dzierlenga, MW; Allen, BC; Clewell, HJ; Longnecker, MP. (2020b). Pharmacokinetic bias analysis of an association between clinical thyroid disease and two perfluoroalkyl substances. Environment International 141: 105784. http://dx.doi.org/10.1016/j.envint.2020.105784

Dzierlenga, MW; Allen, BC; Ward, PL; Clewell, HJ; Longnecker, MP. (2019b). A model of functional thyroid disease status over the lifetime. PLoS ONE 14: e0219769. http://dx.doi.org/10.1371/journal.pone.0219769

Dzierlenga, MW; Moreau, M; Song, G; Mallick, P; Ward, PL; Campbell, JL, et al. (2020c). Quantitative bias analysis of the association between subclinical thyroid disease and two perfluoroalkyl substances in a single study. Environmental Research 182: 109017. http://dx.doi.org/10.1016/j.envres.2019.109017

Ebert, A; Allendorf, F; Berger, U; Goss, KU; Ulrich, N. (2020). Membrane/water partitioning and permeabilities of perfluoroalkyl acids and four of their alternatives and the effects on toxicokinetic behavior. Environmental Science and Technology 54: 5051-5061.

Eggert, A; Cisneros-Montalvo, S; Anandan, S; Musilli, S; Stukenborg, JB; Adamsson, A, et al. (2019). The effects of perfluorooctanoic acid (PFOA) on fetal and adult rat testis. Reproductive Toxicology 90: 68-76. http://dx.doi.org/10.1016/j.reprotox.2019.08.005

Ehresman, DJ; Froehlich, JW; Olsen, GW; Chang, SC; Butenhoff, JL. (2007). Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. Environmental Research 103: 176-184. http://dx.doi.org/10.1016/j.envres.2006.06.008

Eick, SM; Demicco, E; Valeri, L; Woodruff, TJ; Morello-Frosch, R; Hom Thepaksorn, EK, et al. (2020). Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. Environmental Health 19: 100-100. http://dx.doi.org/10.1186/s12940-020-00654-2

Elcombe, BM. Compositions Comprising Perfluorooctanoic Acid, (World Intellectual Property Organization2013). https://patentimages.storage.googleapis.com/24/ee/73/f58267c7d70dde/WO2011101643A1.pdf

Elcombe, CR; Elcombe, BM; Farrar, DG; Foster, JR. (2007). Characterization of ammonium perfluorooctanoic acid (APFO) induced hepatomegaly in rats. Toxicology 240: 172-173. http://dx.doi.org/10.1016/j.tox.2007.06.052

Elcombe, CR; Elcombe, BM; Foster, JR; Farrar, DG; Jung, R; Chang, SC, et al. (2010). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. Archives of Toxicology 84: 787-798. http://dx.doi.org/10.1007/s00204-010-0572-2

Elcombe, CR; Peffer, RC; Wolf, DC; Bailey, J; Bars, R; Bell, D, et al. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator [Review]. Critical Reviews in Toxicology 44: 64-82. http://dx.doi.org/10.3109/10408444.2013.835786

Eldasher, LM; Wen, X; Little, MS; Bircsak, KM; Yacovino, LL; Aleksunes, LM. (2013). Hepatic and renal Bcrp transporter expression in mice treated with perfluorooctanoic acid. Toxicology 306: 108-113. http://dx.doi.org/10.1016/j.tox.2013.02.009

Elmore, SA; Dixon, D; Hailey, JR; Harada, H; Herbert, RA; Maronpot, RR, et al. (2016). Recommendations from the INHAND Apoptosis/Necrosis Working Group. Toxicologic Pathology 44. http://dx.doi.org/https://doi.org/10.1177/0192623315625850

EMEA. (2008). Non-clinical guideline on drug induced hepatotoxicity. (Doc. Ref. EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003355.pdf

EMEA. (2010). Reflection paper on non-clinical evaluation of drug-induced liver injury (DILI). (EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094591.pdf

Emmett, EA; Zhang, H; Shofer, FS; Freeman, D; Rodway, NV; Desai, C; Shaw, LM. (2006). Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters. Journal of Occupational and Environmental Medicine 48: 771-779. http://dx.doi.org/10.1097/01.jom.0000233380.13087.37

Ericson, I; Gómez, M; Nadal, M; van Bavel, B; Lindström, G; Domingo, JL. (2007). Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: a pilot study. Environment International 33: 616-623. http://dx.doi.org/10.1016/j.envint.2007.01.003

Eriksen, KT; Raaschou-Nielsen, O; Mclaughlin, JK; Lipworth, L; Tjønneland, A; Overvad, K; Sørensen, M. (2013). Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PLoS ONE 8: e56969. http://dx.doi.org/10.1371/journal.pone.0056969

Eriksen, KT; Sørensen, M; Mclaughlin, JK; Lipworth, L; Tjønneland, A; Overvad, K; Raaschou-Nielsen, O. (2009). Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. Journal of the National Cancer Institute 101: 605-609. http://dx.doi.org/10.1093/jnci/djp041

Ernst, A; Brix, N; Lauridsen, LLB; Olsen, J; Parner, ET; Liew, Z, et al. (2019). Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the danish national birth cohort. Environmental Health Perspectives 127: 17004.

Erol, E; Kumar, LS; Cline, GW; Shulman, GI; Kelly, DP; Binas, B. (2004). Liver fatty acid binding protein is required for high rates of hepatic fatty acid oxidation but not for the action of PPARalpha in fasting mice. FASEB Journal 18: 347-349. http://dx.doi.org/10.1096/fj.03-0330fje

Espindola-Santos, AD; Meyer, A; Dabkiewicz, VE; Câmara, VDM; Asmus, CIR, F. (2021). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonic acid in pregnant women: Maternal predictors and associations with birth outcomes in the PIPA Project. Journal of Obstetrics and Gynaecology Research 47: 3107-3118. http://dx.doi.org/10.1111/jog.14883

Fàbrega, F; Kumar, V; Benfenati, E; Schuhmacher, M; Domingo, JL; Nadal, M. (2015). Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. Toxicological and Environmental Chemistry 97: 814-827. http://dx.doi.org/10.1080/02772248.2015.1060976

Fàbrega, F; Kumar, V; Schuhmacher, M; Domingo, JL; Nadal, M. (2014). PBPK modeling for PFOS and PFOA: validation with human experimental data. Toxicology Letters 230: 244-251. http://dx.doi.org/10.1016/j.toxlet.2014.01.007

Falkner, B; Gidding, SS; Baker-Smith, CM; Brady, TM; Flynn, JT; Malle, LM, et al. (2023). Pediatric Primary Hypertension: An Underrecognized Condition: A Scientific Statement From the American Heart Association [Review]. Hypertension 80: e101-e111. http://dx.doi.org/10.1161/HYP.0000000000000228

Fan, H; Ducatman, A; Zhang, J. (2014). Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population. Environmental Research 135: 70-75.

Fan, Y; Lu, C; Li, X; Xu, Q; Zhang, Y; Yang, X, et al. (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environmental Pollution 266 Pt 2: 115138. http://dx.doi.org/10.1016/j.envpol.2020.115138

Fasano, WJ; Kennedy, GL; Szostek, B; Farrar, DG; Ward, RJ; Haroun, L; Hinderliter, PM. (2005). Penetration of ammonium perfluorooctanoate through rat and human skin in vitro. Drug and Chemical Toxicology 28: 79-90. http://dx.doi.org/10.1081/DCT-200039707

Fassler, CS; Pinney, SE; Xie, C; Biro, FM; Pinney, SM. (2019). Complex relationships between perfluorooctanoate, body mass index, insulin resistance and serum lipids in young girls. Environmental Research 176: 108558.

FDA. (2002). Guidance for industry: immunotoxicology evaluation of investigational new drugs.

FDA. (2009). Drug-induced liver injury: Premarketing clinical evaluation. (Docket no. FDA-2008-D-0128). Rockville, MD: U.S. Department of Health and Human Services, Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/drug-induced-liver-injury-premarketing-clinical-evaluation

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2008a). Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental Health Perspectives 116: 1391-1395. http://dx.doi.org/10.1289/ehp.11277

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2010a). Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scandinavian Journal of Work, Environment and Health 36: 413-421. http://dx.doi.org/10.5271/sjweh.2908

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2010b). Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environmental Research 110: 773-777. http://dx.doi.org/10.1016/j.envres.2010.08.004

Fei, C; Mclaughlin, JK; Tarone, RE; Olsen, J. (2007). Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. Environmental Health Perspectives 115: 1677-1682. http://dx.doi.org/10.1289/ehp.10506

Fei, C; Mclaughlin, JK; Tarone, RE; Olsen, J. (2008b). Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. American Journal of Epidemiology 168: 66-72. http://dx.doi.org/10.1093/aje/kwn095

Felter, SP; Foreman, JE; Boobis, A; Corton, JC; Doi, AM; Flowers, L, et al. (2018). Human relevance of rodent liver tumors: key insights from a toxicology forum workshop on nongenotoxic modes of

action. Regulatory Toxicology and Pharmacology 92: 1-7. http://dx.doi.org/https://doi.org/10.1016/j.yrtph.2017.11.003

Fenton, SE. (2006). Endocrine-disrupting compounds and mammary gland development: Early exposure and later life consequences [Review]. Endocrinology 147: S18-S24. http://dx.doi.org/10.1210/en.2005-1131

Fenton, SE; Ducatman, A; Boobis, A; DeWitt, JC; Lau, C; Ng, C, et al. (2021). Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research [Review]. Environmental Toxicology and Chemistry 40: 606-630. http://dx.doi.org/10.1002/etc.4890

Fenton, SE; Reiner, JL; Nakayama, SF; Delinsky, AD; Stanko, JP; Hines, EP, et al. (2009). Analysis of PFOA in dosed CD-1 mice. Part 2: Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups. Reproductive Toxicology 27: 365-372. http://dx.doi.org/10.1016/j.reprotox.2009.02.012

Fernandez, E, .; Perez, R, .; Hernandez, A, .; Tejada, P, .; Arteta, M, .; Ramos, J, .T. (2011). Factors and Mechanisms for Pharmacokinetic Differences between Pediatric Population and Adults. Pharmaceutics 3: 53-72. http://dx.doi.org/10.3390/pharmaceutics3010053

Fernández Freire, P; Pérez Martin, JM; Herrero, O; Peropadre, A; de La Peña, E; Hazen, MJ. (2008). In vitro assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid. Toxicology In Vitro 22: 1228-1233. http://dx.doi.org/10.1016/j.tiv.2008.04.004

Filgo, AJ; Quist, EM; Hoenerhoff, MJ; Brix, AE; Kissling, GE; Fenton, SE. (2015). Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARalpha is not required. Toxicologic Pathology 43: 558-568. http://dx.doi.org/10.1177/0192623314558463

Fisher, M; Arbuckle, TE; Wade, M; Haines, DA. (2013). Do perfluoroalkyl substances affect metabolic function and plasma lipids?--Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environmental Research 121: 95-103. http://dx.doi.org/10.1016/j.envres.2012.11.006

Fitz-Simon, N; Fletcher, T; Luster, MI; Steenland, K; Calafat, AM; Kato, K; Armstrong, B. (2013). Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology 24: 569-576. http://dx.doi.org/10.1097/EDE.0b013e31829443ee

Fletcher, T; Galloway, TS; Melzer, D; Holcroft, P; Cipelli, R; Pilling, LC, et al. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environment International 57-58: 2-10. http://dx.doi.org/10.1016/j.envint.2013.03.008

Florentin, A; Deblonde, T; Diguio, N; Hautemaniere, A; Hartemann, P. (2011). Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: cytotoxicity but no genotoxicity? International Journal of Hygiene and Environmental Health 214: 493-499. http://dx.doi.org/10.1016/j.ijheh.2011.05.010

Forsthuber, M; Kaiser, AM; Granitzer, S; Hassl, I; Hengstschläger, M; Stangl, H; Gundacker, C. (2020). Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environment International 137: 105324.

Fragki, S; Dirven, H; Fletcher, T; Grasl-Kraupp, B; Bjerve Gützkow, K; Hoogenboom, R, et al. (2021). Systemic PFOS and PFOA exposure and disturbed lipid homeostasis in humans: what do we know and what not? Critical Reviews in Toxicology141-164. http://dx.doi.org/10.1080/10408444.2021.1888073

Franco, ME; Fernandez-Luna, MT; Ramirez, AJ; Lavado, R. (2020a). Metabolomic-based assessment reveals dysregulation of lipid profiles in human liver cells exposed to environmental obesogens. Toxicology and Applied Pharmacology 398: 115009. http://dx.doi.org/10.1016/j.taap.2020.115009

Franco, ME; Sutherland, GE; Fernandez-Luna, MT; Lavado, R. (2020b). Altered expression and activity of phase I and II biotransformation enzymes in human liver cells by perfluorooctanoate (PFOA)

and perfluorooctane sulfonate (PFOS). Toxicology 430: 152339. http://dx.doi.org/10.1016/j.tox.2019.152339

Franken, C; Koppen, G; Lambrechts, N; Govarts, E; Bruckers, L; Den Hond, E, et al. (2017). Environmental exposure to human carcinogens in teenagers and the association with DNA damage. Environmental Research 152: 165-174. http://dx.doi.org/10.1016/j.envres.2016.10.012

Frisbee, SJ; Shankar, A; Knox, SS; Steenland, K; Savitz, DA; Fletcher, T; Ducatman, AM. (2010). Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Archives of Pediatrics and Adolescent Medicine 164: 860-869. http://dx.doi.org/10.1001/archpediatrics.2010.163

Fry, K; Power, MC. (2017). Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environmental Health: A Global Access Science Source 16: 105.

Fu, J; Gao, Y; Cui, L; Wang, T; Liang, Y; Qu, G, et al. (2016). Occurrence, temporal trends, and half-lives of perfluoroalkyl acids (PFAAs) in occupational workers in China. Scientific Reports 6: 38039.

Fujii, Y; Harada, KH; Kobayashi, H; Haraguchi, K; Koizumi, A. (2020). Lactational transfer of long-chain perfluorinated carboxylic acids in mice: A method to directly collect milk and evaluate chemical transferability. Toxics 8.

Fujii, Y; Niisoe, T; Harada, KH; Uemoto, S; Ogura, Y; Takenaka, K; Koizumi, A. (2015). Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. Journal of Occupational Health 57: 1-12.

Galazka, A; Kardymowicz, B. (1989). Immunity against diphtheria in adults in Poland. Epidemiology and Infection 103: 587-593. http://dx.doi.org/10.1017/s0950268800030983

Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. (1993). The immunological basis for immunization module 2 : Diphtheria. (WHO/EPI/Gen/93.11-18). Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. http://apps.who.int/iris/bitstream/handle/10665/58891/WHO-EPI-GEN-93.12-mod2-eng.pdf?sequence=38&isAllowed=y

Gallo, V; Leonardi, G; Genser, B; Lopez-Espinosa, MJ; Frisbee, SJ; Karlsson, L, et al. (2012). Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environmental Health Perspectives 120: 655-660. http://dx.doi.org/10.1289/ehp.1104436

Gao, B; He, X; Liu, W; Zhang, H; Saito, N; Tsuda, S. (2015a). Distribution of perfluoroalkyl compounds in rats: Indication for using hair as bioindicator of exposure. Journal of Exposure Science and Environmental Epidemiology 25: 632-638.

Gao, K, e; Zhuang, T; Liu, X; Fu, J; Zhang, J; Fu, J, ie, et al. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. Environmental Science and Technology 53: 6529-6538.

Gao, Y; Fu, J; Cao, H; Wang, Y; Zhang, A; Liang, Y, et al. (2015b). Differential Accumulation and Elimination Behavior of Perfluoroalkyl Acid Isomers in Occupational Workers in a Manufactory in China. Environmental Science and Technology 49: 6953-6962.

Gardener, H; Sun, Q; Grandjean, P. (2021). PFAS concentration during pregnancy in relation to cardiometabolic health and birth outcomes. Environmental Research 192: 110287. http://dx.doi.org/10.1016/j.envres.2020.110287

Garry, VF; Nelson, RL. (1981). An assay of cell transformation and cytotoxicity in C3H 10T 1/2 clonal cell line for the test chemical T-2942 CoC. (EPA-AR-226-0428). Minneapolis, MN: Stone Research Laboratories.

Gaylord, A; Berger, KI; Naidu, M; Attina, TM; Gilbert, J; Koshy, TT, et al. (2019). Serum perfluoroalkyl substances and lung function in adolescents exposed to the World Trade Center disaster. Environmental Research 172: 266-272.

Gaylord, A; Trasande, L; Kannan, K; Thomas, KM; Lee, S; Liu, M; Levine, J. (2020). Persistent organic pollutant exposure and celiac disease: A pilot study. Environmental Research 186: 109439.

Gazouli, M; Yao, ZX; Boujrad, N; Corton, JC; Culty, M; Papadopoulos, V. (2002). Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: Role of the peroxisome proliferator-activator receptor alpha. Endocrinology 143: 2571-2583. http://dx.doi.org/10.1210/endo.143.7.8895

Geiger, SD; Xiao, J; Ducatman, A; Frisbee, S; Innes, K; Shankar, A. (2014a). The association between PFOA, PFOS and serum lipid levels in adolescents. Chemosphere 98: 78-83. http://dx.doi.org/10.1016/j.chemosphere.2013.10.005

Geiger, SD; Xiao, J; Shankar, A. (2014b). No association between perfluoroalkyl chemicals and hypertension in children. Integrated Blood Pressure Control 7: 1-7. http://dx.doi.org/10.2147/IBPC.S47660

Genser, B; Teles, CA; Barreto, ML; Fischer, JE. (2015). Within- and between-group regression for improving the robustness of causal claims in cross-sectional analysis. Environmental Health 14: 60. http://dx.doi.org/10.1186/s12940-015-0047-2

Genuis, SJ; Birkholz, D; Ralitsch, M; Thibault, N. (2010). Human detoxification of perfluorinated compounds. Public Health 124: 367-375. http://dx.doi.org/10.1016/j.puhe.2010.03.002

Genuis, SJ; Liu, Y; Genuis, QI; Martin, JW. (2014). Phlebotomy treatment for elimination of perfluoroalkyl acids in a highly exposed family: a retrospective case-series. PLoS ONE 9: e114295.

Getz, GS; Reardon, CA. (2012). Animal models of atherosclerosis [Review]. Arteriosclerosis Thrombosis and Vascular Biology 32: 1104-1115. http://dx.doi.org/10.1161/ATVBAHA.111.237693

Ghisari, M; Long, M; Røge, DM; Olsen, J; Bonefeld-Jørgensen, EC. (2017). Polymorphism in xenobiotic and estrogen metabolizing genes, exposure to perfluorinated compounds and subsequent breast cancer risk: A nested case-control study in the Danish National Birth Cohort. Environmental Research 154: 325-333.

Gibson, SJ; Johnson, JD. (1979). Absorption of FC-143-14C in Rats After a Single Oral Dose. (USEPA Public Docket AR-226-0455). St. Paul, MN: Riker Laboratories, Inc. Subsidiary of 3M company.

Gilbert, SG; Weiss, B. (2006). A rationale for lowering the blood lead action level from 10 to 2 microg/dL [Review]. NeuroToxicology 27: 693-701. http://dx.doi.org/10.1016/j.neuro.2006.06.008

Gilliland, FD; Mandel, JS. (1993). Mortality among employees of a perfluorooctanoic acid production plant. Journal of Occupational Medicine 35: 950-954. http://dx.doi.org/10.1097/00043764-199309000-00020

Giménez-Bastida, JA; Surma, M; Zieliński, H. (2015). In vitro evaluation of the cytotoxicity and modulation of mechanisms associated with inflammation induced by perfluorooctanesulfonate and perfluorooctanoic acid in human colon myofibroblasts CCD-18Co. Toxicology In Vitro 29: 1683-1691. http://dx.doi.org/10.1016/j.tiv.2015.07.001

Girardi, P; Merler, E. (2019). A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Environmental Research 179: 108743.

Glassmeyer, ST; Furlong, ET; Kolpin, DW; Batt, AL; Benson, R; Boone, JS, et al. (2017). Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States. Science of the Total Environment 581-582: 909-922. http://dx.doi.org/10.1016/j.scitotenv.2016.12.004

Gleason, JA; Cooper, KR; Klotz, JB; Post, GB; Van Orden, G; New Jersey Drinking Water Quality Institute (NJDWQI). (2017). Health-based maximum contaminant level support document: Perfluorooctanoic acid (PFOA): Appendix A. https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf

Gleason, JA; Post, GB; Fagliano, JA. (2015). Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environmental Research 136: 8-14. http://dx.doi.org/10.1016/j.envres.2014.10.004

Gluckman, PD; Hanson, MA; Cooper, C; Thornburg, KL. (2008). Effect of in utero and early-life conditions on adult health and disease. New England Journal of Medicine 359: 61-73. http://dx.doi.org/10.1056/NEJMra0708473

Glynn, A; Berger, U; Bignert, A; Ullah, S; Aune, M; Lignell, S; Darnerud, PO. (2012). Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environmental Science and Technology 46: 9071-9079. http://dx.doi.org/10.1021/es301168c

Goeden, HM; Greene, CW; Jacobus, JA. (2019). A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. Journal of Exposure Science and Environmental Epidemiology 29: 183-195. http://dx.doi.org/10.1038/s41370-018-0110-5

Goff, DC; Lloyd-Jones, DM; Bennett, G; Coady, S; D'Agostino, RB; Gibbons, R, et al. (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 129: S49-S73. http://dx.doi.org/10.1161/01.cir.0000437741.48606.98

Gogola, J; Hoffmann, M; Nimpsz, S; Ptak, A. (2020a). Disruption of 17β-estradiol secretion by persistent organic pollutants present in human follicular fluid is dependent on the potential of ovarian granulosa tumor cell lines to metabolize estrogen. Molecular and Cellular Endocrinology 503: 110698. http://dx.doi.org/10.1016/j.mce.2019.110698

Gogola, J; Hoffmann, M; Ptak, A. (2019). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of granulosa tumor spheroids via GPR30 and IGF1R but not via the classic estrogen receptors. Chemosphere 217: 100-110. http://dx.doi.org/10.1016/j.chemosphere.2018.11.018

Gogola, J; Hoffmann, M; Ptak, A. (2020b). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate IGF1 secretion by adult ovarian granulosa cell tumor spheroids and thereby increase proliferation of non-cancer ovarian granulosa cells. Toxicology In Vitro 65: 104769. http://dx.doi.org/10.1016/j.tiv.2020.104769

Gomis, MI; Vestergren, R; Macleod, M; Mueller, JF; Cousins, IT. (2017). Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling. Environment International 108: 92-102. http://dx.doi.org/10.1016/j.envint.2017.08.002

Gomis, MI; Vestergren, R; Nilsson, H; Cousins, IT. (2016). Contribution of Direct and Indirect Exposure to Human Serum Concentrations of Perfluorooctanoic Acid in an Occupationally Exposed Group of Ski Waxers. Environmental Science and Technology 50: 7037-7046. http://dx.doi.org/10.1021/acs.est.6b01477

Goodrich, JA; Walker, D; Lin, X; Wang, H; Lim, T; Mcconnell, R, et al. (2022). Exposure to perfluoroalkyl substances and risk of hepatocellular carcinoma in a multiethnic cohort. 4: 100550. http://dx.doi.org/10.1016/j.jhepr.2022.100550

Gorrochategui, E; Pérez-Albaladejo, E; Casas, J; Lacorte, S; Porte, C. (2014). Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. Toxicology and Applied Pharmacology 277: 124-130. http://dx.doi.org/10.1016/j.taap.2014.03.012

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Chen, CJ; Ito, S, et al. (2017). Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environment International 104: 132-138.

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Kobayashi, S; Chen, CJ, et al. (2016). Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environment International 94: 124-132.

Govarts, E; Iszatt, N; Trnovec, T; de Cock, M; Eggesbø, M; Palkovicova Murinova, L, et al. (2018). Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven European birth cohorts. Environment International 115: 267-278.

Govarts, E; Remy, S; Bruckers, L; Den Hond, E; Sioen, I; Nelen, V, et al. (2016). Combined effects of prenatal exposures to environmental chemicals on birth weight. International Journal of Environmental Research and Public Health 13: n/a.

Governini, L; Guerranti, C; De Leo, V; Boschi, L; Luddi, A; Gori, M, et al. (2015). Chromosomal aneuploidies and DNA fragmentation of human spermatozoa from patients exposed to perfluorinated compounds. Andrologia 47: 1012-1019. http://dx.doi.org/10.1111/and.12371

Graber, JM; Alexander, C; Laumbach, RJ; Black, K; Strickland, PO; Georgopoulos, PG, et al. (2019). Per and polyfluoroalkyl substances (PFAS) blood levels after contamination of a community water supply and comparison with 2013-2014 NHANES. Journal of Exposure Science and Environmental Epidemiology 29: 172-182.

Grandjean, P; Andersen, EW; Budtz-Jørgensen, E; Nielsen, F; Mølbak, K; Weihe, P; Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA: Journal of the American Medical Association 307: 391-397. http://dx.doi.org/10.1001/jama.2011.2034

Grandjean, P; Budtz-Jørgensen, E. (2013). Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environmental Health: A Global Access Science Source 12: 35. http://dx.doi.org/10.1186/1476-069X-12-35

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Budtz-Jørgensen, E. (2017a). Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. Environmental Health Perspectives 125: 077018.

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Timmermann, A; Budtz-Jørgensen, E. (2017b). Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. Journal of Immunotoxicology 14: 188-195.

Grandjean, P; Timmermann, CAG; Kruse, M; Nielsen, F; Vinholt, PJ; Boding, L, et al. (2020). Severity of COVID-19 at elevated exposure to perfluorinated alkylates. PLoS ONE 15: e0244815. http://dx.doi.org/10.1371/journal.pone.0244815

Granum, B; Haug, LS; Namork, E; Stølevik, SB; Thomsen, C; Aaberge, IS, et al. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. Journal of Immunotoxicology 10: 373-379. http://dx.doi.org/10.3109/1547691X.2012.755580

Greenland, S; Longnecker, MP. (1992). Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. American Journal of Epidemiology 135: 1301-1309.

Griffith, FD; Long, JE. (1980). Animal toxicity studies with ammonium perfluorooctanoate. American Industrial Hygiene Association Journal 41: 576-583. http://dx.doi.org/10.1080/15298668091425301

Gross, RS; Ghassabian, A; Vandyousefi, S; Messito, MJ; Gao, C; Kannan, K; Trasande, L. (2020). Persistent organic pollutants exposure in newborn dried blood spots and infant weight status: A case-control study of low-income Hispanic mother-infant pairs. Environmental Pollution 267: 115427. http://dx.doi.org/10.1016/j.envpol.2020.115427

Gundacker, C; Graf-Rohrmeister, K; Gencik, M; Hengstschläger, M; Holoman, K; Rosa, P, et al. (2021). Gene variants determine placental transfer of perfluoroalkyl substances (PFAS), mercury (Hg) and lead (Pb), and birth outcome: Findings from the Ummuki Bratislava-Vienna study. Frontiers in Genetics 12: 664946. http://dx.doi.org/10.3389/fgene.2021.664946

Guo, H; Chen, J; Zhang, H; Yao, J; Sheng, N; Li, Q, et al. (2021a). Exposure to genX and its novel analogs disrupts hepatic bile acid metabolism in male mice. Environmental Science & Technology. http://dx.doi.org/10.1021/acs.est.1c02471

Guo, H; Wang, J; Yao, J; Sun, S; Sheng, N; Zhang, X, et al. (2019). Comparative hepatotoxicity of novel PFOA alternatives (perfluoropolyether carboxylic acids) on male mice. Environmental Science and Technology 53: 3929-3937.

Guo, H; Zhang, H; Sheng, N; Wang, J; Chen, J; Dai, J. (2021b). Perfluorooctanoic acid (PFOA) exposure induces splenic atrophy via overactivation of macrophages in male mice. Journal of Hazardous Materials 407: 124862. http://dx.doi.org/10.1016/j.jhazmat.2020.124862

Guruge, KS; Yeung, LW; Yamanaka, N; Miyazaki, S; Lam, PK; Giesy, JP, et al. (2006). Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicological Sciences 89: 93-107. http://dx.doi.org/10.1093/toxsci/kfj011

Guyatt, HL; Snow, RW. (2004). Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clinical Microbiology Reviews 17: 760-769. http://dx.doi.org/10.1128/cmr.17.4.760-769.2004

Gyllenhammar, I; Diderholm, B; Gustafsson, J; Berger, U; Ridefelt, P; Benskin, JP, et al. (2018). Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environment International 111: 191-199.

Gyllenhammar, I; lynn, A; Lignell, S; Aune, M; Cantillana, T; Fridén, U; Lampa, E. (2017). Evaluation of the relationship between maternal body burden of POPs during pregnancy and lactation period and the health of their children. (2215-17-008). Swedish Environmental Protection Agency.

Hack, M; Flannery, DJ; Schluchter, M; Cartar, L; Borawski, E; Klein, N. (2002). Outcomes in young adulthood for very-low-birth-weight infants. New England Journal of Medicine 346: 149-157. http://dx.doi.org/10.1056/NEJMoa010856

Hack, M; Klein, NK; Taylor, HG. (1995). Long-term developmental outcomes of low birth weight infants. The Future of Children 5: 176-196.

Hagenaars, A; Vergauwen, L; Benoot, D; Laukens, K; Knapen, D. (2013). Mechanistic toxicity study of perfluorooctanoic acid in zebrafish suggests mitochondrial dysfunction to play a key role in PFOA toxicity. Chemosphere 91: 844-856. http://dx.doi.org/10.1016/j.chemosphere.2013.01.056

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A, et al. (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicologic Pathology 40: 971-994. http://dx.doi.org/10.1177/0192623312448935

Hamm, MP; Cherry, NM; Chan, E; Martin, JW; Burstyn, I. (2010). Maternal exposure to perfluorinated acids and fetal growth. Journal of Exposure Science and Environmental Epidemiology 20: 589-597. http://dx.doi.org/10.1038/jes.2009.57

Han, X; Kemper, RA; Jepson, GW. (2005). Subcellular distribution and protein binding of perfluorooctanoic acid in rat liver and kidney. Drug and Chemical Toxicology 28: 197-209. http://dx.doi.org/10.1081/DCT-52547

Han, X; Meng, L; Zhang, G; Li, Y; Shi, Y; Zhang, Q; Jiang, G. (2021). Exposure to novel and legacy per- and polyfluoroalkyl substances (PFASs) and associations with type 2 diabetes: A case-control study in East China. Environment International 156: 106637. http://dx.doi.org/10.1016/j.envint.2021.106637

Han, X; Snow, TA; Kemper, RA; Jepson, GW. (2003). Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16: 775-781. http://dx.doi.org/10.1021/tx034005w

Hanahan, D. (2022). Hallmarks of cancer: New dimensions [Review]. Cancer Discovery 12: 31-46. http://dx.doi.org/10.1158/2159-8290.CD-21-1059

Hanahan, D; Weinberg, RA. (2000). The hallmarks of cancer [Review]. Cell 100: 57-70. http://dx.doi.org/10.1016/S0092-8674(00)81683-9

Hanahan, D; Weinberg, RA. (2011). Hallmarks of cancer: The next generation [Review]. Cell 144: 646-674. http://dx.doi.org/10.1016/j.cell.2011.02.013

Hansen, KJ; Johnson, HO; Eldridge, JS; Butenhoff, JL; Dick, LA. (2002). Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. Environmental Science and Technology 36: 1681-1685. http://dx.doi.org/10.1021/es010780r

Hardell, E; Kärrman, A; van Bavel, B; Bao, J; Carlberg, M; Hardell, L. (2014). Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63: 35-39. http://dx.doi.org/10.1016/j.envint.2013.10.005

Haug, LS; Huber, S; Becher, G; Thomsen, C. (2011). Characterisation of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. Environment International 37: 687-693. http://dx.doi.org/10.1016/j.envint.2011.01.011

Hayashi, M. (2016). The micronucleus test-most widely used in vivo genotoxicity test [Review]. Genes and Environment 38: 18. http://dx.doi.org/10.1186/s41021-016-0044-x

He, X; Liu, Y; Xu, B; Gu, L; Tang, W. (2018). PFOA is associated with diabetes and metabolic alteration in US men: National Health and Nutrition Examination Survey 2003-2012. Science of the Total Environment 625: 566-574.

Hessel, EVS; Tonk, ECM; Bos, PMJ; Van Loveren, H; Piersma, AH. (2015). Developmental immunotoxicity of chemicals in rodents and its possible regulatory impact [Review]. Critical Reviews in Toxicology 45: 68-82. http://dx.doi.org/10.3109/10408444.2014.959163

Hill, AB. (1965). The environment and disease: Association or causation? Proceedings of the Royal Society of Medicine 58: 295-300.

Hinderliter, PM; Delorme, MP; Kennedy, GL. (2006a). Perfluorooctanoic acid: Relationship between repeated inhalation exposures and plasma PFOA concentration in the rat. Toxicology 222: 80-85. http://dx.doi.org/10.1016/j.tox.2006.01.029

Hinderliter, PM; Han, X; Kennedy, GL; Butenhoff, JL. (2006b). Age effect on perfluorooctanoate (PFOA) plasma concentration in post-weaning rats following oral gavage with ammonium perfluorooctanoate (APFO). Toxicology 225: 195-203. http://dx.doi.org/10.1016/j.tox.2006.06.002

Hinderliter, PM; Mylchreest, E; Gannon, SA; Butenhoff, JL; Kennedy, GL. (2005). Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. Toxicology 211: 139-148. http://dx.doi.org/10.1016/j.tox.2005.03.010

Hjermitslev, MH; Long, M; Wielsøe, M; Bonefeld-Jørgensen, EC. (2019). Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. Science of the Total Environment 698: 134118.

Hocevar, SE; Kamendulis, LM; Hocevar, BA. (2020). Perfluorooctanoic acid activates the unfolded protein response in pancreatic acinar cells. Journal of Biochemical and Molecular Toxicology 34: e22561. http://dx.doi.org/10.1002/jbt.22561

Honda-Kohmo, K; Hutcheson, R; Innes, KE; Conway, BN. (2019). Perfluoroalkyl substances are inversely associated with coronary heart disease in adults with diabetes. Journal of Diabetes and its Complications 33: 407-412.

Hossein-Khannazer, N; Zian, Z; Bakkach, J; Kamali, AN; Hosseinzadeh, R; Anka, AU, et al. (2021). Features and roles of T helper 22 cells in immunological diseases and malignancies [Review]. Scandinavian Journal of Immunology 93: e13030. http://dx.doi.org/10.1111/sji.13030

Hotez, P. (2019). America and Europe's new normal: the return of vaccine-preventable diseases. 85: 912-914. http://dx.doi.org/10.1038/s41390-019-0354-3

Hu, J; Li, J; Wang, J; Zhang, A; Dai, J. (2014). Synergistic effects of perfluoroalkyl acids mixtures with J-shaped concentration-responses on viability of a human liver cell line. Chemosphere 96: 81-88. http://dx.doi.org/10.1016/j.chemosphere.2013.07.033

Hu, Q; Franklin, JN; Bryan, I; Morris, E; Wood, A; Dewitt, JC. (2012). Does developmental exposure to perflurooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders? NeuroToxicology 33: 1491-1498.

Hu, Q; Strynar, MJ; Dewitt, JC. (2010). Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? Journal of Immunotoxicology 7: 344-349. http://dx.doi.org/10.3109/1547691X.2010.520045

Hu, XDC; Tokranov, AK; Liddie, J; Zhang, XM; Grandjean, P; Hart, JE, et al. (2019). Tap Water Contributions to Plasma Concentrations of Poly- and Perfluoroalkyl Substances (PFAS) in a

Nationwide Prospective Cohort of U.S. Women. Environmental Health Perspectives 127: 67006. http://dx.doi.org/10.1289/EHP4093

Hu, XZ; Hu, DC. (2009). Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. Archives of Toxicology 83: 851-861. http://dx.doi.org/10.1007/s00204-009-0441-z

Huang, H; Yu, K; Zeng, X; Chen, Q; Liu, Q; Zhao, Y, et al. (2020). Association between prenatal exposure to perfluoroalkyl substances and respiratory tract infections in preschool children. Environmental Research 191: 110156. http://dx.doi.org/10.1016/j.envres.2020.110156

Huang, JS; Borensztajn, J; Reddy, JK. (2011). Hepatic lipid metabolism. In SPS Monga (Ed.), Molecular pathology of liver diseases (pp. 133–146). Boston, MA: Springer. http://dx.doi.org/10.1007/978-1-4419-7107-4_10

Huang, M; Jiao, J; Zhuang, P; Chen, X; Wang, J; Zhang, Y. (2018). Serum polyfluoroalkyl chemicals are associated with risk of cardiovascular diseases in national US population. Environment International 119: 37-46.

Huang, Q; Zhang, J; Martin, FL; Peng, S; Tian, M; Mu, X; Shen, H. (2013). Perfluorooctanoic acid induces apoptosis through the p53-dependent mitochondrial pathway in human hepatic cells: a proteomic study. Toxicology Letters 223: 211-220. http://dx.doi.org/10.1016/j.toxlet.2013.09.002

Huang, Q; Zhang, J; Peng, S; Du, M; Ow, S; Pu, H, et al. (2014). Proteomic analysis of perfluorooctane sulfonate-induced apoptosis in human hepatic cells using the iTRAQ technique. Journal of Applied Toxicology 34: 1342-1351. http://dx.doi.org/10.1002/jat.2963

Huck, I; Beggs, K; Apte, U. (2018). Paradoxical protective effect of perfluorooctanesulfonic acid against high-fat diet-induced hepatic steatosis in mice. International Journal of Toxicology 37: 383-392. http://dx.doi.org/10.1177/1091581818790934

Hui, Z; Li, R; Chen, L. (2017). The impact of exposure to environmental contaminant on hepatocellular lipid metabolism. Gene 622: 67-71. http://dx.doi.org/10.1016/j.gene.2017.04.024

Humblet, O; Diaz-Ramirez, LG; Balmes, JR; Pinney, SM; Hiatt, RA. (2014). Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). Environmental Health Perspectives 122: 1129-1133. http://dx.doi.org/10.1289/ehp.1306606

Hundley, SG; Sarrif, AM; Kennedy, GL. (2006). Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. Drug and Chemical Toxicology 29: 137-145. http://dx.doi.org/10.1080/01480540600561361

Huo, X; Huang, R; Gan, Y; Luo, K; Aimuzi, R; Nian, M, et al. (2020a). Perfluoroalkyl substances in early pregnancy and risk of hypertensive disorders of pregnancy: A prospective cohort study. Environment International 138: 105656.

Huo, X; Zhang, L; Huang, R; Feng, L; Wang, W; Zhang, J; Cohort, SB. (2020b). Perfluoroalkyl substances exposure in early pregnancy and preterm birth in singleton pregnancies: a prospective cohort study. Environmental Health: A Global Access Science Source 19: 60.

Hurley, S; Goldberg, D; Wang, M; Park, JS; Petreas, M; Bernstein, L, et al. (2018). Breast cancer risk and serum levels of per- and poly-fluoroalkyl substances: a case-control study nested in the California Teachers Study. Environmental Health: A Global Access Science Source 17: 83.

Hutcheson, R; Innes, K; Conway, B. (2020). Perfluoroalkyl substances and likelihood of stroke in persons with and without diabetes. Diabetes and Vascular Disease Research 17: 1-8.

IARC (International Agency for Research on Cancer). (2016). Some chemicals used as solvents and in polymer manufacture Perfluorooctanoic acid. In IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyons, France: World Health Organization. http://monographs.iarc.fr/ENG/Monographs/vol110/mono110-01.pdf

IARC. (2019). Preamble to the IARC Monographs. In IARC Monographs on the Identification of Carcinogenic Hazards to Humans. Lyon, France. https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf

Impinen, A; Longnecker, MP; Nygaard, UC; London, SJ; Ferguson, KK; Haug, LS; Granum, B. (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy

and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. Environment International 124: 462-472.

Impinen, A; Nygaard, UC; Lødrup Carlsen, KC; Mowinckel, P; Carlsen, KH; Haug, LS; Granum, B. (2018). Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environmental Research 160: 518-523.

Innes, KE; Wimsatt, JH; Frisbee, S; Ducatman, AM. (2014). Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer 14: 45. http://dx.doi.org/10.1186/1471-2407-14-45

Ioannou, GN; Boyko, EJ; Lee, SP. (2006a). The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. American Journal of Gastroenterology 101: 76-82. http://dx.doi.org/10.1111/j.1572-0241.2005.00341.x

Ioannou, GN; Weiss, NS; Boyko, EJ; Mozaffarian, D; Lee, SP. (2006b). Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. Hepatology 43: 1145-1151. http://dx.doi.org/10.1002/hep.21171

IPCS. (2012). Harmonization project document no. 10: Guidance for immunotoxicity risk assessment for chemicals. Geneva, Switzerland: World Health Organization. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Ito, S; Alcorn, J. (2003). Xenobiotic transporter expression and function in the human mammary gland. Advanced drug delivery reviews 55: 653-665.

Itoh, H; Harada, KH; Kasuga, Y; Yokoyama, S; Onuma, H; Nishimura, H, et al. (2021). Serum perfluoroalkyl substances and breast cancer risk in Japanese women: A case-control study. Science of the Total Environment 800: 149316. http://dx.doi.org/10.1016/j.scitotenv.2021.149316

ITRC (Interstate Technology & Regulatory Council). (2020). PFAS technical and regulatory guidance document and fact sheets [Website]. https://pfas-1.itrcweb.org/

Jackson-Browne, MS; Eliot, M; Patti, M; Spanier, AJ; Braun, JM. (2020). PFAS (per- and polyfluoroalkyl substances) and asthma in young children: NHANES 2013-2014. International Journal of Hygiene and Environmental Health 229: 113565.

Jacquet, N; Maire, MA; Rast, C; Bonnard, M; Vasseur, P. (2012). Perfluorooctanoic acid (PFOA) acts as a tumor promoter on Syrian hamster embryo (SHE) cells. Environmental Science and Pollution Research 19: 2537-2549. http://dx.doi.org/10.1007/s11356-012-0968-z

Jain, RB. (2014). Contribution of diet and other factors to the levels of selected polyfluorinated compounds: data from NHANES 2003-2008. International Journal of Hygiene and Environmental Health 217: 52-61. http://dx.doi.org/10.1016/j.ijheh.2013.03.008

Jain, RB. (2019). Concentration of selected liver enzymes across the stages of glomerular function: The associations with PFOA and PFOS. Heliyon 5: e02168.

Jain, RB. (2020a). Impact of the co-occurrence of obesity with diabetes, anemia, hypertension, and albuminuria on concentrations of selected perfluoroalkyl acids. Environmental Pollution 266 Pt. 2: 115207.

Jain, RB. (2020b). Variabilities in concentrations of selected perfluoroalkyl acids among normotensives and hypertensives across various stages of glomerular function. Archives of Environmental and Occupational Health 76: 1-11.

Jain, RB; Ducatman, A. (2018). Associations between lipid/lipoprotein levels and perfluoroalkyl substances among US children aged 6-11 years. Environmental Pollution 243: 1-8.

Jain, RB; Ducatman, A. (2019a). Perfluoroalkyl acids serum concentrations and their relationship to biomarkers of renal failure: Serum and urine albumin, creatinine, and albumin creatinine ratios across the spectrum of glomerular function among US adults. Environmental Research 174: 143-151.

Jain, RB; Ducatman, A. (2019b). Roles of gender and obesity in defining correlations between perfluoroalkyl substances and lipid/lipoproteins. Science of the Total Environment 653: 74-81.

Jain, RB; Ducatman, A. (2019c). Selective associations of recent low concentrations of perfluoroalkyl substances with liver function biomarkers: nhanes 2011 to 2014 data on us adults aged ≥20 years. Journal of Occupational and Environmental Medicine 61: 293-302.

Jain, RB; Ducatman, A. (2020). Associations between apolipoprotein B and selected perfluoroalkyl substances among diabetics and nondiabetics. Environmental Science and Pollution Research International 2020: 13819-13828. http://dx.doi.org/10.1007/s11356-020-11593-3

JAMA. (2002). JAMA patient page. Low birth weight. JAMA: Journal of the American Medical Association 287: 270.

Jantzen, CE; Annunziato, KA; Bugel, SM; Cooper, KR. (2016a). PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. Aquatic Toxicology 175: 160-170. http://dx.doi.org/10.1016/j.aquatox.2016.03.026

Jantzen, CE; Annunziato, KM; Cooper, KR. (2016b). Behavioral, morphometric, and gene expression effects in adult zebrafish (Danio rerio) embryonically exposed to PFOA, PFOS, and PFNA. Aquatic Toxicology 180: 123-130. http://dx.doi.org/10.1016/j.aquatox.2016.09.011

Jantzen, CE; Toor, F; Annunziato, KA; Cooper, KR. (2017). Effects of chronic perfluorooctanoic acid (PFOA) at low concentration on morphometrics, gene expression, and fecundity in zebrafish (Danio rerio). Reproductive Toxicology 69: 34-42. http://dx.doi.org/10.1016/j.reprotox.2017.01.009

Jarvis, AL; Justice, JR; Elias, MC; Schnitker, B; Gallagher, K. (2021). Perfluorooctane Sulfonate in US Ambient Surface Waters: A Review of Occurrence in Aquatic Environments and Comparison to Global Concentrations. Environmental Toxicology and Chemistry 40: 2425-2442. http://dx.doi.org/10.1002/etc.5147

Jensen, RC; Andersen, MS; Larsen, PV; Glintborg, D; Dalgård, C; Timmermann, CAG, et al. (2020). Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study. Environmental Health Perspectives 128: 77001.

Jensen, TK; Andersen, LB; Kyhl, HB; Nielsen, F; Christesen, HT; Grandjean, P. (2015). Association between Perfluorinated Compound Exposure and Miscarriage in Danish Pregnant Women. PLoS ONE 10: e0123496.

Ji, J; Song, L; Wang, J; Yang, Z; Yan, H; Li, T, et al. (2021). Association between urinary per- and poly-fluoroalkyl substances and COVID-19 susceptibility. Environment International 153: 106524. http://dx.doi.org/10.1016/j.envint.2021.106524

Jiang, W; Deng, Y; Song, Z; Xie, Y; Gong, L; Chen, Y; Kuang, H. (2020). Gestational Perfluorooctanoic Acid Exposure Inhibits Placental Development by Dysregulation of Labyrinth Vessels and uNK Cells and Apoptosis in Mice. Frontiers in Physiology 11: 51.

Jiang, W; Zhang, Y; Zhu, L; Deng, J. (2014). Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environment International 64: 40-47.

Jin, H; Zhang, Y; Jiang, W; Zhu, L; Martin, JW. (2016). Isomer-Specific Distribution of Perfluoroalkyl Substances in Blood. Environmental Science and Technology 50: 7808-7815.

Jin, R; Mcconnell, R; Catherine, C; Xu, S; Walker, DI; Stratakis, N, et al. (2020). Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environment International 134: 105220.

Johanson, CE. (1979). Distribution of fluid between extracellular and intracellular compartments in the heart, lungs, liver and spleen of neonatal rats. 36: 282-289. http://dx.doi.org/10.1159/000241241

Johnson, PI; Sutton, P; Atchley, DS; Koustas, E; Lam, J; Sen, S, et al. (2014). The navigation guide - evidence-based medicine meets environmental health: Systematic review of human evidence for PFOA effects on fetal growth [Review]. Environmental Health Perspectives 122: 1028-1039. http://dx.doi.org/10.1289/ehp.1307893

Jusko, TA; Oktapodas, M; Palkovičová Murinová, L; Babinská, K; Babjaková, J; Verner, MA, et al. (2016). Demographic, reproductive, and dietary determinants of perfluorooctane sulfonic (PFOS) and perfluorooctanoic acid (PFOA) concentrations in human colostrum. Environmental Science and Technology 50: 7152-7162. http://dx.doi.org/10.1021/acs.est.6b00195

Kamendulis, LM; Hocevar, JM; Stephens, M; Sandusky, GE; Hocevar, BA. (2022). Exposure to perfluorooctanoic acid leads to promotion of pancreatic cancer. Carcinogenesis. http://dx.doi.org/10.1093/carcin/bgac005

Kamendulis, LM; Wu, Q; Sandusky, GE; Hocevar, BA. (2014). Perfluorooctanoic acid exposure triggers oxidative stress in the mouse pancreas. Toxicology Reports 1: 513-521. http://dx.doi.org/10.1016/j.toxrep.2014.07.015

Kang, H; Choi, K; Lee, HS; Kim, DH; Park, NY; Kim, S; Kho, Y. (2016a). Elevated levels of short carbon-chain PFCAs in breast milk among Korean women: Current status and potential challenges. Environmental Research 148: 351-359. http://dx.doi.org/10.1016/j.envres.2016.04.017

Kang, H; Lee, HK; Moon, HB; Kim, S; Lee, J; Ha, M, et al. (2018). Perfluoroalkyl acids in serum of Korean children: Occurrences, related sources, and associated health outcomes. Science of the Total Environment 645: 958-965.

Kang, JS; Choi, JS; Park, JW. (2016b). Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells. Chemosphere 155: 436-443. http://dx.doi.org/10.1016/j.chemosphere.2016.04.070

Kang, Q; Gao, F; Zhang, X; Wang, L; Liu, J; Fu, M, et al. (2020). Nontargeted identification of per- and polyfluoroalkyl substances in human follicular fluid and their blood-follicle transfer. Environment International 139: 105686.

Kapraun, DF; Zurlinden, TJ; Verner, M-A; Chiang, C; Dzierlenga, MW; Carlson, LM, et al. (2022). A Generic Pharmacokinetic Model for Quantifying Mother-to-Offspring Transfer of Lipophilic Persistent Environmental Chemicals. Toxicological Sciences.

Kasarala, G; Tillmann, HL. (2016). Standard liver tests. Clinical Liver Disease 8: 13-18. http://dx.doi.org/10.1002/cld.562

Kashino, I; Sasaki, S; Okada, E; Matsuura, H; Goudarzi, H; Miyashita, C, et al. (2020). Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environment International 136: 105355.

Kato, K; Wong, LY; Chen, A; Dunbar, C; Webster, GM; Lanphear, BP; Calafat, AM. (2014). Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environmental Science and Technology 48: 9600-9608.

Kato, K; Wong, LY; Jia, LT; Kuklenyik, Z; Calafat, AM. (2011). Trends in exposure to polyfluoroalkyl chemicals in the US population: 1999-2008. Environmental Science & Technology 45: 8037-8045. http://dx.doi.org/10.1021/es1043613

Kavlock, RJ; Allen, BC; Faustman, EM; Kimmel, CA. (1995). Dose-response assessments for developmental toxicity. IV. Benchmark doses for fetal weight changes. Toxicological Sciences 26: 211-222. http://dx.doi.org/10.1006/faat.1995.1092

Kawamoto, K; Oashi, T; Oami, K; Liu, W; Jin, YH; Saito, N, et al. (2010). Perfluorooctanoic acid (PFOA) but not perfluorooctane sulfonate (PFOS) showed DNA damage in comet assay on Paramecium caudatum. Journal of Toxicological Sciences 35: 835-841. http://dx.doi.org/10.2131/jts.35.835

Kemper, R. (2003). Perfluorooctanoic acid: Toxicokinetics in the rat. (DuPont 7473; US EPA Public Docket Administrative Record AR-226-1499). E.I. du Pont de Nemours and Company, Haskell Laboratory for Health and Environmental Sciences.

Kennedy, GL. (1985). Dermal toxicity of ammonium perfluorooctanoate. Toxicology and Applied Pharmacology 81: 348-355. http://dx.doi.org/10.1016/0041-008X(85)90172-3

Kerstner-Wood, C; Coward, L; Gorman, G; Southern Research Institute. (2003). Protein binding of perfluorobutane sulfonate, perfluorohexane sulfonate, perfluorooctane sulfonate and perfluorooctanoate to plasma (human, rat, and monkey), and various human-derived plasma protein fractions. Study ID 9921.7 [TSCA Submission]. (8EHQ-04-15845A; 88040000364). St. Paul, MN: 3M Company.

Khalil, N; Ducatman, AM; Sinari, S; Billheimer, D; Hu, C; Littau, S; Burgess, JL. (2020). Per- and polyfluoroalkyl substance and cardio metabolic markers in firefighters. Journal of Occupational and Environmental Medicine 62: 1076-1081. http://dx.doi.org/10.1097/JOM.0000000000002062

Khalil, N; Ebert, JR; Honda, M; Lee, M; Nahhas, RW; Koskela, A, et al. (2018). Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8-12 year old children: A pilot study. Environmental Research 160: 314-321.

Ki, SH; Park, O; Zheng, M; Morales-Ibanez, O; Kolls, JK; Bataller, R; Gao, B. (2010). Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. Hepatology 52: 1291-1300. http://dx.doi.org/10.1002/hep.23837

Kielsen, K; Shamim, Z; Ryder, LP; Nielsen, F; Grandjean, P; Budtz-Jørgensen, E; Heilmann, C. (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. Journal of Immunotoxicology 13: 270-273. http://dx.doi.org/10.3109/1547691X.2015.1067259

Kim, HC; Nam, CM; Jee, SH; Han, KH; Oh, DK; Suh, I. (2004). Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. British Medical Journal (Clinical Research Edition) 328: 983. http://dx.doi.org/10.1136/bmj.38050.593634.63

Kim, JH; Qu, A; Reddy, JK; Gao, B; Gonzalez, FJ. (2014). Hepatic oxidative stress activates the Gadd45b gene by way of degradation of the transcriptional repressor STAT3. Hepatology 59: 695-704. http://dx.doi.org/10.1002/hep.26683

Kim, RB. (2003). Organic anion-transporting polypeptide (OATP) transporter family and drug disposition [Review]. European Journal of Clinical Investigation 33 Suppl 2: 1-5. http://dx.doi.org/10.1046/j.1365-2362.33.s2.5.x

Kim, S; Choi, K; Ji, K; Seo, J; Kho, Y; Park, J, et al. (2011a). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environmental Science and Technology 45: 7465-7472. http://dx.doi.org/10.1021/es202408a

Kim, SJ; Heo, SH; Lee, DS; Hwang, IG; Lee, YB; Cho, HY. (2016). Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. Food and Chemical Toxicology 97: 243-255.

Kim, SK; Lee, KT; Kang, CS; Tao, L; Kannan, K; Kim, KR, et al. (2011b). Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environmental Pollution 159: 169-174. http://dx.doi.org/10.1016/j.envpol.2010.09.008

Kim, WR; Flamm, SL; Di Bisceglie, AM; Bodenheimer, HC. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47: 1363-1370. http://dx.doi.org/10.1002/hep.22109

Kim, YR; White, N; Bräunig, J; Vijayasarathy, S; Mueller, JF; Knox, CL, et al. (2020). Per- and poly-fluoroalkyl substances (PFASs) in follicular fluid from women experiencing infertility in Australia. Environmental Research 190: 109963.

Kimura, O; Fujii, Y; Haraguchi, K; Kato, Y; Ohta, C; Koga, N; Endo, T. (2017). Uptake of perfluorooctanoic acid by Caco-2 cells: Involvement of organic anion transporting polypeptides. Toxicology Letters 277: 18-23.

Kingsley, SL; Kelsey, KT; Butler, R; Chen, A; Eliot, MN; Romano, ME, et al. (2017). Maternal serum PFOA concentration and DNA methylation in cord blood: A pilot study. Environmental Research 158: 174-178. http://dx.doi.org/10.1016/j.envres.2017.06.013

Kingsley, SL; Walker, DI; Calafat, AM; Chen, A; Papandonatos, GD; Xu, Y, et al. (2019). Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 15: 95. http://dx.doi.org/10.1007/s11306-019-1560-z

Kishi, R; Nakajima, T; Goudarzi, H; Kobayashi, S; Sasaki, S; Okada, E, et al. (2015). The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: the hokkaido study. Environmental Health Perspectives 123: 1038-1045.

Klaassen, CD. (2013). Casarett & Doull's toxicology: The basic science of poisons. In CD Klaassen (Ed.), (8th ed.). New York, NY: McGraw-Hill Education. https://www.mheducation.com/highered/product/casarett-doull-s-toxicology-basic-science-poisons-9th-edition-klaassen/9781259863745.html

Klaassen, CD; Aleksunes, LM. (2010). Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacological reviews 62: 1-96. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835398/

Klaunig, JE; Babich, MA; Baetcke, KP; Cook, JC; Corton, JC; David, RM, et al. (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance [Review]. Critical Reviews in Toxicology 33: 655-780. http://dx.doi.org/10.1080/713608372

Klaunig, JE; Hocevar, BA; Kamendulis, LM. (2012). Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance [Review]. Reproductive Toxicology 33: 410-418. http://dx.doi.org/10.1016/j.reprotox.2011.10.014

Knox, SS; Jackson, T; Javins, B; Frisbee, SJ; Shankar, A; Ducatman, AM. (2011). Implications of early menopause in women exposed to perfluorocarbons. Journal of Clinical Endocrinology and Metabolism 96: 1747-1753. http://dx.doi.org/10.1210/jc.2010-2401

Kobayashi, S; Azumi, K; Goudarzi, H; Araki, A; Miyashita, C; Kobayashi, S, et al. (2017). Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: The Hokkaido Study. Journal of Exposure Science and Environmental Epidemiology 27: 251-259.

Kobayashi, S; Sata, F; Goudarzi, H; Araki, A; Miyashita, C; Sasaki, S, et al. (2021). Associations among perfluorooctanesulfonic/perfluorooctanoic acid levels, nuclear receptor gene polymorphisms, and lipid levels in pregnant women in the Hokkaido study. Scientific Reports 11: 9994. http://dx.doi.org/10.1038/s41598-021-89285-2

Kobayashi, S; Sata, F; Ikeda-Araki, A; Miyashita, C; Goudarzi, H; Iwasaki, Y, et al. (2022). Relationships between maternal perfluoroalkyl substance levels, polymorphisms of receptor genes, and adverse birth outcomes in the Hokkaido birth cohort study, Japan. Reproductive Toxicology 107: 112-122. http://dx.doi.org/10.1016/j.reprotox.2021.12.004

Konwick, BJ; Tomy, GT; Ismail, N; Peterson, JT; Fauver, RJ; Higginbotham, D; Fisk, AT. (2008). Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. Environmental Toxicology and Chemistry 27: 2011-2018. http://dx.doi.org/10.1897/07-659.1

Koshy, TT; Attina, TM; Ghassabian, A; Gilbert, J; Burdine, LK; Marmor, M, et al. (2017). Serum perfluoroalkyl substances and cardiometabolic consequences in adolescents exposed to the World Trade Center disaster and a matched comparison group. Environment International 109: 128-135.

Koskela, A; Ducatman, A; Schousboe, JT; Nahhas, RW; Khalil, N. (2022). Perfluoroalkyl Substances and Abdominal Aortic Calcification. Journal of Occupational and Environmental Medicine. http://dx.doi.org/10.1097/JOM.0000000000002479

Kotlarz, N; Mccord, J; Collier, D; Lea, CS; Strynar, M; Lindstrom, AB, et al. (2020). Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. Environmental Health Perspectives 128: 77005.

Kuczmarski, RJ; Ogden, CL; Guo, SS; Grummer-Strawn, LM; Flegal, KM; Mei, Z, et al. (2002). 2000 CDC growth charts for the United States: Methods and development. (Vital and Health Statistics:

Series 11, No. 246). Hyattsville, MD: National Center for Health Statistics. https://www.cdc.gov/nchs/data/series/sr_11/sr11_246.pdf

Kudo, N; Katakura, M; Sato, Y; Kawashima, Y. (2002). Sex hormone-regulated renal transport of perfluorooctanoic acid. Chemico-Biological Interactions 139: 301-316. http://dx.doi.org/10.1016/S0009-2797(02)00006-6

Kummu, M; Sieppi, E; Koponen, J; Laatio, L; Vã¤Hã¤Kangas, K; Kiviranta, H, et al. (2015). Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. Placenta 36: 1185-1191.

Kusuhara, H; Sugiyama, Y. (2009). In vitro-in vivo extrapolation of transporter-mediated clearance in the liver and kidney [Review]. Drug Metabolism and Pharmacokinetics 24: 37-52. http://dx.doi.org/10.2133/dmpk.24.37

Kvalem, HE; Nygaard, UC; Lødrup Carlsen, KC; Carlsen, KH; Haug, LS; Granum, B. (2020). Perfluoroalkyl substances, airways infections, allergy and asthma related health outcomes - Implications of gender, exposure period and study design. Environment International 134: 105259.

Kwiatkowski, CF; Andrews, DQ; Birnbaum, LS; Bruton, TA; Dewitt, JC; Knappe, DRU, et al. (2020). Scientific Basis for Managing PFAS as a Chemical Class. Environmental Science & Technology Letters 7: 532-543. http://dx.doi.org/10.1021/acs.estlett.0c00255

Kwo, PY; Cohen, SM; Lim, JK. (2017). ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. American Journal of Gastroenterology 112: 18-35. http://dx.doi.org/10.1038/ajg.2016.517

Kwon, EJ; Shin, JS; Kim, BM; Shah-Kulkarni, S; Park, H; Kho, YL, et al. (2016). Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. Journal of Occupational and Environmental Medicine 58: e198-e205.

Lange, F; Schmidt, C; Brauch, H-J. (2006). Perfluoroalkylcarboxylates and -sulfonates: Emerging Contaminants for Drinking Water Supplies? Nieuwegein, The Netherlands: Association of River Waterworks – RIWA. https://www.riwa-rijn.org/wp-content/uploads/2015/05/137_ptfe_report.pdf

Laptook, AR; O'Shea, TM; Shankaran, S; Bhaskar, B; Network, NN. (2005). Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents. Pediatrics 115: 673-680. http://dx.doi.org/10.1542/peds.2004-0667

Larroque, B; Bertrais, S; Czernichow, P; Léger, J. (2001). School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study. Pediatrics 108: 111-115. http://dx.doi.org/10.1542/peds.108.1.111

Lau, C; Thibodeaux, JR; Hanson, RG; Narotsky, MG; Rogers, JM; Lindstrom, AB; Strynar, MJ. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicological Sciences 90: 510-518.

Launay-Vacher, V; Izzedine, H; Karie, S; Hulot, JS; Baumelou, A; Deray, G. (2006). Renal tubular drug transporters. Nephron Physiology 103: p97-106.

Lauritzen, HB; Larose, TL; Øien, T; Sandanger, TM; Odland, JØ; van de Bor, M; Jacobsen, GW. (2017). Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatric Research 81: 33-42.

Lawlor, TE. (1995). Mutagenicity test with T-6342 in the Salmonella–Escherichia coli/mammalian-microsome reverse mutation assay. (CHV Study No. 17073-0-409; EPA-AR-226-0436). Vienna, VA: Corning Hazleton.

Lawlor, TE. (1996). Mutagenicity test with T-6564 in the Salmonella–Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay. (CHV Study No. 17750-0-409R; EPA-AR-226-0432). Vienna, VA: Corning Hazleton.

Leary, DB; Takazawa, M; Kannan, K; Khalil, N. (2020). Perfluoroalkyl substances and metabolic syndrome in firefighters a pilot study. Journal of Occupational and Environmental Medicine 62: 52-57. http://dx.doi.org/10.1097/JOM.0000000000001756

Lee, ES; Han, S; Oh, JE. (2016). Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reproductive Toxicology 59: 53-59.

Lee, JK; Lee, S; Baek, MC; Lee, BH; Lee, HS; Kwon, TK, et al. (2017a). Association between perfluorooctanoic acid exposure and degranulation of mast cells in allergic inflammation. Journal of Applied Toxicology 37: 554-562. http://dx.doi.org/10.1002/jat.3389

Lee, JS; Ward, WO; Liu, J; Ren, H; Vallanat, B; Delker, D; Corton, JC. (2011). Hepatic xenobiotic metabolizing enzyme and transporter gene expression through the life stages of the mouse. PLoS ONE 6: e24381. http://dx.doi.org/10.1371/journal.pone.0024381

Lee, JW; Choi, K; Park, K; Seong, C; Yu, SD; Kim, P. (2020). Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: A review [Review]. Science of the Total Environment 707: 135334. http://dx.doi.org/10.1016/j.scitotenv.2019.135334

Lee, S; Kim, S; Park, J; Kim, HJ; Choi, G; Choi, S, et al. (2017b). Perfluoroalkyl substances (PFASs) in breast milk from Korea: Time-course trends, influencing factors, and infant exposure. Science of the Total Environment 612: 286-292.

Lee, TH; Kim, WR; Benson, JT; Therneau, TM; Melton, LJ. (2008). Serum aminotransferase activity and mortality risk in a United States community. Hepatology 47: 880-887. http://dx.doi.org/10.1002/hep.22090

Lee, YA; Kim, JH; Jung, HW; Lim, YH; Bae, S; Kho, Y, et al. (2018). The serum concentrations of perfluoroalkyl compounds were inversely associated with growth parameters in 2-year old children. Science of the Total Environment 628-629: 226-232.

Lee, YJ; Kim, MK; Bae, J; Yang, JH. (2013). Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere 90: 1603-1609.

Lehmann, GM; Verner, MA; Luukinen, B; Henning, C; Assimon, SA; Lakind, JS, et al. (2014). Improving the risk assessment of lipophilic persistent environmental chemicals in breast milk [Review]. Critical Reviews in Toxicology 44: 600-617. http://dx.doi.org/10.3109/10408444.2014.926306

Lehner, R; Quiroga, AD. (2016). Chapter 5: Fatty acid handling in mammalian cells. In ND Ridgway; RS McLeod (Eds.), Biochemistry of lipids, lipoproteins and membranes (6th ed., pp. 149-184). Amsterdam, Netherlands: Elsevier. http://dx.doi.org/10.1016/b978-0-444-63438-2.00005-5

Lenters, V; Portengen, L; Rignell-Hydbom, A; Jönsson, BA; Lindh, CH; Piersma, AH, et al. (2016). Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environmental Health Perspectives 124: 365-372.

Leonard, RC; Kreckmann, KH; Sakr, CJ; Symons, JM. (2008). Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Annals of Epidemiology 18: 15-22. http://dx.doi.org/10.1016/j.annepidem.2007.06.011

Leung, YK; Ouyang, B; Niu, L; Xie, C; Ying, J; Medvedovic, M, et al. (2018). Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics 13: 290-300. http://dx.doi.org/10.1080/15592294.2018.1445901

Li, CH; Ren, XM; Guo, LH. (2019a). Adipogenic Activity of Oligomeric Hexafluoropropylene Oxide (Perfluorooctanoic Acid Alternative) through Peroxisome Proliferator-Activated Receptor γ Pathway. Environmental Science & Technology 53: 3287-3295. http://dx.doi.org/10.1021/acs.est.8b06978

Li, D; Song, P; Liu, L; Wang, X. (2018a). Perfluorooctanoic acid exposure during pregnancy alters the apoptosis of uterine cells in pregnant mice. International Journal of Clinical and Experimental Pathology 11: 5602-5611.

Li, D; Zhang, L; Zhang, Y; Guan, S; Gong, X; Wang, X. (2019b). Maternal exposure to perfluorooctanoic acid (PFOA) causes liver toxicity through PPAR-α pathway and lowered histone acetylation in female offspring mice. Environmental Science and Pollution Research 26: 18866-18875.

Li, H; Hammarstrand, S; Midberg, B; Xu, Y; Li, Y; Olsson, DS, et al. (2022). Cancer incidence in a Swedish cohort with high exposure to perfluoroalkyl substances in drinking water. Environmental Research 204: 112217. http://dx.doi.org/10.1016/j.envres.2021.112217

Li, J; Cai, D; Chu, C; Li, QQ; Zhou, Y; Hu, LW, et al. (2020a). Transplacental Transfer of Per- and Polyfluoroalkyl Substances (PFASs): Differences between Preterm and Full-Term Deliveries and Associations with Placental Transporter mRNA Expression. Environmental Science and Technology 54: 5062-5070.

Li, K; Gao, P; Xiang, P; Zhang, X; Cui, X; Ma, LQ. (2017a). Molecular mechanisms of PFOA-induced toxicity in animals and humans: Implications for health risks [Review]. Environment International 99: 43-54. http://dx.doi.org/10.1016/j.envint.2016.11.014

Li, K; Li, C; Yu, NY; Juhasz, AL; Cui, XY; Ma, LQ. (2015). In vivo bioavailability and in vitro bioaccessibility of perfluorooctanoic acid (PFOA) in food matrices: correlation analysis and method development. Environmental Science and Technology 49: 150-158.

Li, K; Sun, J; Yang, J; Roberts, SM; Zhang, X; Cui, X, et al. (2017b). Molecular Mechanisms of Perfluorooctanoate-Induced Hepatocyte Apoptosis in Mice Using Proteomic Techniques. Environmental Science and Technology 51: 11380-11389.

Li, M; Zeng, XW; Qian, ZM; Vaughn, MG; Sauvé, S; Paul, G, et al. (2017c). Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environment International 102: 1-8.

Li, N; Liu, Y; Papandonatos, GD; Calafat, AM; Eaton, CB; Kelsey, KT, et al. (2021). Gestational and childhood exposure to per- and polyfluoroalkyl substances and cardiometabolic risk at age 12 years. Environment International 147: 106344. http://dx.doi.org/10.1016/j.envint.2020.106344

Li, X; Bao, C; Ma, Z; Xu, B; Ying, X; Liu, X; Zhang, X. (2018b). Perfluorooctanoic acid stimulates ovarian cancer cell migration, invasion via ERK/NF-κB/MMP-2/-9 pathway. Toxicology Letters 294: 44-50. http://dx.doi.org/10.1016/j.toxlet.2018.05.009

Li, X; Wang, Z; Klaunig, JE. (2019c). The effects of perfluorooctanoate on high fat diet induced non-alcoholic fatty liver disease in mice. Toxicology 416: 1-14. http://dx.doi.org/10.1016/j.tox.2019.01.017

Li, Y; Barregard, L; Xu, Y; Scott, K; Pineda, D; Lindh, CH, et al. (2020b). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environmental Health: A Global Access Science Source 19: 33.

Li, Y; Fletcher, T; Mucs, D; Scott, K; Lindh, CH; Tallving, P; Jakobsson, K. (2018c). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occupational and Environmental Medicine 75: 46-51.

Li, Y, ing; Mucs, D, aniel; Scott, K, ristin; Lindh, C, hristian; Tallving, P, ia; Fletcher, T, ony; Jakobsson, K, ristina. (2017d). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. (2:2017). Gothenburg, Sweden: Gothenburg University, Unit for Occupational & Environmental Medicine. http://hdl.handle.net/2077/52661

Li, Y; Oliver, DP; Kookana, RS. (2018d). A critical analysis of published data to discern the role of soil and sediment properties in determining sorption of per and polyfluoroalkyl substances (PFASs) [Review]. Science of the Total Environment 628-629: 110-120. http://dx.doi.org/10.1016/j.scitotenv.2018.01.167

Li, Y; Ramdhan, DH; Naito, H; Yamagishi, N; Ito, Y; Hayashi, Y, et al. (2011). Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARα. Toxicology Letters 205: 265-272. http://dx.doi.org/10.1016/j.toxlet.2011.06.015

Liang, L; Pan, Y; Bin, L; Liu, Y; Huang, W; Li, R; Lai, KP. (2021). Immunotoxicity mechanisms of perfluorinated compounds PFOA and PFOS [Review]. Chemosphere 291: 132892. http://dx.doi.org/10.1016/j.chemosphere.2021.132892

Liang, X; Xie, G; Wu, X; Su, M; Yang, B. (2019). Effect of prenatal PFOS exposure on liver cell function in neonatal mice. Environmental Science and Pollution Research 26: 18240-18246.

Liao, S; Yao, W; Cheang, I; Tang, X; Yin, T; Lu, X, et al. (2020). Association between perfluoroalkyl acids and the prevalence of hypertension among US adults. Ecotoxicology and Environmental Safety 196: 110589.

Liew, Z; Luo, J; Nohr, EA; Bech, BH; Bossi, R; Arah, OA; Olsen, J. (2020). Maternal Plasma Perfluoroalkyl Substances and Miscarriage: A Nested Case-Control Study in the Danish National Birth Cohort. Environmental Health Perspectives 128: 47007.

Lin, CY; Chen, PC; Lin, YC; Lin, LY. (2009). Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32: 702-707. http://dx.doi.org/10.2337/dc08-1816

Lin, CY; Chen, PC; Lo, SC; Torng, PL; Sung, FC; Su, TC. (2016). The association of carotid intima-media thickness with serum Level of perfluorinated chemicals and endothelium-platelet microparticles in adolescents and young adults. Environment International 94: 292-299.

Lin, CY; Lee, HL; Hwang, YT; Su, TC. (2020a). The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. Environmental Research 182: 109064.

Lin, CY; Lin, LY; Chiang, CK; Wang, WJ; Su, YN; Hung, KY; Chen, PC. (2010). Investigation of the Associations Between Low-Dose Serum Perfluorinated Chemicals and Liver Enzymes in US Adults. American Journal of Gastroenterology 105: 1354-1363. http://dx.doi.org/10.1038/ajg.2009.707

Lin, CY; Lin, LY; Wen, TW; Lien, GW; Chien, KL; Hsu, SH, et al. (2013). Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. International Journal of Cardiology 168: 3309-3316.

Lin, HW; Feng, HX; Chen, L; Yuan, XJ; Tan, Z. (2020b). Maternal exposure to environmental endocrine disruptors during pregnancy is associated with pediatric germ cell tumors. Nagoya Journal of Medical Science 82: 323-333.

Lin, P; Cardenas, A; Hauser, R; Gold, DR; Kleinman, K; Hivert, MF, et al. (2019). Per- and polyfluoroalkyl substances and blood lipid levels in pre-diabetic adults-longitudinal analysis of the diabetes prevention program outcomes study. Environment International 129: 343-353.

Lin, PD; Cardenas, A; Hauser, R; Gold, DR; Kleinman, KP; Hivert, MF, et al. (2020c). Per- and polyfluoroalkyl substances and blood pressure in pre-diabetic adults-cross-sectional and longitudinal analyses of the diabetes prevention program outcomes study. Environment International 137: 105573.

Lin, T; Zhang, Y; Ding, X; Huang, T; Zhang, W; Zou, W, et al. (2020d). Perfluorooctanoic acid induces cytotoxicity in spermatogonial GC-1 cells. Chemosphere 260: 127545. http://dx.doi.org/10.1016/j.chemosphere.2020.127545

Lin, TW; Chen, MK; Lin, CC; Chen, MH; Tsai, MS; Chan, DC, et al. (2020e). Association between exposure to perfluoroalkyl substances and metabolic syndrome and related outcomes among older residents living near a Science Park in Taiwan. International Journal of Hygiene and Environmental Health 230: 113607. http://dx.doi.org/10.1016/j.ijheh.2020.113607

Lind, DV; Priskorn, L; Lassen, TH; Nielsen, F; Kyhl, HB; Kristensen, DM, et al. (2017a). Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reproductive Toxicology 68: 200-206.

Lind, PM; Salihovic, S; van Bavel, B; Lind, L. (2017b). Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environmental Research 152: 157-164.

Lindstrom, AB; Strynar, MJ; Libelo, EL. (2011). Polyfluorinated compounds: past, present, and future [Review]. Environmental Science and Technology 45: 7954-7961. http://dx.doi.org/10.1021/es2011622

Lira, PI; Ashworth, A; Morris, SS. (1996). Low birth weight and morbidity from diarrhea and respiratory infection in northeast Brazil. Journal of Pediatrics 128: 497-504. http://dx.doi.org/10.1016/s0022-3476(96)70360-8

Liu, CY; Chen, PC; Lien, PC; Liao, YP. (2018a). Prenatal perfluorooctyl sulfonate exposure and Alu DNA hypomethylation in cord blood. International Journal of Environmental Research and Public Health 15: 1066. http://dx.doi.org/10.3390/ijerph15061066

Liu, G; Dhana, K; Furtado, JD; Rood, J; Zong, G; Liang, L, et al. (2018b). Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Medicine 15: e1002502.

Liu, G; Zhang, B; Hu, Y; Rood, J; Liang, L; Qi, L, et al. (2020a). Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environmental Health: A Global Access Science Source 19: 5.

Liu, H; Chen, Q; Lei, L; Zhou, W; Huang, L; Zhang, J; Chen, D. (2018c). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. Environmental Pollution 235: 446-452. http://dx.doi.org/10.1016/j.envpol.2017.12.095

Liu, H; Wang, J; Sheng, N; Cui, R; Pan, Y; Dai, J. (2017a). Acot1 is a sensitive indicator for PPARα activation after perfluorooctanoic acid exposure in primary hepatocytes of Sprague-Dawley rats. Toxicology In Vitro 42: 299-307. http://dx.doi.org/10.1016/j.tiv.2017.05.012

Liu, HS; Wen, LL; Chu, PL; Lin, CY. (2018d). Association among total serum isomers of perfluorinated chemicals, glucose homeostasis, lipid profiles, serum protein and metabolic syndrome in adults: NHANES, 2013-2014. Environmental Pollution 232: 73-79.

Liu, J; Li, J; Liu, Y; Chan, HM; Zhao, Y; Cai, Z; Wu, Y. (2011). Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environment International 37: 1206-1212. http://dx.doi.org/10.1016/j.envint.2011.05.001

Liu, J; Liu, S; Huang, Z; Fu, Y; Fei, J; Liu, X; He, Z. (2020b). Associations between the serum levels of PFOS/PFOA and IgG N-glycosylation in adult or children. Environmental Pollution 265: 114285. http://dx.doi.org/10.1016/j.envpol.2020.114285

Liu, M; Zhang, G; Meng, L; Han, X; Li, Y; Shi, Y, et al. (2021). Associations between novel and legacy per- and polyfluoroalkyl substances in human serum and thyroid cancer: A case and healthy population in Shandong Province, East China. Environmental Science & Technology. http://dx.doi.org/10.1021/acs.est.1c02850

Liu, QS; Hao, F; Sun, Z; Long, Y; Zhou, Q; Jiang, G. (2018e). Perfluorohexadecanoic acid increases paracellular permeability in endothelial cells through the activation of plasma kallikrein-kinin system. Chemosphere 190: 191-200. http://dx.doi.org/10.1016/j.chemosphere.2017.10.002

Liu, QS; Sun, Y; Qu, G; Long, Y; Zhao, X; Zhang, A, et al. (2017b). Structure-Dependent Hematological Effects of Per- and Polyfluoroalkyl Substances on Activation of Plasma Kallikrein-Kinin System Cascade. Environmental Science & Technology 51: 10173-10183. http://dx.doi.org/10.1021/acs.est.7b02055

Liu, RC; Hurtt, ME; Cook, JC; Biegel, LB. (1996). Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. Fundamental and Applied Toxicology 30: 220-228. http://dx.doi.org/10.1006/faat.1996.0059

Liu, SH; Kuiper, JR; Chen, Y; Feuerstahler, L; Teresi, J; Buckley, JP. (2022). Developing an exposure burden score for chemical mixtures using item response theory, with applications to PFAS mixtures. Environmental Health Perspectives 130: 117001. http://dx.doi.org/10.1289/EHP10125

Liu, W; Irudayaraj, J. (2020). Perfluorooctanoic acid (PFOA) exposure inhibits DNA methyltransferase activities and alters constitutive heterochromatin organization. Food and Chemical Toxicology 141: 111358. http://dx.doi.org/10.1016/j.fct.2020.111358

Liu, W; Xu, C; Sun, X, i; Kuang, H; Kuang, X; Zou, W, et al. (2016). Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. Toxicology Research 5: 224-234. http://dx.doi.org/10.1039/c5tx00260e

Liu, W; Yang, B; Wu, L; Zou, W; Pan, X; Zou, T, et al. (2015). Involvement of NRF2 in Perfluorooctanoic Acid-Induced Testicular Damage in Male Mice. Biology of Reproduction 93: 41. http://dx.doi.org/10.1095/biolreprod.115.128819

Liu, X; Chen, D; Wang, B; Xu, F; Pang, Y; Zhang, L, et al. (2020c). Does Low Maternal Exposure to Per- and Polyfluoroalkyl Substances Elevate the Risk of Spontaneous Preterm Birth? A Nested Case-Control Study in China. Environmental Science and Technology 54: 8259-8268.

Liu, Z; Que, S; Xu, J; Peng, T. (2014). Alanine aminotransferase-old biomarker and new concept: a review. International Journal of Medical Sciences 11: 925-935. http://dx.doi.org/10.7150/ijms.8951

Lloyd-Jones, DM; Huffman, MD; Karmali, KN; Sanghavi, DM; Wright, JS; Pelser, C, et al. (2017). Estimating Longitudinal Risks and Benefits From Cardiovascular Preventive Therapies Among Medicare Patients: The Million Hearts Longitudinal ASCVD Risk Assessment Tool: A Special Report From the American Heart Association and American College of Cardiology [Review]. Circulation 135: e793-e813. http://dx.doi.org/10.1161/CIR.0000000000000467

Loccisano, AE; Campbell, JL; Butenhoff, JL; Andersen, ME; Clewell, HJ. (2012a). Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33: 468-490. http://dx.doi.org/10.1016/j.reprotox.2011.07.003

Loccisano, AE; Campbell, JL, Jr; Andersen, ME; Clewell, HJ, III. (2011). Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology and Pharmacology 59: 157-175. http://dx.doi.org/10.1016/j.yrtph.2010.12.004

Loccisano, AE; Campbell, JL, Jr; Butenhoff, JL; Andersen, ME; Clewell, HJ, III. (2012b). Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33: 452-467. http://dx.doi.org/10.1016/j.reprotox.2011.04.006

Loccisano, AE; Longnecker, MP; Campbell, JL, Jr; Andersen, ME; Clewell, HJ, III. (2013). Development of pbpk models for pfoa and pfos for human pregnancy and lactation life stages. Journal of Toxicology and Environmental Health, Part A: Current Issues 76: 25-57. http://dx.doi.org/10.1080/15287394.2012.722523

Looker, C; Luster, MI; Calafat, AM; Johnson, VJ; Burleson, GR; Burleson, FG; Fletcher, T. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicological Sciences 138: 76-88. http://dx.doi.org/10.1093/toxsci/kft269

Lopez-Espinosa, MJ; Carrizosa, C; Luster, MI; Margolick, JB; Costa, O; Leonardi, GS; Fletcher, T. (2021). Perfluoroalkyl substances and immune cell counts in adults from the Mid-Ohio Valley (USA). Environment International 156: 106599. http://dx.doi.org/10.1016/j.envint.2021.106599

Lopez-Espinosa, MJ; Mondal, D; Armstrong, BG; Eskenazi, B; Fletcher, T. (2016). Perfluoroalkyl Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional Analysis within the C8 Health Project. Environmental Health Perspectives 124: 1269-1275.

Lorber, M; Eaglesham, GE; Hobson, P; Toms, LM; Mueller, JF; Thompson, JS. (2015). The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. Chemosphere 118: 170-177. http://dx.doi.org/10.1016/j.chemosphere.2014.07.093

Lorber, M; Egeghy, PP. (2011). Simple intake and pharmacokinetic modeling to characterize exposure of Americans to perfluoroctanoic acid, PFOA. Environmental Science and Technology 45: 8006-8014. http://dx.doi.org/10.1021/es103718h

Loret de Mola, C; de França, GV; Quevedo Lde, A; Horta, BL. (2014). Low birth weight, preterm birth and small for gestational age association with adult depression: systematic review and meta-analysis. British Journal of Psychiatry 205: 340-347. http://dx.doi.org/10.1192/bjp.bp.113.139014

Lou, I; Wambaugh, JF; Lau, C; Hanson, RG; Lindstrom, AB; Strynar, MJ, et al. (2009). Modeling single and repeated dose pharmacokinetics of PFOA in mice. Toxicological Sciences 107: 331-341. http://dx.doi.org/10.1093/toxsci/kfn234

Louisse, J; Rijkers, D; Stoopen, G; Janssen, A; Staats, M; Hoogenboom, R, et al. (2020). Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorononanoic acid (PFNA) increase triglyceride levels and decrease cholesterogenic gene expression in human HepaRG liver cells. Archives of Toxicology 94: 3137-3155. http://dx.doi.org/10.1007/s00204-020-02808-0

Loveless, SE; Hoban, D; Sykes, G; Frame, SR; Everds, NE. (2008). Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. Toxicological Sciences 105: 86-96. http://dx.doi.org/10.1093/toxsci/kfn113

Lu, H; Zhang, H; Gao, J; Li, Z; Bao, S; Chen, X, et al. (2019). Effects of perfluorooctanoic acid on stem Leydig cell functions in the rat. Environmental Pollution 250: 206-215. http://dx.doi.org/10.1016/j.envpol.2019.03.120

Lu, Y; Pan, Y; Sheng, N; Zhao, AZ; Dai, J. (2016). Perfluorooctanoic acid exposure alters polyunsaturated fatty acid composition, induces oxidative stress and activates the AKT/AMPK pathway in mouse epididymis. Chemosphere 158: 143-153.

Luebker, DJ; Hansen, KJ; Bass, NM; Butenhoff, JL; Seacat, AM. (2002). Interactions of fluorochemicals with rat liver fatty acid-binding protein. Toxicology 176: 175-185. http://dx.doi.org/10.1016/S0300-483X(02)00081-1

Lum, KJ; Sundaram, R; Barr, DB; Louis, TA; Buck Louis, GM. (2017). Perfluoroalkyl Chemicals, Menstrual Cycle Length, and Fecundity: Findings from a Prospective Pregnancy Study. Epidemiology 28: 90-98.

Lundin, JI; Alexander, BH; Olsen, GW; Church, TR. (2009). Ammonium Perfluorooctanoate Production and Occupational Mortality. Epidemiology 20: 921-928. http://dx.doi.org/10.1097/EDE.0b013e3181b5f395

Luo, D; Wu, W; Pan, Y; Du, B; Shen, M; Zeng, L. (2021). Associations of prenatal exposure to per- and polyfluoroalkyl substances with the neonatal birth size and hormones in the growth hormone/insulin-like growth factor axis. Environmental Science & Technology 55: 11859-11873. http://dx.doi.org/10.1021/acs.est.1c02670

Luster, MI; Johnson, VJ; Yucesoy, B; Simeonova, PP. (2005). Biomarkers to assess potential developmental immunotoxicity in children. Toxicology and Applied Pharmacology 206: 229-236. http://dx.doi.org/10.1016/j.taap.2005.02.010

Lv, D; Gu, Y; Guo, M; Hou, P; Li, Y; Wu, R. (2019). Perfluorooctanoic acid exposure induces apoptosis in SMMC-7721 hepatocellular cancer cells. Environmental Pollution 247: 509-514. http://dx.doi.org/10.1016/j.envpol.2019.01.035

Ma, S; Xu, C; Ma, J; Wang, Z; Zhang, Y; Shu, Y; Mo, X. (2019). Association between perfluoroalkyl substance concentrations and blood pressure in adolescents. Environmental Pollution 254: 112971.

Ma, Z; Liu, X; Li, F; Wang, Y; Xu, Y; Zhang, M, et al. (2016). Perfluorooctanoic acid induces human Ishikawa endometrial cancer cell migration and invasion through activation of ERK/mTOR signaling. Oncotarget 7: 66558-66568. http://dx.doi.org/10.18632/oncotarget.11684

Macgillivray, DM; Kollmann, TR. (2014). The role of environmental factors in modulating immune responses in early life [Review]. Frontiers in Immunology 5: 434. http://dx.doi.org/10.3389/fimmu.2014.00434

Macmanus-Spencer, LA; Tse, ML; Hebert, PC; Bischel, HN; Luthy, RG. (2010). Binding of perfluorocarboxylates to serum albumin: a comparison of analytical methods. Analytical Chemistry 82: 974-981. http://dx.doi.org/10.1021/ac902238u

Macon, MB; Fenton, SE. (2013). Endocrine disruptors and the breast: Early life effects and later life disease [Review]. Journal of Mammary Gland Biology and Neoplasia 18: 43-61. http://dx.doi.org/10.1007/s10911-013-9275-7

Macon, MB; Villanueva, LR; Tatum-Gibbs, K; Zehr, RD; Strynar, MJ; Stanko, JP, et al. (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. Toxicological Sciences 122: 134-145.

Maekawa, R; Ito, R; Iwasaki, Y; Saito, K; Akutsu, K; Takatori, S, et al. (2017). Evidence of exposure to chemicals and heavy metals during pregnancy in Japanese women. Reproductive Medicine and Biology 16: 337-348.

Maestri, L; Negri, S; Ferrari, M; Ghittori, S; Fabris, F; Danesino, P; Imbriani, M. (2006). Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Communications in Mass Spectrometry 20: 2728-2734. http://dx.doi.org/10.1002/rcm.2661

Maher, JM; Aleksunes, LM; Dieter, MZ; Tanaka, Y; Peters, JM; Manautou, JE; Klaassen, CD. (2008). Nrf2- and PPAR alpha-mediated regulation of hepatic Mrp transporters after exposure to perfluorooctanoic acid and perfluorodecanoic acid. Toxicological Sciences 106: 319-328. http://dx.doi.org/10.1093/toxsci/kfn177

Maisonet, M; Terrell, ML; Mcgeehin, MA; Christensen, KY; Holmes, A; Calafat, AM; Marcus, M. (2012). Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environmental Health Perspectives 120: 1432-1437. http://dx.doi.org/10.1289/ehp.1003096

Malhotra, P; Gill, RK; Saksena, S; Alrefai, WA. (2020). Disturbances in Cholesterol Homeostasis and Non-alcoholic Fatty Liver Diseases [Review]. Frontiers in Medicine 7: 467. http://dx.doi.org/10.3389/fmed.2020.00467

Maloney, EK; Waxman, DJ. (1999). trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. Toxicology and Applied Pharmacology 161: 209-218. http://dx.doi.org/10.1006/taap.1999.8809

Mamsen, LS; Björvang, RD; Mucs, D; Vinnars, MT; Papadogiannakis, N; Lindh, CH, et al. (2019). Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environment International 124: 482-492.

Mamsen, LS; Jönsson, BAG; Lindh, CH; Olesen, RH; Larsen, A; Ernst, E, et al. (2017). Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Science of the Total Environment 596-597: 97-105.

Mancini, FR; Cano-Sancho, G; Gambaretti, J; Marchand, P; Boutron-Ruault, MC; Severi, G, et al. (2020). Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort. International Journal of Cancer 146: 917-928.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D, et al. (2017a). Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environment International 108: 278-284.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D, et al. (2017b). Prenatal exposure to perfluoroalkyl substances and cardiometabolic risk in children from the Spanish INMA birth cohort study. Environmental Health Perspectives 125: 097018.

Manzano-Salgado, CB; Granum, B; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Gascón, M, et al. (2019). Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in children from a Spanish birth cohort study. International Journal of Hygiene and Environmental Health 222: 945-954.

Marks, KJ; Cutler, AJ; Jeddy, Z; Northstone, K; Kato, K; Hartman, TJ. (2019). Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. International Journal of Hygiene and Environmental Health 222: 889-895.

Martin, MT; Brennan, RJ; Hu, W; Ayanoglu, E; Lau, C; Ren, H, et al. (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. Toxicological Sciences 97: 595-613. http://dx.doi.org/10.1093/toxsci/kfm065

Mashayekhi, V; Tehrani, KH; Hashemzaei, M; Tabrizian, K; Shahraki, J; Hosseini, MJ. (2015). Mechanistic approach for the toxic effects of perfluorooctanoic acid on isolated rat liver and brain mitochondria. Human & Experimental Toxicology 34: 985-996. http://dx.doi.org/10.1177/0960327114565492

Mathiesen, UL; Franzén, LE; Frydén, A; Foberg, U; Bodemar, G. (1999). The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. Scandinavian Journal of Gastroenterology 34: 85-91. http://dx.doi.org/10.1080/00365529950172880

Matilla-Santander, N; Valvi, D; Lopez-Espinosa, MJ; Manzano-Salgado, CB; Ballester, F; Ibarluzea, J, et al. (2017). Exposure to Perfluoroalkyl Substances and Metabolic Outcomes in Pregnant Women: Evidence from the Spanish INMA Birth Cohorts. Environmental Health Perspectives 125: 117004.

Matkowskyj, KA; Bai, H; Liao, J; Zhang, W; Li, H; Rao, S, et al. (2014). Aldoketoreductase family 1B10 (AKR1B10) as a biomarker to distinguish hepatocellular carcinoma from benign liver lesions. Human Pathology 45: 834-843. http://dx.doi.org/10.1016/j.humpath.2013.12.002

Mattsson, K; Rignell-Hydbom, A; Holmberg, S; Thelin, A; Jönsson, BA; Lindh, CH, et al. (2015). Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environmental Research 142: 148-154.

McComb, J; Mills, IG; Berntsen, HF; Ropstad, E; Verhaegen, S; Connolly, L. (2019). Human-based exposure levels of perfluoroalkyl acids may induce harmful effects to health by disrupting major components of androgen receptor signalling in vitro. Exposure and Health 12: 527-538. http://dx.doi.org/10.1007/s12403-019-00318-8

Mcdonough, CA; Li, W; Bischel, HN; De Silva, AO; Dewitt, JC. (2022). Widening the Lens on PFASs: Direct Human Exposure to Perfluoroalkyl Acid Precursors (pre-PFAAs). Environmental Science & Technology 56: 6004-6013. http://dx.doi.org/10.1021/acs.est.2c00254

McIntire, DD; Bloom, SL; Casey, BM; Leveno, KJ. (1999). Birth weight in relation to morbidity and mortality among newborn infants. New England Journal of Medicine 340: 1234-1238.

Melnikov, F; Botta, D; White, CC; Schmuck, SC; Winfough, M; Schaupp, CM, et al. (2018). Kinetics of Glutathione Depletion and Antioxidant Gene Expression as Indicators of Chemical Modes of Action Assessed in vitro in Mouse Hepatocytes with Enhanced Glutathione Synthesis. Chemical Research in Toxicology 32: 421-436. http://dx.doi.org/10.1021/acs.chemrestox.8b00259

Meng, Q; Inoue, K; Ritz, B; Olsen, J; Liew, Z. (2018). Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the danish national birth cohort. International Journal of Environmental Research and Public Health 15: 1832.

Mi, X; Yang, YQ; Zeeshan, M; Wang, ZB; Zeng, XY; Zhou, Y, et al. (2020). Serum levels of per- and polyfluoroalkyl substances alternatives and blood pressure by sex status: Isomers of C8 health project in China. Chemosphere 261: 127691.

Miao, C; Ma, J; Zhang, Y; Chu, Y; Li, J; Kuai, R, et al. (2015). Perfluorooctanoic acid enhances colorectal cancer DLD-1 cells invasiveness through activating NF-κB mediated matrix metalloproteinase-2/-9 expression. International Journal of Clinical and Experimental Pathology 8: 10512-10522.

Midic, U; Goheen, B; Vincent, KA; Vandevoort, CA; Latham, KE. (2018). Changes in gene expression following long-term in vitro exposure of macaca mulatta trophoblast stem cells to biologically relevant levels of endocrine disruptors. Reproductive Toxicology 77: 154-165. http://dx.doi.org/10.1016/j.reprotox.2018.02.012

Mills, KH; Dungan, LS; Jones, SA; Harris, J. (2013). The role of inflammasome-derived IL-1 in driving IL-17 responses [Review]. Journal of Leukocyte Biology 93: 489-497. http://dx.doi.org/10.1189/jlb.1012543

Min, JY; Lee, KJ; Park, JB; Min, KB. (2012). Perfluorooctanoic acid exposure is associated with elevated homocysteine and hypertension in US adults. Occupational and Environmental Medicine 69: 658-662. http://dx.doi.org/10.1136/oemed-2011-100288

Minata, M; Harada, KH; Kärrman, A; Hitomi, T; Hirosawa, M; Murata, M, et al. (2010). Role of peroxisome proliferator-activated receptor-alpha in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. Industrial Health 48: 96-107. http://dx.doi.org/10.2486/indhealth.48.96

Minatoya, M; Itoh, S; Miyashita, C; Araki, A; Sasaki, S; Miura, R, et al. (2017). Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environmental Research 156: 175-182.

Mitro, SD; Sagiv, SK; Fleisch, AF; Jaacks, LM; Williams, PL; Rifas-Shiman, SL, et al. (2020). Pregnancy per- and polyfluoroalkyl substance concentrations and postpartum health in project viva: A prospective cohort. Journal of Clinical Endocrinology and Metabolism 105: e3415–e3426.

Miura, R; Araki, A; Miyashita, C; Kobayashi, S; Kobayashi, S; Wang, SL, et al. (2018). An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: The Hokkaido study. Environment International 115: 21-28. http://dx.doi.org/10.1016/j.envint.2018.03.004

Mobacke, I; Lind, L; Dunder, L; Salihovic, S; Lind, PM. (2018). Circulating levels of perfluoroalkyl substances and left ventricular geometry of the heart in the elderly. Environment International 115: 295-300.

Mogensen, UB; Grandjean, P; Heilmann, C; Nielsen, F; Weihe, P; Budtz-Jørgensen, E. (2015a). Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. Environmental Health: A Global Access Science Source 14: 47.

Mogensen, UB; Grandjean, P; Nielsen, F; Weihe, P; Budtz-Jørgensen, E. (2015b). Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates. Environmental Science and Technology 49: 10466-10473.

Mondal, D; Weldon, RH; Armstrong, BG; Gibson, LJ; Lopez-Espinosa, MJ; Shin, HM; Fletcher, T. (2014). Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environmental Health Perspectives 122: 187-192.

Monroy, R; Morrison, K; Teo, K; Atkinson, S; Kubwabo, C; Stewart, B; Foster, WG. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental Research 108: 56-62. http://dx.doi.org/10.1016/j.envres.2008.06.001

Mora, AM; Fleisch, AF; Rifas-Shiman, SL; Woo Baidal, JA; Pardo, L; Webster, TF, et al. (2018). Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine aminotransferase levels. Environment International 111: 1-13.

Mora, S. (2016). Nonfasting for Routine Lipid Testing: From Evidence to Action. JAMA Internal Medicine 176: 1005-1006. http://dx.doi.org/10.1001/jamainternmed.2016.1979

Mordenti, J; Chen, SA; Moore, JA; Ferraiolo, BL; Green, JD. (1991). Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. Pharmaceutical Research 8: 1351–1359. http://dx.doi.org/10.1023/a:1015836720294

Murli, H. (1995). Mutagenicity test on T-6342 in an in vivo mouse micronucleus assay. (EPA-AR-226-0435). Vienna, VA: Corning Hazleton.

Murli, H. (1996a). Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. (EPA-AR-226-0434). Vienna, VA: Corning Hazleton.

Murli, H. (1996b). Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. (EPA-AR-226-0433). Vienna, VA: Corning Hazleton.

Murli, H. (1996c). Mutagenicity test on T-6564 in an in vivo mouse micronucleus assay. (CHV Study No. 17750-0-455; EPA-AR-226-0430). Vienna, VA: Corning Hazleton.

Murli, H. (1996d). Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. (CHV Study No. 17750-0-437CO; EPA-AR-226-0431). Vienna, VA: Corning Hazleton.

Myers, LP. (2018). Clinical immunotoxicology. In JC DeWitt; CE Rockwell; CC Bowman (Eds.), Immunotoxicity testing: Methods and protocols (2nd ed., pp. 15–26). Totowa, NJ: Humana Press. http://dx.doi.org/10.1007/978-1-4939-8549-4_2

Mylchreest, E. (2003). PFOA: Lactational and Placental Transport Pharmacokinetic Study in Rats. (DuPont-13309). Newark, DE: Haskell Laboratory for Health and Environmental Sciences.

Nakagawa, H; Hirata, T; Terada, T; Jutabha, P; Miura, D; Harada, KH, et al. (2008). Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 103: 1-8. http://dx.doi.org/10.1111/j.1742-7843.2007.00155.x

Nakagawa, H; Terada, T; Harada, KH; Hitomi, T; Inoue, K; Inui, K; Koizumi, A. (2009). Human organic anion transporter hOAT4 is a transporter of perfluorooctanoic acid. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 105: 136-138. http://dx.doi.org/10.1111/j.1742-7843.2009.00409.x

Nakayama, S; Strynar, MJ; Helfant, L; Egeghy, P; Ye, X; Lindstrom, AB. (2007). Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. Environmental Science and Technology 41: 5271-5276. http://dx.doi.org/10.1021/es070792y

NASEM. (2021). Review of U.S. EPA's ORD staff handbook for developing IRIS assessments: 2020 version. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/26289

Natarajan, AT; Darroudi, F. (1991). Use of human hepatoma cells for in vitro metabolic activation of chemical mutagens/carcinogens. Mutagenesis 6: 399-404.

NCBI. (2022). PubChem: Compound summary: Perfluorooctanoic acid. Available online at https://pubchem.ncbi.nlm.nih.gov/compound/Perfluorooctanoic-acid (accessed April 21, 2022).

NCHS. (2019). Health, United States – Data Finder. 2019: Table 23. Hyattsville, MD. https://www.cdc.gov/nchs/hus/contents2019.htm

Negri, E; Metruccio, F; Guercio, V; Tosti, L; Benfenati, E; Bonzi, R, et al. (2017). Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data [Review]. Critical Reviews in Toxicology 47: 482-508. http://dx.doi.org/10.1080/10408444.2016.1271972

Nelson, JW; Hatch, EE; Webster, TF. (2010). Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General US Population. Environmental Health Perspectives 118: 197-202. http://dx.doi.org/10.1289/ehp.0901165

Nelson, JW; Scammell, MK; Hatch, EE; Webster, TF. (2012). Social disparities in exposures to bisphenol A and polyfluoroalkyl chemicals: a cross-sectional study within NHANES 2003-2006. Environmental Health 11: 10. http://dx.doi.org/10.1186/1476-069X-11-10

Neumann, J; Rose-Sperling, D; Hellmich, UA. (2017). Diverse relations between ABC transporters and lipids: An overview [Review]. Biochimica et Biophysica Acta Biomembranes 1859: 605-618. http://dx.doi.org/10.1016/j.bbamem.2016.09.023

Ngueta, G; Longnecker, MP; Yoon, M; Ruark, CD; Clewell, HJ; Andersen, ME; Verner, MA. (2017). Quantitative bias analysis of a reported association between perfluoroalkyl substances (PFAS) and endometriosis: The influence of oral contraceptive use. Environment International 104: 118-121. http://dx.doi.org/10.1016/j.envint.2017.03.023

Nguyen, TMH; Bräunig, J; Thompson, K; Thompson, J; Kabiri, S; Navarro, DA, et al. (2020). Influences of Chemical Properties, Soil Properties, and Solution pH on Soil-Water Partitioning Coefficients of Per- and Polyfluoroalkyl Substances (PFASs). Environmental Science & Technology 54: 15883-15892. http://dx.doi.org/10.1021/acs.est.0c05705

Nian, M; Li, QQ; Bloom, M; Qian, ZM; Syberg, KM; Vaughn, MG, et al. (2019). Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. Environmental Research 172: 81-88.

NLM. (2022a). ChemIDplus: Perfluorooctanoic acid. Available online at https://chem.nlm.nih.gov/chemidplus/name/pfoa

NLM. (2022b). PubChem Hazardous Substances Data Bank (HSDB) Annotation Record for Perfluorooctanoic acid. Washington, DC: National Institutes of Health, Department of Health and Human Services. Retrieved from https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7137

Nolan, LA; Nolan, JM; Shofer, FS; Rodway, NV; Emmett, EA. (2009). The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reproductive Toxicology 27: 231-238. http://dx.doi.org/10.1016/j.reprotox.2008.11.001

Nolan, LA; Nolan, JM; Shofer, FS; Rodway, NV; Emmett, EA. (2010). Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reproductive Toxicology 29: 147-155. http://dx.doi.org/10.1016/j.reprotox.2009.10.012

NOTOX. (2000). Evaluation of the ability of T-7524 to induce chromosome aberrations in cultured peripheral human lymphocytes. (NOTOX Project Number 292062). Hertogenbosch, The Netherlands: NOTOX.

NTP. (2016). NTP Monograph: Immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). Research Triangle Park, NC: U.S. Department of Health and Human Services, Office of Health Assessment and Translation. https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf

NTP. (2019). NTP technical report on the toxicity studies of perfluoroalkyl carboxylates (perfluorohexanoic acid, perfluorooctanoic acid, perfluorononanoic acid, and perfluorodecanoic acid) administered by gavage to Sprague Dawley (Hsd:Sprague Dawley SD) rats [NTP]. (Toxicity Report 97). Research Triangle Park, NC.

NTP. (2020). NTP technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague Dawley (Hsd:Sprague Dawley SD) rats [NTP]. (Technical Report 598). Research Triangle Park, NC.

O'Malley, KD; Ebbins, KL. (1981). Repeat application 28 day percutaneous absorption study with T-2618CoC in albino rabbits. (USEPA Administrative Record 226-0446). St. Paul, MN: Riker Laboratories, Inc.

Obourn, JD; Frame, SR; Bell, RH; Longnecker, DS; Elliott, GS; Cook, JC. (1997). Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. Toxicology and Applied Pharmacology 145: 425-436. http://dx.doi.org/10.1006/taap.1997.8210

OECD. (2018). Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). (ENV/JM/MONO(2018)7). http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/

OEHHA. (2004). Public Health Goal for Arsenic in Drinking Water: Arsenic. Sacramento, CA: Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Ojo, AF; Peng, C; Ng, JC. (2020). Combined effects and toxicological interactions of perfluoroalkyl and polyfluoroalkyl substances mixtures in human liver cells (HepG2). Environmental Pollution 263: 114182. http://dx.doi.org/10.1016/j.envpol.2020.114182

Okada, E; Sasaki, S; Saijo, Y; Washino, N; Miyashita, C; Kobayashi, S, et al. (2012). Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environmental Research 112: 118-125. http://dx.doi.org/10.1016/j.envres.2011.10.003

Olsen, G, .W., B.,urlew, M.,.M.; Burris, J, .M.; Mandel, J, .H. (2001a). A Longitudinal Analysis of Serum Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997 and 2000 Fluorochemical Medical Surveillance Program. Final Report. (Epidemiology, 220-3W-05). St. Paul, MN: 3M Company. https://www.ag.state.mn.us/Office/Cases/3M/docs/PTX/PTX1799.pdf

Olsen, GW; Burlew, MM; Burris, JM; Mandel, JH. (2001b). A cross-sectional analysis of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee

participants of the 2000 antwerp and decatur fluorochemical medical surveillance program. Final report. St. Paul, MN: 3M Company.

Olsen, GW; Burris, JM; Burlew, MM; Mandel, JH. (1998). 3M final report: an epidemiologic investigation of plasma cholecystokinin, hepatic function and serum perfluorooctanoic acid levels in production workers. (U.S. Environmental Protection Agency Administrative Record 226-0476). 3M Company.

Olsen, GW; Burris, JM; Burlew, MM; Mandel, JH. (2000). Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug and Chemical Toxicology 23: 603-620. http://dx.doi.org/10.1081/DCT-100101973

Olsen, GW; Burris, JM; Burlew, MM; Mandel, JH. (2003). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. Journal of Occupational and Environmental Medicine 45: 260-270. http://dx.doi.org/10.1097/01.jom.0000052958.59271.10

Olsen, GW; Ehresman, DJ; Buehrer, BD; Gibson, BA; Butenhoff, JL; Zobel, LR. (2012). Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. Journal of Occupational and Environmental Medicine 54: 974-983.

Olsen, GW; Hansen, KJ; Clemen, LA; Burris, JM; Mandel, JH. (2001c). Identification of Fluorochemicals in Human Tissue. (U.S. Environmental Protection Agency Administrative Record 226-1030a022). 3M.

Olsen, GW; Zobel, LR. (2007). Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. International Archives of Occupational and Environmental Health 81: 231-246. http://dx.doi.org/10.1007/s00420-007-0213-0

Omoike, OE; Pack, RP; Mamudu, HM; Liu, Y; Strasser, S; Zheng, S, et al. (2020). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environmental Research 196: 110361. http://dx.doi.org/10.1016/j.envres.2020.110361

Omoike, OE; Pack, RP; Mamudu, HM; Liu, Y; Wang, L. (2021). A cross-sectional study of the association between perfluorinated chemical exposure and cancers related to deregulation of estrogen receptors. Environmental Research 196: 110329. http://dx.doi.org/10.1016/j.envres.2020.110329

Ong, KK; Dunger, DB. (2002). Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Practice and Research: Clinical Endocrinology and Metabolism 16: 191-207. http://dx.doi.org/10.1053/beem.2002.0195

Oppi, S; Lüscher, TF; Stein, S. (2019). Mouse models for atherosclerosis research- Which is my line? [Review]. Frontiers in Cardiovascular Medicine 6: 46. http://dx.doi.org/10.3389/fcvm.2019.00046

Orbach, SM; Ehrich, MF; Rajagopalan, P. (2018). High-throughput toxicity testing of chemicals and mixtures in organotypic multi-cellular cultures of primary human hepatic cells. Toxicology In Vitro 51: 83-94. http://dx.doi.org/10.1016/j.tiv.2018.05.006

Oshida, K; Vasani, N; Jones, C; Moore, T; Hester, S; Nesnow, S, et al. (2015a). Identification of chemical modulators of the constitutive activated receptor (CAR) in a gene expression compendium. Nuclear Receptor Signaling 13: e002. http://dx.doi.org/10.1621/nrs.13002

Oshida, K; Vasani, N; Thomas, RS; Applegate, D; Rosen, M; Abbott, B, et al. (2015b). Identification of modulators of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) in a mouse liver gene expression compendium. PLoS ONE 10: e0112655. http://dx.doi.org/10.1371/journal.pone.0112655

Oshida, K; Waxman, DJ; Corton, JC. (2016). Chemical and hormonal effects on STAT5b-dependent sexual dimorphism of the liver transcriptome. PLoS ONE 11: e0150284. http://dx.doi.org/10.1371/journal.pone.0150284

Osmond, C; Barker, DJ. (2000). Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women [Review]. Environmental Health Perspectives 108: 545-553. http://dx.doi.org/10.2307/3454545

Osorio-Yáñez, C; Sanchez-Guerra, M; Cardenas, A; Lin, PID; Hauser, R; Gold, DR, et al. (2021). Per- and polyfluoroalkyl substances and calcifications of the coronary and aortic arteries in adults with prediabetes: Results from the diabetes prevention program outcomes study. Environment International 151: 106446. http://dx.doi.org/10.1016/j.envint.2021.106446

OSTP. (2023). Per- and polyfluoroalkyl substances (PFAS) report. National Science and Technology Council, Joint Subcommittee on Environment, Innovation, and Public Health Per- and Polyfluoroalkyl Substances Strategy Team. https://www.whitehouse.gov/wp-content/uploads/2023/03/OSTP-March-2023-PFAS-Report.pdf?trk=public_post_comment-text

Osuna, CE; Grandjean, P; Weihe, P; El-Fawal, HA. (2014). Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicological Sciences 142: 158-166. http://dx.doi.org/10.1093/toxsci/kfu163

Ou, Y; Zeng, X; Lin, S; Bloom, MS; Han, F; Xiao, X, et al. (2021). Gestational exposure to perfluoroalkyl substances and congenital heart defects: A nested case-control pilot study. Environment International 154: 106567. http://dx.doi.org/10.1016/j.envint.2021.106567

Ouidir, M; Mendola, P; Louis, GMB; Kannan, K; Zhang, C; Tekola-Ayele, F. (2020). Concentrations of persistent organic pollutants in maternal plasma and epigenome-wide placental DNA methylation. Clinical Epigenetics 12: 103. http://dx.doi.org/10.1186/s13148-020-00894-6

Pan, Y; Zhu, Y; Zheng, T; Cui, Q; Buka, SL; Zhang, B, et al. (2017). Novel Chlorinated Polyfluorinated Ether Sulfonates and Legacy Per-/Polyfluoroalkyl Substances: Placental Transfer and Relationship with Serum Albumin and Glomerular Filtration Rate. Environmental Science and Technology 51: 634-644.

Panaretakis, T; Shabalina, IG; Grandér, D; Shoshan, MC; Depierre, JW. (2001). Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma HepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid. Toxicology and Applied Pharmacology 173: 56-64. http://dx.doi.org/10.1006/taap.2001.9159

Papadopoulou, E; Stratakis, N; Basagaña, X; Brantsæter, AL; Casas, M; Fossati, S, et al. (2021). Prenatal and postnatal exposure to PFAS and cardiometabolic factors and inflammation status in children from six European cohorts. Environment International 157: 106853. http://dx.doi.org/10.1016/j.envint.2021.106853

Park, JH; Choi, J; Jun, DW; Han, SW; Yeo, YH; Nguyen, MH. (2019a). Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study. Journal of Clinical Medicine 8. http://dx.doi.org/10.3390/jcm8091445

Park, MH; Gutiérrez-García, AK; Choudhury, M. (2019b). Mono-(2-ethylhexyl) phthalate aggravates inflammatory response via sirtuin regulation and inflammasome activation in RAW 264.7 cells. Chemical Research in Toxicology 32: 935-942. http://dx.doi.org/10.1021/acs.chemrestox.9b00101

Park, SK; Peng, Q; Ding, N; Mukherjee, B; Harlow, SD. (2019c). Determinants of per- and polyfluoroalkyl substances (PFAS) in midlife women: Evidence of racial/ethnic and geographic differences in PFAS exposure. Environmental Research 175: 186-199. http://dx.doi.org/10.1016/j.envres.2019.05.028

Pastoor, TP; Lee, KP; Perri, MA; Gillies, PJ. (1987). Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Experimental and Molecular Pathology 47: 98-109. http://dx.doi.org/10.1016/0014-4800(87)90011-6

Pecquet, AM; Maier, A; Kasper, S; Sumanas, S; Yadav, J. (2020). Exposure to perfluorooctanoic acid (PFOA) decreases neutrophil migration response to injury in zebrafish embryos. BMC Research Notes 13: 408. http://dx.doi.org/10.1186/s13104-020-05255-3

Peng, S; Yan, L; Zhang, J; Wang, Z; Tian, M; Shen, H. (2013). An integrated metabonomics and transcriptomics approach to understanding metabolic pathway disturbance induced by perfluorooctanoic acid. Journal of Pharmaceutical and Biomedical Analysis 86: 56-64. http://dx.doi.org/10.1016/j.jpba.2013.07.014

Pennings, JLA; Jennen, DGJ; Nygaard, UC; Namork, E; Haug, LS; van Loveren, H; Granum, B. (2016). Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. Journal of Immunotoxicology 13: 173-180. http://dx.doi.org/10.3109/1547691X.2015.1029147

Peraza, MA; Burdick, AD; Marin, HE; Gonzalez, FJ; Peters, JM. (2006). The toxicology of ligands for peroxisome proliferator-activated receptors (PPAR) [Review]. Toxicological Sciences 90: 269-295. http://dx.doi.org/10.1093/toxsci/kfj062

Pérez, F; Nadal, M; Navarro-Ortega, A; Fàbrega, F; Domingo, JL; Barceló, D; Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. Environment International 59: 354-362.

Perkins, RG; Butenhoff, JL; Kennedy, GL; Palazzolo, MJ. (2004). 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug and Chemical Toxicology 27: 361-378.

Perng, We; Rifas-Shiman, SL; Kramer, MS; Haugaard, LK; Oken, E; Gillman, MW; Belfort, MB. (2016). Early Weight Gain, Linear Growth, and Mid-Childhood Blood Pressure A Prospective Study in Project Viva. Hypertension 67: 301-308. http://dx.doi.org/10.1161/HYPERTENSIONAHA.115.06635

Peropadre, A; Freire, PF; Hazen, MJ. (2018). A moderate exposure to perfluorooctanoic acid causes persistent DNA damage and senescence in human epidermal HaCaT keratinocytes. Food and Chemical Toxicology 121: 351-359. http://dx.doi.org/10.1016/j.fct.2018.09.020

Petersen, MS; Halling, J; Jørgensen, N; Nielsen, F; Grandjean, P; Jensen, TK; Weihe, P. (2018). Reproductive function in a population of young Faroese men with elevated exposure to polychlorinated biphenyls (pcbs) and perfluorinated alkylate substances (pfas). International Journal of Environmental Research and Public Health 15: n/a.

Pierozan, P; Cattani, D; Karlsson, O. (2020). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) induce epigenetic alterations and promote human breast cell carcinogenesis in vitro. Archives of Toxicology 94: 3893-3906. http://dx.doi.org/10.1007/s00204-020-02848-6

Pierozan, P; Jerneren, F; Karlsson, O. (2018). Perfluorooctanoic acid (PFOA) exposure promotes proliferation, migration and invasion potential in human breast epithelial cells. Archives of Toxicology 92: 1729-1739. http://dx.doi.org/10.1007/s00204-018-2181-4

Pilkerton, CS; Hobbs, GR; Lilly, C; Knox, SS. (2018). Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. PLoS ONE 13: e0203330.

Pirali, B; Negri, S; Chytiris, S; Perissi, A; Villani, L; La Manna, L, et al. (2009). Perfluorooctane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. Thyroid 19: 1407-1412. http://dx.doi.org/10.1089/thy.2009.0174

Pitter, G; Zare Jeddi, M; Barbieri, G; Gion, M; Fabricio, ASC; Daprà, F, et al. (2020). Perfluoroalkyl substances are associated with elevated blood pressure and hypertension in highly exposed young adults. Environmental Health: A Global Access Science Source 19: 102. http://dx.doi.org/10.1186/s12940-020-00656-0

Pizzurro, DM; Seeley, M; Kerper, LE; Beck, BD. (2019). Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria [Review]. Regulatory Toxicology and Pharmacology 106: 239-250.

Podder, A, .; Sadmani, A, .; Reinhart, D, .; Chang, N, . B.; Goel, R, . (2021). Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: A transboundary review of their occurrences and toxicity effects. Journal of Hazardous Materials 419: 126361. http://dx.doi.org/10.1016/j.jhazmat.2021.126361

Poothong, S; Thomsen, C; Padilla-Sanchez, JA; Papadopoulou, E; Haug, LS. (2017). Distribution of novel and well-known poly- and perfluoroalkyl substances (PFASs) in human serum, plasma, and whole blood. Environmental Science and Technology 51: 13388-13396.

Porpora, MG; Lucchini, R; Abballe, A; Ingelido, AM; Valentini, S; Fuggetta, E, et al. (2013). Placental transfer of persistent organic pollutants: a preliminary study on mother-newborn pairs. International Journal of Environmental Research and Public Health 10: 699-711.

Portier, K; Tolson, JK; Roberts, SM. (2007). Body weight distributions for risk assessment. Risk Analysis 27: 11-26. http://dx.doi.org/10.1111/j.1539-6924.2006.00856.x

Post, GB; Birnbaum, LS; Dewitt, JC; Goeden, H; Heiger-Bernays, WJ; Schlezinger, JJ. (2022). Letter to the editors regarding "The conundrum of the PFOA human half-life, an international collaboration" [Letter]. Regulatory Toxicology and Pharmacology 134: 105240. http://dx.doi.org/10.1016/j.yrtph.2022.105240

Post, GB; Cohn, PD; Cooper, KR. (2012). Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature [Review]. Environmental Research 116: 93-117. http://dx.doi.org/10.1016/j.envres.2012.03.007

Pouwer, MG; Pieterman, EJ; Chang, SC; Olsen, GW; Caspers, MPM; Verschuren, L, et al. (2019). Dose effects of ammonium perfluorooctanoate on lipoprotein metabolism in apoe*3-leiden.cetp mice. Toxicological Sciences 168: 519-534.

Predieri, B; Iughetti, L; Guerranti, C; Bruzzi, P; Perra, G; Focardi, SE. (2015). High Levels of Perfluorooctane Sulfonate in Children at the Onset of Diabetes. International Journal of Endocrinology 2015: 234358.

Pritchard, JA. (1965). Changes in the blood volume during pregnancy and delivery [Review]. Anesthesiology 26: 393-399. http://dx.doi.org/10.1097/00000542-196507000-00004

Qazi, MR; Abedi, MR; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2010). Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. International Immunopharmacology 10: 1420-1427. http://dx.doi.org/10.1016/j.intimp.2010.08.009

Qazi, MR; Bogdanska, J; Butenhoff, JL; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2009). High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 262: 207-214. http://dx.doi.org/10.1016/j.tox.2009.06.010

Qazi, MR; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2012). High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. Food and Chemical Toxicology 50: 2955-2963. http://dx.doi.org/10.1016/j.fct.2012.06.023

Qin, P; Liu, R; Pan, X; Fang, X; Mou, Y. (2010). Impact of carbon chain length on binding of perfluoroalkyl acids to bovine serum albumin determined by spectroscopic methods. Journal of Agricultural and Food Chemistry 58: 5561-5567. http://dx.doi.org/10.1021/jf100412q

Quist, EM; Filgo, AJ; Cummings, CA; Kissling, GE; Hoenerhoff, MJ; Fenton, SE. (2015). Hepatic mitochondrial alteration in CD-1 mice associated with prenatal exposures to low doses of perfluorooctanoic acid. Toxicologic Pathology 43: 546-557. http://dx.doi.org/10.1177/0192623314551841

Rainieri, S; Conlledo, N; Langerholc, T; Madorran, E; Sala, M; Barranco, A. (2017). Toxic effects of perfluorinated compounds at human cellular level and on a model vertebrate. Food and Chemical Toxicology 104: 14-25. http://dx.doi.org/10.1016/j.fct.2017.02.041

Raleigh, KK; Alexander, BH; Olsen, GW; Ramachandran, G; Morey, SZ; Church, TR, et al. (2014). Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occupational and Environmental Medicine 71: 500-506. http://dx.doi.org/10.1136/oemed-2014-102109

Rantakokko, P; Männistö, V; Airaksinen, R; Koponen, J; Viluksela, M; Kiviranta, H; Pihlajamäki, J. (2015). Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: A cohort study. Environmental Health: A Global Access Science Source 14: 79.

Rashid, F; Ramakrishnan, A; Fields, C; Irudayaraj, J. (2020). Acute PFOA exposure promotes epigenomic alterations in mouse kidney tissues. Toxicology Reports 7: 125-132. http://dx.doi.org/10.1016/j.toxrep.2019.12.010

Rebholz, SL; Jones, T; Herrick, RL; Xie, C; Calafat, AM; Pinney, SM; Woollett, LA. (2016). Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice. Toxicology Reports 3: 46-54. http://dx.doi.org/10.1016/j.toxrep.2015.11.004

Remucal, CK. (2019). Spatial and temporal variability of perfluoroalkyl substances in the Laurentian Great Lakes [Review]. Environmental Science: Processes & Impacts 21: 1816-1834. http://dx.doi.org/10.1039/c9em00265k

Reyes, L; Mañalich, R. (2005). Long-term consequences of low birth weight [Review]. Kidney International Supplement 68: S107-S111. http://dx.doi.org/10.1111/j.1523-1755.2005.09718.x

Risnes, KR; Vatten, LJ; Baker, JL; Jameson, K; Sovio, U; Kajantie, E, et al. (2011). Birthweight and mortality in adulthood: a systematic review and meta-analysis [Review]. International Journal of Epidemiology 40: 647-661. http://dx.doi.org/10.1093/ije/dyq267

Robinson, MW; Harmon, C; O'Farrelly, C. (2016). Liver immunology and its role in inflammation and homeostasis [Review]. Cellular and Molecular Immunology 13: 267-276. http://dx.doi.org/10.1038/cmi.2016.3

Robledo, CA; Yeung, E; Mendola, P; Sundaram, R; Maisog, J; Sweeney, AM, et al. (2015). Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environmental Health Perspectives 123: 88-94. http://dx.doi.org/10.1289/ehp.1308016

Romano, ME; Xu, Y; Calafat, AM; Yolton, K; Chen, A; Webster, GM, et al. (2016). Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. Environmental Research 149: 239-246.

Rooney, JP; Oshida, K; Kumar, R; Baldwin, WS; Corton, JC. (2019). Chemical Activation of the Constitutive Androstane Receptor Leads to Activation of Oxidant-Induced Nrf2. Toxicological Sciences 167: 172-189. http://dx.doi.org/10.1093/toxsci/kfy231

Rose, K; Wolf, KK; Ukairo, O; Moore, A; Gaffney, J; Bradford, BU, et al. (2016). Nuclear Receptor-Mediated Gene Expression Changes in a Human Hepatic Micropatterned Coculture Model After Treatment with Hepatotoxic Compounds. Applied in Vitro Toxicology 2: 8-16. http://dx.doi.org/10.1089/aivt.2015.0024

Rosen, MB; Abbott, BD; Wolf, DC; Corton, JC; Wood, CR; Schmid, JE, et al. (2008a). Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. Toxicologic Pathology 36: 592-607. http://dx.doi.org/10.1177/0192623308318208

Rosen, MB; Das, KP; Rooney, J; Abbott, B; Lau, C; Corton, JC. (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387: 95-107. http://dx.doi.org/10.1016/j.tox.2017.05.013

Rosen, MB; Das, KP; Wood, CR; Wolf, CJ; Abbott, BD; Lau, C. (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology 308: 129-137. http://dx.doi.org/10.1016/j.tox.2013.03.011

Rosen, MB; Lee, JS; Ren, H; Vallanat, B; Liu, J; Waalkes, MP, et al. (2008b). Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. Toxicological Sciences 103: 46-56. http://dx.doi.org/10.1093/toxsci/kfn025

Rosenmai, AK; Ahrens, L; le Godec, T; Lundqvist, J; Oskarsson, A. (2018). Relationship between peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl substances in HepG2 cells. Journal of Applied Toxicology 38: 219-226. http://dx.doi.org/10.1002/jat.3515

Rosenmai, AK; Nielsen, FK; Pedersen, M; Hadrup, N; Trier, X; Christensen, JH; Vinggaard, AM. (2013). Fluorochemicals used in food packaging inhibit male sex hormone synthesis. Toxicology and Applied Pharmacology 266: 132-142. http://dx.doi.org/10.1016/j.taap.2012.10.022

Rotander, A; Toms, LM; Aylward, L; Kay, M; Mueller, JF. (2015). Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environment International 82: 28-34.

Roth, K; Yang, Z; Agarwal, M; Liu, W; Peng, Z; Long, Z, e, et al. (2021). Exposure to a mixture of legacy, alternative, and replacement per- and polyfluoroalkyl substances (PFAS) results in sex-dependent modulation of cholesterol metabolism and liver injury. Environment International 157: 106843. http://dx.doi.org/10.1016/j.envint.2021.106843

Ruark, CD; Song, G; Yoon, M; Verner, MA; Andersen, ME; Clewell, HJ; Longnecker, MP. (2017). Quantitative bias analysis for epidemiological associations of perfluoroalkyl substance serum concentrations and early onset of menopause. Environment International 99: 245-254. http://dx.doi.org/10.1016/j.envint.2016.11.030

Ruggiero, MJ; Miller, H; Idowu, JY; Zitzow, JD; Chang, SC; Hagenbuch, B. (2021). Perfluoroalkyl Carboxylic Acids Interact with the Human Bile Acid Transporter NTCP. Livers 1: 221-229.

Ruhl, CE; Everhart, JE. (2009). Elevated Serum Alanine Aminotransferase and gamma-Glutamyltransferase and Mortality in the United States Population. Gastroenterology 136: 477-485. http://dx.doi.org/10.1053/j.gastro.2008.10.052

Ruhl, CE; Everhart, JE. (2013). The Association of Low Serum Alanine Aminotransferase Activity With Mortality in the US Population. American Journal of Epidemiology 178: 1702-1711. http://dx.doi.org/10.1093/aje/kwt209

Russell, MH; Waterland, RL; Wong, F. (2015). Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid (PFOA). Chemosphere 129: 210-216. http://dx.doi.org/10.1016/j.chemosphere.2014.07.061

Sadhu, D. (2002). CHO/HGPRT forward mutation assay – ISO (T6.889.7). (US EPA AR-226-1101). Bedford, MA: Toxicon Corporation.

Saejia, P; Lirdprapamongkol, K; Svasti, J; Paricharttanakul, NM. (2019). Perfluorooctanoic Acid Enhances Invasion of Follicular Thyroid Carcinoma Cells Through NF-κB and Matrix Metalloproteinase-2 Activation. Anticancer Research 39: 2429-2435. http://dx.doi.org/10.21873/anticanres.13360

Sagiv, SK; Rifas-Shiman, SL; Fleisch, AF; Webster, TF; Calafat, AM; Ye, X, et al. (2018). Early Pregnancy Perfluoroalkyl Substance Plasma Concentrations and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? American Journal of Epidemiology 187: 793-802.

Sakr, CJ; Kreckmann, KH; Green, JW; Gillies, PJ; Reynolds, JL; Leonard, RC. (2007a). Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonia perfluorooctanoate or APFO) as part of a general health survey in a cohorent of occupational exposed workers. Journal of Occupational and Environmental Medicine 49: 1086-1096. http://dx.doi.org/10.1097/JOM.0b013e318156eca3

Sakr, CJ; Leonard, RC; Kreckmann, KH; Slade, MD; Cullen, MR. (2007b). Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. Journal of Occupational and Environmental Medicine 49: 872-879. http://dx.doi.org/10.1097/JOM.0b013e318124a93f

Sakr, CJ; Symons, JM; Kreckmann, KH; Leonard, RC. (2009). Ischaemic heart disease mortality study among workers with occupational exposure to ammonium perfluorooctanoate. Occupational and Environmental Medicine 66: 699-703. http://dx.doi.org/10.1136/oem.2008.041582

Salihovic, S; Stubleski, J; Kärrman, A; Larsson, A; Fall, T; Lind, L; Lind, PM. (2018). Changes in markers of liver function in relation to changes in perfluoroalkyl substances - A longitudinal study. Environment International 117: 196-203.

Salimi, A; Nikoosiar Jahromi, M; Pourahmad, J. (2019). Maternal exposure causes mitochondrial dysfunction in brain, liver, and heart of mouse fetus: An explanation for perfluorooctanoic acid induced abortion and developmental toxicity. Environmental Toxicology 34: 878-885.

Salvalaglio, M; Muscionico, I; Cavallotti, C. (2010). Determination of energies and sites of binding of PFOA and PFOS to human serum albumin. Journal of Physical Chemistry B 114: 14860-14874. http://dx.doi.org/10.1021/jp106584b

Sanchez Garcia, D; Sjödin, M; Hellstrandh, M; Norinder, U; Nikiforova, V; Lindberg, J, et al. (2018). Cellular accumulation and lipid binding of perfluorinated alkylated substances (PFASs) - A comparison with lysosomotropic drugs. Chemico-Biological Interactions 281: 1-10.

Savitz, DA; Stein, CR; Bartell, SM; Elston, B; Gong, J; Shin, HM; Wellenius, GA. (2012a). Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. Epidemiology 23: 386-392. http://dx.doi.org/10.1097/EDE.0b013e31824cb93b

Savitz, DA; Stein, CR; Elston, B; Wellenius, GA; Bartell, SM; Shin, HM, et al. (2012b). Relationship of perfluorooctanoic Acid exposure to pregnancy outcome based on birth records in the mid-ohio valley. Environmental Health Perspectives 120: 1201-1207. http://dx.doi.org/10.1289/ehp.1104752

Schaaf, MJM. (2017). Nuclear receptor research in zebrafish [Review]. Journal of Molecular Endocrinology 59: R65-R76. http://dx.doi.org/10.1530/JME-17-0031

Schiller, JS; Lucas, JW; Ward, BW; Peregoy, JA. (2012). Summary health statistics for U.S. adults: National Health Interview Survey, 2010. (DHHS Publication No. (PHS) 2012–1580). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_252.pdf

Schlezinger, JJ; Puckett, H; Oliver, J; Nielsen, G; Heiger-Bernays, W; Webster, S. (2020). Perfluorooctanoic acid activates multiple nuclear receptor pathways and skews expression of genes regulating cholesterol homeostasis in liver of humanized PPARα mice fed an American diet. Toxicology and Applied Pharmacology 405: 115204. http://dx.doi.org/10.1016/j.taap.2020.115204

Selgrade, MK. (2007). Immunotoxicity: The risk is real [Review]. Toxicological Sciences 100: 328-332. http://dx.doi.org/10.1093/toxsci/kfm244

Seo, SH; Son, MH; Choi, SD; Lee, DH; Chang, YS. (2018). Influence of exposure to perfluoroalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects. Environment International 113: 149-161.

Shah, D. (2009). Healthy worker effect phenomenon. Indian Journal of Occupational and Environmental Medicine 13: 77-79. http://dx.doi.org/10.4103/0019-5278.55123

Shan, G; Wang, Z, hi; Zhou, L; Du, P, in; Luo, X; Wu, Q; Zhu, L. (2016). Impacts of daily intakes on the isomeric profiles of perfluoroalkyl substances (PFASs) in human serum. Environment International 89-90: 62-70. http://dx.doi.org/10.1016/j.envint.2016.01.002

Shan, G; Ye, M; Zhu, B; Zhu, L. (2013). Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. Chemosphere 93: 2101-2107. http://dx.doi.org/10.1016/j.chemosphere.2013.07.054

Shane, HL; Baur, R; Lukomska, E; Weatherly, L; Anderson, SE. (2020). Immunotoxicity and allergenic potential induced by topical application of perfluorooctanoic acid (PFOA) in a murine model. Food and Chemical Toxicology 136: 111114. http://dx.doi.org/10.1016/j.fct.2020.111114

Shankar, A; Xiao, J; Ducatman, A. (2012). Perfluorooctanoic acid and cardiovascular disease in US adults. Archives of Internal Medicine 172: 1397-1403.

Shao, X; Ji, F; Wang, Y; Zhu, L; Zhang, Z; Du, X, et al. (2018). Integrative Chemical Proteomics-Metabolomics Approach Reveals Acaca/Acacb as Direct Molecular Targets of PFOA. Analytical Chemistry 90: 11092-11098. http://dx.doi.org/10.1021/acs.analchem.8b02995

Shearer, JJ; Callahan, CL; Calafat, AM; Huang, WY; Jones, RR; Sabbisetti, VS, et al. (2021). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. Journal of the National Cancer Institute 113: 580-587. http://dx.doi.org/10.1093/jnci/djaa143

Shen, M; Xiao, Y; Huang, Y; Jing, D; Su, J; Luo, D, et al. (2022). Perfluoroalkyl substances are linked to incident chronic spontaneous urticaria: A nested case-control study. Chemosphere 287: 132358. http://dx.doi.org/10.1016/j.chemosphere.2021.132358

Sheng, N; Cui, R; Wang, J; Guo, Y; Wang, J; Dai, J. (2018). Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid binding protein. Archives of Toxicology 92: 359-369. http://dx.doi.org/10.1007/s00204-017-2055-1

Shi, LC; Zheng, JJ; Yan, SK; Li, YX; Wang, YJ; Liu, XB; Xiao, CX. (2020). Exposure to Perfluorooctanoic Acid Induces Cognitive Deficits via Altering Gut Microbiota Composition, Impairing Intestinal Barrier Integrity, and Causing Inflammation in Gut and Brain. Journal of Agricultural and Food Chemistry 68: 13916-13928. http://dx.doi.org/10.1021/acs.jafc.0c05834

Shi, Y; Yang, L; Li, J; Lai, J; Wang, Y; Zhao, Y; Wu, Y. (2017). Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 169: 396-402.

Shih, YH; Blomberg, AJ; Bind, MA; Holm, D; Nielsen, F; Heilmann, C, et al. (2021). Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. Journal of Immunotoxicology 18: 85-92. http://dx.doi.org/10.1080/1547691X.2021.1922957

Shin, HM, oo; Vieira, VM; Ryan, PB; Steenland, K; Bartell, SM. (2013). Retrospective Exposure Estimation and Predicted versus Observed Serum Perfluorooctanoic Acid Concentrations for Participants in the C8 Health Project (vol 119, pg 1760, 2011). Environmental Health Perspectives 121: A113-A113.

Shin, HM; Vieira, VM; Ryan, PB; Steenland, K; Bartell, SM. (2011). Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environmental Health Perspectives 119: 1760-1765. http://dx.doi.org/10.1289/ehp.1103729

Shinwell, ES; Shlomo, M. (2003). Measured length of normal term infants changes over the first two days of life. Journal of Pediatric Endocrinology & Metabolism 16: 537-540. http://dx.doi.org/10.1515/jpem.2003.16.4.537

Shoaff, J; Papandonatos, GD; Calafat, AM; Chen, A; Lanphear, BP; Ehrlich, S, et al. (2018). Prenatal exposure to perfluoroalkyl substances: Infant birth weight and early life growth. Environmental Epidemiology 2: e010.

Sibai, BM; Frangieh, A. (1995). Maternal adaptation to pregnancy. Current Opinion in Obstetrics and Gynecology 7: 420-426. http://dx.doi.org/10.1097/00001703-199512000-00003

Silverstein, RL; Febbraio, M. (2009). CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior [Review]. Science Signaling 2: re3. http://dx.doi.org/10.1126/scisignal.272re3

Simpson, C; Winquist, A; Lally, C; Steenland, K. (2013). Relation between perfluorooctanoic acid exposure and strokes in a large cohort living near a chemical plant. Environmental Research 127: 22-28. http://dx.doi.org/10.1016/j.envres.2013.10.002

Singer, AB; Whitworth, KW; Haug, LS; Sabaredzovic, A; Impinen, A; Papadopoulou, E; Longnecker, MP. (2018). Menstrual cycle characteristics as determinants of plasma concentrations of perfluoroalkyl substances (PFASs) in the Norwegian Mother and Child Cohort (MoBa study). Environmental Research 166: 78-85. http://dx.doi.org/10.1016/j.envres.2018.05.019

Sinisalu, L; Sen, P; Salihović, S; Virtanen, SM; Hyöty, H; Ilonen, J, et al. (2020). Early-life exposure to perfluorinated alkyl substances modulates lipid metabolism in progression to celiac disease. Environmental Research 188: 109864. http://dx.doi.org/10.1016/j.envres.2020.109864

Sinisalu, L; Yeung, LWY; Wang, J; Pan, Y; Dai, J; Hyötyläinen, T. (2021). Prenatal exposure to poly-/per-fluoroalkyl substances is associated with alteration of lipid profiles in cord-blood. Metabolomics 17: 103. http://dx.doi.org/10.1007/s11306-021-01853-9

Skuladottir, M; Ramel, A; Rytter, D; Haug, LS; Sabaredzovic, A; Bech, BH, et al. (2015). Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environmental Research 143: 33-38.

Smit, LA; Lenters, V; Høyer, BB; Lindh, CH; Pedersen, HS; Liermontova, I, et al. (2015). Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 70: 653-660.

Smith, CJ; Ryckman, KK; Barnabei, VM; Howard, BV; Isasi, CR; Sarto, GE, et al. (2016a). The impact of birth weight on cardiovascular disease risk in the Women's Health Initiative. Nutrition, Metabolism & Cardiovascular Diseases 26: 239-245. http://dx.doi.org/10.1016/j.numecd.2015.10.015

Smith, MT; Guyton, KZ; Gibbons, CF; Fritz, JM; Portier, CJ; Rusyn, I, et al. (2016b). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis [Review]. Environmental Health Perspectives 124: 713-721. http://dx.doi.org/10.1289/ehp.1509912

Smith, MT; Guyton, KZ; Kleinstreuer, N; Borrel, A; Cardenas, A; Chiu, WA, et al. (2020). The Key Characteristics of Carcinogens: Relationship to the Hallmarks of Cancer, Relevant Biomarkers, and Assays to Measure Them [Editorial]. Cancer Epidemiology Biomarkers and Prevention. http://dx.doi.org/10.1158/1055-9965.EPI-19-1346

Smith, PJ; Humiston, SG; Marcuse, EK; Zhao, Z; Dorell, CG; Howes, C; Hibbs, B. (2011). Parental delay or refusal of vaccine doses, childhood vaccination coverage at 24 months of age, and the Health Belief Model. 126: 135-146. http://dx.doi.org/10.1177/00333549111260S215

Smithwick, M; Norstrom, RJ; Mabury, SA; Solomon, K; Evans, TJ; Stirling, I, et al. (2006). Temporal trends of perfluoroalkyl contaminants in polar bears (Ursus maritimus) from two locations in the North American Arctic, 1972-2002. Environmental Science and Technology 40: 1139-1143. http://dx.doi.org/10.1021/es051750h

Son, HY; Kim, SH; Shin, HI; Bae, HI; Yang, JH. (2008). Perfluorooctanoic acid-induced hepatic toxicity following 21-day oral exposure in mice. Archives of Toxicology 82: 239-246. http://dx.doi.org/10.1007/s00204-007-0246-x

Son, HY; Lee, S; Tak, EN; Cho, HS; Shin, HI; Kim, SH; Yang, JH. (2009). Perfluorooctanoic acid alters T lymphocyte phenotypes and cytokine expression in mice. Environmental Toxicology 24: 580-588. http://dx.doi.org/10.1002/tox.20459

Song, M, i-Kyung; Cho, Y, oon; Jeong, S, eung-Chan; Ryu, J, ae-Chun. (2016). Analysis of gene expression changes in relation to hepatotoxicity induced by perfluorinated chemicals in a human hepatoma cell line. Toxicology and Environmental Health Sciences 8: 114–127. http://dx.doi.org/10.1007/s13530-016-0269-x

Song, P; Li, D; Wang, X; Zhong, X. (2018). Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice. Andrologia 50: e13059.

Song, P; Li, D; Wang, X; Zhong, X. (2019). Lycopene protects from perfluorooctanoic acid induced liver damage and uterine apoptosis in pregnant mice. International Journal of Clinical and Experimental Medicine 12: 212-219.

Sørli, JB; Låg, M; Ekeren, L; Perez-Gil, J; Haug, LS; Da Silva, E, et al. (2020). Per- and polyfluoroalkyl substances (PFASs) modify lung surfactant function and pro-inflammatory responses in human bronchial epithelial cells. Toxicology In Vitro 62: 104656. http://dx.doi.org/10.1016/j.tiv.2019.104656

Souza, MCO; Saraiva, MCP; Honda, M; Barbieri, MA; Bettiol, H; Barbosa, F; Kannan, K. (2020). Exposure to per- and polyfluorinated alkyl substances in pregnant Brazilian women and its association with fetal growth. Environmental Research 187: 109585.

Spliethoff, HM; Tao, L; Shaver, SM; Aldous, KM; Pass, KA; Kannan, K; Eadon, GA. (2008). Use of newborn screening program blood spots for exposure assessment: declining levels of perluorinated compounds in New York State infants. Environmental Science and Technology 42: 5361-5367. http://dx.doi.org/10.1021/es8006244

Spratlen, MJ; Perera, FP; Lederman, SA; Robinson, M; Kannan, K; Herbstman, J; Trasande, L. (2020). The association between perfluoroalkyl substances and lipids in cord blood. Journal of Clinical Endocrinology and Metabolism 105: 43-54.

Starling, AP; Adgate, JL; Hamman, RF; Kechris, K; Calafat, AM; Dabelea, D. (2019). Prenatal exposure to per- and polyfluoroalkyl substances and infant growth and adiposity: The healthy start study. Environment International 131: 104983.

Starling, AP; Adgate, JL; Hamman, RF; Kechris, K; Calafat, AM; Ye, X; Dabelea, D. (2017). Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: Examining mediation by maternal fasting glucose in the healthy start study. Environmental Health Perspectives 125: 067016.

Starling, AP; Engel, SM; Whitworth, KW; Richardson, DB; Stuebe, AM; Daniels, JL, et al. (2014). Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. Environment International 62: 104-112. http://dx.doi.org/10.1016/j.envint.2013.10.004

Steenland, K; Barry, V; Savitz, D. (2018a). Serum perfluorooctanoic acid and birthweight: an updated meta-analysis with bias analysis. Epidemiology 29: 765-776. http://dx.doi.org/10.1097/EDE.0000000000000903

Steenland, K; Fletcher, T; Stein, CR; Bartell, SM; Darrow, L; Lopez-Espinosa, MJ, et al. (2020). Review: Evolution of evidence on PFOA and health following the assessments of the C8 Science Panel [Review]. Environment International 145: 106125. http://dx.doi.org/10.1016/j.envint.2020.106125

Steenland, K; Hofmann, JN; Silverman, DT; Bartell, SM. (2022). Risk assessment for PFOA and kidney cancer based on a pooled analysis of two studies. Environment International 167: 107425. http://dx.doi.org/10.1016/j.envint.2022.107425

Steenland, K; Kugathasan, S; Barr, DB. (2018b). PFOA and ulcerative colitis. Environmental Research 165: 317-321.

Steenland, K; Tinker, S; Frisbee, S; Ducatman, A; Vaccarino, V. (2009). Association of Perfluorooctanoic Acid and Perfluorooctane Sulfonate With Serum Lipids Among Adults Living Near a Chemical Plant. American Journal of Epidemiology 170: 1268-1278. http://dx.doi.org/10.1093/aje/kwp279

Steenland, K; Woskie, S. (2012). Cohort mortality study of workers exposed to perfluorooctanoic acid. American Journal of Epidemiology 176: 909-917. http://dx.doi.org/10.1093/aje/kws171

Steenland, K; Zhao, L; Winquist, A. (2015). A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occupational and Environmental Medicine 72: 373-380.

Steenland, K; Zhao, L; Winquist, A; Parks, C. (2013). Ulcerative Colitis and Perfluorooctanoic Acid (PFOA) in a Highly Exposed Population of Community Residents and Workers in the Mid-Ohio Valley. Environmental Health Perspectives 121: 900-905.

Stein, CR; Ge, Y; Wolff, MS; Ye, X; Calafat, AM; Kraus, T; Moran, TM. (2016a). Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. Environmental Research 149: 171-178.

Stein, CR; Mcgovern, KJ; Pajak, AM; Maglione, PJ; Wolff, M. (2016b). Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatric Research 79: 348-357. http://dx.doi.org/10.1038/pr.2015.213

Stein, CR; Savitz, DA; Dougan, M. (2009). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. American Journal of Epidemiology 170: 837-846. http://dx.doi.org/10.1093/aje/kwp212

Stein, CR; Savitz, DA; Elston, B; Thorpe, PG; Gilboa, SM. (2014). Perfluorooctanoate exposure and major birth defects. Reproductive Toxicology 47: 15-20. http://dx.doi.org/10.1016/j.reprotox.2014.04.006

Stengel, D; Wahby, S; Braunbeck, T. (2018). In search of a comprehensible set of endpoints for the routine monitoring of neurotoxicity in vertebrates: sensory perception and nerve transmission in

zebrafish (Danio rerio) embryos. Environmental Science and Pollution Research 25: 4066-4084. http://dx.doi.org/10.1007/s11356-017-0399-y

Suh, CH; Cho, NK; Lee, CK; Lee, CH; Kim, DH; Kim, JH, et al. (2011). Perfluorooctanoic acid-induced inhibition of placental prolactin-family hormone and fetal growth retardation in mice. Molecular and Cellular Endocrinology 337: 7-15. http://dx.doi.org/10.1016/j.mce.2011.01.009

Sun, Q; Zong, G; Valvi, D; Nielsen, F; Coull, B; Grandjean, P. (2018). Plasma concentrations of perfluoroalkyl substances and risk of Type 2 diabetes: A prospective investigation among U.S. women. Environmental Health Perspectives 126: 037001.

Sun, S; Guo, H, ua; Wang, J; Dai, J. (2019). Hepatotoxicity of perfluorooctanoic acid and two emerging alternatives based on a 3D spheroid model. Environmental Pollution 246: 955-962. http://dx.doi.org/10.1016/j.envpol.2018.12.065

Takacs, ML; Abbott, BD. (2007). Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicological Sciences 95: 108-117. http://dx.doi.org/10.1093/toxsci/kfl135

Takagi, A; Sai, K; Umemura, T; Hasegawa, R; Kurokawa, Y. (1991). Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. Cancer Letters 57: 55-60. http://dx.doi.org/10.1016/0304-3835(91)90063-N

Tan, YM; Clewell, HJ; Andersen, ME. (2008). Time dependencies in perfluorooctylacids disposition in rat and monkeys: a kinetic analysis. Toxicology Letters 177: 38-47. http://dx.doi.org/10.1016/j.toxlet.2007.12.007

Tanner, EM; Bornehag, CG; Gennings, C. (2020). Dynamic growth metrics for examining prenatal exposure impacts on child growth trajectories: Application to perfluorooctanoic acid (PFOA) and postnatal weight gain. Environmental Research 182: 109044.

Tao, L; Kannan, K; Wong, CM; Arcaro, KF; Butenhoff, JL. (2008). Perfluorinated compounds in human milk from Massachusetts, USA. Environmental Science and Technology 42: 3096-3101. http://dx.doi.org/10.1021/es702789k

Taylor, KW; Hoffman, K; Thayer, KA; Daniels, JL. (2014). Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environmental Health Perspectives 122: 145-150. http://dx.doi.org/10.1289/ehp.1306707

Taylor, KW; Joubert, BR; Braun, JM; Dilworth, C; Gennings, C; Hauser, R, et al. (2016). Statistical Approaches for Assessing Health Effects of Environmental Chemical Mixtures in Epidemiology: Lessons from an Innovative Workshop. Environmental Health Perspectives 124: A227-A229. http://dx.doi.org/10.1289/EHP547

Thayer, KA; Angrish, M; Arzuaga, X; Carlson, LM; Davis, A; Dishaw, L, et al. (2022). Systematic evidence map (SEM) template: report format and methods used for the US EPA integrated risk information system (iris) program, provisional peer reviewed toxicity value (PPRTV) program, and other "fit for purpose" literature-based human health analyses (manuscript-in-progress) (pp. 1-69). Thayer, KA; Angrish, M; Arzuaga, X; Carlson, LM; Davis, A; Dishaw, L; Druwe, I; Gibbons, C; Glenn, B; Jones, R; Kaiser, JP; Keshava, C; Keshava, N; Kraft, A; Lizarraga, L; Persad, A; Radke, EG; Rice, G; Schulz, B; Shaffer, R; Shannon. T; Shapiro, A; Thacker, S; Vulimiri, S; Williams, AJ; Woodall, G; Yost, E; Blain, R; Duke, K; Goldstone, AE; Hartman, P; Hobbie, K; Ingle, B; Lemeris, C; Lin, C; Lindahl, A; McKinley, K; Soleymani, P; Vetter, N.

Thompson, J; Lorber, M; Toms, LM; Kato, K; Calafat, AM; Mueller, JF. (2010a). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. Environment International 36: 390-397. http://dx.doi.org/10.1016/j.envint.2010.02.008

Thompson, J; Lorber, M; Toms, LML; Kato, K; Calafat, AM; Mueller, JF. (2010b). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid (vol 36, pg 390, 2010). Environment International 36: 647-648. http://dx.doi.org/10.1016/j.envint.2010.05.008

Thomsen, C; Haug, LS; Stigum, H; Frøshaug, M; Broadwell, SL; Becher, G. (2010). Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. Environmental Science and Technology 44: 9550-9556. http://dx.doi.org/10.1021/es1021922

Thorsdottir, I; Birgisdottir, BE. (1998). Different weight gain in women of normal weight before pregnancy: postpartum weight and birth weight. Obstetrics and Gynecology 92: 377-383.

Tian, M; Reichetzeder, C; Li, J; Hocher, B. (2019). Low birth weight, a risk factor for diseases in later life, is a surrogate of insulin resistance at birth. Journal of Hypertension 37: 2123-2134. http://dx.doi.org/10.1097/HJH.0000000000002156

Tian, Y; Miao, M; Ji, H; Zhang, X; Chen, A; Wang, Z, et al. (2020). Prenatal exposure to perfluoroalkyl substances and cord plasma lipid concentrations. Environmental Pollution 268: 115426. http://dx.doi.org/10.1016/j.envpol.2020.115426

Timmermann, CA; Budtz-Jørgensen, E; Jensen, TK; Osuna, CE; Petersen, MS; Steuerwald, U, et al. (2017a). Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. Journal of Immunotoxicology 14: 39-49.

Timmermann, CA; Budtz-Jørgensen, E; Petersen, MS; Weihe, P; Steuerwald, U; Nielsen, F, et al. (2017b). Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reproductive Toxicology 68: 164-170.

Timmermann, CAG; Jensen, KJ; Nielsen, F; Budtz-Jørgensen, E; van Der Klis, F; Benn, CS, et al. (2020). Serum Perfluoroalkyl Substances, Vaccine Responses, and Morbidity in a Cohort of Guinea-Bissau Children. Environmental Health Perspectives 128: 87002.

Timmermann, CAG; Pedersen, HS; Weihe, P; Bjerregaard, P; Nielsen, F; Heilmann, C; Grandjean, P. (2021). Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environmental Research 203: 111712. http://dx.doi.org/10.1016/j.envres.2021.111712

Trefts, E; Gannon, M; Wasserman, DH. (2017). The liver. Current Biology 27: R1147-R1151. http://dx.doi.org/10.1016/j.cub.2017.09.019

Tsai, MS; Chang, SH; Kuo, WH; Kuo, CH; Li, SY; Wang, MY, et al. (2020). A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environment International 142: 105850.

Tucker, DK; Macon, MB; Strynar, MJ; Dagnino, S; Andersen, E; Fenton, SE. (2014). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reproductive Toxicology 54: 26-36. http://dx.doi.org/10.1016/j.reprotox.2014.12.002

U.S. EPA. (1991). Guidelines for developmental toxicity risk assessment. Federal Register 56: 63798-63826.

U.S. EPA. (2002a). Hepatocellular hypertrophy. HED guidance document #G2002.01 [EPA Report]. Washington, DC.

U.S. EPA. (2002b). A review of the reference dose and reference concentration processes. (EPA630P02002F). Washington, DC. https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

U.S. EPA. (2003). Toxicological review of methyl ethyl ketone (CAS No. 78-93-3): In support of summary information on the Integrated Risk Information System (IRIS). (EPA 635/R-03/009). Washington, DC. http://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0071tr.pdf

U.S. EPA. (2004). Toxicological review of boron and compounds. In support of summary information on the Integrated Risk Information System (IRIS) [EPA Report]. (EPA/635/04/052). Washington, DC: U.S. Environmental Protection Agency, IRIS. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P1006CK9.txt

U.S. EPA. (2005a). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf

U.S. EPA. (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens [EPA Report]. (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/supplemental-guidance-assessing-susceptibility-early-life-exposure-carcinogens

U.S. EPA. (2009). Final Contaminant Candidate List 3 Chemicals: Screening to a PCCL. (815R09007). http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P10056VO.txt

U.S. EPA. (2011a). Age Dependent Adjustment Factor (ADAF) application [EPA Report]. Washington, DC.

U.S. EPA. (2011b). Exposure factors handbook: 2011 edition [EPA Report]. (EPA/600/R-090/052F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252

U.S. EPA. (2012a). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

U.S. EPA. (2012b). Toxicological review of tetrahydrofuran. In support of summary information on the integrated risk information system (IRIS) (pp. 1-207). (EPA/635/R-11/006F). Washington, DC.

U.S. EPA. (2013). America's children and the environment [EPA Report] (3rd ed.). (EPA 240-R-13-001). Washington, D.C. http://www.epa.gov/ace

U.S. EPA. (2014). Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation [EPA Report]. (EPA/100/R-14/002F). Washington, DC: Risk Assessment Forum, Office of the Science Advisor. https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf

U.S. EPA. (2016a). Drinking water health advisory for perfluorooctanoic acid (PFOA) [EPA Report]. (EPA 822-R-16-005). Washington, DC: U.S. Environmental Protection Agency, Office of Water. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final_508.pdf

U.S. EPA. (2016b). Health effects support document for perfluorooctane sulfonate (PFOS) [EPA Report]. (EPA 822-R-16-002). Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division. https://www.epa.gov/sites/production/files/2016-05/documents/pfos_hesd_final_508.pdf

U.S. EPA. (2016c). Health effects support document for perfluorooctanoic acid (PFOA) [EPA Report]. (EPA 822-R-16-003). Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf

U.S. EPA. (2018). An umbrella Quality Assurance Project Plan (QAPP) for PBPK models [EPA Report]. (ORD QAPP ID No: B-0030740-QP-1-1). Research Triangle Park, NC.

U.S. EPA. (2020a). ORD staff handbook for developing IRIS assessments (public comment draft) [EPA Report]. (EPA/600/R-20/137). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Center for Public Health and Environmental Assessment. https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=350086

U.S. EPA. (2020b). Systematic review protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS assessments: Supplemental information appendix A [EPA Report]. (EPA/635/R-20/131). Washington, DC: US EPA, ORD, CPHEA, Integrated Risk Information System. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1010Y0L.txt

U.S. EPA. (2021a). 2010/2015 PFOA stewardship program. Available online at http://www2.epa.gov/assessing-and-managing-chemicals-under-tsca/20102015-pfoa-stewardship-program; https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/pfoa-stewardship-program-baseline-year-summary-report

U.S. EPA. (2021b). EPA's policy on children's health.

U.S. EPA. (2021c). EXTERNAL PEER REVIEW DRAFT: Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water [EPA Report]. Washington, DC: U.S. Environmental Protection Agency (EPA). https://sab.epa.gov/ords/sab/f?p=100:18:16490947993:::RP,18:P18_ID:2601

U.S. EPA. (2021d). Final Regulatory Determination 4 Support Document [EPA Report]. (EPA 815R21001). U.S. Environmental Protection Agency (EPA). https://www.regulations.gov/document/EPA-HQ-OW-2019-0583-0284

U.S. EPA. (2021e). Human health toxicity values for hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (CASRN 13252-13-6 and CASRN 62037-80-3). Also known as "GenX chemicals." Final report [EPA Report]. (EPA-822R-21-010). Washington, DC: U.S. Environmental Protection Agency, Office of Water.

U.S. EPA. (2021f). Human health toxicity values for perfluorobutane sulfonic acid (CASRN 375-73-5) and related compound potassium perfluorobutane sulfonate (CASRN 29420-49-3) [EPA Report]. (EPA/600/R-20/345F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=350888

U.S. EPA. (2021g). Revisions to the Unregulated Contaminant Monitoring Rule (UCMR 5) for Public Water Systems and Announcement of Public Meetings. Federal Register 86: 73131-73157.

U.S. EPA. (2022a). Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA). (EPA-842-D-22-001). Washington, DC: U.S. Environmental Protection Agency, Office of Water. https://www.epa.gov/system/files/documents/2022-04/pfoa-report-2022.pdf

U.S. EPA. (2022b). Draft Economic Analysis for the Proposed PFAS Rule. Washington, DC. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/10369698C3 -

U.S. EPA (U.S. Environmental Protection Agency). (2022c). IRIS toxicological review of perfluorobutanoic acid (PFBA, CASRN 375-22-4) and related salts [EPA Report]. (EPA/635/R-22/277Fa). Washington, D.C.: U.S. Environmental Protection Agency. https://iris.epa.gov/ChemicalLanding/&substance_nmbr=701

U.S. EPA. (2022d). ORD staff handbook for developing IRIS assessments [EPA Report]. (EPA 600/R-22/268). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Center for Public Health and Environmental Assessment. https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=356370

U.S. EPA. (2022e). Review of EPA's Analysis to Support EPA's National Primary Drinking Water Rulemaking for PFAS. (EPA-SAB-22-008). U.S. Environmental Protection Agency, Science Advisory Board.

U.S. EPA. (2023a). Public comment draft: toxicity assessment and proposed maximum contaminant level goal for perfluorooctanoic acid (PFOA) in drinking water. (EPA/822/P-23/005). https://www.epa.gov/system/files/documents/2023-03/MAIN_Proposed%20MCLG%20for%20PFOA%20in%20Drinking%20Water_3.9.23_For%20Proposal.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2023b). Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Salts (Final Report, 2023) [EPA Report]. (EPA/635/R-23/027F). https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=357314

U.S. EPA. (2024a). Appendix - Office of Water Final Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA). (815R24008).

U.S. EPA. (2024b). Economic analysis for the final per- and polyfluoroalkyl substances National Primary Drinking Water Regulation [EPA Report]. (EPA 815-R-24-001).

U.S. EPA. (2024c). EPA's responses to public comments on the proposed PFAS National Primary Drinking Water Regulation [EPA Report]. (EPA-815-R-24-005).

U.S. EPA. (2024d). PFAS National Primary Drinking Water Regulation rulemaking. Final rule. Federal Register [pending publication].

U.S. EPA. (2024e). PFAS occurrence and contaminant background support document for the final PFAS NPDWR [EPA Report]. (EPA-815-R-24-013).

U.S. EPA OW. (2023). EPA response to final Science Advisory Board recommendations (August 2022) on four draft support documents for the EPA's proposed PFAS National Primary Drinking Water Regulation [EPA Report]. (EPA Document No. 815D23001). Washington, DC: UlS. Environmental Protection Agency, Office of Water. https://www.epa.gov/system/files/documents/2023-03/Response%20to%20SAB%20recommendations_3.9.23_For%20Proposal_1.pdf

Valenti, L; Pelusi, S; Bianco, C; Ceriotti, F; Berzuini, A; Iogna Prat, L, et al. (2021). Definition of Healthy Ranges for Alanine Aminotransferase Levels: A 2021 Update. Hepatology Communications 5: 1824-1832. http://dx.doi.org/https://doi.org/10.1002/hep4.1794

Valvi, D; Oulhote, Y; Weihe, P; Dalgård, C; Bjerve, KS; Steuerwald, U; Grandjean, P. (2017). Gestational diabetes and offspring birth size at elevated environmental pollutant exposures. Environment International 107: 205-215. http://dx.doi.org/10.1016/j.envint.2017.07.016

van Esterik, JC; Sales, LB; Dollé, ME; Håkansson, H; Herlin, M; Legler, J; van der Ven, LT. (2015). Programming of metabolic effects in C57BL/6JxFVB mice by in utero and lactational exposure to perfluorooctanoic acid. Archives of Toxicology 90: 701-715.

Varshavsky, JR; Robinson, JF; Zhou, Y; Puckett, KA; Kwan, E; Buarpung, S, et al. (2021). Organophosphate Flame Retardants, Highly Fluorinated Chemicals, and Biomarkers of Placental Development and Disease during Mid-Gestation. Toxicological Sciences 181: 215-228. http://dx.doi.org/10.1093/toxsci/kfab028

Verner, MA; Loccisano, AE; Morken, NH; Yoon, M; Wu, H; Mcdougall, R, et al. (2015). Associations of Perfluoroalkyl Substances (PFAS) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK). Environmental Health Perspectives 123: 1317-1324. http://dx.doi.org/10.1289/ehp.1408837

Verner, MA; Ngueta, G; Jensen, ET; Fromme, H; Vöelkel, W; Nygaard, UC, et al. (2016). A simple pharmacokinetic model of prenatal and postnatal exposure to perfluoroalkyl substances (PFASs). Environmental Science and Technology 50: 978-986. http://dx.doi.org/10.1021/acs.est.5b04399

Vestergren, R; Cousins, I; Trudel, D; Wormuth, M; Scheringer, M. (2008). Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. Chemosphere 73: 1617-1624. http://dx.doi.org/10.1016/j.chemosphere.2008.08.011

Vesterholm Jensen, D; Christensen, J; Virtanen, HE; Skakkebæk, NE; Main, KM; Toppari, J, et al. (2014). No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. Reproduction 147: 411-417.

Vieira, VM; Hoffman, K; Shin, HM; Weinberg, JM; Webster, TF; Fletcher, T. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environmental Health Perspectives 121: 318-323. http://dx.doi.org/10.1289/ehp.1205829

Vilanova, CS; Hirakata, VN; de Souza Buriol, VC; Nunes, M; Goldani, MZ; da Silva, CH. (2019). The relationship between the different low birth weight strata of newborns with infant mortality and the influence of the main health determinants in the extreme south of Brazil. Population Health Metrics 17: 15. http://dx.doi.org/10.1186/s12963-019-0195-7

vonderEmbse, AN; DeWitt, JC. (2018). Developmental immunotoxicity (DIT) testing: Current recommendations and the future of DIT testing. In JC DeWitt; CE Rockwell; CC Bowman (Eds.), Immunotoxicity testing: Methods and protocols (2nd ed., pp. 47-56). Totowa, NJ: Humana Press. http://dx.doi.org/10.1007/978-1-4939-8549-4_4

Wambaugh, JF; Setzer, RW; Pitruzzello, AM; Liu, J; Reif, DM; Kleinstreuer, NC, et al. (2013). Dosimetric anchoring of in vivo and in vitro studies for perfluorooctanoate and perfluorooctanesulfonate. Toxicological Sciences 136: 308-327. http://dx.doi.org/10.1093/toxsci/kft204

Wan, C; Han, R; Liu, L; Zhang, F; Li, F; Xiang, M; Ding, W. (2016). Role of miR-155 in fluorooctane sulfonate-induced oxidative hepatic damage via the Nrf2-dependent pathway. Toxicology and Applied Pharmacology 295: 85-93.

Wang, B; Fu, J; Gao, K; Liu, Q; Zhuang, L; Zhang, G, et al. (2021). Early pregnancy loss: Do Per- and polyfluoroalkyl substances matter? Environment International 157: 106837. http://dx.doi.org/10.1016/j.envint.2021.106837

Wang, B; Zhang, R; Jin, F; Lou, H; Mao, Y; Zhu, W, et al. (2017). Perfluoroalkyl substances and endometriosis-related infertility in Chinese women. Environment International 102: 207-212.

Wang, H; Du, H; Yang, J; Jiang, H; O, K; Xu, L, et al. (2019). PFOS, PFOA, estrogen homeostasis, and birth size in Chinese infants. Chemosphere 221: 349-355.

Wang, J; Pan, Y; Cui, Q; Yao, B; Wang, J; Dai, J. (2018). Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. Environmental Science and Technology 52: 13553-13561.

Wang, J; Zhang, Y; Zhang, W; Jin, Y; Dai, J. (2012). Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. Environmental Science and Technology 46: 9274-9281.

Wang, L; Wang, Y; Liang, Y; Li, J; Liu, Y; Zhang, J, et al. (2013). Specific accumulation of lipid droplets in hepatocyte nuclei of PFOA-exposed BALB/c mice. Scientific Reports 3: 2174. http://dx.doi.org/10.1038/srep02174

Wang, Y; Adgent, M; Su, PH; Chen, HY; Chen, PC; Hsiung, CA; Wang, SL. (2016). Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan maternal and infant cohort study. Environmental Health Perspectives 124: 1794-1800.

Wang, Y; Wang, L; Li, J; Liang, Y; Ji, H; Zhang, J, et al. (2014). The mechanism of immunosuppression by perfluorooctanoic acid in BALB/c mice. Toxicology Research 3: 205-213. http://dx.doi.org/10.1039/c3tx50096a

Wang, Y; Zhang, C. (2019). The Roles of Liver-Resident Lymphocytes in Liver Diseases [Review]. Frontiers in Immunology 10: 1582. http://dx.doi.org/10.3389/fimmu.2019.01582

Wang, Z; Shi, R; Ding, G; Yao, Q; Pan, C; Gao, Y; Tian, Y. (2022). Association between maternal serum concentration of perfluoroalkyl substances (PFASs) at delivery and acute infectious diseases in infancy. Chemosphere 289: 133235. http://dx.doi.org/10.1016/j.chemosphere.2021.133235

Warembourg, C; Maitre, L, ea; Tamayo-Uria, I; Fossati, S; Roumeliotaki, T; Aasvang, GM, et al. (2019). Early-life environmental exposures and blood pressure in children. Journal of the American College of Cardiology 74: 1317-1328.

Washino, N; Saijo, Y; Sasaki, S; Kato, S; Ban, S; Konishi, K, et al. (2009). Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environmental Health Perspectives 117: 660-667. http://dx.doi.org/10.1289/ehp.11681

Watkins, DJ; Wellenius, GA; Butler, RA; Bartell, SM; Fletcher, T; Kelsey, KT. (2014). Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. Environment International 63: 71-76. http://dx.doi.org/10.1016/j.envint.2013.10.018

Weaver, YM; Ehresman, DJ; Butenhoff, JL; Hagenbuch, B. (2010). Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. Toxicological Sciences 113: 305-314. http://dx.doi.org/10.1093/toxsci/kfp275

Wedemeyer, H; Hofmann, WP; Lueth, S; Malinski, P; Thimme, R; Tacke, F; Wiegand, J. (2010). [ALT screening for chronic liver diseases: scrutinizing the evidence] [Review]. Zeitschrift für Gastroenterologie 48: 46-55. http://dx.doi.org/10.1055/s-0028-1109980

Weiss, JM; Andersson, PL; Lamoree, MH; Leonards, PEG; van Leeuwen, SPJ; Hamers, T. (2009). Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. Toxicological Sciences 109: 206-216. http://dx.doi.org/10.1093/toxsci/kfp055

Weisskopf, MG; Seals, RM; Webster, TF. (2018). Bias amplification in epidemiologic analysis of exposure to mixtures. Environmental Health Perspectives 126. http://dx.doi.org/10.1289/EHP2450

Wen, HJ; Wang, SL; Chen, PC; Guo, YL. (2019a). Prenatal perfluorooctanoic acid exposure and glutathione s-transferase T1/M1 genotypes and their association with atopic dermatitis at 2 years of age. PLoS ONE 14: e0210708.

Wen, HJ; Wang, SL; Chuang, YC; Chen, PC; Guo, YL. (2019b). Prenatal perfluorooctanoic acid exposure is associated with early onset atopic dermatitis in 5-year-old children. Chemosphere 231: 25-31.

Wen, X; Baker, AA; Klaassen, CD; Corton, JC; Richardson, JR; Aleksunes, LM. (2019c). Hepatic carboxylesterases are differentially regulated in PPARα-null mice treated with perfluorooctanoic acid. Toxicology 416: 15-22. http://dx.doi.org/10.1016/j.tox.2019.01.014

Wen, Y; Mirji, N; Irudayaraj, J. (2020). Epigenetic toxicity of PFOA and GenX in HepG2 cells and their roles in lipid metabolism. Toxicology In Vitro 65: 104797. http://dx.doi.org/10.1016/j.tiv.2020.104797

White, SS; Kato, K; Jia, LT; Basden, BJ; Calafat, AM; Hines, EP, et al. (2009). Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. Reproductive Toxicology 27: 289-298. http://dx.doi.org/10.1016/j.reprotox.2008.11.054

White, SS; Stanko, JP; Kato, K; Calafat, AM; Hines, EP; Fenton, SE. (2011). Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environmental Health Perspectives 119: 1070-1076. http://dx.doi.org/10.1289/ehp.1002741

Whitworth, KW; Haug, LS; Baird, DD; Becher, G; Hoppin, JA; Skjaerven, R, et al. (2012). Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. American Journal of Epidemiology 175: 1209-1216. http://dx.doi.org/10.1093/aje/kwr459

WHO. (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. https://apps.who.int/iris/handle/10665/330098

WHO. (2014). Global nutrition targets 2025: Low birth weight policy brief. Geneva. http://who.int/nutrition/publications/globaltargets2025_policybrief_lbw/en/

WHO. (2018a). 2018 Global reference list of 100 core health indicators (plus health-related SDGs). Geneva. https://apps.who.int/iris/handle/10665/259951

WHO. (2018b). The immunological basis for immunization series. Module 3: Tetanus. Geneva. https://apps.who.int/iris/bitstream/handle/10665/275340/9789241513616-eng.pdf?sequence=1&isAllowed=y

WHO; FAO (World Health Organization and Food and Agriculture Organization of the United Nations). (2009). Principles and methods for the risk assessment of chemicals in food [WHO EHC]. (EHC 240). Geneva, Switzerland: World Health Organization. https://www.who.int/publications/i/item/9789241572408

WHO; UNICEF. (2004). Low birthweight: country, regional and global estimates. World Health Organization and United Nations Children's Fund (UNICEF). https://apps.who.int/iris/handle/10665/43184

WHO & FAO. (2020). Section 4.5: Genotoxicity. In Principles and methods for the risk assessment of chemicals in food. Geneva, Switzerland: World Health Organization. https://cdn.who.int/media/docs/default-source/food-safety/publications/section4-5-genotoxicity.pdf?sfvrsn=8ec3434_2

Wielsøe, M; Kern, P; Bonefeld-Jørgensen, EC. (2017). Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environmental Health: A Global Access Science Source 16: 56.

Wielsøe, M; Long, M; Ghisari, M; Bonefeld-Jørgensen, EC. (2015). Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. Chemosphere 129: 239-245. http://dx.doi.org/10.1016/j.chemosphere.2014.10.014

Wikström, S; Hussein, G; Lingroth Karlsson, A; Lindh, CH; Bornehag, CG. (2021). Exposure to perfluoroalkyl substances in early pregnancy and risk of sporadic first trimester miscarriage. Scientific Reports 11: 3568. http://dx.doi.org/10.1038/s41598-021-82748-6

Wikström, S; Lin, PI; Lindh, CH; Shu, H; Bornehag, CG. (2020). Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatric Research 87: 1093-1099.

Winquist, A; Steenland, K. (2014). Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environmental Health Perspectives 122: 1299-1305. http://dx.doi.org/10.1289/ehp.1307943

Witt, KL; Knapton, A; Wehr, CM; Hook, GJ; Mirsalis, J; Shelby, MD; Macgregor, JT. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environmental and Molecular Mutagenesis 36: 163-194. http://dx.doi.org/10.1002/1098-2280(2000)36:3<163::AID-EM1>3.0.CO;2-P

Wolf, CJ; Fenton, SE; Schmid, JE; Calafat, AM; Kuklenyik, Z; Bryant, XA, et al. (2007). Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicological Sciences 95: 462-473.

Wolf, CJ; Rider, CV; Lau, C; Abbott, BD. (2014). Evaluating the additivity of perfluoroalkyl acids in binary combinations on peroxisome proliferator-activated receptor-α activation. Toxicology 316: 43-54. http://dx.doi.org/10.1016/j.tox.2013.12.002

Wolf, DC; Moore, T; Abbott, BD; Rosen, MB; Das, KP; Zehr, RD, et al. (2008). Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR-alpha knockout and wild-type mice. Toxicologic Pathology 36: 632-639. http://dx.doi.org/10.1177/0192623308318216

Woods, MM; Lanphear, BP; Braun, JM; McCandless, LC. (2017). Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: A Bayesian analysis of the HOME Study. Environmental Health: A Global Access Science Source 16: 115. http://dx.doi.org/10.1186/s12940-017-0332-3

Workman, CE; Becker, AB; Azad, MB; Moraes, TJ; Mandhane, PJ; Turvey, SE, et al. (2019). Associations between concentrations of perfluoroalkyl substances in human plasma and maternal, infant, and home characteristics in Winnipeg, Canada. Environmental Pollution 249: 758-766.

Worley, RR; Moore, SM; Tierney, BC; Ye, X; Calafat, AM; Campbell, S, et al. (2017a). Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. Environment International 106: 135-143.

Worley, RR; Yang, X; Fisher, J. (2017b). Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. Toxicology and Applied Pharmacology 330: 9-21. http://dx.doi.org/10.1016/j.taap.2017.07.001

Wu, H; Yoon, M; Verner, MA; Xue, J; Luo, M, an; Andersen, ME, et al. (2015). Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environment International 82: 61-68. http://dx.doi.org/10.1016/j.envint.2015.05.006

Wu, K; Xu, X; Peng, L; Liu, J; Guo, Y; Huo, X. (2012). Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environment International 48: 1-8.

Wu, LL; Gao, HW; Gao, NY; Chen, FF; Chen, L. (2009). Interaction of perfluorooctanoic acid with human serum albumin. BMC Structural Biology 9: 31. http://dx.doi.org/10.1186/1472-6807-9-31

Wu, X; Xie, G; Xu, X; Wu, W; Yang, B. (2018). Adverse bioeffect of perfluorooctanoic acid on liver metabolic function in mice. Environmental Science and Pollution Research 25: 4787-4793. http://dx.doi.org/10.1007/s11356-017-0872-7

Xiao, C; Grandjean, P; Valvi, D; Nielsen, F; Jensen, TK; Weihe, P; Oulhote, Y. (2019). Associations of exposure to perfluoroalkyl substances with thyroid hormone concentrations and birth size. Journal of Clinical Endocrinology and Metabolism 105: 735-745.

Xu, C; Yin, S; Liu, Y; Chen, F; Zhong, Z; Li, F, et al. (2019a). Prenatal exposure to chlorinated polyfluoroalkyl ether sulfonic acids and perfluoroalkyl acids: Potential role of maternal determinants and associations with birth outcomes. Journal of Hazardous Materials 380: 120867.

Xu, H; Mao, Y; Hu, Y; Xu, B. (2020a). Association between exposure to polyfluoroalkyl chemicals and increased fractional exhaled nitric oxide in adults. Environmental Research In Press: 110450. http://dx.doi.org/10.1016/j.envres.2020.110450

Xu, M; Liu, G; Li, M; Huo, M; Zong, W; Liu, R. (2020b). Probing the Cell Apoptosis Pathway Induced by Perfluorooctanoic Acid and Perfluorooctane Sulfonate at the Subcellular and Molecular Levels. Journal of Agricultural and Food Chemistry 68: 633-641. http://dx.doi.org/10.1021/acs.jafc.9b07072

Xu, M; Wan, J; Niu, Q; Liu, R. (2019b). PFOA and PFOS interact with superoxide dismutase and induce cytotoxicity in mouse primary hepatocytes: A combined cellular and molecular methods. Environmental Research 175: 63-70. http://dx.doi.org/10.1016/j.envres.2019.05.008

Xu, Y; Fletcher, T; Pineda, D; Lindh, CH; Nilsson, C; Glynn, A, et al. (2020c). Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. Environmental Health Perspectives 128: 77004.

Xu, Y; Li, Y; Scott, K; Lindh, CH; Jakobsson, K; Fletcher, T, et al. (2020d). Inflammatory bowel disease and biomarkers of gut inflammation and permeability in a community with high exposure to perfluoroalkyl substances through drinking water. Environmental Research 181: 108923.

Yahia, D; El-Nasser, MA; Abedel-Latif, M; Tsukuba, C; Yoshida, M; Sato, I; Tsuda, S. (2010). Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. Journal of Toxicological Sciences 35: 527-533. http://dx.doi.org/10.2131/jts.35.527

Yahia, D; Haruka, I; Kagashi, Y; Tsuda, S. (2016). 8-Hydroxy-2'-deoxyguanosine as a biomarker of oxidative DNA damage induced by perfluorinated compounds in TK6 cells. Environmental Toxicology 31: 192-200. http://dx.doi.org/10.1002/tox.22034

Yamaguchi, M; Arisawa, K; Uemura, H; Katsuura-Kamano, S; Takami, H; Sawachika, F, et al. (2013). Consumption of seafood, serum liver enzymes, and blood levels of PFOS and PFOA in the Japanese population. Journal of Occupational Health 55: 184-194. http://dx.doi.org/10.1539/joh.12-0264-OA

Yan, S; Wang, J; Dai, J. (2015a). Activation of sterol regulatory element-binding proteins in mice exposed to perfluorooctanoic acid for 28 days. Archives of Toxicology 89: 1569-1578.

Yan, S; Wang, J; Zhang, W; Dai, J. (2014). Circulating microRNA profiles altered in mice after 28 d exposure to perfluorooctanoic acid. Toxicology Letters 224: 24-31.

Yan, S; Zhang, H; Guo, X; Wang, J; Dai, J. (2017). High perfluorooctanoic acid exposure induces autophagy blockage and disturbs intracellular vesicle fusion in the liver. Archives of Toxicology 91: 247-258.

Yan, S; Zhang, H; Wang, J; Zheng, F; Dai, J. (2015b). Perfluorooctanoic acid exposure induces endoplasmic reticulum stress in the liver and its effects are ameliorated by 4-phenylbutyrate. Free Radical Biology and Medicine 87: 300-311. http://dx.doi.org/10.1016/j.freeradbiomed.2015.06.043

Yang, B; Zou, W; Hu, Z; Liu, F; Zhou, L; Yang, S, et al. (2014). Involvement of oxidative stress and inflammation in liver injury caused by perfluorooctanoic acid exposure in mice. BioMed Research International 2014: 409837. http://dx.doi.org/10.1155/2014/409837

Yang, BY; Wu, J; Niu, X; He, C; Bloom, MS; Abudoukade, M, et al. (2022). (In Press) Low-level environmental per- and polyfluoroalkyl substances and preterm birth: A nested case-control study among a Uyghur population in northwestern China. Exposure and Health. http://dx.doi.org/10.1007/s12403-021-00454-0

Yang, C; Tan, YS; Harkema, JR; Haslam, SZ. (2009a). Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains. Reproductive Toxicology 27: 299-306. http://dx.doi.org/10.1016/j.reprotox.2008.10.003

Yang, CH; Glover, KP; Han, X. (2009b). Organic anion transporting polypeptide (Oatp) 1a1-mediated perfluorooctanoate transport and evidence for a renal reabsorption mechanism of Oatp1a1 in renal elimination of perfluorocarboxylates in rats. Toxicology Letters 190: 163-171. http://dx.doi.org/10.1016/j.toxlet.2009.07.011

Yang, CH; Glover, KP; Han, X. (2010). Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. Toxicological Sciences 117: 294-302. http://dx.doi.org/10.1093/toxsci/kfq219

Yang, D; Han, J; Hall, DR; Sun, J; Fu, J; Kutarna, S, et al. (2020a). Nontarget Screening of Per- and Polyfluoroalkyl Substances Binding to Human Liver Fatty Acid Binding Protein. Environmental Science and Technology 54: 5676-5686.

Yang, J; Wang, H; Du, H; Fang, H; Han, M; Xu, L, et al. (2020b). Serum perfluoroalkyl substances in relation to lipid metabolism in Chinese pregnant women. Chemosphere 273: 128566. http://dx.doi.org/10.1016/j.chemosphere.2020.128566

Yang, Q; Abedi-Valugerdi, M; Xie, Y; Zhao, XY; Möller, G; Nelson, BD; Depierre, JW. (2002a). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. International Immunopharmacology 2: 389-397. http://dx.doi.org/10.1016/S1567-5769(01)00164-3

Yang, Q; Guo, X; Sun, P; Chen, Y; Zhang, W; Gao, A. (2018). Association of serum levels of perfluoroalkyl substances (PFASs) with the metabolic syndrome (MetS) in Chinese male adults: A cross-sectional study. Science of the Total Environment 621: 1542-1549.

Yang, Q; Xie, Y; Alexson, SE; Nelson, BD; Depierre, JW. (2002b). Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochemical Pharmacology 63: 1893-1900. http://dx.doi.org/10.1016/S0006-2952(02)00923-1

Yang, Q; Xie, Y; Depierre, JW. (2000). Effects of peroxisome proliferators on the thymus and spleen of mice. Clinical and Experimental Immunology 122: 219-226. http://dx.doi.org/10.1046/j.1365-2249.2000.01367.x

Yang, Q; Xie, Y; Eriksson, AM; Nelson, BD; Depierre, JW. (2001). Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. Biochemical Pharmacology 62: 1133-1140. http://dx.doi.org/10.1016/s0006-2952(01)00752-3

Yang, Y; Lv, QY; Guo, LH; Wan, B; Ren, XM; Shi, YL; Cai, YQ. (2017). Identification of protein tyrosine phosphatase SHP-2 as a new target of perfluoroalkyl acids in HepG2 cells. Archives of Toxicology 91: 1697-1707. http://dx.doi.org/10.1007/s00204-016-1836-2

Yao, Q; Gao, Y; Zhang, Y; Qin, K; Liew, Z; Tian, Y. (2021). Associations of paternal and maternal per- and polyfluoroalkyl substances exposure with cord serum reproductive hormones, placental steroidogenic enzyme and birth weight. Chemosphere 285: 131521. http://dx.doi.org/10.1016/j.chemosphere.2021.131521

Yao, X; Zhong, L. (2005). Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. Mutation Research 587: 38-44. http://dx.doi.org/10.1016/j.mrgentox.2005.07.010

Yarahalli Jayaram, V; Baggavalli, S; Reddy, D; Sistla, S; Malempati, R. (2018). Effect of endosulfan and bisphenol A on the expression of SUMO and UBC9. Drug and Chemical Toxicology 43: 1-8. http://dx.doi.org/10.1080/01480545.2018.1526179

Ye, WL; Chen, ZX; Xie, YQ; Kong, ML; Li, QQ; Yu, S, et al. (2021). Associations between serum isomers of perfluoroalkyl acids and metabolic syndrome in adults: Isomers of C8 Health Project in China. Environmental Research 196: 110430. http://dx.doi.org/10.1016/j.envres.2020.110430

Yeung, EH; Bell, EM; Sundaram, R; Ghassabian, A; Ma, W; Kannan, K; Louis, GM. (2019). Examining endocrine disruptors measured in newborn dried blood spots and early childhood growth in a prospective cohort. Obesity 27: 145-151.

Ylinen, M; Kojo, A; Hanhijärvi, H; Peura, P. (1990). Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bulletin of Environmental Contamination and Toxicology 44: 46-53. http://dx.doi.org/10.1007/BF01702360

Yu, CH; Riker, CD; Lu, SE; Fan, ZT. (2020). Biomonitoring of emerging contaminants, perfluoroalkyl and polyfluoroalkyl substances (PFAS), in New Jersey adults in 2016-2018. International Journal of Hygiene and Environmental Health 223: 34-44. http://dx.doi.org/10.1016/j.ijheh.2019.10.008

Yu, N; Wei, S; Li, M; Yang, J; Li, K; Jin, L, et al. (2016). Effects of Perfluorooctanoic Acid on Metabolic Profiles in Brain and Liver of Mouse Revealed by a High-throughput Targeted Metabolomics Approach. Scientific Reports 6: 23963. http://dx.doi.org/10.1038/srep23963

Yu, S; Feng, WR; Liang, ZM; Zeng, XY; Bloom, MS; Hu, GC, et al. (2021). Perfluorooctane sulfonate alternatives and metabolic syndrome in adults: New evidence from the Isomers of C8 Health Project in China. Environmental Pollution 283: 117078. http://dx.doi.org/10.1016/j.envpol.2021.117078

Yue, Y; Sun, Y; Yan, X; Liu, J; Zhao, S; Zhang, J. (2016). Evaluation of the binding of perfluorinated compound to pepsin: Spectroscopic analysis and molecular docking. Chemosphere 161: 475-481.

Zahm, S; Bonde, JP; Chiu, WA; Hoppin, J; Kanno, J; Abdallah, M, et al. (2023). Carcinogenicity of perfluorooctanoic acid and perfluorooctanesulfonic acid. The Lancet Oncology. http://dx.doi.org/10.1016/S1470-2045(23)00622-8

Zaïr, ZM; Eloranta, JJ; Stieger, B; Kullak-Ublick, GA. (2008). Pharmacogenetics of OATP (SLC21/SLCO), OAT and OCT (SLC22) and PEPT (SLC15) transporters in the intestine, liver and kidney. Pharmacogenomics 9: 597-624.

Zare Jeddi, M; Dalla Zuanna, T; Barbieri, G; Fabricio, ASC; Daprà, F; Fletcher, T, et al. (2021). Associations of Perfluoroalkyl Substances with Prevalence of Metabolic Syndrome in Highly Exposed Young Adult Community Residents-A Cross-Sectional Study in Veneto Region, Italy. International Journal of Environmental Research and Public Health 18: 1194. http://dx.doi.org/10.3390/ijerph18031194

Zareitalabad, P; Siemens, J; Hamer, M; Amelung, W. (2013). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients [Review]. Chemosphere 91: 725-732. http://dx.doi.org/10.1016/j.chemosphere.2013.02.024

Zeleke, BM; Zelalem, M; Mohammed, N, . (2012). Incidence and correlates of low birth weight at a referral hospital in Northwest Ethiopia. Pan African Medical Journal 12: 4.

Zenewicz, LA; Yancopoulos, GD; Valenzuela, DM; Murphy, AJ; Karow, M; Flavell, RA. (2007). Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity 27: 647-659. http://dx.doi.org/10.1016/j.immuni.2007.07.023

Zeng, X; Chen, Q; Zhang, X; Li, H; Liu, Q; Li, C, et al. (2019a). Association between prenatal exposure to perfluoroalkyl substances and asthma-related diseases in preschool children. Environmental Science and Pollution Research 26: 29639-29648.

Zeng, XW; Bloom, MS; Dharmage, SC; Lodge, CJ; Chen, D; Li, S, et al. (2019b). Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study. Science of the Total Environment 663: 60-67.

Zeng, XW; Li, QQ; Chu, C; Ye, WL; Yu, S; Ma, H, et al. (2020). Alternatives of perfluoroalkyl acids and hepatitis B virus surface antibody in adults: Isomers of C8 Health Project in China. Environmental Pollution 259: 113857.

Zeng, XW; Qian, Z; Emo, B; Vaughn, M; Bao, J; Qin, XD, et al. (2015). Association of polyfluoroalkyl chemical exposure with serum lipids in children. Science of the Total Environment 512-513: 364-370.

Zhang, H; Cui, R; Guo, X; Hu, J; Dai, J. (2016a). Low dose perfluorooctanoate exposure promotes cell proliferation in a human non-tumor liver cell line. Journal of Hazardous Materials 313: 18-28. http://dx.doi.org/10.1016/j.jhazmat.2016.03.077

Zhang, H; Fang, W; Wang, D; Gao, N; Ding, Y; Chen, C. (2014a). The role of interleukin family in perfluorooctanoic acid (PFOA)-induced immunotoxicity. Journal of Hazardous Materials 280: 552-560. http://dx.doi.org/10.1016/j.jhazmat.2014.08.043

Zhang, H; Lu, Y; Luo, B; Yan, S; Guo, X; Dai, J. (2014b). Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery. Journal of Proteome Research 13: 3370-3385.

Zhang, L; Ren, XM; Guo, LH. (2013a). Structure-based investigation on the interaction of perfluorinated compounds with human liver fatty acid binding protein. Environmental Science and Technology 47: 11293-11301. http://dx.doi.org/10.1021/es4026722

Zhang, R; Zhang, H; Chen, B; Luan, T. (2020a). Fetal bovine serum attenuating perfluorooctanoic acid-inducing toxicity to multiple human cell lines via albumin binding. Journal of Hazardous Materials 389: 122109.

Zhang, S; Kang, Q; Peng, H; Ding, M; Zhao, F; Zhou, Y, et al. (2019). Relationship between perfluorooctanoate and perfluorooctane sulfonate blood concentrations in the general population and routine drinking water exposure. Environment International 126: 54-60. http://dx.doi.org/10.1016/j.envint.2019.02.009

Zhang, T; Qin, X. (2014). Assessment of fetal exposure and maternal elimination of perfluoroalkyl substances. Environmental Science: Processes & Impacts 16: 1878-1881.

Zhang, T; Sun, H; Lin, Y; Qin, X; Zhang, Y; Geng, X; Kannan, K. (2013b). Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environmental Science and Technology 47: 7974-7981.

Zhang, T; Sun, H; Qin, X; Gan, Z; Kannan, K. (2015). PFOS and PFOA in paired urine and blood from general adults and pregnant women: assessment of urinary elimination. Environmental Science and Pollution Research 22: 5572-5579.

Zhang, X; Lohmann, R; Dassuncao, C; Hu, XC; Weber, AK; Vecitis, CD; Sunderland, EM. (2016b). Source attribution of poly- and perfluoroalkyl substances (PFASs) in surface waters from Rhode Island and the New York metropolitan area. Environmental Science & Technology Letters 3: 316-321. http://dx.doi.org/10.1021/acs.estlett.6b00255

Zhang, Y; Beesoon, S; Zhu, L; Martin, JW. (2013c). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environmental Science and Technology 47: 10619-10627.

Zhang, Y; Beesoon, S; Zhu, L; Martin, JW. (2013d). Isomers of perfluorooctanesulfonate and perfluorooctanoate and total perfluoroalkyl acids in human serum from two cities in North China. Environment International 53: 9-17. http://dx.doi.org/10.1016/j.envint.2012.12.007

Zhang, Y; Cao, X; Chen, L; Qin, Y; Xu, Y; Tian, Y; Chen, L. (2020b). Exposure of female mice to perfluorooctanoic acid suppresses hypothalamic kisspeptin-reproductive endocrine system through enhanced hepatic fibroblast growth factor 21 synthesis, leading to ovulation failure and prolonged dioestrus. Journal of Neuroendocrinology 32: e12848.

Zhang, Y; Le, Y; Bu, P; Cheng, X. (2020c). Regulation of Hox and ParaHox genes by perfluorochemicals in mouse liver. Toxicology 441: 152521. http://dx.doi.org/10.1016/j.tox.2020.152521

Zhang, Y; Mustieles, V; Wang, YX; Sun, Q; Coull, B; Sun, Y, et al. (2023). Red blood cell folate modifies the association between serum per- and polyfluoroalkyl substances and antibody concentrations in U.S. adolescents. Environmental Science & Technology 57: 2445-2456. http://dx.doi.org/10.1021/acs.est.2c07152

Zhang, YM; Dong, XY; Fan, LJ; Zhang, ZL; Wang, Q; Jiang, N; Yang, XS. (2017). Poly- and perfluorinated compounds activate human pregnane X receptor. Toxicology 380: 23-29. http://dx.doi.org/10.1016/j.tox.2017.01.012

Zhang, YM; Wang, T; Yang, XS. (2020d). An in vitro and in silico investigation of human pregnane X receptor agonistic activity of poly- and perfluorinated compounds using the heuristic method-best subset and comparative similarity indices analysis. Chemosphere 240: 124789. http://dx.doi.org/10.1016/j.chemosphere.2019.124789

Zhao, G; Wang, J; Wang, X; Chen, S; Zhao, Y; Gu, F, et al. (2011). Mutagenicity of PFOA in mammalian cells: role of mitochondria-dependent reactive oxygen species. Environmental Science & Technology 45: 1638-1644. http://dx.doi.org/10.1021/es1026129

Zhao, L; Zhang, Y; Zhu, L; Ma, X; Wang, Y; Sun, H; Luo, Y. (2017a). Isomer-Specific Transplacental Efficiencies of Perfluoroalkyl Substances in Human Whole Blood. Environmental Science & Technology Letters 4: 391-398.

Zhao, W; Zitzow, JD; Weaver, Y; Ehresman, DJ; Chang, SC; Butenhoff, JL; Hagenbuch, B. (2017b). Organic anion transporting polypeptides contribute to the disposition of perfluoroalkyl acids in humans and rats. Toxicological Sciences 156: 84-95.

Zhong, Y; Shen, L; Ye, X; Zhou, D; He, Y; Zhang, H. (2020). Mechanism of immunosuppression in zebrafish (Danio rerio) spleen induced by environmentally relevant concentrations of perfluorooctanoic acid. Chemosphere 249: 126200. http://dx.doi.org/10.1016/j.chemosphere.2020.126200

Zhou, R; Cheng, W; Feng, Y; Wei, H; Liang, F; Wang, Y. (2017a). Interactions between three typical endocrine-disrupting chemicals (EDCs) in binary mixtures exposure on myocardial differentiation of mouse embryonic stem cell. Chemosphere 178: 378-383. http://dx.doi.org/10.1016/j.chemosphere.2017.03.040

Zhou, Y; Bao, WW; Qian, ZM; Dee Geiger, S; Parrish, KL; Yang, BY, et al. (2017b). Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: A case-control study of children. Environmental Research 159: 158-163.

Zhou, Y; Hu, LW; Qian, ZM; Geiger, SD; Parrish, KL; Dharmage, SC, et al. (2017c). Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children. Scientific Reports 7: 899.

Zhu, Y; Qin, XD; Zeng, XW; Paul, G; Morawska, L; Su, MW, et al. (2016). Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. Science of the Total Environment 559: 166-173.

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FINAL
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Acronyms and Abbreviations

AASLD	American Association for the Study of Liver Diseases
ABC	ATP-binding cassette transporter
ACG	American College of Gastroenterology
ADME	absorption, distribution, metabolism, and excretion
AF:CB	amniotic fluid and cord blood ratio
AFFF	aqueous film forming foam
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	alanine aminotransferase
APOB	apolipoprotein B
ApoC-III	apolipoprotein C-III
ASBT	apical sodium-dependent bile salt transporter
AST	aspartate aminotransferase
ATF	activating transcription factor
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BK	bradykinin
BM	bone marrow
BMD	benchmark dose
BMD_{10}	dose corresponding to a 10% change in response
BMDL	benchmark dose lower limits
$BMDL_{10}$	dose level corresponding to the 95% lower confidence limit of a 10% change
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BWT	birthweight
BW	body weight
C_{last7}	average concentration over the final week of study
CAD	coronary artery disease
CalEPA	California Environmental Protection Agency
CAMK	calcium/calmodulin dependent protein kinase
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CAT	catalase
C_{avg}	average blood concentration
$C_{avg,pup,gest}$	area under the curve normalized per day during gestation
$C_{avg,pup,gest,lact}$	area under the curve normalized dose per day during gestation/lactation
$C_{avg,pup,lact}$	area under the curve normalized per day during lactation
CCL	Contaminant Candidate List
CD	celiac disease
CDC	Centers for Disease Control and Prevention
C-F	carbon-fluorine
CHD	coronary heart disease
CHDS	Child Health and Development Studies

CHF	congestive heart failure
CHO	Chinese hamster ovary
CI	confidence interval
CIMT	carotid artery intima-media thickness
C_{max}	maximum blood concentration
CRP	C-reactive protein
CSF	cancer slope factor
CSM	cholestyramine
CVD	cardiovascular disease
CYP	cytochrome P450 aromatase
CYTL	cytokine like
DBP	diastolic blood pressure
DCFDA	2,7-2,7-dichlorofluorescein diacetate
DDIT	DNA damage inducible transcript
DE	differentially expressed
DIPP	Diabetes Prediction and Prevention
DMR	differentially methylated region
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program and Outcomes Study
DTH	delayed-type hypersensitivity response
DWI-BW	body weight-based drinking water intake
EC	effect concentration
EC_{50}	half maximal effective concentration
ECM	extracellular matrix
ESC-CM	embryonic stem cell-derived cardiomyocyte
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ER	estrogen receptor
ERK	extracellular signal-regulated protein kinase
F_1	first generation
F_2	second generation
FGF	fibroblast growth factor
f_{oc}	soil organic carbon fraction
FXII	Hageman factor XII
GBCA	Genetic and Biomarkers study for Childhood Asthma
GD	gestational day
GH	growth hormone
GF	glomerular filtration
GGT	γ-glutamyltransferase
GI	gastrointestinal
glst	generalized least-squares for trend
GSSG	glutathione disulfide
GSH	glutathione
GSH-Px	glutathione peroxidase
HAWC	Health Assessment Workspace Collaborative
HDL	high density lipoprotein cholesterol
HED	human equivalent dose
HERO	Health and Environmental Research Online
HESD	Health Effects Support Document
HFD	high fat diet
HFMD	hand, foot, and mouth disease
HFPO	hexafluoropropylene oxide
Hib	*Haemophilus influenzae type b*

HIV	human immunodeficiency virus
HK	high-molecular-weight kininogen
HMOX	heme oxygenase
HMVEC	human microvascular endothelial cells
HNF	hepatocyte nuclear factor
HOME	Health Outcomes and Measures of the Environment
HR	Hazard Ratio
HRL	health reference level
HSA	human serum albumin
HUVEC	human umbilical cord endothelial cell
ICAM	intracellular adhesion molecule
iCOS	inducible co-stimulator
iCOSL	inducible co-stimulator ligand
IDL	intermediate density lipoprotein
IgE	immunoglobulin E
IGF	insulin-like growth factors
IgG	immunoglobulin G
IgM	immunoglobulin M
IHD	ischemic heart disease
IL	interleukin
IP	intraperitoneal
IPA	Ingenuity Pathway Analysis
IPCS	International Programme on Chemical Safety
IQR	interquartile range
IRIS	Integrated Risk Information System
IV	intravenous
JNK	c-JUN amino-terminal kinase
KC	Kupffer cell
KEGG	Kyoto Encyclopedia of Genes and Genomes
KKS	kallikrein-kinin system
K_H	Henry's Law Constant
KM	Kunming mice
$K_{mem/w}$	membrane/water partition coefficients
KO	knockout
K_{oc}	organic carbon-water partitioning coefficient
K_{ow}	octanol-water partition coefficient
LBW	low birthweight
LC	lethal concentration
LCM	liver capsular macrophages
LC-MS	liquid chromatography–mass spectrometry
LD	lactational day
LDL	low density lipoprotein cholesterol
L-FABP	liver fatty acid binding protein
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest observed effect concentration
LOD	limit of detection
LPS	lipopolysaccharide
LSEC	liver sinusoidal endothelial cell
LXR	liver X receptor
LYZ	lysozyme
MAIT	mucosal invariant T
MALDI	Matrix-Assisted Laser Desorption/Ionization
MAM	mitochondria-associated endoplasmic reticulum membrane
MAPK	mitogen-activated protein kinase
MCLG	Maximum Contaminant Level Goal

MDA	malondialdehyde
MDH	Minnesota Department of Health
MDM	monocyte-derived macrophages
mEB	mouse embryoid body
MEF	mouse embryonic fibroblast
MeFOSAA	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
MEHP	mono-(2-ethylhexyl)phthalate
Me-PFOSA-AcOH	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
miRNA	micro ribonucleic acid
MMR	measles, mumps, and rubella
MOA	mode of action
mPLP	mouse prolactin-like protein
MRL	Minimum Reporting Level
mRNA	messenger ribonucleic acid
MRP	multidrug resistance-associated protein
MS	multiple sclerosis
MTTP	microsomal triglyceride transfer protein
MWCNT	multi-walled carbon nanotube
NAFLD	non-alcoholic fatty liver disease
NCBI	National Center for Biotechnology Information
NCEH	Neutral Cholesterol Ester Hydrolase
NCI	National Cancer Institute
NF	nuclear factor
NHANES	National Health and Nutrition Examination Survey
NK	natural killer
NOAEL	no-observed-adverse-effect level
NOD	non-obese diabetic
NOS	nitric oxide synthase
NPDWR	National Primary Drinking Water Regulation
NFR	nuclear factor-erythroid factor
NSC	neural stem cells
NT	not tested
NTCP	sodium/taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OAT	organic anion transporter
OATP	organic anion transporting polypeptides
OECD	Organisation for Economic and Co-operation and Development
OR	odds ratio
OVA	ovalbumin
P_0	parental generation
PBL	peripheral blood leukocytes
PBPK	physiologically based pharmacokinetic
PcG	Polycomb group
PCM	peritoneal macrophages
PCNA	proliferating cell nuclear antigen
PDTC	pyrrolidine dithiocarbamate
PECAM-1	platelet endothelial cell adhesion molecule

PECO	Population, Exposure, Comparator, and Outcome
PFAA	perfluoroalkyl acids
PFAS	perfluoroalkyl and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFC	plaque forming cell
PFCA	perfluorinated carboxylic acids
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFSA	perfluorosulfonic acid
PHA	phytohemagglutinin
P_{ion}	anionic permeability
PK	pharmacokinetic
P_{milk}	milk:blood PFOS partition coefficient
PND	postnatal day
PNW	postnatal week
POD	point of departure
POD_{HED}	point of departure human equivalent dose
POUNDS-Lost	Prevention of Obesity Using Novel Dietary Strategies Lost
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PR	progesterone receptor
PRR	pattern recognition receptor
PSA	prostate specific antigen
PTB	preterm birth
PTGS	prostaglandin-endoperoxide synthase
PWS	public water systems
PXR	pregnane X receptor
QA	Quality Assurance
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RAR	retinoic acid receptor
RfD	reference dose
R_{fm}	ratio of the concentrations in the fetus(es) and the mother during pregnancy
r^{i}_{milk}	species-specific milk consumption rate during lactation for the i^{th} week of lactation
RNS	reactive nitrogen species
ROS	reactive oxygen species
R_{PM}	ratio of PFOS in placenta relative to maternal serum
RSC	relative source contribution
RSV	respiratory syncytial virus
RXR	retinoid X receptor
SAB	Science Advisory Board
SBP	systolic blood pressure
SD	standard deviation
SDWA	Safe Drinking Water Act
SES	socioeconomic status
SGA	small for gestational age
SGP	sphingosine-1-posphate lyase
SHE	Syrian hamster embryo
SIRT	sirtuin
SOD	superoxide dismutase
SRBC	sheep red blood cell
T1D	type 1 diabetes
T-AOC	total antioxidant capacity
TBARS	thiobarbituric acid-reactive substances
TC	total cholesterol

TCR	T cell receptor
TG	triglycerides
THEMIS	thymocyte selection associated
TLR	toll-like receptor
TLT	TREM-like transcript cells
TNF	tumor necrosis factor
TNP	trinitrophenyl
TSCATS	Toxic Substance Control Act Test Submissions
TTE	transplacental transfer efficiencies
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UC	ulcerative colitis
UCMR 3	Third Unregulated Contaminant Monitoring Rule
UF	uncertainty factors
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL extrapolation uncertainty factor
UF_S	uncertainty factor for extrapolation from a subchronic to a chronic exposure duration
UF_{TOT}	total uncertainty factors
UV-vis	ultraviolet visible
V_d	volume of distribution
Vfil	filtrate volume
VLDL	very low-density lipoprotein cholesterol
WBC	white blood cell
WHO	World Health Organization
WNT	wingless-related integration site
WoS	Web of Science
WT	wild type
WTCHR	World Trade Center Health Registry
ZFL	zebrafish liver line

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final toxicity values for *perfluorooctane sulfonic acid (PFOS), including all isomers and nonmetal salts.* The toxicity assessment for PFOS is a scientific report that describes the evaluation of the available animal toxicity and human epidemiology data in order to characterize the noncancer and cancer human health hazards. This assessment also includes f*inal toxicity values* associated with noncancer health effects (i.e., oral reference doses, or RfDs) and cancer effects (i.e., cancer slope factors, or CSFs) following oral PFOS exposure. It is not a risk assessment, as it does not include an exposure assessment or an overall risk characterization nor does it address the legal, policy, social, economic, or technical considerations involved in risk management. The PFOS toxicity assessment can be used by EPA, states, Tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, the potential risk associated with human exposures to PFOS, its isomers, and its nonmetal salts.

This final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) per- and polyfluoroalkyl substances (PFAS) Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 (U.S. EPA, 2022e) as well as feedback from the public comment period (U.S. EPA, 2024c). This final assessment builds upon the literature review presented in the *2016 Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (hereafter referred to as the 2016 PFOS HESD) (U.S. EPA, 2016b) and is an update of the *SAB review draft, Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2022b) and the subsequent *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023).

PFOS is a member of the PFAS group. These manufactured chemicals have a history of industrial and consumer use in the United States and are considered persistent chemicals based on their physicochemical properties. Some of the human health concerns about exposure to PFOS and other PFAS stem from their resistance to hydrolysis, photolysis, metabolism, and microbial degradation in the environment and in the human body. PFAS are not naturally occurring; they are manmade compounds that have been used widely over the past several decades in industrial applications and consumer products since many PFAS have repellant and surfactant properties. Frequently used as emulsifiers and as stain-, oil-, or water-repellents, PFAS are found in a variety of environmental media and in tissues of organisms, including humans.

Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007, EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (U.S. EPA, 2016a). Manufacturers have since shifted to alternative short-chain PFAS, such as perfluorobutane sulfonic acid (PFBS) (3M, 2002). However, PFOS remains persistent in environmental media because it is resistant to environmental degradation processes.

The purpose of this human health toxicity assessment is to derive toxicity values pertaining to oral exposure for PFOS. The development of this toxicity assessment relied on a robust systematic review process, based on the EPA peer-reviewed human health risk assessment methodology outlined in the EPA *ORD Staff Handbook for Developing IRIS Assessments* (U.S. EPA, 2022d), to identify human epidemiological, animal toxicological, mechanistic, and toxicokinetic data relevant to oral exposure. The PFOS systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) was developed prior to the initiation of this assessment largely mirrors the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments* (U.S. EPA, 2020b). The protocol outlines the scoping and problem-formulation efforts and describes the systematic review, including study quality evaluation, and the dose-response methods used to conduct this assessment. The final assessment incorporates peer-reviewed studies captured from: EPA's 2016 PFOS HESD (U.S. EPA, 2016b), literature searches of scientific databases and gray literature from 2013 through February 2023, the SAB PFAS Review Panel recommendations, and public comment. Consistent with the analysis provided in the peer-reviewed draft assessment (U.S. EPA, 2022b) and with recommendations from external peer review (i.e., the SAB PFAS Review Panel; (U.S. EPA, 2022e)), this final assessment focused on qualitative and quantitative assessments of five "priority" health outcome categories based on those with the strongest weight of evidence. These five priority health outcomes are cancer, hepatic, developmental, cardiovascular, and immune. The results of the systematic literature reviews and qualitative assessments for the remaining "nonpriority" health outcomes are presented in the Appendix accompanying this final assessment (U.S. EPA, 2024a).

Qualitative Assessment of Noncancer Effects

Overall, the available ***evidence indicates*** that PFOS exposure is likely to cause hepatic, immunological, cardiovascular, and developmental effects in humans given sufficient exposure conditions (e.g., at measured levels in humans as low as 0.57 to 5.0 ng/mL and at administered doses in animals as low as 0.0017 to 0.4 mg/kg/day). These judgments are based on data from epidemiological studies of infants, children, adolescents, pregnant individuals, and nonpregnant adults, as well as short-term (28-day), subchronic (90-day), developmental (gestational), and chronic (2-year) oral-exposure studies in rodents. For hepatic effects, the primary support is evidence of increased serum liver enzyme levels (i.e., alanine transaminase (ALT)) in humans and coherent evidence of hepatotoxicity in animals, including increased liver weights and hepatocellular hypertrophy accompanied by necrosis, inflammation, or increased liver enzyme levels that indicate liver injury. For immunological effects, the primary support is evidence of developmental immunosuppression in humans, specifically decreased antibody response to vaccination against tetanus, diphtheria, and rubella in children, and evidence of immunosuppression and other types of immunotoxicity in studies of adult animals, including decreased plaque forming cell response to sheep red blood cells, extramedullary hematopoiesis in the spleen, reduced spleen and thymus weights, changes in immune cell populations, and decreased splenic and thymic cellularity. For cardiovascular effects, the primary support is evidence of increased serum lipids levels in humans and alterations to lipid homeostasis in animals. For developmental effects, the primary evidence is decreased birth weight in human infants and decreased fetal and maternal weight in animal studies. According to the protocol described in Appendix A (U.S. EPA, 2024a) and aligned with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d), selected quantitative data in medium and high

confidence studies from these identified hazards were used to derive toxicity values (see Table ES-1). Specific criteria for data and study selection are provided in Appendix A (U.S. EPA, 2024a) and Section 4.1.

Quantitative Assessment of Noncancer Effects and Oral RfD Derivation

EPA followed agency guidelines and methodologies for risk assessment in determining points of departure (PODs) for the derivation of the RfDs for PFOS (U.S. EPA, 2022d, 2014, 2012a, 2011b, 2002b) and performed modeling following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For data from epidemiological studies, the dose-response modeling approach was selected based on the health outcome and available data. A hybrid modeling approach, which estimated the probability of responses at specified exposure levels above the control, was conducted when clinically adverse outcome levels could be defined (i.e., for developmental, hepatic, and cardiovascular effects) following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For other outcomes (i.e., immune effects), study results from multivariate models were used to define a benchmark response (BMR). For data from animal toxicological studies, EPA conducted benchmark dose modeling, when possible, to empirically model the dose-response relationship in the range of observed data. When BMDLs could not be derived, EPA used a no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) approach.

PODs were converted to external POD human equivalent doses (POD_{HEDS}) using pharmacokinetic modeling (see Section 4.1.3). Consistent with the recommendations presented in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), EPA considered the database of information to inform the application of uncertainty factors (UFs) to POD_{HEDS} to address intraspecies variability, interspecies variability, extrapolation from a LOAEL to NOAEL, extrapolation from a subchronic to a chronic exposure duration, and database deficiencies. EPA derived and considered multiple candidate RfDs from both human epidemiological and animal toxicological studies across the four priority noncancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental) (see Figure ES-1 for candidate RfD values). Additional details on candidate RfD derivation for PFOS are available in Section 4.1.

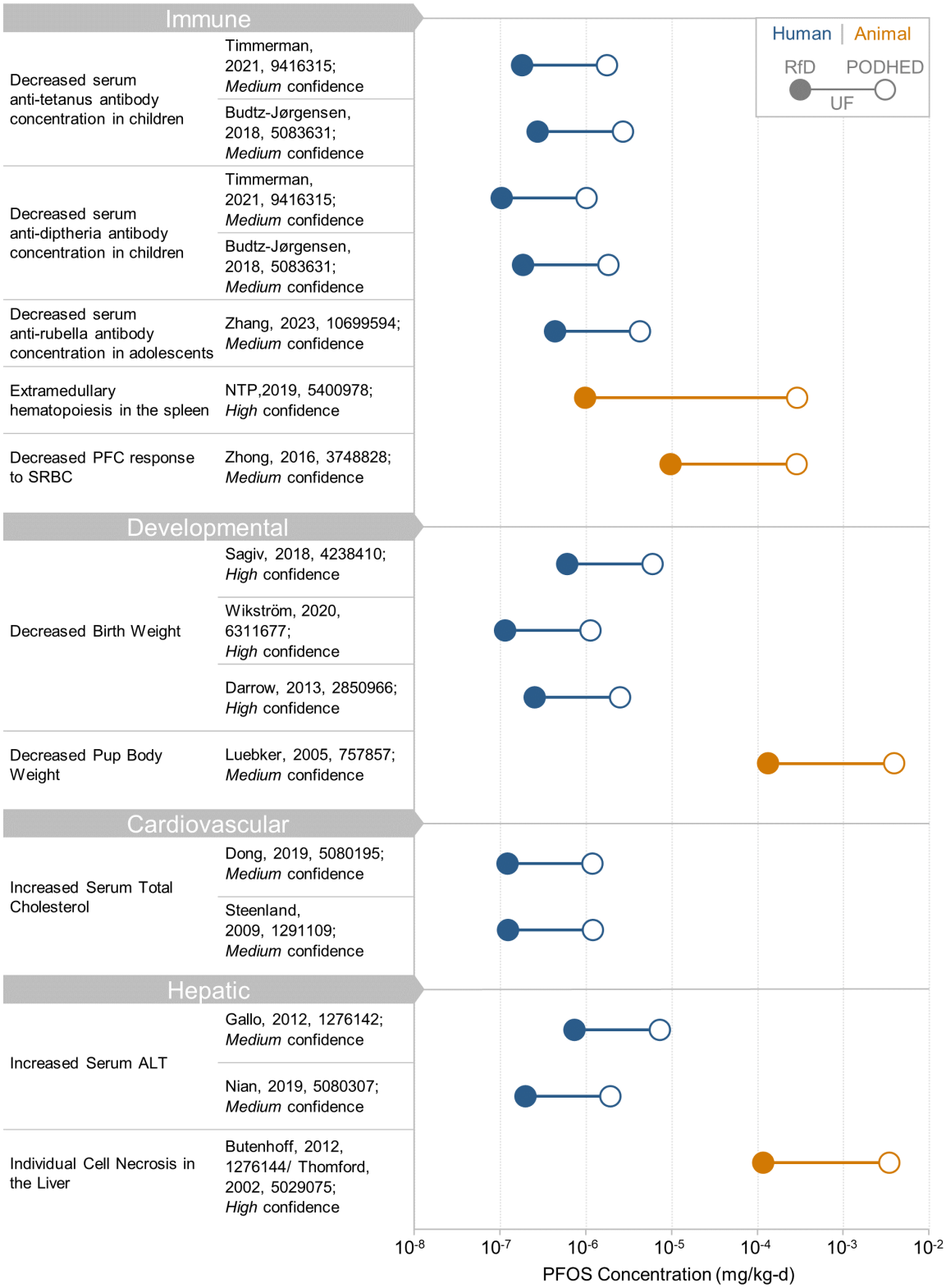

Figure ES-1. Schematic Depicting Candidate RfDs Derived From Epidemiological and Animal Toxicological Studies of PFOS

See text and Figure 4-3 in Section 4.1 for additional detail on dose-response modeling for PFOS studies.

The co-critical effects for the oral RfD of 1 x 10^{-7} mg/kg/day were decreased infant birth weight (Wikström et al., 2020) and increased total cholesterol in adults (Dong et al., 2019) (see Table ES-1). These co-critical effects were selected based on the procedures outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and were consistent with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d). The RfD was derived by using a total UF of 10 to account for intraspecies variability (UF_H). Notably, the RfD is protective of effects that may occur in sensitive populations (i.e., embryo and fetus, infants, and young children), as well as hepatic effects in adults that may result from PFOS exposure. As one of the co-critical effects identified for PFOS is a developmental endpoint and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOS is applicable to both short-term and chronic risk assessment scenarios.

Qualitative Carcinogenicity Assessment

Consistent with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), EPA reviewed the available data and conducted a weight of evidence evaluation across the human epidemiological and animal toxicological studies and concluded that PFOS is *Likely to Be Carcinogenic to Humans* via the oral route of exposure (see Section 3.5). Epidemiological studies provided evidence of bladder, prostate, liver, kidney, and breast cancers in humans, although evidence was limited or mixed for some cancer types. Animal toxicological studies supported findings from human studies. Bioassays conducted in Sprague-Dawley rats reported hepatocellular tumors, pancreatic islet cell tumors, and thyroid follicular cell tumors after chronic oral exposure. Some studies observed multisite tumorigenesis (liver and pancreas) in male and female rats. PFOS exposure is associated with multiple key characteristics of carcinogenicity (Smith et al., 2016b). Available mechanistic data suggest that multiple modes of action (MOAs) play a role in pancreatic and hepatic tumorigenesis associated with PFOS exposure in animal models. A full MOA analysis, including in-depth discussions on the potential MOAs for kidney and testicular tumors, as well as discussions on the potential MOAs and human relevance for pancreatic and liver tumors observed in rats, is presented in Section 3.5.4.2.

Quantitative Cancer Assessment and CSF Derivation

EPA followed agency guidelines for risk assessment in deriving CSFs for PFOS (U.S. EPA, 2022d, 2012a, 2005a). EPA selected *medium* and *high* confidence studies for derivation that met criteria outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and Section 4.1.1, conducted benchmark dose modeling (U.S. EPA, 2012a), and used the same pharmacokinetic modeling approach as described for the derivation of noncancer RfDs above (see Section 4.2.2). Data from epidemiological studies were not suitable for CSF derivation. From the studies that met the criteria, EPA used multistage models to derive and consider multiple candidate CSFs from animal toxicological studies across multiple tissue types or organ systems (i.e., liver and pancreas). Multistage cancer models were used to predict the doses at which the selected BMR for tumor incidence would occur. BMDLs for each tumor type served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. Candidate CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type.

The oral slope factor of 39.5 $(mg/kg/day)^{-1}$ for hepatocellular adenomas and carcinomas in female rats from Butenhoff et al. (2012)/Thomford (2002b) was selected as the basis of the overall CSF for PFOS (see Table ES-1; rationale in Section 4.2). Per EPA's *Guidelines for*

Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005a, b), age-dependent adjustment factors were not applied during CSF derivation because there was a lack of information to support a mutagenic MOA for PFOS, and the available evidence was insufficient to assess susceptibility to cancer following PFOS exposure during early life. Additional detail on candidate CSF derivation and CSF selection is provided in Table 4-12 in Section 4.2.

Final Toxicity Values for PFOS

Table ES-1. Final Toxicity Values for PFOS

Toxicity Value Type	Critical Effect(s)	Study, Confidence	Strain/Species, Sex, Age	Toxicity Value[a]
Reference Dose	Co-critical effects: decreased birth weight in infants; increased serum total cholesterol in adults	Wikström et al. (2020), *High*; Dong et al. (2019), *Medium*	Human, male and female, PFOS concentrations in first and second trimesters; Human, male and female, 20–80 years	1×10^{-7} (mg/kg/d)
Cancer Slope Factor	Combined hepatocellular adenomas and carcinomas	Butenhoff et al. (2012)/Thomford (2002b)[b], *High*	Sprague-Dawley rats, female	39.5 $(mg/kg/d)^{-1}$

Notes:
[a] Reference doses were rounded to one significant figure.
[b] Butenhoff et al. (2012) and Thomford (2002b) reported data from the same experiment.

1 Background

1.1 Purpose of This Document

The primary purpose of this toxicity assessment for perfluorooctane sulfonic acid (PFOS) is to describe the best available science on the human health effects associated with PFOS exposure and the derivation of toxicity values (i.e., noncancer reference doses (RfDs) and cancer slope factors (CSFs)). The latest health science on PFOS was identified, evaluated using systematic review methods, and described, and subsequently, a cancer classification was assigned and toxicity values were developed. The final cancer classification and cancer and noncancer toxicity values in this assessment build on the work described in the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023), *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b), and the *Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)* (U.S. EPA, 2016b). This final toxicity assessment for PFOS reflects expert scientific recommendations from the U.S. Environmental Protection Agency (EPA) Science Advisory Board (SAB) (U.S. EPA, 2022e) and public comments received on the draft assessment (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114; U.S. EPA (2024c)).

In addition to documenting EPA's basis for the cancer classification and toxicity values, this document serves to:

- Describe and document transparently the literature searches conducted and systematic review methods used to identify health effects information (epidemiological and animal toxicological studies and physiologically based pharmacokinetic models) in the literature (Sections 2 and 3; Appendices A and B, (U.S. EPA, 2024a)).
- Describe and document literature screening methods, including use of the Populations, Exposures, Comparators, and Outcomes (PECO) criteria and the process for tracking studies throughout the literature screening (Section 2; Appendix A, (U.S. EPA, 2024a)).
- Identify epidemiological and animal toxicological literature that reports health effects after exposure to PFOS (and its related salts) as outlined in the PECO criteria (Section 3).
- Describe and document the study quality evaluations conducted on epidemiological and animal toxicological studies considered potentially useful for point-of-departure (POD) derivation (Section 3).
- Describe and document the data from all epidemiological studies and animal toxicological studies that were considered for POD derivation (Section 3).
- Synthesize and document the adverse health effects evidence across studies. The assessment focuses on synthesizing the available evidence for five priority health outcomes that were found to have the strongest weight of evidence, as recommended by the SAB – developmental, hepatic, immune, and cardiovascular effects, and cancer (Section 3) – and also provides supplemental syntheses of evidence for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects, reproductive effects in males or females, and general toxicity (Appendix C, (U.S. EPA, 2024a)).

- Evaluate and document the available mechanistic information (including toxicokinetic understanding) associated with PFOS exposure to inform interpretation of findings related to potential health effects in studies of humans and animals, with a focus on five priority health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer) (Section 3).
- Develop and document strength of evidence judgments across studies (or subsets of studies) separately for epidemiological, animal toxicological, and mechanistic lines of evidence for the five priority health outcomes (Section 3).
- Develop and document integrated expert judgments across evidence streams (i.e., epidemiological, animal toxicological, and mechanistic streams) as to whether and to what extent the evidence supports that exposure to PFOS has the potential to be hazardous to humans (Section 3).
- Determine the cancer classification for PFOS using a weight-of-evidence approach (Section 3.5.5).
- Describe and document the attributes used to evaluate and select studies for derivation of toxicity values. These attributes are considered in addition to the study confidence evaluation domains and enable extrapolation to relevant exposure levels (e.g., studies with exposure levels near the range of typical environmental human exposures, broad exposure range, or multiple exposure levels) (Section 4).
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation (Section 4).
- Derive candidate RfDs (Section 4.1) and CSFs (Section 4.2), select the final RfD (Section 4.1.6) and CSF (Section 4.2.3) for PFOS, and describe the rationale.
- Characterize hazards (e.g., uncertainties, data gaps) (Sections 3, 4, and 5).

1.2 Background on Per-and Polyfluoroalkyl Substances

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals that share a common structure of a chain of linked carbon and fluorine atoms. The PFAS group includes PFOS, perfluorooctanoic acid (PFOA), and thousands of other chemicals. There is no consensus definition of PFAS as a class of chemicals (OSTP, 2023). Consistent with three related structural definitions associated with EPA's identification of PFAS included in the fifth Contaminant Candidate List[1] (CCL), the universe of environmentally relevant PFAS – including parent chemicals, metabolites, and degradants – is approximately 15,000 compounds.[2] The 2018 Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)* includes over 4,700 PFAS (OECD, 2018).

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States since the 1950's. PFAS have strong, stable carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Buck et al., 2011; Beach et al., 2006). The chemical structures of PFAS enable

[1] The CCL is a list, published every 5 years, of unregulated contaminants that are not subject to any current proposed or promulgated NPDWRs, are known or anticipated to occur in public water systems, and might require regulation under SDWA.
[2] See the EPA List of PFAS Structures available at: https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCT.

them to repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties. These properties make PFAS useful for commercial and industrial applications and make many PFAS extremely persistent in the human body and the environment (Kwiatkowski et al., 2020; Calafat et al., 2019; Calafat et al., 2007). Because of their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many different PFAS co-occur in exposure media (e.g., air, water, ice, sediment) as well as in tissues and blood of aquatic and terrestrial organisms, including humans.

With regard to structure, there are many families or classes of PFAS, each containing many individual structural homologues that can exist as either branched-chain or straight-chain isomers (Buck et al., 2011). These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl acids (PFAAs), fluorotelomer-based substances, and per- and polyfluoroalkyl ethers. PFOS belongs to the PFAA family of the non-polymer PFAS category and is among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review, see Podder et al. (2021)).

1.3 Chemical Identity

PFOS is a perfluoroalkyl sulfonate that was used as an aqueous dispersion agent and emulsifier in a variety of water-, oil-, and stain-repellent products (e.g., agricultural chemicals, alkaline cleaners, carpets, firefighting foam, floor polish, textiles) (NLM, 2022). It can exist in linear- or branched-chain isomeric form. PFOS is a strong acid that is generally present as the sulfonate anion at typical environmental pH values. Therefore, this assessment applies to all isomers of PFOS, as well as nonmetal salts of PFOS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body).

PFOS is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and it dissipates by advection, dispersion, and sorption to particulate matter. PFOS has low volatility in its ionized form but can adsorb to particles and be deposited on the ground and into water bodies. Because of its persistence, it can be transported long distances in air or water, as evidenced by detections of PFOS in arctic media and biota, including polar bears, oceangoing birds, and fish found in remote areas (Lindstrom et al., 2011; Smithwick et al., 2006).

Physical and chemical properties and other reference information for PFOS are provided in Table 1-1. However, there is uncertainty in the estimation, measurement, and/or applicability of certain physical/chemical properties of PFOS in drinking water, including the K_{oc} (Nguyen et al., 2020b; Li et al., 2018c), octanol-water partition coefficient (K_{ow}), and Henry's Law Constant (K_H) (NCBI, 2022; ATSDR, 2021). For example, for K_{ow}, the Agency for Toxic Substances and Disease Registry (ATSDR) (2021) reported that a value could not be measured because PFOS is expected to form multiple layers in octanol/water mixtures.

For a more detailed discussion related to the chemical and physical properties and environmental fate of PFOS, please see the *PFAS Occurrence and Contaminant Background Support Document for the Final PFAS National Primary Drinking Water Regulation*(U.S. EPA, 2024e), the 2016 *PFOS Health Effects Support Document* (U.S. EPA, 2016b), and the *Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctane Sulfonate (PFOS)* (U.S. EPA, 2022a).

Table 1-1. Chemical and Physical Properties of PFOS

Property	PFOS, Acidic Form; Experimental Average	Source
Chemical Abstracts Service Registry Number (CASRN)[a]	1763-23-1	NLM (2022)
Chemical Abstracts Index Name	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid	
Synonyms	Perfluorooctane sulfonic acid; heptadecafluoro-1-octane sulfonic acid; PFOS acid	EPA CompTox Chemicals Dashboard
Chemical Formula	$C_8HF_{17}O_3S$	NLM (2022)
Molecular Weight	500.13 g/mol	NLM (2022)
Color/Physical State	Liquid	NLM (2022)
Boiling Point	249°C	NLM (2022)
Melting Point	>400°C	ATSDR (2021) (potassium salt)
Vapor Pressure	0.002 mm Hg at 25°C	NLM (2022) (estimated)
Henry's Law Constant (K_H)	4.1E-04 atm-m^3/mol at 25°C	NLM (2022) (estimated from vapor pressure and water solubility)
K_{oc}	1,000 ± 5.0 L/kg (mean of values ± 1 standard deviation of selected values)	Zareitalabad et al. (2013) (converted from log K_{oc} to K_{oc})
Log K_{ow}	4.49	NLM (2022) (estimated)
Solubility in Water	0.0032 mg/L at 25°C; 570 mg/L	NLM (2022) (estimated) ATSDR (2021) (potassium salt in pure water)

Notes: CASRN = Chemical Abstracts Service Registry Number; K_{oc} = organic carbon-water partitioning coefficient; K_{ow} = octanol-water partition coefficient.
[a] The CASRN given is for linear PFOS, but the toxicity studies are based on both linear and branched; thus, this assessment applies to all isomers of PFOS.

1.4 Occurrence Summary

1.4.1 Biomonitoring

The U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOS has been detected in up to 98% of serum samples taken in biomonitoring studies that are representative of the U.S. general population. Blood levels of PFOS declined by >85% between 1999 and 2018, presumably because of restrictions on its commercial usage in the United States (CDC, 2017). However, studies of residents in locations of suspected PFAS contamination show higher serum levels of PFAS, including PFOS, compared with the general U.S. population as reported by NHANES (ATSDR, 2022; Table 17-6 in ITRC, 2020; Kotlarz et al., 2020; Yu et al., 2020).

Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007, EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (U.S. EPA, 2016a). Manufacturers have since shifted to alternative short-chain PFAS, such as perfluorobutane sulfonic acid (PFBS) (3M, 2002). Additionally, other PFAS were found in human blood samples

from recent (2011–2016) NHANES surveys (e.g., perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoDA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), perfluorononanoic acid (PFNA), and 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH or MeFOSAA)). There is less publicly available information on the occurrence and health effects of these replacement PFAS than for PFOS, PFOA, and other members of the carboxylic acid and sulfonate PFAS categories.

1.4.2 Ambient Water

Among the PFAS with established analytical methods for detection, PFOS is one of the dominant PFAS compounds detected in ambient water both in the United States and worldwide (Remucal, 2019; Dinglasan-Panlilio et al., 2014; Zareitalabad et al., 2013; Benskin et al., 2012; Ahrens, 2011; Nakayama et al., 2007). Although it has a history of wide usage and is highly persistent in aquatic environments, current information on the distribution of PFOS in surface waters of the United States is somewhat limited; most published PFOS ambient water occurrence data focuses on regions with known PFAS use or occurrence. These regions are primarily freshwater systems in eastern states, including the Mississippi River, Great Lakes, Cape Fear Drainage Basin, and waterbodies near Decatur, Alabama, and in northern Georgia (Jarvis et al., 2021). Additional monitoring has been conducted in areas of known aqueous film-forming foam use.

In a recent review, Jarvis et al. (2021) found that concentrations of PFOS in global surface waters ranged over eight orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels (range: 0.074–8,970,000 ng/L, arithmetic mean: 786.77 ng/L, geometric mean: 5.468 ng/L, median: 3.6 ng/L). Although these calculated concentrations are not necessarily representative of all the measured PFOS concentrations in U.S. surface waters, the majority of PFOS concentrations reported (approximately 91%) are less than 300 ng/L. Figure 1-1 (excerpted from Jarvis et al. (2021)) shows the distribution of PFOA concentrations (ng/L) measured in surface waters for each U.S. state or waterbody (excluding the Great Lakes) with reported data in the publicly available literature.

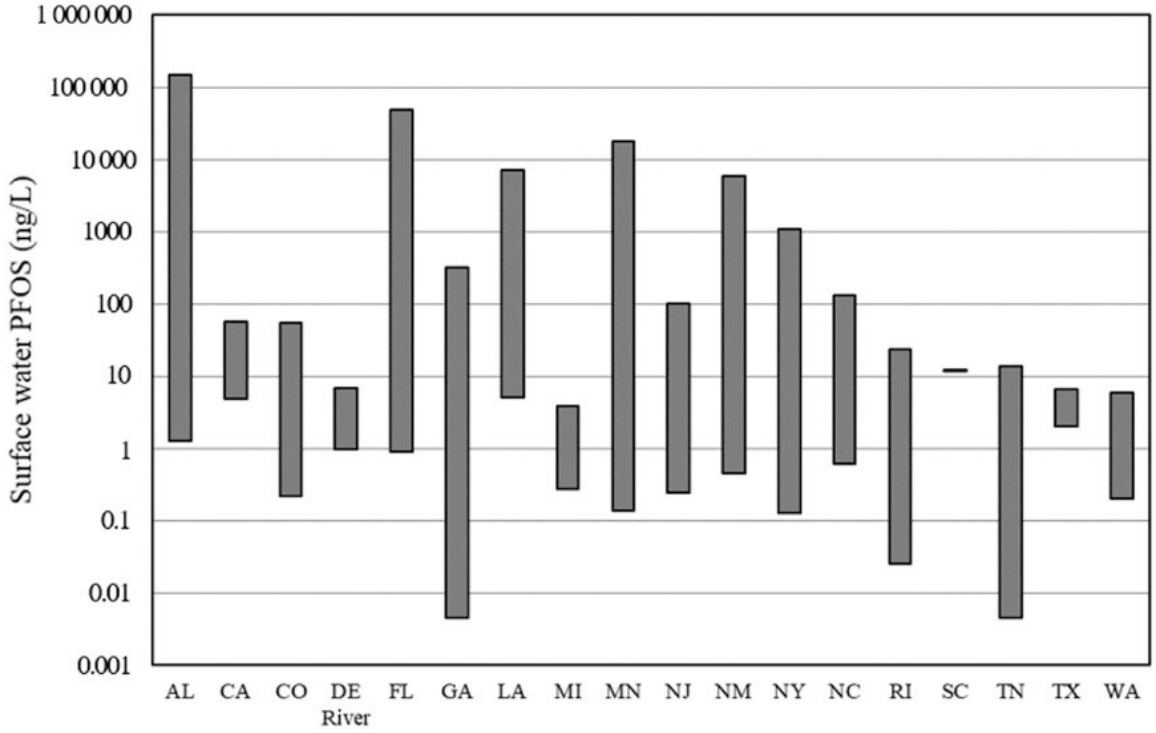

Figure 1-1. Distribution of PFOS Concentrations in Surface Waters by State/Waterbody (Excluding Great Lakes) in the United States (Jarvis et al., 2021)

1.4.3 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOS. Serum PFOS concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges.

EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are suspected to be present in drinking water and do not have health-based standards set under the Safe Drinking Water Act (SDWA). Under the UCMR, drinking water is monitored from public water systems (PWSs), specifically community water systems and non-transient, non-community water systems. The UCMR improves EPA's understanding of the frequency and concentrations of contaminants of concern occurring in the nation's drinking water systems. The first four UCMRs collected data from a census of large water systems (serving more than 10,000 people) and from a statistically representative sample of small water systems (serving 10,000 or fewer people). UCMR 3 monitoring occurred between 2013 and 2015 and is currently the most comprehensive nationally representative finished water dataset for PFOS (U.S. EPA, 2024d, e). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed. PFOS was found in 292 samples at 95 systems above the UCMR 3 minimum reporting level (40 ng/L). These systems serve a population of approximately 10.4 million people located in 28 states, Tribes, or U.S. territories (U.S. EPA, 2024d, e).

More recent state data were collected using newer EPA-approved analytical methods and some state results reflect lower reporting limits than those in the UCMR 3. State data are available from 32 states: Alabama, Arizona, California, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri,

New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin (U.S. EPA, 2024d, e). State results show continued occurrence of PFOS in multiple geographic locations. These data also show PFOS occurrence at lower concentrations and significantly greater frequencies than were measured under the UCMR 3, likely because the more recent monitoring was able to rely on more sensitive analytical methods (U.S. EPA, 2024d, e). More than one-third of states that conducted nontargeted monitoring detected PFOA and/or PFOS at more than 25% of systems (U.S. EPA, 2024d, e). Among the detections, PFOS concentrations ranged from 0.24 to 650 ng/L with a range of median concentrations from 1.21 to 12.1 ng/L (U.S. EPA, 2024d, e). Monitoring data for PFOA and PFOS from states that conducted targeted monitoring efforts, including 15 states, demonstrate results consistent with the nontargeted state monitoring. Within the 20 states that conducted nontargeted monitoring, there are 1,260 systems with results above 4.0 ng/L and 1,577 systems with results above 4.0 ng/L (U.S. EPA, 2024d, e). These systems serve populations of 12.5 and 14.4 million people, respectively. Monitoring data for PFOS from states that conducted targeted sampling efforts showed additional systems exceeding 4 ng/L (U.S. EPA, 2024d, e).

Finally, the fifth UCMR (UCMR 5) was published in December 2021 and requires sample collection and analysis for 29 PFAS, including PFOS, between January 2023 and December 2025 using drinking water analytical methods developed by EPA (U.S. EPA, 2021e). The UCMR 5 defined the minimum reporting level at 4 ng/L for PFOS using EPA Method 533, which is lower than the 40 ng/L used in the UCMR 3 with EPA Method 537 (U.S. EPA, 2021e). Therefore, the UCMR 5 will be able to provide nationally representative occurrence data for PFOS at lower detection concentrations. While the complete UCMR 5 dataset is not currently available, the small subset of data released (7% of the total results that EPA expects to receive) as of July 2023 is consistent with the results of UCMR 3 and the state data described above (U.S. EPA, 2024d, e).

Likewise, Glassmeyer et al. (2017) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging chemical and microbial contaminants, including 11 PFAS. PFOS was reported in source water at 88% of systems, with a median concentration of 2.28 ng/L and maximum concentration of 48.30 ng/L. Similarly, in treated drinking water, PFOS was detected in 80% of systems, with a median concentration of 1.62 ng/L and maximum concentration of 36.90 ng/L.

1.5 History of EPA's Human Health Assessment for PFOS

EPA developed an HESD for PFOS after it was listed on the third CCL (CCL 3) in 2009 (U.S. EPA, 2009). An HESD is synonymous with a toxicity assessment in that they both describe the assessment of cancer and noncancer health effects and derive toxicity values. The 2016 PFOS HESD was peer reviewed in 2014 and revised based on consideration of peer reviewers' comments, public comments, and additional studies published through December 2015. The resulting *Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (U.S. EPA, 2016b) was published in 2016 and described the assessment of cancer and noncancer health effects and the derivation of a noncancer RfD for PFOS.

EPA initiated an update to the 2016 PFOS HESD in 2021 when the agency made a determination to regulate PFOS with a national primary drinking water regulation (NPDWR) (U.S. EPA,

2021c). The initial update of the 2016 PFOS HESD was the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021b). This assessment described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, a relative source contribution (RSC), and cancer classification, which would subsequently be used to prepare draft and final toxicity assessments. The agency sought peer review from the EPA SAB PFAS Review Panel on key scientific issues, including the systematic review approach for evaluating health effects studies, the derivation of oral toxicity values, the RSC, and the cancer classification for PFOS.

The SAB provided draft recommendations on June 3, 2022, and final recommendations on August 23, 2022 (U.S. EPA, 2022e). To be responsive to the SAB recommendations, EPA developed a detailed response to comment document (USEPA OOW, 2023) and addressed every recommendation from the SAB in the development of the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023). Briefly, EPA:

- updated and expanded the scope of the studies included in the assessment;
- expanded the systematic review steps beyond study quality evaluation to include evidence integration to ensure consistent hazard decisions across health outcomes;
- separated hazard identification and dose-response assessment;
- added protocols for all steps of the systematic review and more transparently described the protocols;
- evaluated alternative pharmacokinetic models and further validated the selected model;
- conducted additional dose-response analyses using additional studies and endpoints;
- evaluated and integrated mechanistic information;
- strengthened the weight-of-evidence discussion for cancer effects and rationale for the cancer classification;
- strengthened the rationales for selection of PODs for the noncancer health outcomes; and
- clarified language related to the RSC determination, including the relevance of drinking water exposures and the relationship between the RfD and the RSC.

EPA then released the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* for a 60-day public comment period. These assessments described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, an RSC, and cancer classification for PFOS.

EPA incorporated feedback from public comment into the assessment and developed a detailed response to public comment document (U.S. EPA, 2024c). Briefly, EPA has improved descriptions of rationale and added clarifications related to the systematic review protocol used for this assessment, study and endpoint selection for POD derivation, and the modeling choices related to toxicity value derivation. Therefore, this *Final Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* incorporates feedback from external peer review and public comment and supersedes all other health effects documents produced by the EPA Office of Water for PFOS.

2 Summary of Assessment Methods

This section summarizes the methods used for the systematic review of the health effects literature for all isomers of PFOS, as well as nonmetal salts of PFOS, that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body). The purposes of this systematic review were to identify the best available and most relevant health effects literature, to evaluate studies for quality, and to subsequently identify health effects and studies for dose-response assessment. A detailed description of these methods is provided as a protocol in Appendix A, (U.S. EPA, 2024a).

2.1 Introduction to the Systematic Review Assessment Methods

The methods used to conduct the systematic review for PFOS are consistent with the methods described in the draft and final *EPA ORD Staff Handbook for Developing IRIS Assessments* (U.S. EPA, 2022d, 2020a) (hereafter referred to as the Integrated Risk Information System (IRIS) Handbook) and a companion publication (Thayer et al., 2022). EPA's IRIS Handbook has incorporated feedback from the National Academy of Sciences (NAS) at workshops held in 2018 and 2019 and was well regarded by the NAS review panel for reflecting "significant improvements made by EPA to the IRIS assessment process, including systematic review methods for identifying chemical hazards" (NASEM, 2021). Furthermore, EPA's IRIS program has used the IRIS Handbook to develop toxicological reviews for numerous chemicals, including some PFAS (U.S. EPA, 2023, 2022c). Though the IRIS Handbook was finalized concurrently with the development of this assessment, the revisions in the final IRIS Handbook compared to the draft version do not conflict with the methods used in this assessment. The assessment team concluded that implementing these minor changes in study quality evaluation between the draft and final IRIS Handbook versions would not change the assessment conclusions. Therefore, EPA considers the methods described herein to be consistent with the final IRIS Handbook and cites this version accordingly. Additionally, the methods used to conduct the systematic review are also consistent with and largely mirror the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

For this updated PFOS toxicity assessment, systematic review methods were consistent with those in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b) for the steps of literature search; screening; study quality evaluation; data extraction; display of study evaluation results; synthesis of human and experimental animal data; and evidence integration for all health outcomes through the 2020 literature searches, as presented in the preliminary analyses of the 2021 *Proposed Approaches To The Derivation Of A Draft Maximum Contaminant Level Goal For Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) In Drinking Water* draft document that was reviewed by the Science Advisory Board (SAB) (U.S. EPA, 2022d, 2021b). The EPA then focused the remaining steps of the systematic review process (synthesis and integration of mechanistic data; derivation of toxicity values) on health outcomes with the strongest weight of evidence based on the conclusions presented in the 2021 draft documents, and consistent with the recommendations of the SAB (U.S. EPA, 2022e). These five "priority" health outcomes are developmental, hepatic, immune, cardiovascular, and cancer. The updated systematic review focused on the priority health outcomes was published in

2023 as the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023).

The following subsections provide a summary of methods used to search for and screen identified literature, evaluate the identified studies to characterize study quality, extract data, and select studies for dose-response analysis. Extracted data are available in interactive visual formats (see Section 3) and can be downloaded in open access, interactive formats. The full systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) provides a detailed description of the systematic review methods that were used. The protocol also includes the description of the problem formulation and key science issues guiding this assessment.

2.1.1 Literature Database

The EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this PFOS toxicity assessment based on three main data streams: 1) literature published from 2013 through February 6, 2023 identified via literature searches conducted in 2019, 2020, 2022 and 2023 of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g., searches of the gray literature, studies shared with EPA by the SAB, studies submitted through public comment), and 3) literature identified in EPA's 2016 *Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (U.S. EPA, 2016b). All of these streams are described in detail below.

For the literature searches, the search strings focused on the chemical name (PFOS and its related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, *in vitro*, *in silico*) or health outcomes. The EPA conducted a literature search in 2019 (covering January 2013 through April 11, 2019), which was subsequently updated by a search covering April 2019 through September 3, 2020 prior to SAB review of the draft assessment (2020 literature search), a third search covering September 2020 through February 3, 2022 prior to release of the draft assessment for public comment (2022 literature search), and a final supplemental search covering February 4, 2022 through February 6, 2023.

The publicly available databases listed below were searched for literature containing the chemical search terms outlined in Appendix A (U.S. EPA, 2024a):

- Web of Science™ (WoS) (Thomson Reuters),
- PubMed® (National Library of Medicine),
- ToxLine (incorporated into PubMed post 2019), and
- TSCATS (Toxic Substances Control Act Test Submissions).

The search strings and literature sources searched are described in Appendix A (U.S. EPA, 2024a).

For the second data stream, other review efforts and searches of publicly available sources were used to identify relevant studies (see Appendix A, (U.S. EPA, 2024a)), as listed below:

- studies cited in assessments published by other U.S. federal, international, and/or U.S. state agencies (this included assessments by ATSDR (ATSDR, 2021) and California Environmental Protection Agency (CalEPA, 2021)),

- studies identified during mechanistic or toxicokinetic evidence synthesis (i.e., during manual review of reference lists of relevant mechanistic and toxicokinetic studies deemed relevant after screening against mechanistic- and ADME-specific PECO criteria),
- studies identified by the SAB in their final report dated August 23, 2022 (U.S. EPA, 2022e), and
- studies submitted through public comment by May 2023 (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

For the third data stream, EPA relied on epidemiological and animal toxicological literature synthesized in the 2016 PFOS HESD to identify studies relevant to the five priority health outcomes, as recommended by SAB and consistent with preliminary conclusions from EPA's analysis in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water*. The 2016 PFOS HESD contained a summary of all relevant literature identified in searches conducted through 2013. EPA's 2016 PFOS HESD relied on animal toxicological studies for quantitative analyses whereas epidemiology studies were considered qualitatively, as a supporting line of evidence. This updated assessment includes epidemiological studies that were identified and presented in the 2016 PFOS HESD for the five priority health outcomes. It also includes "key" animal toxicological studies from the 2016 PFOS HESD, which includes studies that were selected in 2016 for dose-response modeling. The details of the studies included from the 2016 PFOS HESD are described in Appendix A (U.S. EPA, 2024a).

All studies identified through the data streams outlined above were uploaded into the publicly available Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608).

EPA has continued to monitor the literature published since February 2023 for other potentially relevant studies. Potentially relevant studies identified after February 2023 that were not recommended by the SAB in their final report or via public comment are not included as part of the evidence base for this updated assessment but are provided in a repository detailing the results and potential impacts of new literature on the assessment (see Appendix A, (U.S. EPA, 2024a)).

2.1.2 Literature Screening

This section summarizes the methods used to screen the identified health effects, mechanistic, and absorption, distribution, metabolism, excretion (ADME) literature. Briefly, the EPA used populations, exposures, comparators, and outcomes (PECO) criteria to screen the literature identified from the literature sources outlined above in order to prioritize studies for dose-response assessment and to identify studies containing supplemental information such as mechanistic studies that could inform the mode of action analyses. The PECO criteria used for screening the health effects, toxicokinetic, and mechanistic literature are provided in Appendix A (U.S. EPA, 2024a).

Consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b), studies identified in the literature searches and stored in HERO were imported into the SWIFT Review software platform and the software was used to identify those studies most

likely to be relevant to human health risk assessment. Studies captured then underwent title and abstract screening by at least two independent reviewers using screening tools consistent with the IRIS Handbook ((U.S. EPA, 2022d); DistillerSR or SWIFT ActiveScreener software), and studies that passed this screening underwent full-text review by at least two independent reviewers. Health effects studies that met PECO inclusion criteria following both title and abstract screening and full-text review underwent study quality evaluation as described below (Section 2.1.3). Studies that were tagged as containing relevant PBPK models were sent to the modeling technical experts for scientific and technical review. Studies tagged as supplemental and containing potentially relevant mechanistic or ADME (or toxicokinetic) data following title and abstract and full-text level screening underwent further screening using mechanistic- or ADME-specific PECO criteria, and those deemed relevant underwent light data extraction of key study elements (e.g., extraction of information about the tested species or population, mechanistic or ADME endpoints evaluated, dose levels tested; see Appendix A, (U.S. EPA, 2024a)). Supplemental studies that were identified as mechanistic or ADME during screening did not undergo study quality evaluation.

For the supplemental literature search conducted in 2023 and literature received through public comment, studies were screened for relevancy and considered for potential impact on the toxicity assessments for PFOS. Consistent with the IRIS Handbook (U.S. EPA, 2022d), the studies identified after February 3, 2022, including studies recommended via public comment, were "considered for inclusion only if they [were] directly relevant to the assessment PECO criteria and [were] expected to potentially impact assessment conclusions or address key uncertainties" (U.S. EPA, 2022d). For the purposes of this assessment, the EPA defined impacts on the assessment conclusions as data from a study (or studies) that, if incorporated into the assessment, have the potential to significantly affect (i.e., by an order of magnitude or more) the final toxicity values (i.e., RfDs and CSFs) or alter the cancer classification for PFOS (see Appendix A, (U.S. EPA, 2024a)).

2.1.3 Study Quality Evaluation for Epidemiological Studies and Animal Toxicological Studies

Study quality evaluations were performed consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). For study quality evaluation of the PECO-relevant human epidemiological and animal toxicological studies (i.e., studies identified in the four literature searches (all health outcomes for the 2019 and 2020 searches; the five priority health outcomes for the 2022 search; studies impacting assessment conclusions within the five priority health outcomes for the 2023 search (see Appendix A, (U.S. EPA, 2024a))), studies recommended by the SAB, studies recommended via public comment that reported potentially significant results on one or more of the five priority health outcomes, epidemiological studies from the 2016 PFOS HESD that reported results on one or more of the five priority health outcomes, and key animal toxicological studies from the 2016 PFOS HESD), two independent primary reviewers followed by a quality assurance (QA) reviewer assigned ratings about the reliability of study results (*good, adequate, deficient* (or *"not reported"*), or *critically deficient*) for different evaluation domains as described in the IRIS Handbook (U.S. EPA, 2022d) (see Appendix A, (U.S. EPA, 2024a)). These study quality evaluation domains are listed below and

details about the domains, including prompting questions and suggested considerations, are described in Appendix A (U.S. EPA, 2024a).

- Epidemiological study quality evaluation domains: participant selection; exposure measurement criteria; outcome ascertainment; potential confounding; analysis; selective reporting; and study sensitivity.
- Animal toxicological study quality evaluation domains: reporting quality; allocation; observational bias/blinding; confounding/variable control; reporting and attrition bias; chemical administration and characterization; exposure timing, frequency, and duration; endpoint sensitivity and specificity; and results presentation.

The independent reviewers performed study quality evaluations using a structured platform housed within EPA's Health Assessment Workplace Collaboration (HAWC; https://hawcproject.org/). Once the individual domains were rated, reviewers independently evaluated the identified strengths and limitations of each study to reach an overall classification on study confidence of *high, medium, low*, or *uninformative* for each PECO-relevant endpoint evaluated in the study consistent with the IRIS Handbook (U.S. EPA, 2022d). A study can be given an overall *mixed* confidence rating if different PECO-relevant endpoints within the study receive different confidence ratings (e.g., *medium* and *low* confidence ratings).

2.1.4 Data Extraction

Data extraction was conducted for all relevant human epidemiological and animal toxicological studies determined to be of *medium* and *high* confidence after study quality evaluation. Due to the abundance of *medium* and *high* confidence studies in this database, data were only extracted from *low* confidence epidemiological studies when data were limited for a health outcome or when there was a notable effect, consistent with the IRIS Handbook (U.S. EPA, 2022d). Studies evaluated as being *uninformative* for an endpoint were not considered further when characterizing that endpoint and therefore did not undergo data extraction. All health endpoints were considered for extraction, regardless of the magnitude of effect or statistical significance of the response relative to the control group. The level of detail in data extractions for different endpoints within a study could differ based on how the data were presented for each outcome (i.e., ranging from a narrative summary to a full extraction of dose-response effect size information).

Extractions were conducted using DistillerSR for epidemiological studies and HAWC for animal toxicological studies. An initial reviewer conducted the extraction, followed by a second reviewer conducting an independent QA who confirmed accuracy and edited/corrected the extraction as needed. Discrepancies in data extraction were resolved by discussion and confirmation within the extraction team.

Data extracted from epidemiology studies included population, study design, year of data collection, exposure measurement, and quantitative data from statistical models. Data extracted from statistical models reported in the studies included the health effect category, endpoint measured, sample size, description of effect estimate, covariates, and model comments. Data extracted from animal toxicological studies included information on the experimental design and exposure duration, species and number of animals tested, dosing regime, and endpoints

measured. Further information about data extraction can be found in Appendix A (U.S. EPA, 2024a).

2.1.5 Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes. Evidence synthesis refers to the process of analyzing the results of the available studies (including their strengths and weaknesses) for consistency and coherence, often by evidence stream (e.g., human or animal) and health outcome (i.e., an organ- or organ system-level category of related health effects and endpoints). In evidence integration, the evidence across streams is considered together and integrated to develop judgments (for each health outcome) about whether the chemical in question poses a hazard to human health. Consistent with the IRIS Handbook, groups of related outcomes within a health outcome category were considered together as a unit of analysis during evidence synthesis and evidence integration (U.S. EPA, 2022d). For example, birth weight, birth length, and head circumference were all considered under the unit of analysis of the fetal growth restriction.

Evidence syntheses are summary discussions of the body of evidence for each evidence stream (i.e., human and animal) for each health outcome analyzed. The available human and animal health effects evidence were synthesized separately, with each synthesis resulting in a summary discussion of the available evidence. For the animal toxicological evidence stream, evidence synthesis included consideration of studies rated *high* and *medium* confidence. For the epidemiological evidence stream, evidence synthesis was based primarily on studies of *high* and *medium* confidence, including discussion of study quality considerations, according to the recommendations of the SAB (U.S. EPA, 2022e). Consistent with the IRIS Handbook (U.S. EPA, 2022d), *low* confidence epidemiological studies and results were used only in a supporting role and given less weight during evidence synthesis and integration compared to *high* or *medium* confidence studies. *Low* confidence epidemiological studies were included in evidence syntheses in order to capture all of the available data for PFOS in the weight of evidence analyses. As described above, *uninformative* studies were not extracted or included in the evidence syntheses. Results from epidemiological studies were discussed within sections organized by population type, including children, general population adults, pregnant women, and occupational populations. Childhood was defined as the effect of environmental exposure during early life: from conception, infancy, early childhood and through adolescence until 21 years of age (U.S. EPA, 2021a). Epidemiological studies were excluded from the evidence synthesis narrative if they included data that were reported in multiple studies (e.g., overlapping NHANES studies). Studies reporting results from the same cohort and on the same health outcome as another study were considered overlapping evidence, and, to avoid duplication or overrepresentation of results from the same group of participants, these additional studies were not discussed in the evidence synthesis narrative. In cases of overlapping studies, the study with the largest number of participants and/or the most accurate outcome measures was given preference. For the five priority health outcomes, EPA also developed mechanistic syntheses.

For evidence integration, conclusions regarding the strength of evidence were drawn for each health outcome across human and animal evidence streams. For the five priority health outcomes, this included consideration of epidemiological studies identified in the 2016 PFOS HESD, as well as mechanistic evidence. The evidence integration provides a summary of the causal interpretations between PFOS exposure and health effects based on results of the available

epidemiological and animal toxicological studies, in addition to the available mechanistic evidence. Considerations when evaluating the available studies included risk of bias, sensitivity, consistency, strength (effect magnitude) and precision, biological gradient/dose-response, coherence, and mechanistic evidence related to biological plausibility. The judgments were directly informed by the evidence syntheses and based on structured review of an adapted set of considerations for causality first introduced by Austin Bradford Hill (Hill, 1965).

The evidence integration was conducted according to guidance outlined in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). The evidence integration included evidence stream evaluation, in which the qualitative summaries on the strength of evidence from studies in animals and humans were evaluated, and subsequent inference across all evidence streams. Human relevance of animal models as well as mechanistic evidence to inform mode of action were considered. Evidence integration produced an overall judgment about whether sufficient or insufficient evidence of an association with PFOS exposure exists for each human health outcome, as well as the rationale for each judgment. The potential evidence integration judgments for characterizing human health effects are ***evidence demonstrates, evidence indicates (likely), evidence suggests, evidence inadequate,*** and ***strong evidence supports no effect.*** Considerations for each evidence integration judgment are summarized within corresponding evidence integration sections in an evidence profile table (EPT). EPTs were organized by evidence stream (i.e., human, animal, and mechanistic, respectively), and, within evidence streams, units of analysis with the strongest evidence were presented first.

Additional details about evidence synthesis and integration are summarized in Appendix A (U.S. EPA, 2024a).

2.2 Dose-Response Assessment

Evidence synthesis and integration enabled identification of the health outcomes with the strongest weight of evidence supporting causal relationships between PFOS exposure and adverse health effects, as well as the most sensitive cancer and noncancer endpoints within those health outcomes. Dose-response modeling was performed for endpoints within health outcomes with data warranting evidence integration conclusions of *evidence demonstrates* and *evidence indicates (likely)* for noncancer endpoints and carcinogenicity descriptors of *Carcinogenic to Humans* and *Likely to be Carcinogenic to Humans*. EPA identified specific studies for dose-response modeling and POD derivation following attributes described in Table 7-2 of the IRIS Handbook (U.S. EPA, 2022d). Examples of study attributes evaluated included study design characteristics, study confidence, and data availability, among others (see Appendix A, (U.S. EPA, 2024a)). Human epidemiological and animal toxicological studies that were consistent with the overall weight of evidence for a specific endpoint were considered for dose-response. Additionally, for human evidence, all *high* or *medium* confidence studies pertaining to a specific endpoint were considered; for animal evidence, only animal toxicological studies with at least two PFOS exposure groups that were of *high* or *medium* confidence were considered. Relevance of the endpoint or species reported by animal toxicological studies to human health effects was also considered. Additional information on study selection is provided in Appendix A (U.S. EPA, 2024a).

2.2.1 Approach to POD and Candidate RfD Derivation for Noncancer Health Outcomes

The current recommended EPA human health risk assessment approach for noncancer POD derivation described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* includes selection of a benchmark response (BMR), analysis of dose and response within the observed dose range, followed by extrapolation to lower exposure levels (U.S. EPA, 2002b). For noncancer health outcomes, EPA performed dose-response assessments to define PODs, including low-dose extrapolation, when feasible, and applied uncertainty factors (UFs) to those PODs to derive candidate RfDs. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002b). For PFOS, multiple candidate RfDs were derived within a health outcome as described in Section 4.

For PFOS animal toxicological studies, EPA attempted benchmark dose (BMD) modeling on all studies considered for dose-response to refine the POD. BMD modeling was performed after converting the administered dose reported by the study to an internal dose using a pharmacokinetic model (see Section 4.1.3 for additional details). This approach resulted in dose levels corresponding to specific response levels near the low end of the observable range of the data and identified the lower limits of the BMDs (BMDLs) which serve as potential PODs (U.S. EPA, 2012a). EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (i.e., BMD) that corresponds to a predetermined level of response (i.e., benchmark response or BMR). For dichotomous data, the BMR is typically set at either 5% or 10% above the background or the response of the control group. For continuous data, a BMR of one-half or one standard deviation from the control mean is typically used when there are no outcome-specific data to indicate what level of response is biologically significant (U.S. EPA, 2012a). For dose-response data for which BMD modeling did not produce an adequate model fit, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD. However, a POD derived using a BMD approach typically provides a higher level of confidence in the conclusions for any individual case, as the BMDL takes into account all the data from the dose-response curve, incorporates the evaluation of the uncertainty in the BMD, and is related to a known and predefined potential effect size (i.e., the BMR) (U.S. EPA, 2022d, 2012a). For noncancer endpoints, there were several factors considered when selecting the final model and BMD/BMDL, including the type of measured response variable (i.e., dichotomous or continuous), experimental design, and covariates (U.S. EPA, 2012a). However, as there is currently no prescriptive hierarchy, selection of model types was often based on the goodness-of-fit and was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. The *Benchmark Dose Technical Guidance* provides a "BMD Decision Tree" to assist in model selection (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

For the epidemiological studies considered for dose-response assessment, EPA used multiple modeling approaches to determine PODs, depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies,

EPA used a hybrid modeling approach that involves estimating the incidence of individuals above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control (U.S. EPA, 2012a) because the EPA was able to define a level considered clinically adverse for these outcomes (see Appendix E, (U.S. EPA, 2024a)). As sensitivity analyses for comparison purposes, EPA also performed BMD modeling and provided study LOAELs/NOAELs as PODs for the epidemiological hepatic and serum lipid dose-response studies. For the immune studies, for which a clinically defined adverse level is not established, EPA used multivariate models provided in the studies and determined a BMR according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

After POD derivation, EPA used a pharmacokinetic model for human dosimetry to estimate human equivalent doses (HEDs) from both animal and epidemiological studies. A pharmacokinetic model for human dosimetry is used to simulate the HED from the animal PODs and is also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal dose POD obtained from dose-response modeling (Section 4.1.3). Based on the available data, a serum PFOS concentration was identified as a suitable internal dosimetry target for the human and animal endpoints of interest. Next, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HEDS}) to account for five possible areas of uncertainty and variability: human variation, extrapolation from animals to humans, extrapolation to chronic exposure duration, the type of POD being used for reference value derivation, and extrapolation to a minimal level of risk (if not observed in the data set). UFs used in this assessment were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). For additional detail on UFs, see Appendix A (U.S. EPA, 2024a). The POD_{HED} for a particular candidate RfD is divided by the composite UFs.

The general steps for deriving an RfD for PFOS are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints affected by exposure to PFOS. This step involves selecting the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study confidence, and characterized for adverse health outcomes, the risk assessor selects health endpoints/outcomes judged to be relevant to human health and among the most sensitive, defined as effects observed in the lower exposure range. Considerations that might influence selection of endpoints include whether data have dose-response information, magnitude of response, adversity of effect, and consistency across studies.

Step 1a (for dose-response data from a study in an animal model): Convert administered dose to an internal dose. A pharmacokinetic model is used to predict the internal dose (in the animals used in the toxicity studies) that would correspond to the administered dose used in the study (see 4.1.3 for additional detail). A number of dose-metrics across life stages are selected for simulation in a mouse, rat, or monkey. Concentrations of PFOS in blood are considered for all the internal dose-metrics.

Step 2: Conduct dose-response modeling. See above and Appendix E (U.S. EPA, 2024a) for study-specific details.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (e.g., BMDL, NOAEL) is converted to an HED following the method described in Section 4.1.3.

Step 4: Select appropriate UFs and provide rationale for UF selection. UFs are applied in accordance with EPA methodology considering variations in sensitivity among humans, differences between animals and humans (if applicable), the duration of exposure in the critical study compared to the lifetime of the species studied, and the completeness of the epidemiological or animal toxicological database (U.S. EPA, 2002b).

Step 5: Calculate the chronic RfD. The RfD is calculated by dividing the POD_{HED} by the composite (total) UF specific to that POD_{HED}.

$$RfD = \left(\frac{POD_{HED}}{UF_C}\right)$$

where:

POD_{HED} = calculated from the internal dose POD using the human pharmacokinetic (PK) model presented in Section 4.1.3.2.

UF_C = Composite (total) UF calculated by multiplying the selected individual UFs for variations in sensitivity among humans, differences between animals and humans, duration of exposure in the critical study compared to the lifetime of the species studied, and completeness of the toxicology database, in accordance with EPA methodology (U.S. EPA, 2002b).

2.2.2 Cancer Assessment

2.2.2.1 Approach for Cancer Classification

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, a descriptive weight of evidence expert judgment is made, based on all available animal, human, and mechanistic data, as to the likelihood that a contaminant is a human carcinogen and the conditions under which the carcinogenic effects may be expressed (U.S. EPA, 2005a). A narrative is developed to provide a complete description of the weight of evidence and conditions of carcinogenicity. The potential carcinogenicity descriptors (presented in the 2005 guidelines) are:

- Carcinogenic to Humans
- Likely to Be Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not Likely to Be Carcinogenic to Humans

More than one carcinogenicity descriptor can be applied if a chemical's carcinogenic effects differ by dose, exposure route, or mode of action (MOA)[3]. For example, a chemical may be

[3]MOA is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. It is contrasted with "mechanism of action," which implies a more detailed understanding and description of events.

carcinogenic to humans above but not below a specific dose level if a key event in tumor formation does not occur below that dose. MOA information informs both the qualitative and quantitative aspects of the assessment, including the human relevance of tumors observed in animals. The MOA analysis must be conducted separately for each target organ/tissue type (U.S. EPA, 2005a).

2.2.2.2 Derivation of Candidate Cancer Slope Factors

EPA's 2005 *Guidelines for Carcinogen Risk Assessment* recommends a two-step process for the quantitation of cancer risk as a CSF. A CSF is a plausible upper bound lifetime cancer risk from chronic ingestion of a chemical per unit of mass consumed per unit body weight per day (mg/kg-day) (U.S. EPA, 2005a). First, a model is used to fit a dose-response curve to the data, based on the doses and associated tumors observed (U.S. EPA, 2005a). In the second step of quantitation, the POD is extrapolated to the low-dose region of interest for environmental exposures. The approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). When evidence indicates that a chemical causes cancer through a mutagenic MOA (i.e., mutation of deoxyribonucleic acid (DNA)) or the MOA for carcinogenicity is not known, the linear approach is used, and the extrapolation is performed by drawing a line (on a graph of dose vs. response) from the POD to the origin (zero dose, zero tumors). The slope of the line (Δresponse/Δdose) gives rise to the CSF, which can be interpreted as the risk per mg/kg/day.

For animal toxicological studies, EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). First, a PK model converted the administered dose reported by the study to an internal dose (see Section 4.1.3 for additional details). Then, BMDS fits multistage models, the preferred model type (U.S. EPA, 2012a), to the data and the model is used to identify a POD for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD_{10} (the dose corresponding to a 10% increase in tumors) and the $BMDL_{10}$ (the 95% lower confidence limit for that dose) are also reported and are often used as the POD. Similar to noncancer PODs, selection of model types is often based on the goodness-of-fit (U.S. EPA, 2012a). For PFOS, after a POD was determined, a PK model was used to calculate the HED for animal oral exposures (POD_{HED}). The CSF is derived by dividing the BMR by the POD_{HED}. See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

In addition, according to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), affirmative determination of a mutagenic MOA (as opposed to defaulting to a mutagenic MOA based on insufficient data or limited data indicating potential mutagenicity) indicates the potential for higher cancer risks from an early-life exposure compared to the same exposure during adulthood, and so requires that the application of age-dependent adjustment factors (ADAFs) be considered in the quantification of risk to account for additional sensitivity of children. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposure to a mutagenic chemical.

In cases for which a chemical is shown to cause cancer via an MOA that is not linear at low doses, and the chemical does not demonstrate mutagenic or other activity consistent with

linearity at low doses, a nonlinear extrapolation is conducted. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* state that "where tumors arise through a nonlinear MOA, an oral RfD or inhalation reference concentration, or both, should be developed in accordance with EPA's established practice of developing such values, taking into consideration the factors summarized in the characterization of the POD" (U.S. EPA, 2005a). In these cases, an RfD-like value is calculated based on the key event[4] for carcinogenesis or the tumor response.

2.2.3 Selecting Health Outcome-Specific and Overall Toxicity Values

The next step is to select a health outcome-specific toxicity value for each hazard (cancer and noncancer) identified in the assessment. This selection can be based on the study confidence considerations, the most sensitive outcome, a clustering of values, or a combination of such factors; the rationale for the selection is presented in the assessment. Key considerations for candidate value selection are described in the IRIS Handbook (U.S. EPA, 2022e) and include: 1) the weight of evidence for the specific effect or health outcome; 2) study confidence; 3) sensitivity and basis of the POD; and 4) uncertainties in modeling or extrapolations. The value selected as the organ/system-specific toxicity value is discussed in the assessment.

The selection of final toxicity values for noncancer and cancer effects involves the study preferences described above, consideration of overall toxicity, study confidence, and confidence in each value, including the strength of various dose-response analyses and the possibility of basing a more robust result on multiple data sets. The values selected as the overall RfD and CSF are discussed in Section 4.

[4]The key event is defined as an empirically observed precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element.

3 Results of the Health Effects Systematic Review and Toxicokinetics Methods

3.1 Literature Search and Screening Results

Studies referenced in this assessment are cited as "Author Last Name, Publication Year, HERO ID" and are available in EPA HERO: A Database of Scientific Studies and References. The HERO ID is a unique identifier for studies available in HERO. Additional study metadata are publicly available and can be obtained by searching for the HERO ID on the public-facing webpage available here: https://hero.epa.gov/.

The three database searches yielded 7,160 unique records (combined for PFOA and PFOS) prior to running SWIFT Review. Table 3-1 shows the results from database searches conducted in April 2019, September 2020, and February 2022, and February 2023.

Table 3-1. Database Literature Search Results

Database	Date Run: Results
WoS	4/10/2019: 3,081 results 9/3/2020: 1,286 results 2/2/2022: 1,021 results 2/6/2023: 966 results
PubMed	4/10/2019: 2,191 results 9/3/2020: 811 results 2/2/2022: 1,728 results 2/6/2023: 719 results
TOXLINE	4/10/2019: 60 results
TSCATS	4/11/2019: 0 results
Total number of references from all databases for all searches[a]	4/2019: 3,382 results 9/2020: 1,153 results 2/2022: 1,858 results 2/2023: 1,153 results
Total number of references after running SWIFT Review[a]	4/2019: 1,977 results 9/2020: 867 results 2/2022: 1,370 results 2/2023: 881 results
Total number of unique references moved to screening[b]	**4,802**

Notes:
[a] The number of studies includes duplicate references across search dates due to overlap between search years.
[b] Duplicates across search dates removed.

The additional sources of literature outlined in Section 2.1.1 (i.e., assessments published by other agencies, studies identified during mechanistic or toxicokinetic syntheses, studies identified by the Science Advisory Board (SAB), and EPA's 2016 Health Effects Support Documents (HESDs) for perfluorooctanoic acid (PFOA) (U.S. EPA, 2016c) and perfluorooctane sulfonate (PFOS) (U.S. EPA, 2016b)) yielded 238 unique records (combined for PFOA and PFOS).

The 4,802 studies captured with the SWIFT Review evidence streams filters and the 238 records identified from additional sources yield a total of 5,011 unique studies. These 5,011 studies were moved to the next stage of screening (title and abstract screening using either DistillerSR or

SWIFT ActiveScreener). Of the 5,011 unique studies, 1,062 moved on to full-text level review, 1,697 were excluded during title and abstract screening, and 2,252 were tagged as containing potentially relevant supplemental material. Of the 1,062 screened at the full-text level, 760 were considered to meet PECO eligibility criteria (see Appendix A, (U.S. EPA, 2024a)) and included relevant information on PFOS. The 760 studies that were determined to meet PECO criteria after full-text level screening included 429 epidemiological (human) studies, 45 animal toxicological studies, 11 physiologically based pharmacokinetic (PBPK) studies, and 275 studies that were not extracted (e.g., *low* confidence studies, meta-analyses, studies from the 2022 and 2023 searches that did not evaluate effects on one of the priority health outcomes). An additional 16 PBPK studies were identified during the toxicokinetic screening for a total of 27 PBPK studies. Details of the literature search and screening process are shown in Figure 3-1.

The 429 epidemiological studies and 45 animal toxicological studies relevant to PFOS underwent study quality evaluation and were subsequently considered for data extraction as outlined in Sections 2.1.3 and 2.1.4 (see Appendix A, (U.S. EPA, 2024a)). The results of the health outcome-specific study quality evaluations and data extractions are described in Sections 3.4 and 3.5.

Additionally, the 27 studies tagged as containing relevant PBPK models for PFOS were reviewed by pharmacokinetic (PK) subject matter experts for inclusion consideration. The included studies are summarized in Section 3.3.2 and parameters described in these studies were considered for incorporation into the animal and human PK models, which are summarized in Section 4.1.3.

Finally, the 104 toxicokinetic and 305 mechanistic studies identified as relevant for PFOS moved on to a limited data extraction as described in the Appendix (U.S. EPA, 2024a). The toxicokinetic studies pertaining to ADME are synthesized in Section 3.3.1. The mechanistic studies relevant to the five priority health outcomes are synthesized in Sections 3.4 and 3.5 and were considered as part of the evidence integration.

In addition to the studies identified through database searches and the other sources outlined above, public comments submitted in response to the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023) included 944 studies, relevant to PFOA and/or PFOS, which were reviewed for relevance to the toxicity assessment. Of the 944 studies, 297 were duplicates of studies included in the toxicity assessment and 31 were duplicates of studies included in the 2016 PFOA or PFOS HESD assessment. The 599 studies that were not identified in the 2016 HESDs and were not included in the toxicity assessments underwent additional review identify studies with that could impact assessment conclusions as outlined in Appendix A.3 (U.S. EPA, 2024a). Ultimately, none of the 599 studies were incorporated in the toxicity assessments upon further screening. The submitted references were either deemed not relevant after secondary review, were supplemental studies (e.g., PFOA or PFOS assessments published by other scientific bodies, mechanistic, ADME, etc), or addressed non- priority health outcomes. The results of this screening can be found in the docket ("Review of Public Comment References Related to PFOA and PFOS Health Effects;" https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

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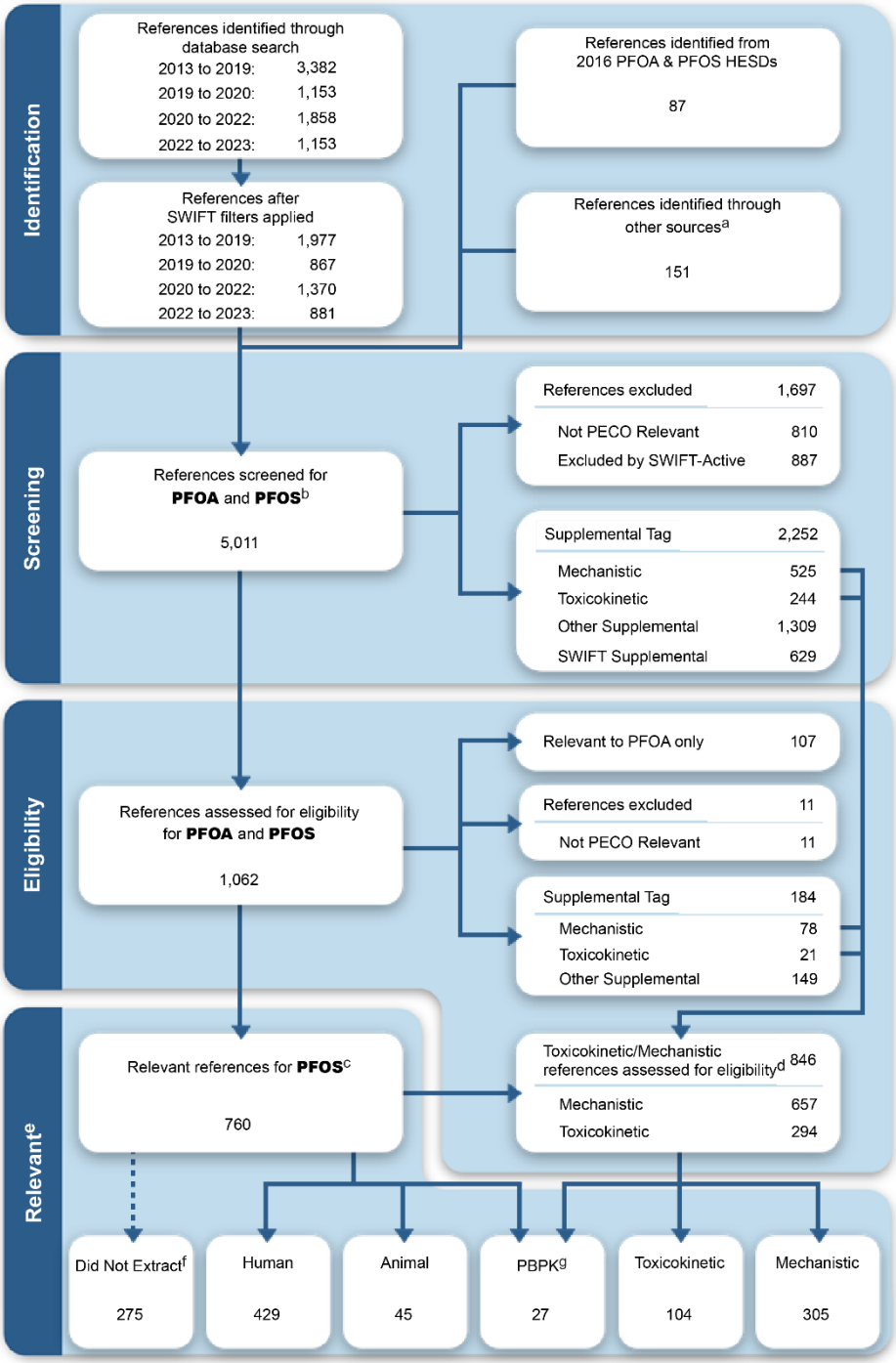


Figure 3-1. Summary of Literature Search and Screening Process for PFOS

Interactive figure and additional study details available on HAWC.
Interactive figure based on work by Magnuson et al. (2022).
"Other sources" include assessments published by other agencies, studies identified during mechanistic or toxicokinetic syntheses, and studies identified by the SAB.
[a] References identified by SAB and through database searches were counted as identified through database search only.
[b] Includes number of unique references after deduplication of studies captured with the SWIFT Review evidence streams filters and records identified from additional sources.
[c] Includes number of unique references considered to meet PECO eligibility criteria at the full-text level and include relevant information on PFOS.

[d] Includes number of unique references identified during title/abstract screening, full-text screening, and data extraction assessed for toxicokinetic and/or mechanistic eligibility.
[e] Only includes references with relevant information on PFOS.
[f] References tagged to 'Not a priority human health system' include those identified in the 2019 search that overlap with 2016 PFOS HESD references or those identified in 2022 and 2023 searches.
[g] Includes 11 PBPK references determined to meet PECO criteria plus an additional 16 PBPK references identified during the toxicokinetic screening.

3.1.1 Results for Epidemiology Studies of PFOS by Health Outcome

Of the 429 epidemiological studies that met the inclusion criteria and underwent extraction, 181 studies had a cohort study design, 169 had a cross-sectional design, 42 had a case-control design, and 37 had other study designs (e.g., nested case-control). Epidemiological studies were categorized into 18 health outcomes. Most studies reported on the developmental (n = 90), cardiovascular (n = 86), metabolic (n = 74), or immune systems (n = 66). Studies that reported outcomes spanning multiple health outcomes were not counted more than once in the grand totals shown in Figure 3-2.

	Study Design				
Health System	Case-control	Cohort	Cross-sectional	Other	**Grand Total**
Cancer	7	3	3	5	18
Cardiovascular	5	19	56	6	86
Dermal	0	1	0	0	1
Developmental	6	58	19	7	90
Endocrine	1	8	20	7	36
Gastrointestinal	1	4	0	0	5
Hematologic	0	0	8	0	8
Hepatic	1	4	18	2	25
Immune	6	32	19	9	66
Metabolic	7	32	31	4	74
Musculoskeletal	0	0	6	2	8
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	1	3	16	0	20
Reproductive, Male	0	7	15	2	24
Reproductive, Female	10	23	19	3	55
Respiratory	1	3	1	0	5
Other	0	2	3	0	5
Grand Total	42	181	169	37	429

Figure 3-2. Summary of Epidemiology Studies of PFOS Exposure by Health System and Study Design[a]

Interactive figure and additional study details available on HAWC.
[a] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.1.2 Results for Animal Toxicological Studies of PFOS by Health Outcome

Of the 45 animal toxicological studies that met the inclusion criteria and underwent extraction, most studies had either short-term (n = 19) or developmental (n = 15) study designs. Approximately equal numbers of studies were conducted in rats (n = 23) and mice (n = 21). The rat studies had short-term (n = 12), developmental (n = 7), chronic (n = 2), reproductive (n = 2), and subchronic (n = 1) study designs. The mouse studies had developmental (n = 8), short-term (n = 7), subchronic (n = 5), or reproductive (n = 1) study designs. The single monkey study used a chronic study design and the single rabbit study used a developmental study design. Animal toxicological studies were categorized into 13 health outcomes. Most studies reported results for the whole body (n = 25; i.e., systemic endpoints such as body weight), hepatic (n = 20), reproductive (n = 19), or developmental (n = 16) systems. Studies that reported outcomes spanning multiple health outcomes, study designs, or species were not counted more than once in the grand totals shown in Figure 3-3.

	Study Design & Species											
	Short-term		Subchronic		Chronic		Developmental			Reproductive		Grand Total
Health System	Mouse	Rat	Mouse	Rat	Monkey	Rat	Mouse	Rabbit	Rat	Mouse	Rat	
Cancer	0	0	0	0	0	1	0	0	0	0	0	1
Cardiovascular	1	2	2	0	1	1	0	0	2	0	1	10
Developmental	0	0	0	0	0	0	8	1	6	0	2	16
Endocrine	1	4	1	0	1	1	2	0	3	0	1	13
Hematologic	1	3	0	0	1	0	0	0	0	0	0	5
Hepatic	2	6	4	0	1	2	4	0	2	0	1	20
Immune	2	4	3	0	1	2	1	0	0	0	0	13
Metabolic	0	3	1	0	0	2	0	0	1	0	1	7
Nervous	2	6	1	0	0	1	1	0	2	0	1	14
Renal	0	3	3	0	1	2	2	0	0	0	0	10
Reproductive	2	3	1	1	1	0	4	1	3	1	2	19
Respiratory	1	1	1	0	0	0	0	0	0	0	0	3
Whole Body	4	7	4	1	1	2	2	1	2	0	2	25
Grand Total	7	12	5	1	1	2	8	1	7	1	2	45

Figure 3-3. Summary of Animal Toxicological Studies of PFOS Exposure by Health System, Study Design, and Species[a,b]

Interactive figure and additional study details available on HAWC.

[a] A study can report on more than one study design and species. Row grand totals represent the number of unique studies and are not a sum of study design and species tags.

[b] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.2 Data Extraction Results

All data from this project are available in the public HAWC (https://hawc.epa.gov/assessment/100500248/) site displayed as exposure-response arrays, forest plots, and evidence maps. Data extracted from the 429 epidemiological studies are available here. Data extracted from the 45 animal toxicological studies are available here. See Sections 3.4 and 3.5 for health outcome-specific data extracted for synthesis development. Additionally, the limited data extractions from the ADME and mechanistic studies can be found here and here, respectively.

3.3 Toxicokinetic Synthesis

As described in Section 3.1, EPA identified 104 and 27 studies containing information relevant to the toxicokinetics and PBPK modeling of PFOS, respectively. The results of these studies are described in the subsections below and additional information related to toxicokinetic characteristics of PFOS can be found in Appendix B (U.S. EPA, 2024a).

3.3.1 ADME

PFOS is resistant to metabolic and environmental degradation due to its strong carbon-fluorine bonds. It is not readily eliminated and can have a long half-life in humans and animals. However, the toxicokinetic profile and the underlying mechanism for the chemical's long half-life are not completely understood. For PFOS, membrane transporter families appear to play an important role in ADME, including organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), multidrug resistance-associated proteins (MRPs), and urate transporters. Transporters play a critical role in GI tract absorption, uptake by tissues, and excretion via bile and the kidney. Limited data are available regarding the transporters for PFOS; however, the toxicokinetic properties of PFOS suggest tissue uptake and renal resorption through facilitated uptake. Some inhibition studies suggest that PFOS transport could involve the same transporters as for PFOA, since PFOS and PFOA have similar chain lengths, renal excretion properties, and liver accumulation.

Animal studies indicate that PFOS is well-absorbed orally and distributes to many tissues and organs. High levels of PFOS are consistently observed in blood and liver. While PFOS can form as a degradation product or metabolite from other per- or polyfluoroalkyl substances, PFOS itself does not undergo further metabolism after absorption takes place. PFAS are known to activate peroxisome proliferator-activated receptor (PPAR) pathways by increasing transcription of genes related to mitochondrial and peroxisomal lipid metabolism, as well as sterol and bile acid biosynthesis. Given the transcriptional activation of many genes in PPARα-null mice, however, other gene products likely modify toxicokinetics of PFOS (Andersen et al., 2008).

3.3.1.1 Absorption

Absorption data are available in laboratory animals for oral (Chang et al., 2012) and inhalation (Rusch, 1979) exposures, and extensive data are available demonstrating the presence of PFOS in human serum. Limited in vitro absorption data are available (see Appendix B, (U.S. EPA, 2024a)).

Since PFOS is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across interface membranes was thought to be dominated by transporters or mechanisms other than simple diffusion across the lipid bilayer. Recent mechanistic studies, however, support transporter-independent uptake through passive diffusion processes. Ebert et al. (2020) determined membrane/water partition coefficients ($K_{mem/w}$) for PFOS and examined passible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. In this system, the partition coefficients were considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes.

Uptake by cells may be influenced by interactions with lipids and serum proteins. PFOS exhibited higher levels of binding to lipids and phospholipids relative to PFOA, which correlated with uptake into lung epithelial cells (Sanchez Garcia et al., 2018). Phospholipophilicity correlated to cellular accumulation better than other lipophilicity measures. The extent to which PFOS phospholipophilicity influences absorption through the GI tract, lungs, or skin is unknown.

While there are no studies available that quantify absorption in humans, extensive data on serum PFOS confirm uptake from the environment but do not establish an exposure route. Studies that provide the basis for human half-life estimates rely on changes in PFOS serum levels over time.

Bioavailability of PFOS after oral exposure is very high in rats. Serum PFOS concentrations after oral dosing were >100% of levels measured after intravenous (IV) dosing, which may reflect enterohepatic absorption that occurs after gavage but not IV administration (Huang et al., 2019; Kim et al., 2016).

3.3.1.2 Distribution

3.3.1.2.1 PFOS Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature regarding the distribution of PFOS in humans and animals are provided in the Appendix B (U.S. EPA, 2024a). Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. Distribution of PFAS to plasma has been reported to be chain length-dependent (Jin et al., 2016). Increasing chain length (from C6 to C11) correlated with an increased mass fraction in human plasma. Among different kinds of human blood samples, PFOS accumulates to highest levels in plasma, followed by whole blood and serum (Forsthuber et al., 2020; Poothong et al., 2017; Jin et al., 2016). Poothong et al. (2017) found that median PFOS concentrations in plasma, serum, and whole blood were 5.24, 4.77, and 2.85 ng/mL, respectively. These findings suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum (Ehresman et al., 2007) will not provide accurate estimates for PFOS.

PFOS is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOS interactions with human serum albumin (HSA) (Liu et al., 2017b; D'Alessandro et al., 2013; Salvalaglio et al., 2010; Chen and Guo, 2009; Zhang et al., 2009). In vitro analyses found that plasma proteins can bind PFOS in plasma from humans, cynomolgus monkeys, and rats (Kerstner-Wood et al., 2003). PFOS was highly bound (99.8%) to albumin and showed affinity for low-density lipoproteins (95.6%) with some binding to alpha-globulins (59.4%) and gamma-globulins (24.1%). HSA-PFOS intermolecular interactions are mediated through van der Waals forces and hydrogen bonds (Chen and Guo, 2009; Zhang et al., 2009). Beesoon and Martin (2015) determined that linear PFOS bound more strongly to calf serum albumin than the branched chain isomers in the order of 3m < 4m < 1m < 5m < 6m (iso) < linear. PFOS binding to HSA results in alterations in the albumin secondary structure and can diminish esterase activity (Liu et al., 2017b), though the extent to which this affects the physiological functions of albumin is unknown. PFOS-mediated conformational changes may also interfere with albumin's ability to transport its natural ligands and pharmaceuticals, including vitamin B_2 (riboflavin) and ibuprofen (D'Alessandro et al., 2013), and may interfere with PFOS uptake into cells (Sheng et al., 2020).

Binding to albumin and other serum proteins may affect transfer of PFOS from maternal blood to the fetus (Gao et al., 2019). Since there is effectively a competition between PFOS binding in maternal serum versus cord blood, lower cord blood albumin levels compared with maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017) found that a high concentration of cord serum albumin was associated with higher PFOS transfer efficiencies, whereas high maternal serum albumin concentration was associated with reduced transfer efficiency.

3.3.1.2.2 PFOS Binding to Intracellular Proteins and Transporters

Within cells, PFOS has been shown to bind to liver fatty acid binding protein (L-FABP) (Yang et al., 2020a; Zhang et al., 2013b; Luebker et al., 2002). L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators (Erol et al., 2004) and constitutes 2%–5% of the cytosolic protein in hepatocytes.

PFOS entry from serum into tissues appears to be controlled by several families of membrane transporters based on extrapolation from PFOA studies and several PFOS-specific studies. Yu et al. (2011) observed that PFOS exposure in rats increased hepatic OATP2 and MRP2 messenger ribonucleic acid (mRNA) expression. Transporters responsible for PFOS transport across the placenta are not well understood, though preliminary studies examining transporter expression identified OAT4 as a candidate receptor (Kummu et al., 2015). Thus far, no functional studies demonstrating a role for these transporters in PFOS uptake in liver or placenta have been identified.

3.3.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOS distributes to a wide range of tissues, organs, and matrices throughout the body. It should be noted, however, that autopsy and surgical tissues may not accurately reflect PFAS tissue distribution in the living body (Cao and Ng, 2021; Maestri et al., 2006). Blood and liver are major sites of PFOS accumulation (Olsen et al., 2001c). Two studies measured PFOS levels in cerebrospinal fluid and serum (Wang et al., 2018; Harada et al., 2007) and in both studies, PFOS levels in cerebrospinal fluid were two orders of magnitude lower than in serum, suggesting that PFOS does not easily cross the adult human blood-brain barrier. In a study of autopsy tissues collected within 24 hours of death, Pérez et al. (2013) found PFOS in the liver (104 ng/g), kidney (75.6 ng/g), lung (29.1 ng/g), and brain (4.9 ng/g), with levels below the limit of detection (LOD) in bone. Another study of post-mortem tissues found varying PFOS levels in different tissues ranging from 1.0 ng/g in skeletal muscle to 13.6 ng/g in liver. PFOS was also detected in brain and basal ganglia, endocrine organs (pituitary, thyroid, pancreas), liver, kidney, and adipose tissue (Maestri et al., 2006). PFOS also accumulates in follicular fluid (Kang et al., 2020) and gonads (Maestri et al., 2006), raising the possibility of reproductive toxicity in humans.

Studies of tissue distribution are available for several species of animals including non-human primates, rats, and mice. Studies of non-human primates indicate PFOS accumulates in serum in a dose-dependent manner (Chang et al., 2017; Seacat et al., 2002). Limited data on liver accumulation of PFOS in monkeys show that PFOS levels in liver were similar or slightly lower than serum levels. Several rodent studies identified high levels of PFOS in blood and liver across a range of dosing regimens and study durations. Whereas monkeys had nearly a 1:1 liver to

serum ratio, rodent models were observed to accumulate far more PFOS in liver than serum (NTP, 2019). Additional studies in rats and mice documented PFOS distribution to a wide range of tissues including kidney, heart, lungs, and spleen. Interestingly, in rodents, PFOS has been measured in moderate quantities in the brain and testicles, indicating that PFOS does cross the blood-brain and blood-testis barriers in rats (Qiu et al., 2013) and mice (Bogdanska et al., 2011; Cui et al., 2009). In fact, one study in rats (Wang et al., 2015a) observed higher PFOS levels in the hippocampus than in serum measured on PND 1 in prenatally exposed rats. Plasma PFOS concentrations were generally similar in males and females. For example, in a 28-day toxicity study, dose-normalized plasma concentrations (μM/mmol/kg/day) in males and females were within 1.5-fold across the dose groups (NTP, 2019). However, some sex-dependent differences in PFOS levels were observed in rodents that varied by species, lifestage, and dose duration (Zhong et al., 2016; Curran et al., 2008; Thomford, 2002b).

3.3.1.2.4 Distribution During Reproduction and Development

Several studies in humans, rats, and mice quantified distribution of PFOS from pregnant females to placenta, cord blood, and amniotic fluid, which demonstrate pathways of distribution to and elimination from fetuses. Accumulation of PFOS in fetal tissues was found to vary by gestational age. New studies also confirm that distribution of PFOS from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding. Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching.

The ratio of PFOS in placenta relative to maternal serum (R_{PM}) ranged from 0.048 to 0.749 (Chen et al., 2017a; Zhang et al., 2013c). Zhang et al. (2015b) observed differential accumulation of PFOS based on branching characteristics. Specifically, R_{PM}s of branched PFOS isomers increased with distance of branching points away from the sulfonate group in the order of iso-PFOS < 4m-PFOS < 3 + 5m-PFOS < 1m-PFOS. Mamsen et al. (2019) demonstrated that gestational age can affect PFOS concentrations in maternal serum and placentas, estimating a placental PFOS accumulation rate of 0.13% per day during gestation.

Several studies reported a strong positive correlation between maternal and cord serum levels of PFOS (Kato et al., 2014; Porpora et al., 2013). The ratio of PFOS in cord serum relative to maternal serum ranged from 0.22 to 0.98 (see Appendix B, (U.S. EPA, 2024a)) and generally increased with gestational age (Li et al., 2020a). Li et al. (2020a) also showed a 6% increase in branched PFOS accumulation compared with linear PFOS isomers. Zhao et al. (2017) observed higher transplacental transfer efficiencies (TTEs) for 1m-, 4m-, 3 + 5m-, and m2-PFOS compared with n-PFOS. Together, these findings indicate that branched isomers of PFOS transfer more efficiently from maternal blood to cord blood compared with linear isomers. In addition to PFOS branching, maternal factors including exposure sources, parity, and other maternal demographics are postulated to influence observed variations in cord:maternal serum ratios (Brochot et al., 2019; Eryasa et al., 2019; Jusko et al., 2016).

Lower PFOS concentrations were measured in amniotic fluid compared with placenta and cord blood (Zhang et al., 2013c). The mean concentration ratio between amniotic fluid and maternal blood (AF:MB) was lower for PFOS (0.0014) than for PFOA (0.13). The mean concentration ratio between amniotic fluid and cord blood (AF:CB) was lower for PFOS (0.0065) than for PFOA (0.023). Authors attributed the differences in ratios between the two compartments to the

solubilities of PFOS and PFOA and their respective protein binding capacities in the two matrices.

PFOS also distributes widely in fetal tissues. Mamsen et al. (2017) measured the concentrations of five PFAS in fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark. The concentration of PFOS decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. In a second study, PFAS levels were measured in embryos and fetuses at gestational weeks 7–42 and in serum from their matched maternal pairs (Mamsen et al., 2019). PFOS accumulated at higher levels in fetal tissues compared with other PFAS chemicals examined in fetal tissues and across trimesters. The concentration of PFAS in fetal tissues fluctuated across trimesters and did not follow any particular trend. For example, PFOS concentration in the liver was higher in the second trimester compared with the third trimester, and lowest in the lung in the second trimester compared with the first and third trimesters.

New studies also confirm that distribution of PFOS from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding (Gyllenhammar et al., 2018a; Cariou et al., 2015; Mogensen et al., 2015b; Mondal et al., 2014). Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching. In the Mondal study (Mondal et al., 2014), mean maternal serum PFOS concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOS than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding (Mogensen et al., 2015b; Mondal et al., 2014). Cariou et al. (2015) reported that PFOS levels in breastmilk were approximately 66-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOS was 0.38 ± 0.16. The authors noted that the transfer rates of PFAS from serum to breastmilk were lower compared with other lipophilic persistent organic pollutants such as polychlorinated biphenyls.

Developmental studies in rodents confirmed PFOS distribution from rat and mouse dams to fetuses and pups, as well as variable PFOS level across many fetal tissues (Ishida et al., 2017; Chen et al., 2012b; Zeng et al., 2011; Borg et al., 2010; Chang et al., 2009; Liu et al., 2009; Luebker et al., 2005a).

3.3.1.2.5 Volume of Distribution in Humans and Animals

In humans, a single volume of distribution (V_d) value of 239 mL/kg has been uniformly applied for most PFOS studies (Thompson et al., 2010a). Gomis et al. (2017) used a V_d of 235 mL/kg by averaging V_d values estimated for both humans and animals. V_d values may be influenced by differences in distribution between males and females, between pregnant and non-pregnant females, and across serum, plasma, and whole blood.

V_d estimates derived in monkeys, mice, and rats vary by species, age, sex, and dosing regimen. For example, Huang et al. (2019) calculated the apparent volume of central and peripheral distribution in rats. In this study, a two-compartment model was the best fit for male rats for both IV and gavage routes of administration and females dosed by the IV route, whereas a one-compartment model was the best fit for female rats dosed by oral gavage. V_d values in females after IV administration were lower than that observed in males in both the central and peripheral compartments. For the oral route, striking sex differences were noted between the central and peripheral compartments. While V_d values were quite similar in males for both compartments,

they were notably higher in the central compartment compared with the peripheral compartment in females. Interestingly, another study found that for PFOS, a classical compartment model was not applicable (Iwabuchi et al., 2017). Rather, the body organs behaved as an assortment of independent one-compartments with a longer elimination half-life in liver than serum in the elimination phase. Further discussion on the V_d for PFOS can be found in Section 5.6.2.

3.3.1.3 Metabolism

Consistent with other reports and reviews (ATSDR, 2021; Pizzurro et al., 2019; U.S. EPA, 2016b), the literature reviewed for this assessment do not provide evidence that PFOS is metabolized in humans, primates, or rodents.

3.3.1.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Most studies have investigated the elimination of PFOS in humans, cynomolgus monkeys, and rats. Available evidence supports urine as the primary route of excretion in most species, though fecal elimination is prominent in rats. In rats, hair is another route of elimination in both males and females. In females, elimination pathways include menstruation, pregnancy (cord blood, placenta, amniotic fluid, and fetal tissues) and lactation (breast milk) (see Appendix B, (U.S. EPA, 2024a)).

3.3.1.4.1 Urinary and Fecal Excretion

Urinary excretion is considered the main route of PFOS excretion in humans. Zhang et al. (2015b) estimated a daily urinary excretion rate of 16% of the estimated total daily intake for PFOS for adults. Zhang et al. (2013d) calculated median renal clearance rates of 0.044 mL/kg/day in young women and 0.024 mL/kg/day in men and older women for total PFOS. In a later study, Fu et al. (2016) estimated a urinary clearance rate 0.010 mL/kg/day (geometric mean for men and women). These studies showed that PFOS daily renal clearance values were significantly lower in males compared with females.

Several studies in rats suggest that the fecal route is as or more important than the urinary route of excretion for PFOS. In a study by Chang et al. (2012), excretion in urine and feces were approximately equivalent when examined 24 and 48 hours after oral gavage administration of ^{14}C-PFOS. A study by Kim et al. (2016) measured the amounts of unchanged PFOS excreted into the urine and the feces of male and female Sprague-Dawley rats for 70 days after a single dose of 2 mg/kg by oral or IV administration (Kim et al., 2016). PFOS levels in urine and feces were similar in both males and females, which correlated to similar half-life estimates for PFOS (26.44 and 28.70 days in males and 23.50 and 24.80 days in females by the oral and IV routes, respectively).

In summary, evidence supports excretion through the fecal route in both animals and humans. Human studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. In rats, however, both urinary and fecal routes play prominent roles in PFOS elimination. There are sex-specific differences in fecal excretion of PFOS. Excretion through the fecal route appears to be more efficient in males compared with females. Also, in male rats, fecal and urinary concentrations were similar after oral but not IV dosing. Finally, exposures to mixtures of PFAS suggest that PFOS in the context of a mixture may be preferentially excreted

through the fecal route. The extent to which resorption by hepatic and enteric routes impacts fecal excretion has not been established in either humans or animals.

3.3.1.4.2 Enterohepatic Resorption

Early evidence of enterohepatic resorption of PFOS was revealed by Johnson et al. (1984), who demonstrated that cholestyramine (CSM) treatment increased mean cumulative ^{14}C elimination in feces by 9.5-fold for male CD rats administered 3.4 mg/kg ^{14}C-PFOS. CSM is a bile acid sequestrant, and its facilitation of PFOS gastrointestinal clearance suggests enterohepatic circulation.

Several studies present evidence of enterohepatic excretion and potential resorption in humans (Genuis et al., 2010; Harada et al., 2007). Harada et al. (2007) estimated a biliary resorption rate of 0.97, which could contribute to the long half-life in humans. Genuis et al. (2010) described a case report of excretion analyzed after inhalation PFOS exposure. After treatment with a bile acid sequestrant CSM for 1 week, PFOS serum levels decreased from 23 ng/g to 14.4 ng/g. Additionally, stool PFOS concentrations increased from undetectable before treatment (LOD = 0.5 ng/g) to 9.06 and 7.94 ng/g in the weeks after treatment, suggesting that it may help with removing PFOS that gains access to the GI tract via bile.

Zhao and colleagues (Zhao et al., 2017; 2015) evaluated enterohepatic transporters identified in liver hepatocytes and intestinal enterocytes in humans and rats. Using in vitro transfection assays, PFOS was found to be a substrate of both sodium-dependent and -independent enterohepatic transporters involved in recirculation of bile acids. With the exception of rat apical sodium-dependent bile salt transporter (ASBT), PFOS was demonstrated to be a substrate for all tested transporters (sodium/taurocholate cotransporting polypeptide (NTCP), OATP1B1, OATP1B3, OATP2B1) as well as organic solute and steroid transporter alpha/beta. Binding efficiency to the enterohepatic transporters was chain-length dependent. NTCP transported PFAS with decreasing affinity but increasing capacity as the chain length increased (Zhao et al., 2015). The opposite trend was seen for OATP-mediated uptake (Zhao et al., 2017). While these in vitro studies demonstrate that PFOS is a substrate of enterohepatic transporters found in the livers and intestines of humans and rats, it is as yet unknown whether and to what extent these transporters function *in vivo*.

Studies describing renal resorption are discussed in Appendix B (U.S. EPA, 2024a).

3.3.1.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

PFOS can readily pass from mothers to their fetuses during gestation and through breast milk during lactation. In conjunction with elimination through menstruation discussed in Section 3.3.1.4.4, females may eliminate PFOS through routes not available to males. The total daily elimination of PFOS in pregnant females was estimated to be 30.1 ng/day, higher than the 11.4 ng/day for PFOA (Zhang and Qin, 2014). The ratio of branched:total PFOS isomers in cord blood was 0.27 and was higher in cord blood compared with maternal blood and placenta. These findings suggest branched PFOS isomers may transfer to the fetus more readily than linear forms. In another study in humans (Zhang et al., 2013c), the mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.1%, respectively, of levels found in the mother's blood, demonstrating that cord blood, placenta, and amniotic fluid are additional routes of elimination in pregnant females. Blood loss during childbirth could be another source of

excretion. Underscoring the importance of pregnancy as a lifestage when excretion is altered, Zhang et al., (2015a) observed that the partitioning ratio of PFOS concentrations between urine and whole blood in pregnant women (0.0004) was lower than the ratio found in non-pregnant women (0.0013) and may be affected by the increase in blood volume during pregnancy (Pritchard, 1965).

Mamsen and colleagues (2017) measured placental samples and fetal organs in relation to maternal plasma levels of five PFAS in 39 Danish women (Mamsen et al., 2017). Fetal organ levels of PFOS were lower than in maternal blood. The average concentration of PFOS was 0.6 ng/g in fetal organs compared with 1.3 ng/g in the placenta and 8.2 ng/g in maternal plasma. Increasing fetal PFOS levels with fetal age suggest that the rate of elimination of PFOS from mother to fetus may increase through the gestational period.

After birth, women can also eliminate PFOS via lactation (Lee et al., 2017; Thomsen et al., 2011; Tao et al., 2008) and it was shown that PFOS levels in breastmilk are affected by parity (Lee et al., 2017; Jusko et al., 2016). In one study, mean PFOS concentrations were 3.67, 1.38, and 0.040 ng/mL in maternal serum, cord serum, and breast milk, respectively (Cariou et al., 2015). The observed ratio of cord serum and maternal serum for PFOS was 0.38 in this study, much lower than the ratio of 0.78 for PFOA. However, the ratio between breast milk and maternal serum was 0.038, essentially the same as PFOA. Thus, PFOS exhibits a low transfer from maternal blood to cord blood and a 10-fold lower transfer from maternal blood to breast milk.

3.3.1.4.4 Other Routes of Elimination

Menstruation may be an important factor in the sex-specific differences observed in PFOS elimination. Wong et al. (2014) estimated that menstrual serum loss is 432 mL/year, which could account for >30% of the difference in the elimination half-life between females and males.

Two studies supported an association between increased serum concentrations of PFOA and PFOS and early menopause (Taylor et al., 2014; Knox et al., 2011). However, a re-analysis of these data (Ruark et al., 2017) suggested that this association could be explained by reverse causality and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Also challenging the assumption that this is due to menstruation, Singer et al. (2018) failed to find evidence of associations between menstrual cycle length and PFAS concentrations. Furthermore, Lorber et al. (2015) suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may also help explain the differences in elimination rates between males and females. Studies providing direct measurements of PFOS in menstrual blood were not identified. However, for PFOS to be selectively retained from the blood lost through menstruation would require a specific mechanism for that process and no such mechanism has been demonstrated or proposed.

Gao et al. (2015) found that hair is a potential route of PFAS elimination in rats. A dose-dependent increase in hair PFOS concentration was observed in all exposed animals. PFOS did not exhibit the sexual dimorphic pattern in hair noted for PFOA. While hair PFOS levels were lower in males compared with females in the low dose group, there were no significant differences in hair PFOS concentrations between males and females in the higher dose groups.

3.3.1.4.5 Half-Life Data

There have been several studies of half-lives in humans all supporting a long residence time for serum PFOS with estimates measured in years rather than months or weeks (see Appendix B, (U.S. EPA, 2024a)). Because there is no evidence that PFOS is metabolized in mammals, half-life determinations are governed by excretion. The calculated PFOS half-lives reported in the literature vary considerably, which poses challenges in predicting both the routes and rates of excretion. Half-life estimates vary considerably by species, being most rapid in rodents (measured in hours to days), followed by primates (measured in days to weeks) and humans (measured in years). Half-life estimates were shorter in human females relative to males, but sex differences were less clear in animal studies.

Human PFOS half-life estimates range from less than 1 year in a single male child of 16 years (Genuis et al., 2014) to up to 60.9 years for males occupationally exposed in a facility in China (Fu et al., 2016) (see Appendix B, (U.S. EPA, 2024a)). With one exception (Genuis et al., 2014), half-lives estimated for males are longer than those estimated for females and show an age-related increase (Zhang et al., 2013d). Also, linear isomers exhibit longer half-lives than branched isomers (Xu et al., 2020c; Zhang et al., 2013d). While most studies were conducted in adults and/or adolescents, at least one study estimated a PFOS half-life of 4.1 years in newborns (Spliethoff et al., 2008).

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOS half-lives along with measured intake and serum and urine PFOS concentrations (Xu et al., 2020c; Worley et al., 2017a; Fu et al., 2016; Zhang et al., 2013e) (see Appendix B, (U.S. EPA, 2024a)). PFOS half-life values among these four studies varied dramatically from 1.04 years in Xu et al. (2020c) to 60.9 years in Fu et al. (2016). These comparisons support principles suggested by the broader literature. First, sex related differences with males exhibiting much longer half-lives compared with females which may, at least in part, relate to menstruation as an important route of elimination in females (especially females of reproductive age) may relate, at least in part, to menstruation as an important route of elimination. Second, Xu et al. (2020c) suggest that linear PFOS molecules exhibit longer half-lives than branched forms, which may reflect differential affinities of linear versus branched forms for resorption transporters. Third, the relationships between blood and urine concentrations are not obvious, underscoring the role of non-urinary routes of excretion and the difficulty in measuring renal resorption. Finally, only two studies estimated PFOS intake in subjects (Xu et al., 2020c; Worley et al., 2017a). Altogether, there is insufficient data to correlate PFOS intake measurements to serum/plasma and urine concentrations. These factors, as well as age and health status of subjects, likely contribute to the variability in PFOS half-life estimates in humans.

In animals, half-life values are reported in days rather than in years. Values in cynomolgus monkeys ranged from 88 to 200 days (Chang et al., 2012; Seacat et al., 2002) and were generally longer than those observed in rodents, but much shorter than values observed in humans. Depending on the experimental conditions, half-lives in rats ranged from 14.5 to 43 days (Huang et al., 2019; Kim et al., 2016; Chang et al., 2012). In contrast to sex-specific differences in half-lives for PFOA, PFOS half-lives showed only minor differences between males and females.

3.3.2 Pharmacokinetic Models

Pharmacokinetic (PK) models are tools for quantifying the relationship between external measures of exposure and internal measures of dose. For this assessment, PK models were evaluated for their ability to allow for 1) cross-species PK extrapolation of animal studies of both cancer and noncancer effects and 2) the estimation of the external dose associated with an internal dose metric that represents the POD calculated from animal toxicological or epidemiological studies. The following sections first describe and evaluate published PK modeling efforts and then present conclusions from analyses that assessed the utility of the models to predict internal doses for use in dose-response assessment.

Numerous PK models for PFOS have been developed and published over the years to characterize the unique ADME described in Section 3.3.1. These approaches can be classified into three categories: classical compartmental models, modified compartmental models, and PBPK models. With classical compartmental modeling, the body is defined as either a one- or two-compartment system with volumes and intercompartmental transfer explicitly fit to the available PFAS PK dataset. Modified compartmental models are more physiologically based in that they attempt to characterize unique aspects of in vivo ADME through protein binding, cardiac output, and known renal elimination from the published literature. However, these models still rely on explicit fitting of data to the non-physiological parameters. Finally, PBPK models describe the tissues and organs of the body as discrete, physiologically based compartments with transport between compartments informed by available data on the physiologically relevant quantifications of blood flow and tissue perfusion. Determining additional, non-physiological parameters typically requires explicitly fitting the PBPK model to time-course concentration data. However, the number of parameters estimated through data fitting is generally fewer than for classical PK or modified compartmental models. A review of the available PK models regarding their ability to predict PFOS ADME is provided below.

3.3.2.1 Classical Compartmental Analysis

The most common approach for the prediction of serum levels of PFOS is to apply a relatively simple one-compartment model. This type of model describes the toxicokinetics of the substance with a single differential equation that describes the rate of change in the amount or concentration of the substance over time as a function of the exposure rate and the clearance rate. This type of model describes the relationship between exposure, serum concentration, and clearance and can be used to predict one of these values when the other two values are set. Additionally, because the model can produce predictions of changes in exposure and serum concentration over time, these models can be applied to fill the temporal gaps around or between measured serum concentrations or exposures.

Some examples of one-compartment models used to predict human exposure from serum concentrations include the work of Dassuncao et al. (2018) who used a model to describe historical changes in exposure in seafood and consumer products, Hu et al. (2019b) who used paired tap water and serum concentration to estimate the proportion of total exposure that originates from drinking water, and Balk et al. (2019) who used measured concentrations in drinking water, dust and air samples, and serum concentrations in developing children (measured at several time points) to assess the relative proportion of exposure that originates from dietary exposure. Zhang et al. (2019) performed a similar study using community tap water

measurements and serum concentrations to estimate the proportion of PFOS exposure that originates from drinking water.

Other applications are used to better understand the toxicokinetics of PFOS in humans by combining estimated exposure values and serum values to estimate clearance and half-life in a population of interest. One example of this type of model application was presented by Worley et al. (2017a) who estimated the half-life of PFOS using exposure predicted from drinking water PFAS concentration in a community with contaminated drinking water. Fu et al. (2016) used paired serum and urine samples from an occupational cohort to estimate the half-life separately from renal clearance (in urine) and in the whole body (in serum). One of the largest challenges in the estimation of half-life is the problem of estimating exposure to PFOS.

One common modification of the one-compartment model is to perform a "steady-state approximation" (i.e., to assume that the rate of change of the serum concentration is zero). This scenario occurs when an individual experiences constant exposure, constant body habitus, and constant clearance over a timespan of several half-lives. Because of the long half-life of PFOS, steady state is a reasonable assumption for adults starting from the age of 25 and above. However, the steady state approximation cannot be applied for ages younger than 21 years of age (EPA defines childhood as <21 years of age; (U.S. EPA, 2021a)) due to ongoing development during childhood and adolescence. This growth dilutes the concentration of the chemical in the body and results in lower levels than would be seen in its absence. Even though pubertal development including skeletal growth typically ends several years prior to the age of 25, there is a period after growth ceases during which PFOS levels increase until the adult steady-state level is reached. The general acceptability of the steady-state assumption in adults has the caveat that pregnancy or breastfeeding will result in changes in serum concentration and will not be accounted for in the steady-state approximation.

When adopting a steady-state assumption, the rate of change in serum levels over time is zero. It follows that the ratio between exposure to the substance and clearance determines the serum concentration. This is the approach used in the 2016 PFOS HESD to determine the constant exposure associated with a serum concentration (U.S. EPA, 2016b). A similar approach was used in the recent toxicity assessment performed by CalEPA (CalEPA, 2021). Publications reporting applications of similar models include the work of Zhang et al. (2015b) who used paired urine and serum data to estimate the total intake of PFOS and compared it to the rate of urinary elimination, and Lorber et al. (2015) who examined the effects of regular blood loss due to phlebotomy on PFOS levels and extrapolated that finding to clearance via menstruation.

In animals, two classical PK models for PFOS have been published since the 2016 PFOS HESD. In Huang et al. (2019), male and female Sprague-Dawley rats were dosed via oral gavage at 2 or 20 mg/kg, through multiple administrations of PFOS at 2 mg/kg/day for five days, or intravenously at 2 mg/kg. Following the administration of PFOS, rats were sacrificed from 5 minutes up to 140 days post-dosing to characterize the biphasic PK curve. Using plasma data from these exposure scenarios, Huang and coworkers developed a two-compartment model to characterize PK parameters of interest such as the alpha- and beta-phase half-life, central and peripheral compartment volumes, and total PFOS clearance. For each dosing scenario, a single set of PK parameters were fit, making extrapolation to other dosing scenarios difficult. However, the authors demonstrate no significant difference between males and females in beta-phase half-

life and overall clearance which is in agreement with previous studies of PFOS PK in rats (Kim et al., 2016).

Gomis et al. (2017) utilized the functional form of a two-compartment model with oral gavage to predict internal dosimetry of PFOS in rats using PK data from Seacat et al. (2003). However, because the scope of the Gomis et al. (2017) study involved predicting internal dose points-of-departure, PK parameters are not presented.

3.3.2.2 Modified Compartmental Models

In addition to the common one-compartment models described above, several models for humans have been developed to extend the simple one-compartment model to describe the PK during pregnancy and lactation. The key factors that must be introduced into the model are the changes in body habitus that occur during pregnancy (e.g., increases in blood plasma volume and body weight), the distribution and transfer of the substance between the maternal and fetal tissues, the transfer from the mother to the infant during nursing, and postnatal development, including growth of the infant during the early period of life. The mathematical formulation of this type of model requires two differential equations, one describing the rate of change in amount or concentration in the mother and one describing the rate of change in infants. One such developmental model with a lactational component was used to predict the maternal serum concentrations and exposure from measurements of PFOS concentrations in breast milk (Abdallah et al., 2020). Verner et al. (2016) presented another developmental model to predict PFOS serum concentrations in the mother and child and predict previous exposure using mother/child paired serum measurements at different times. This model included all the key aspects previously mentioned for developmental PK models. Another unique approach that extended the one-compartment framework was a publication by Shan et al. (2016), who estimated the exposure to specific isomers of PFOS using measurements in food, tap water, and dust to estimate the isomeric profiles of the substances in human serum.

Pharmacokinetic models that can accommodate longer half-life values than would be predicted based on standard ADME concepts and allow for dose-dependent changes in excretion rate compared with the classic 1- or 2- compartment approaches have been published as tools to estimate internal doses for humans, monkeys, mice, and rats (Chou and Lin, 2019; Loccisano et al., 2013; Wambaugh et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011; Andersen et al., 2006). The underlying assumption for all the models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

One of the earliest PK models (Andersen et al., 2006) was developed for PFOS using two dosing situations in cynomolgus monkeys. In the first, three male and three female monkeys received a single IV dose of potassium PFOS at 2 mg/kg (Noker and Gorman, 2003). For oral dosing, groups of four to six male and female monkeys were administered daily oral doses of 0, 0.03, 0.15, or 0.75 mg/kg PFOS for 26 weeks (Seacat et al., 2002). This model was based on the hypothesis that saturable resorption capacity in the kidney would account for the unique half-life properties of PFOS across species. The model structure was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells.

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption and transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOS concentrations than at low concentrations. In addition to decreased renal excretion due to the renal resorption, excretion is also reduced in the model by implementing a constant proportion of PFOS that is bound to protein in plasma and is not available for renal filtration.

The model was parameterized using the body weight and urine output for cynomolgus monkeys (Butenhoff et al., 2004) and a cardiac output of 15 L/h-kg from the literature (Corley et al., 1990). A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were assumed or optimized to fit the PK data for monkeys. In the IV time-course data, some time and/or dose-dependent changes occurred in distribution of PFOS between the blood and tissue compartments, and these changes were less noticeable in the females; therefore, only the female data were used. The simulation captured the overall time-course scenario but did not provide good correspondence with the initial rapid loss from plasma and the apparent rise in plasma concentrations over the first 20 days. For oral dosing, the 0.15 mg/kg dose simulation was uniformly lower, and the 0.75 mg/kg dose simulation was higher than the data. When compared with PFOA, PFOS had a longer terminal half-life and more rapid approach to steady-state with repeated oral administration.

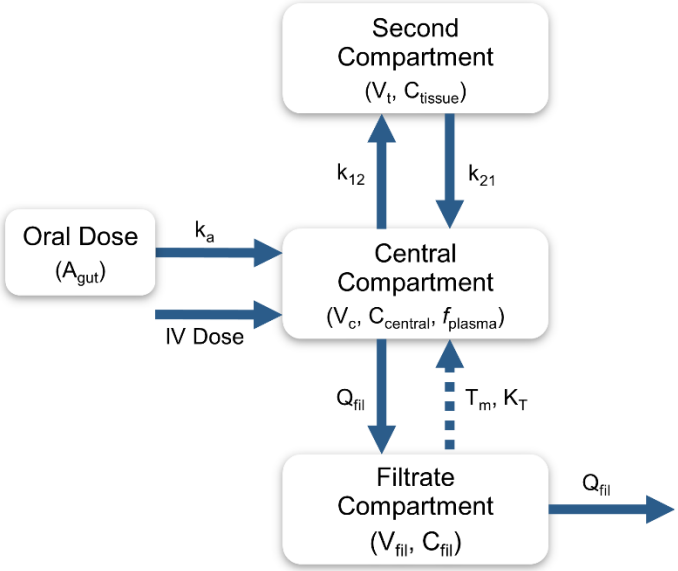


Figure 3-4. Schematic for a Physiologically Motivated Renal Resorption PK Model

Adapted from Wambaugh et al. (2013).

Building on the work of other researchers, Wambaugh et al. (2013) developed and published a PK model to support the development of an EPA RfD for PFOS (U.S. EPA, 2016b). The model was applied to data from studies conducted in monkeys, rats, or mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption term was used. This concept has played a fundamental role in the design of all of

the published PFOS models summarized in this section. The model structure is depicted in Figure 3-4 (adapted from Wambaugh et al. (2013)).

Wambaugh et al. (2013) placed bounds on the estimated values for some parameters of the Andersen et al. (2006) model to support the assumption that serum carries a significant portion of the total PFOS body load. The Andersen et al. (2006) model is a modified two-compartment model in which a primary compartment describes the serum and a secondary deep tissue compartment acts as a specified tissue reservoir. Wambaugh et al. (2013) constrained the total V_d such that the amount in the tissue compartment was not greater than 100 times that in the serum. As a result, the ratio of the two volumes (serum vs. total) was estimated in place of establishing a rate of transfer from the tissue to serum, but the rate of transfer from serum to tissue was also estimated from the data. A nonhierarchical model for parameter values was also assumed. Under this assumption, a single numeric value represents all individuals of the same species, sex, and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters varied for different studies. Measurement errors were assumed to be log-normally distributed. Table 4-3. in Section 4.1.3.1.1 provides the estimated and assumed PK parameters applied in the Wambaugh et al. (2013) model for each of the species evaluated.

The PK data that supported the Wambaugh et al. (2013) analysis were derived from two in vivo PFOS PK studies. The monkey PK data were derived from Seacat et al. (2002) and Chang et al. (2012). Data for the rats (male/females) and mice were both from Chang et al. (2012). The data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and sexes and to identify serum levels associated with the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) external doses. Prior distributions for the parameters were chosen to be broad, log-normal distributions, allowing the fitted parameters to be positive and for the posterior distribution to be primarily informed by the data likelihood rather than by the priors.

3.3.2.3 PBPK Models

An alternative approach to the use of a classical or modified compartmental model is a PBPK model, which describes the changes in substance amount or concentration in a number of discrete tissues. One of the main advantages of a PBPK model are the ability to define many parameters based on physiological data, rather than having to estimate them from chemical-specific data. Such physiological parameters include, for example, organ volumes and the blood flow to different organs; they can be measured relatively easily and are chemical independent. Another advantage is that amount and concentration of the substance can be predicted in specific tissues, in addition to blood. This can be valuable for certain endpoints where it is expected that a tissue concentration would better reflect the relevant dosimetry compared with blood concentration.

The first PBPK model developed for PFOS was reported in a series of publications by Loccisano et al., which together describe the PK of PFOS in rats, monkeys, and humans, in both adult and developmental (for rat and human) scenarios (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011). These models were developed based on an earlier “biologically motivated” model that served as a bridge between a one-compartment model and PBPK by implementing a tissue compartment (similar to a two-compartment model), an absorption

compartment, and a renal filtrate compartment with saturable renal resorption (Tan et al., 2008). The work of Tan et al. (2008) was a development of the earlier work of Andersen et al. (2006) previously discussed. The PBPK model of Loccisano and colleagues then extended this "biologically motivated" model by the addition of discrete tissue compartments, rather than a single compartment representing all tissues.

A series of follow-up studies applied the Loccisano and coauthors' model structure, with extensions, to address how PK variation in human populations could bias the result of the study. This consisted of the work of Wu et al. (2015) who developed a detailed model of adolescent female development during puberty and menstrual clearance of PFOS to investigate the interaction between chemical levels and the timing of menarche, Ruark et al. (2017) who added a detailed description of menopause to evaluate how that affects serum levels and the epidemiological association between early menopause and PFOS levels, Ngueta et al. (2017) who implemented a reduction in menstrual clearance in individuals using oral contraceptives and the interaction between oral contraceptive use, endometriosis, and serum PFOS levels, and Dzierlenga et al. (2020b; 2020c) who applied a model of thyroid disease (Dzierlenga et al., 2019) to describe changes in PFOS renal clearance due to disease state.

In addition to this set of studies, Fabrega et al. (2014) updated the model of Loccisano et al. (2013) for humans by modeling a human population using regional food and drinking water measurements and human tissue data collected from cadavers in a region of Spain. The use of human tissue data is relatively rare due to the challenges in sourcing human tissue but may prove preferable to the assumption that human distribution is similar to distribution in an animal model. However, Fabrega et al. (2014) estimated their tissue to blood partition coefficients from the ratio of tissue concentrations in the cadavers to the average serum concentrations in live volunteers who lived in the same region but were sampled several years earlier (Ericson et al., 2007) and they provided no details on how their renal resorption parameters were estimated from the human blood concentrations. This model was further applied to a population in Norway and extended to other PFAS (Fàbrega et al., 2015).

Brochot et al. (2019) presented the application of a PBPK model for PFOS with gestation and lactation phases to describe development and predicted maternal, infant, and breastmilk concentrations over a variety of scenarios including the prediction of maternal levels across multiple pregnancies.

One of the major challenges in the parameterization of PBPK models for PFOS is the estimation of the chemical-dependent parameters such as those involved in protein binding and renal clearance. One way to investigate this issue is to perform in vitro experiments to help inform the parameters. Worley et al. (2017b) used in vitro measurements of renal transporter activity to describe in detail the various steps involved in the renal filtration, resorption, and excretion of PFOS.

Chou and Lin (2019) developed a PFOS PBPK model for rat, mouse, monkey, and human. Using the model structure of Worley and Fisher (2015), parameters were determined using a hierarchical Bayesian framework to pool datasets across studies for each species. This model reflects saturable resorption in the proximal tubule cells of the kidney and fecal elimination through the bile. While the Bayesian approach is ideal for handling multiple datasets, the method for implementing the Bayesian inference raises questions about the final posterior parameter

distributions. Priors for the hierarchical model were determined using a least-squares fitting method on the most sensitive parameters as opposed to defining priors using information from previous studies and letting the data update those priors to determine the joint posterior distribution of the parameter space. In a subsequent study, Chou and Lin (2021) added a gestation/lactation element to the model and parameterized the gestation/lactation components for rats and humans. This model structure used a three-compartment fetal model during gestation and a physiologically motivated PK model, similar to Wambaugh et al. (2013) with renal resorption, for the infant. Using this model, the authors developed human equivalent doses (HEDs) using interspecies extrapolation of the average serum concentration POD derived from the rat model. While the fits demonstrated good agreement with the evaluation dataset, parameters for only the rat are available for developmental endpoints.

3.4 Noncancer Health Effects Evidence Synthesis and Integration

3.4.1 Hepatic

EPA identified 24 epidemiological studies (30 publications)[5,6] and 25 animal toxicological studies that investigated the association between PFOS and hepatic effects. Of the epidemiological publications, 17 were classified as *medium* confidence, 6 as *low* confidence, and 7 were considered *uninformative* (Section 3.4.1.1). Of the animal toxicological studies, 3 were classified as *high* confidence, 17 as *medium* confidence, and 5 were considered *low* confidence (Section 3.4.1.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.1.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.1.1.1 Introduction and Summary of Evidence from the 2016 PFOS HESD

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Bilirubin and γ-glutamyltransferase (GGT) are also routinely used to evaluate potential hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Elevation of liver serum biomarkers is frequently an indication of liver injury, though not as specific as structural or functional analyses such as histology findings and liver disease.

There are 7 epidemiological studies (8 publications)[6] from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and hepatic effects. Study quality evaluations for these eight studies are shown in Figure 3-5. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-2 and below.

[5] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.
[6] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b); however, data for PFOA and hepatic outcomes is reported in Olsen (2001a). Olsen (2001b) was considered overlapping and not evaluated because data in the technical report was completely described in Olsen (2003).

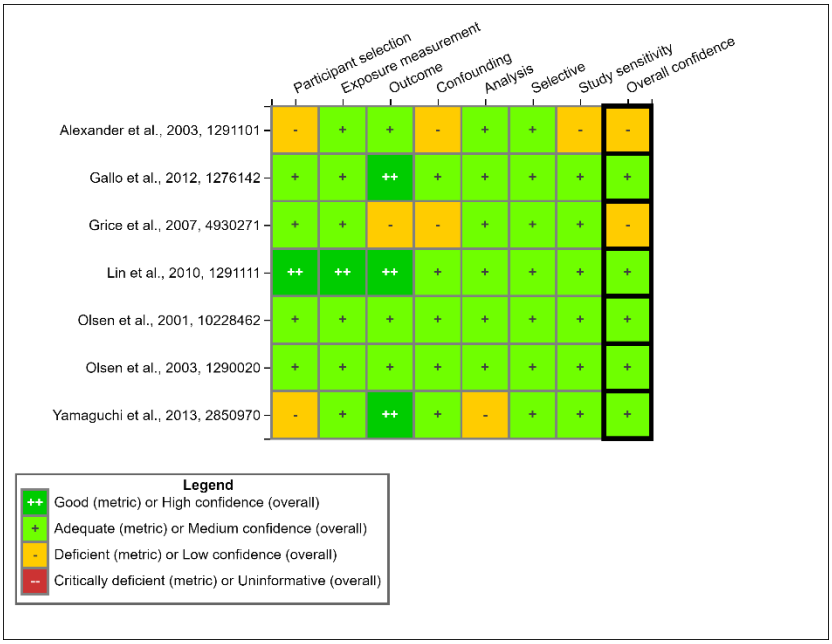


Figure 3-5. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Hepatic Effects Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

The 2016 PFOS HESD (U.S. EPA, 2016b) describes both cross-sectional and longitudinal studies that evaluated PFOS and liver enzymes in adults. Two available cross-sectional studies (Gallo et al., 2012; Lin et al., 2010) reported positive associations between PFOS exposure and ALT in adults of the general population (see Appendix D, (U.S. EPA, 2024a)). Lin et al. (2010) examined 2,216 adults in NHANES (1999–2000, and 2003–2004) and observed that higher serum concentrations of PFOS were associated with abnormal liver enzyme increases in the U.S. general population. With each increase in logPFOS, serum ALT and GGT concentrations (U/L) increased by 1.01 units (SE = 0.53) and 0.01 units (SE 0.03), respectively (Lin et al., 2010). When PFOA, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, one unit increase in serum logPFOS concentration was associated with a decrease of 0.19 units (SE = 0.63, p-value = 0.769) in serum ALT concentration (U/L) and a 0.06 unit (SE = 0.03, p-value = 0.025) decrease in serum log-GGT concentration (U/L). The four PFAS were moderately correlated with one another, with PFOA and PFOS most strongly correlated (Spearman correlation coefficient of 0.68), and PFHxS and PFNA the least correlated (Spearman correlation coefficient of 0.24). Another *medium* confidence cross-sectional study (Yamaguchi et al., 2013) conducted in Japan reported a positive correlation with ALT in addition to factors influencing PFOS exposure.

Gallo et al. (2012) reported an analysis of data from the C8 Health Project, reflective of a highly exposed community. One of the largest studies of PFOS and ALT in adults, Gallo et al. (2012) evaluated 47,092 adults from the C8 Study Project living in communities in Ohio and West Virginia impacted from a manufacturing-related PFOA-contaminated drinking water supply. Natural log transformed serum PFOS concentrations were associated with ln-ALT in linear

regression models (regression coefficient: 0.020; 95% CI: 0.014, 0.026) and with elevated ALT in logistic regression models across deciles of PFOS (OR = 1.13; 95% CI: 1.07, 1.18). There was less consistent evidence of an association between PFOS and GGT or bilirubin in this study.

Both the Gallo et al. (2012) and Lin et al. (2010) studies observed a slight positive association between serum PFOS levels and increased serum ALT values (Figure 3-6). The association between PFOS and increased serum GGT was less defined. Total or direct bilirubin showed no association with PFOS in either study. In the Gallo et al. (2012) study, the cross-sectional design and self-reported lifestyle characteristics are limitations of the study, and while both Lin et al. (2010) and Gallo et al. (2012) showed a trend, it was not large in magnitude.

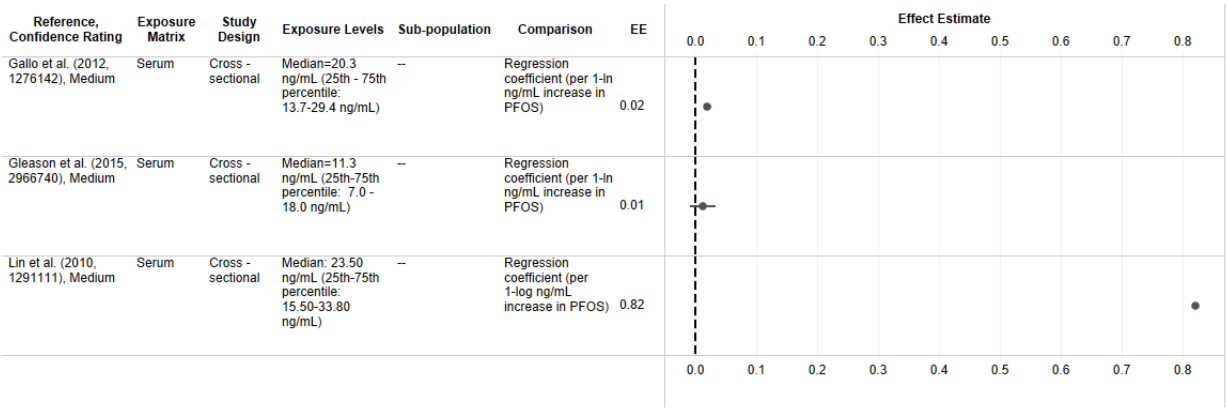


Figure 3-6. Overall ALT Levels from 2016 PFOS HESD Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Several cross-sectional occupational studies in PFOS production workers reported mostly null or inconsistent findings with respect to biomarkers of liver disease. Exposure to PFOA was generally associated with increased ALT concentrations, but findings were inconsistent for some timepoints or in sex-stratified groups (Olsen et al., 2003; Olsen et al., 2001a). Null or inconsistent associations were also reported with GGT and bilirubin. There was no evidence of association with functional hepatic endpoints in these identified studies. No increases in deaths from cirrhosis of the liver were found in workers at the 3M facility in Decatur, Alabama (Alexander et al., 2003). At the same plant, nonsignificant increases in noncancerous liver disease (including cirrhosis) were observed with cumulative exposure to PFOS (Grice et al., 2007).

Table 3-2. Associations Between Elevated Exposure to PFOS and Hepatic Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]
Alexander, 2003, 1291101 *Low*	Cohort	Occupational	NA	NA	NA	NA	–

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]
Gallo, 2012, 1276142 *Medium*	Cross-sectional	Adults	↑↑	NA	–	NA	NA
Grice, 2007, 4930271 *Low*	Cohort	Occupational	NA	NA	NA	NA	↑
Lin, 2010, 1291111 *Medium*	Cross-sectional	Adults	↑↑	NA	–	NA	NA
Olsen, 2001, 10228462 *Medium*	Cohort	Occupational	↑	↑	–	–	NA
Olsen, 2003, 1290020 *Medium*	Cross-sectional	Occupational	↑	–	↑	↑	NA
Yamaguchi, 2013, 2850970 *Medium*	Cross-sectional	Adults and adolescents	↑↑	↑↑	↑↑	NA	NA

Notes: ALP = alkaline phosphatase; ALT = alanine transferase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

3.4.1.1.2 Study Quality Evaluation Results for the Updated Literature Review

There are 17 epidemiological studies (23 publications)[7] from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 17 studies (23 publications) are shown in 3.

Of these, 12 were classified as *medium* confidence, four as *low* confidence, and seven were considered *uninformative.* Of the informative studies, two cross-sectional studies (Nian et al., 2019; van den Dungen et al., 2017), multiple publications of data from NHANES (Omoike et al., 2020; Jain, 2019; Jain and Ducatman, 2019a, c; Liu et al., 2018d; Gleason et al., 2015), one prospective cohort in elderly adults (Salihovic et al., 2018), and one occupational cohort of fluorochemical plant workers (Olsen et al., 2012) examined liver enzymes in adults. In addition, two cross-sectional studies (Rantakokko et al., 2015 Liu, 2018, 4238396) examined functional liver endpoints in adults. In children and adolescents, four studies were available including one cohort study (Mora et al., 2018) and three cross-sectional studies (Jin et al., 2020; Attanasio, 2019; Khalil et al., 2018), with one examining function liver endpoints (Jin et al., 2020). All of the studies measured PFOS exposure using biomarkers in blood. The *uninformative* studies were excluded due to potential confounding (Abraham et al., 2020; Sinisalu et al., 2020; Predieri et al., 2015; Jiang et al., 2014), lack of information on participant selection (Sinisalu et al., 2021), or

[7] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

use of PFAS as the dependent variable (in a publication with a more suitable analysis available (Jain, 2020a) or where the independent variable is a genetic variant and thus not affected by PFAS exposure (Fan et al., 2014)).

Figure 3-7. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Hepatic Effects[a]

Interactive figure and additional study details available on HAWC.

[a] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

3.4.1.1.3 Synthesis of Hepatic Injury From the Updated Literature Review

Results for the eight studies that examined ALT are presented in Appendix D (U.S. EPA, 2024a). Of the available informative studies that measured ALT in adults, statistically significant positive associations between ALT and PFOS (i.e., increases in ALT as a continuous measure with higher PFOS exposure levels) were observed in two of five studies (Nian et al., 2019; Salihovic et al., 2018) and multiple NHANES publications, including all the *medium* confidence studies. However, the positive associations in Jain et al. (2019) were observed only in obese participants (Figure 3-8.). In non-obese participants, associations were generally null, with an inverse association in non-obese participants with glomerular filtration (GF) stage of 3B/4. Among *low* confidence studies in adults, an inverse association ($p < 0.05$) was reported in Olsen et al. (2012) (see Appendix D, (U.S. EPA, 2024a)). However, this analysis differed from the other studies in that the exposure measure used was change in PFOS levels during the study period. In van den Dungen et al. (2017), no association was observed. ALT findings from *low* confidence studies are not included in figures.

In children and adolescents, positive associations were observed in girls in the fourth quartile reported by Attanasio (2019) and in the *low* confidence study in obese children (Khalil et al., 2018). However, inverse associations were observed in Mora et al. (2018), which may indicate that the associations in children are less consistent than in adults or that there are sex differences in children. Insufficient data were available to assess the potential for effect modification by sex.

Six studies examined AST and are presented in Appendix D (U.S. EPA, 2024a). In adults, statistically significant positive associations were observed in the one *medium* confidence study (Nian et al., 2019) and in NHANES studies. Van den Dungen et al. (2017) reported a nonsignificant positive association. No association was observed in Olsen et al. (2012). In children and adolescents, the *medium* confidence study (Attanasio, 2019) also observed a positive association in girls but not boys, while the *low* confidence study (Khalil et al., 2018) reported an inverse association, both not statistically significant. For the other liver enzymes (bilirubin, GGT), results were generally consistent with ALT and AST (Attanasio, 2019; Nian et al., 2019; van den Dungen et al., 2017) with the exception of inverse associations (not statistically significant) for GGT in Jain (2019) and bilirubin in Salihovic et al. (2018).

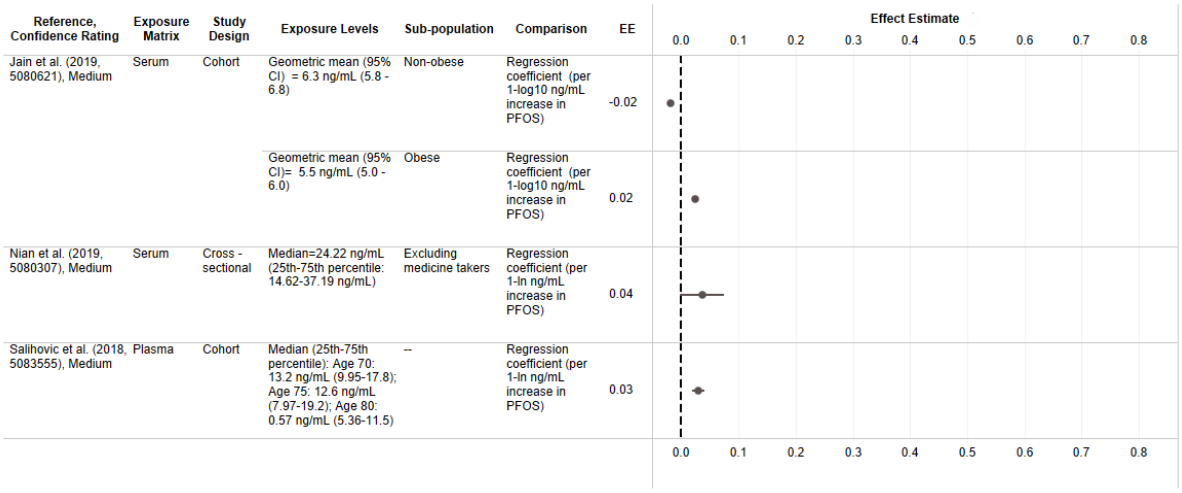


Figure 3-8. Overall ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

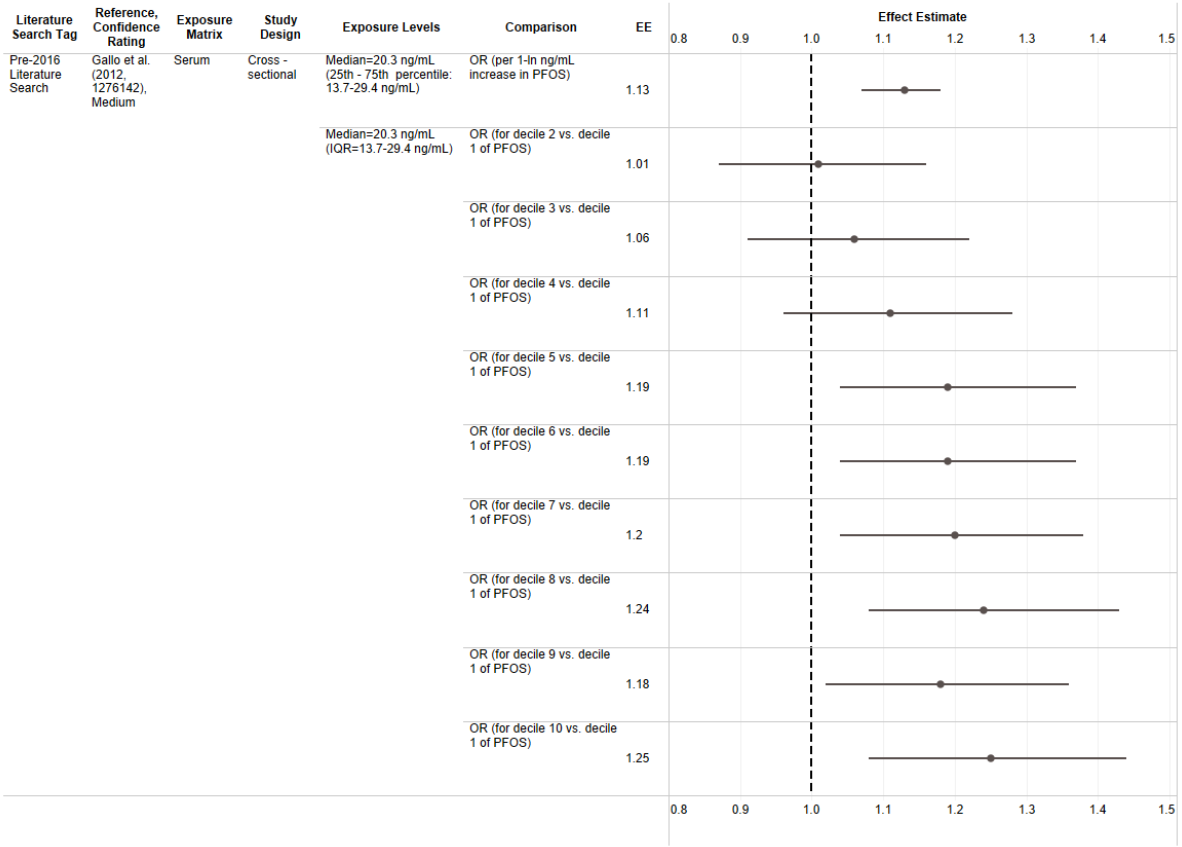


Figure 3-9. Odds of Elevated ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

For functional measures of liver injury, two *medium* confidence studies (one in adults and one in children and adolescents) examined histology endpoints. Both studies examined lobular inflammation. Rantakokko et al. (2015) reported that higher PFOS exposure levels were associated with reduced odds of lobular inflammation, whereas Jin et al. (2020) reported the opposite, with an OR of 2.9 for 2–4 foci versus. none, though the results in the latter study were non-monotonic and both were not statistically significant. Jin et al. (2020) additionally reported higher odds (not statistically significant) of non-alcoholic steatosis ($p < 0.05$), ballooning, fibrosis, and portal inflammation. Lastly, Liu et al. (2018b) examined hepatic fat mass and found no correlation with PFOS exposure.

In summary, across studies in the 2016 PFOS HESD (U.S. EPA, 2016b) and the updated systematic review, there is generally consistent evidence of a positive association between exposure to PFOS and ALT. However, one source of uncertainty in epidemiology studies of PFAS is confounding across the PFAS, as individuals are exposed to a mixture of PFAS and it is difficult to disentangle the effects of the individual contaminants. This cannot be ruled out in this body of evidence given the attenuation of the association in Lin et al. (2010), the only general population study that performed multi-pollutant modeling. In addition, associations for other hepatic outcomes were less consistent, including for functional outcomes such as liver disease. Thus, while there is evidence of an association between PFOS and ALT in epidemiological studies, there is residual uncertainty.

3.4.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 animal toxicological studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and 19 animal toxicological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 25 studies are shown in Figure 3-10 and Figure 3-11.

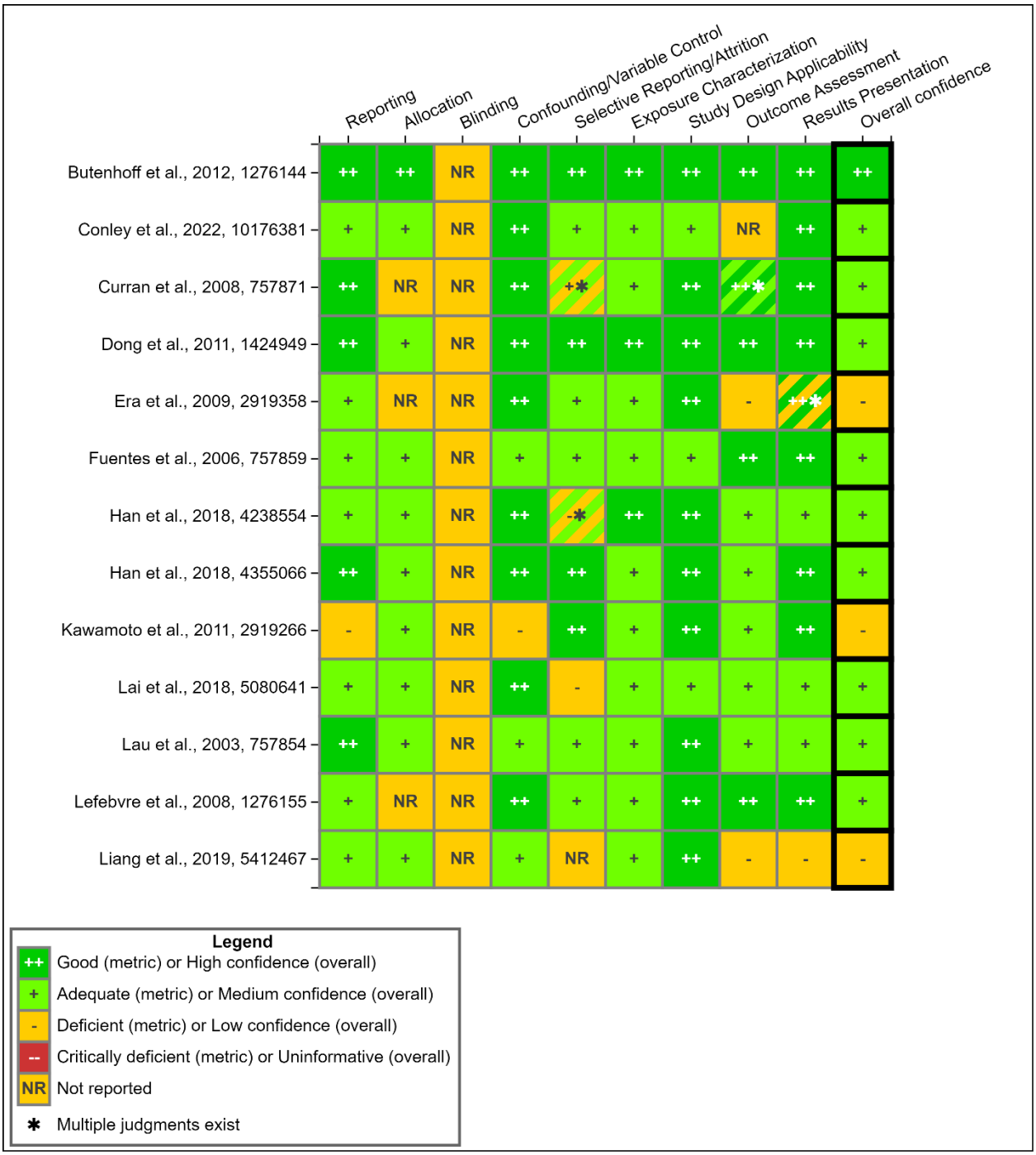


Figure 3-10. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Hepatic Effects[a,b]

Interactive figure and additional study details available on HAWC.
[a] Han et al. (2018a) and Wan et al. (2016) reported on the same hepatic data as Han et al. (2018b).
[b] Lefebvre et al. (2008) reported on the same hepatic data as Curran et al. (2008).

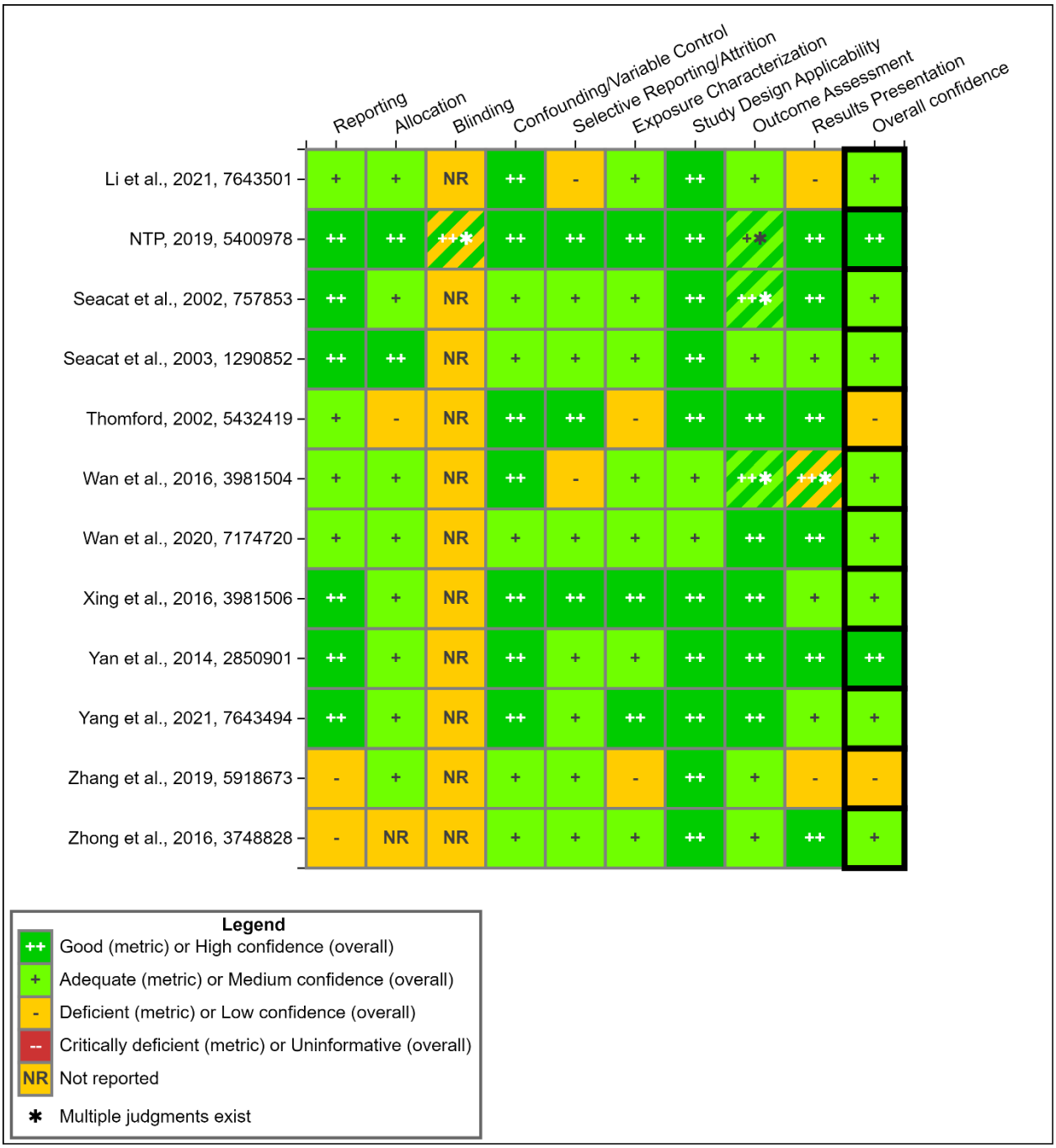


Figure 3-11. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Hepatic Effects (Continued) [a,b]

Interactive figure and additional study details available on HAWC.
[a] Han et al. (2018a) and Wan et al. (2016) reported on the same hepatic data as Han et al. (2018b).
[b] Lefebvre et al. (2008) reported on the same hepatic data as Curran et al. (2008).

Hepatic effects were observed in male and female mice, rats, and monkeys after varying oral PFOS exposure durations and doses. This includes effects such as increased absolute and relative liver weight, altered clinical parameters indicating potential liver injury, and histopathological

alterations of liver tissue. Data from numerous studies provide evidence confirming the liver as a target of PFOS toxicity.

3.4.1.2.1 Liver Weight

Significant increases in liver weight relative to body weight and absolute liver weight were observed in several strains of male and female mice exposed to 1.25–10 mg/kg/day PFOS for short-term, subchronic, and gestational durations (Yang et al., 2021; Lai et al., 2018; Xing et al., 2016; Zhong et al., 2016; Yan et al., 2014; Dong et al., 2011; Lau et al., 2003). In male BALB/c mice, significant increases in both relative and absolute liver weights were observed after a 28-day exposure to PFOS doses of 1.25 and 5 mg/kg/day (Yan et al., 2014). Similarly, two short-term studies in male C57BL/6 mice reported significantly increased relative liver weights following exposures to 2.5 (Yang et al., 2021) or 2.5–10 mg/kg/day PFOS (Xing et al., 2016). In a 60-day study in male C57BL/6 mice, Dong et al. (2011) observed a dose-related increase in relative liver weights; at doses above 0.417 mg/kg/day PFOS, the increases were statistically significant compared with control. In a 7-week gavage study in female CD-1 mice, Lai et al. (2018) reported significant increases in absolute and relative liver weights at 3 mg/kg/day PFOS but not 0.3 mg/kg/day.

Two developmental studies in CD-1 mice observed increased liver weights in the dams following gestational PFOS exposure (Wan et al., 2020; Fuentes et al., 2006). Fuentes et al. (2006) observed significantly increased absolute liver weights in dams exposed to 3 or 6 mg/kg/day PFOS and significantly increased relative liver weights in dams exposed to 6 mg/kg/day PFOS. The dams were exposed from GD 6–18 to 0, 1.5, 3, or 6 mg/kg/day PFOS. Similarly, Wan et al. (2020) reported significantly increased relative liver weights in dams exposed to 3 mg/kg/day PFOS without changes in maternal body weight (absolute liver weight not reported). Dams were exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5–17.5. There was a 10% increase in relative liver weight in the fetuses, but the increase was not statistically significant and may have been related to reduced fetal weight in this group.

Two additional developmental toxicity studies in mice indicate that relative liver weights of pups exposed to PFOS during gestation may increase and then subsequently return to control levels after prolonged cessation of exposure during postnatal development (Zhong et al., 2016; Lau et al., 2003). Zhong et al. (2016) dosed C57BL/6J mouse dams with 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–17. Relative liver weights of male and female pups in the 5 mg/kg/day group were significantly increased at postnatal week 4 (PNW 4), but returned to levels statistically indistinguishable from controls by PNW 8. Similarly, Lau et al. (2003) exposed pregnant CD-1 mice to 0, 1, 5, or 10 mg/kg/day PFOS from GD 1–17 and found significant increases in offspring liver weights in the 5 and 10 mg/kg/day dose groups at PNDs 0 and 7 but not PND 35.

Significant increases in relative and absolute liver weights were also observed in male and female rats exposed to 0.15–20 mg/kg/day PFOS for short-term, chronic, and gestational durations (NTP, 2019; Han et al., 2018b; Wan et al., 2016; Wan et al., 2012; Cui et al., 2009; Curran et al., 2008; Lau et al., 2003; Seacat et al., 2003). An increase in relative liver weight was observed with exposure as low as 0.15 mg/kg/day PFOS administered to female Sprague-Dawley rats for 28 days (Curran et al., 2008). In males from the same study, relative liver weight was significantly increased at 1.33 mg/kg/day. A similar study in Sprague-Dawley rats found that relative and absolute liver weights were increased in both males and females dosed with

≥0.312 mg/kg/day PFOS for 28 days (NTP, 2019). In a 14-week feeding study, Seacat et al. (2003) also observed similar responses in male and female Sprague-Dawley rats, with significant increases in relative liver weight at the highest dose tested in each sex (1.33 and 1.56 mg/kg/day, respectively) and increased absolute liver weight in males at 1.33 mg/kg/day.

In a developmental toxicity study, Lau et al. (2003) observed inconsistent alterations in liver weight across time points in Sprague-Dawley rat offspring exposed to 0, 1, 2, or 3 mg/kg/day PFOS from GD 2–21. Significant increases in relative liver weight were observed in the 2 and 3 mg/kg/day dose groups at PND 5 but not PND 0 or PND 35. No significant changes in relative or absolute liver weights were observed in Sprague-Dawley rat dams following a relatively short 5-day exposure (GD 14–18) to PFOS concentrations of 0, 0.1, 0.3,1, 3, 10, or 30 mg/kg/day (Conley et al., 2022).

In a subchronic study in cynomolgus monkeys, relative and absolute liver weights were increased in males and females dosed with 0.75 mg/kg/day PFOS for 182 days (26 weeks) (Seacat et al., 2002).

3.4.1.2.2 Clinical Chemistry Measures

Increases in serum enzymes including ALT, alkaline phosphatase (ALP), AST, and GGT following PFOS exposure were observed across multiple species, sexes, and exposure paradigms (Figure 3-12 (mice), Figure 3-13 (male rats), Figure 3-14 (female rats)). Serum levels of these enzymes are often useful indicators of hepatic enzyme induction, hepatocellular damage, or hepatobiliary damage, as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood (U.S. EPA, 2002a). Alterations in serum enzyme levels are generally considered to reach biological significance and indicate potential adversity at levels ≥ twofold compared with controls (i.e., ≥ 100% change relative to control response) (Hall et al., 2012; U.S. EPA, 2002a).

Two studies in male mice found statistically and biologically significant increases in serum enzymes indicative of hepatic or hepatobiliary damage after oral PFOS exposure (Figure 3-12) (Xing et al., 2016; Yan et al., 2014). Xing et al. (2016) observed a dose-dependent increase in ALT in male C57BL/6J mice after 30 days of PFOS exposure; ALT levels were increased by 50% and 88% above control in the 5 and 10 mg/kg/day groups, respectively. In comparison, in a study of 28-day exposure to 0, 1.25, or 5 mg/kg/day PFOS in male BALB/c mice, Yan et al. (2014) observed much larger increases in ALT in the 5 mg/kg/day group (> 700% change), though there was no apparent linear dose-response relationship observed across the two tested dose levels. Both Yan et al. (2014) and Xing et al. (2016) observed statistically but not biologically significant increases in AST with increasing PFOS dose (responses did not exceed 50% change from control at any dose level). Xing et al. (2016) observed a similar statistically but not biologically significant increase in ALP level (53% change in the 10 mg/kg/day group). Yan et al. (2014) also reported a large increase in ALP (321% change relative to control) in the 5 mg/kg/day dose group. A statistically and biologically significant dose-dependent increase in GGT was observed by Xing et al. (2016), with an increase of approximately 140% in the lowest dose group (2.5 mg/kg/day) and 535% in the highest dose group (10 mg/kg/day), indicating potential damage to the biliary system (U.S. EPA, 2002a).

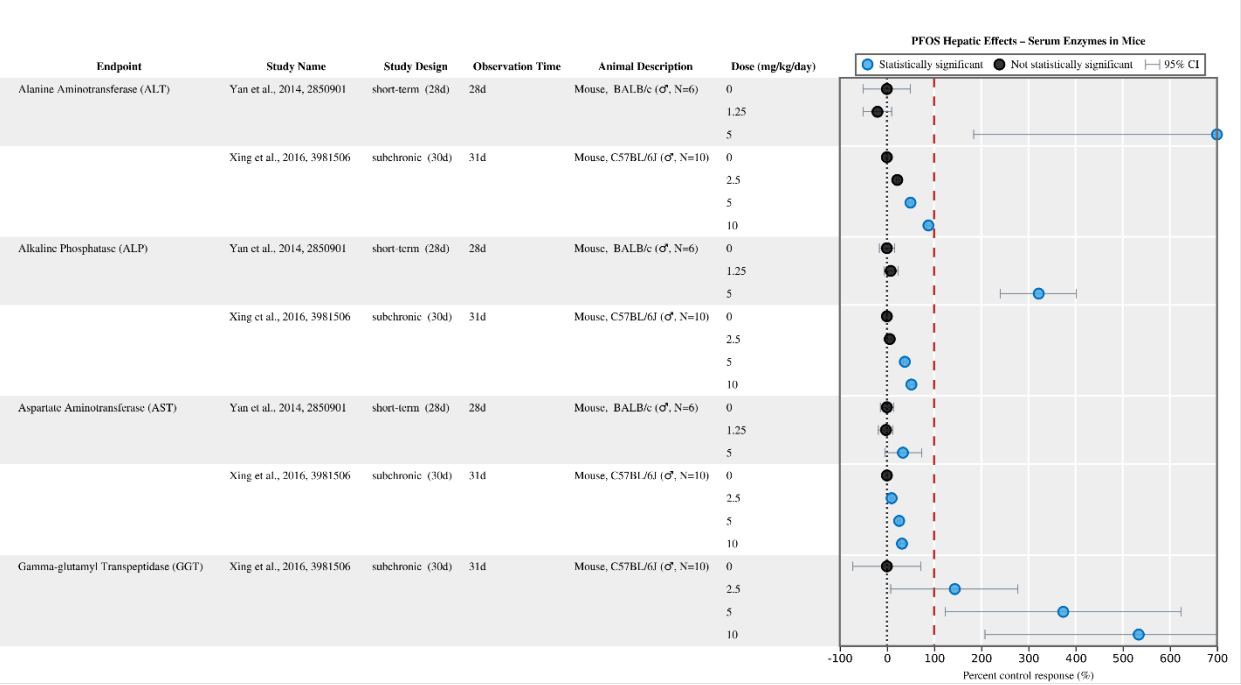


Figure 3-12. Percent Change in Serum Enzyme Levels Relative to Controls in Mice Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase; d = day; CI = confidence interval.
[a] Results for Yan et al. (2014) are presented for 3 dose levels (0, 1.25, and 5 mg/kg/day), and a statistically significant response of 756% occurred at the highest dose for the ALT endpoint alanine aminotransferase. The x axis has been truncated at 700% to allow results at lower doses for other studies and endpoints to be legible.
[b] The red dashed line indicates a 100% increase from the control response.

Multiple studies assessed serum liver enzymes in male and female Sprague-Dawley rats exposed to PFOS for short-term and chronic exposure durations, or in dams following a developmental exposure paradigm (Figure 3-13, Figure 3-14) (Conley et al., 2022; NTP, 2019; Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003).

The NTP (2019), Han et al. (2018b), and Curran et al. (2008) studies reported statistically significant increases in ALT levels in male rats exposed to PFOS for 28 days. However, these increases did not exceed 75% change at even the highest doses tested in each study (5, 10, and 6.34 mg/kg/day, respectively). Seacat et al. (2003) similarly observed statistically but not biologically significant increases in ALT in male rats from the highest dose group (1.33 mg/kg/day) in a 14-week dietary PFOS study. Butenhoff et al. (2012) did not observe consistent dose-related changes in ALT levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks, though this study tested relatively low doses (approximately 0.02 to 1 mg/kg/day).

As with ALT levels, AST levels in male Sprague-Dawley rats exposed to PFOS for varying durations were increased, but the increases did not exceed twofold compared with controls. Han et al. (2018b) reported a statistically significant increase in AST in male rats dosed with

10 mg/kg/day PFOS for 28 days, but the increase was less than a 20% change from the control. Three other 28-day studies assessing AST levels in male rats either reported changes in AST that were not dose-dependent (NTP, 2019) or not statistically significant between treated and control groups (Curran et al., 2008; Seacat et al., 2003). Butenhoff et al. (2012) also did not observe statistically significant changes in AST levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks at doses up to 0.984 mg/kg/day.

NTP (2019) reported statistically significant increases in ALP in male rats after a 28-day PFOS exposure at dose levels as low as 0.625 mg/kg/day. However, these increases only ranged from approximately 15%–35% change across all doses with statistically significant responses. Similarly, Curran et al. (2008) did not observe consistent effects of 28-day dietary consumption of PFOS on ALP levels at dose levels up to approximately 6.34 mg/kg/day in male rats.

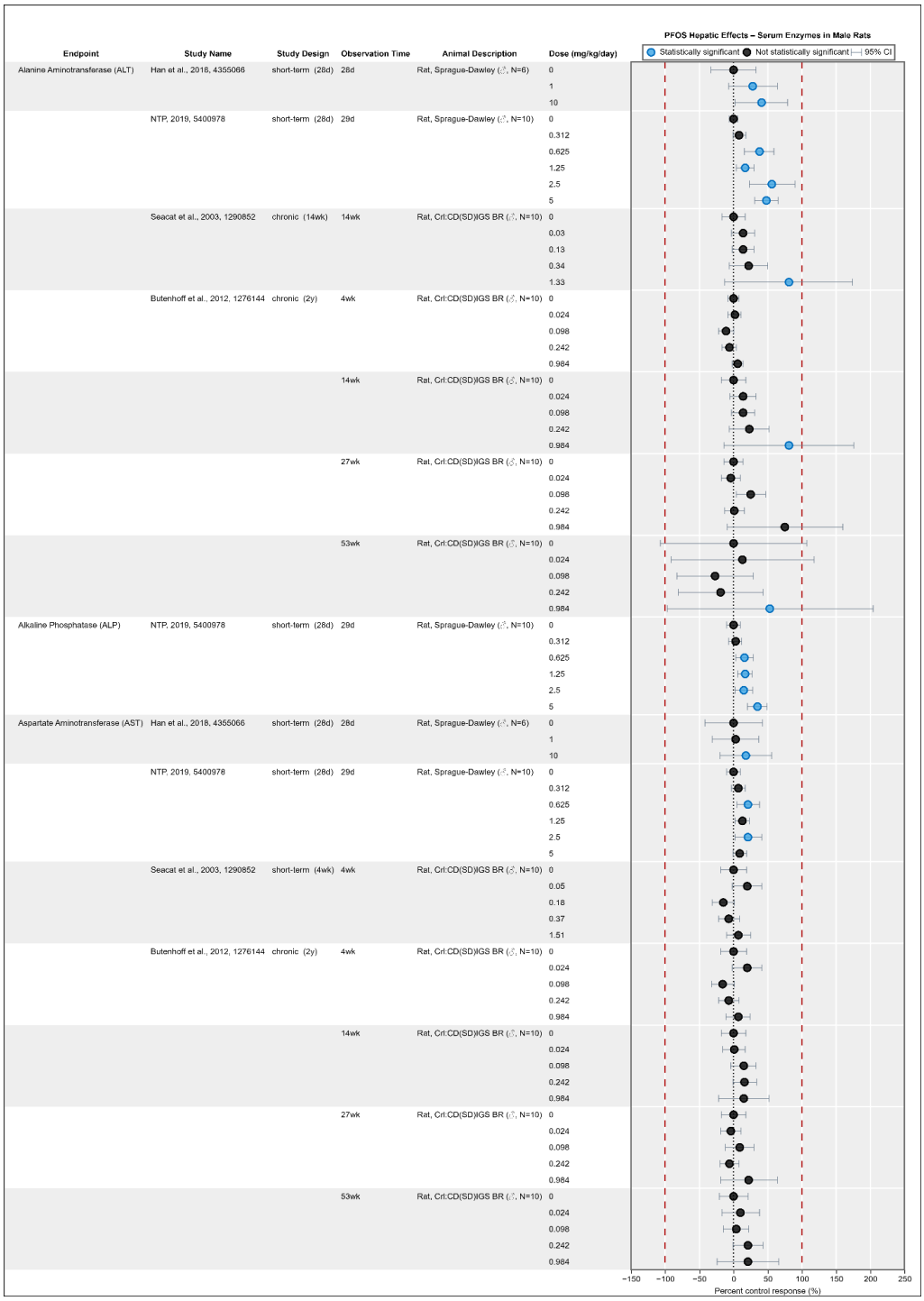


Figure 3-13. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = year; CI = confidence interval.
[a] Two publications Han et al. (2018a) and Wan et al. (2016) reported on the same data as Han et al. (2018b) and are not shown in the figure.
[b] The red dashed lines indicate a 100% increase and decrease from the control response.

As generally observed in male Sprague-Dawley rats, there were also statistically but not biologically significant alterations in serum enzyme levels observed in female Sprague-Dawley rats exposed to PFOS for 4–53 weeks (NTP, 2019; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). In a 28-day study in female rats, NTP (2019) reported dose-dependent increases in ALT, though these increases reached only approximately 62% change with the highest dose tested (10 mg/kg/day). A dietary 28-day study in female rats reported no statistically significant difference between the control group and groups treated with up to ~7.58 mg/kg/day PFOS (Curran et al., 2008). Similarly, Seacat et al. (2003) observed no significant differences in ALT levels of female rats exposed to dietary concentrations of PFOS up to ~1.56 mg/kg/day for 14 weeks. Butenhoff et al. (2012) also did not observe significant changes in ALT levels in female rats exposed to dietary concentrations of PFOS for 4, 14, 27, or 53 weeks with doses up to ~1.25 mg/kg/day and Conley et al. (2022) did not observe effects on ALT levels in female Sprague-Dawley dams treated with up to 30 mg/kg/day PFOS from GD 14–18.

Both Curran et al. (2008) and Butenhoff et al. (2012) observed statistically significant decreases in AST levels of female rats exposed to PFOS for 28 days at the highest dose tested in each study (7.58 and 1.251 mg/kg/day, respectively). These alterations were approximately 25%–26% decreases from control levels in both studies. In contrast, two other 28-day studies in female rats did not observe significant changes in AST levels compared with controls (NTP, 2019; Seacat et al., 2003) and the statistically significant decrease observed by Butenhoff et al. (2012) at the high dose at the 4-week time point were not observed at the 14-, 27-, or 53-week time points. In a developmental exposure paradigm, Conley et al. (2022) observed no significant effect on AST in the serum of Sprague-Dawley dams exposed to PFOS concentrations between 0.1–30 mg/kg/day from GD 14–18.

NTP (2019) reported statistically but not biologically significant increases in ALP at dose levels of 2.5 and 5 mg/kg/day in female rats exposed to PFOS for 28 days (increases did not exceed 35% change with either dose). In another 28-day study, ALP levels in female rats administered up to 7.58 mg/kg/day PFOS were not significantly different from control levels (Curran et al., 2008).

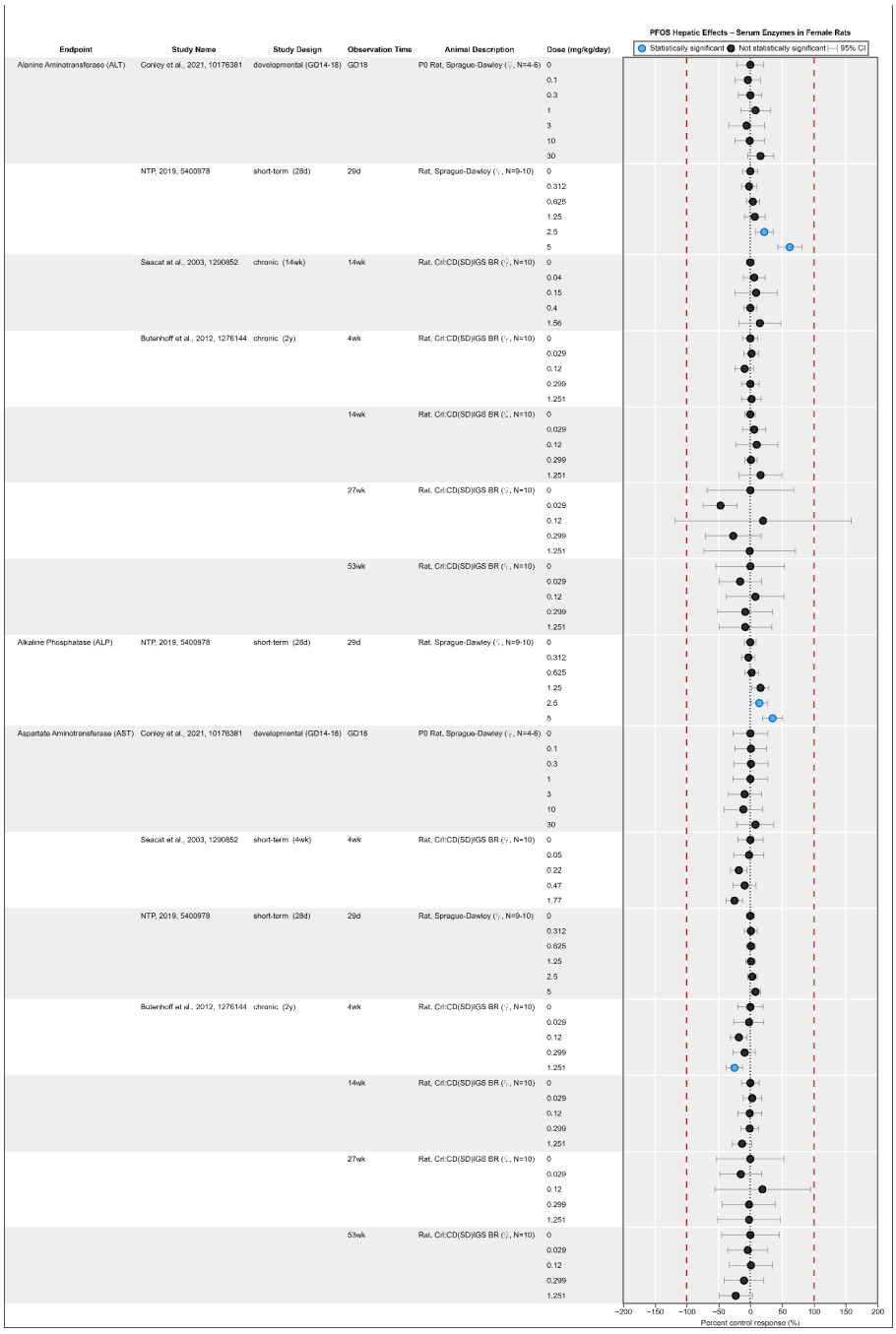


Figure 3-14. Percent Change in Serum Enzyme Levels Relative to Controls in Female Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = year; CI = confidence interval.
[a] Two publications Han et al. (2018a) and Wan et al. (2016) reported on the same data as Han et al. (2018b) and are not shown in the figure.
[b] The red dashed lines indicate a 100% increase or 100% decrease from the control response.

Neither ALT nor ALP were significantly altered in male or female cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al., 2002).

Levels of bilirubin, albumin, and bile salt/acids were also observed to be altered in several studies in mice, rats, and monkeys. However, these clinical chemistry measurements were generally altered at higher concentrations of PFOS than were serum enzymes, and changes were inconsistent across studies. Bilirubin (direct, indirect, or total) was either unchanged or increased in male rats exposed to ≥5 mg/kg/day PFOS and in female rats exposed to ≥2.5 mg/kg/day PFOS (NTP, 2019; Curran et al., 2008; Seacat et al., 2003). Total bilirubin was decreased in male monkeys exposed to 0.75 mg/kg/day for 91–182 days, but there was no statistically significant response in female monkeys (Seacat et al., 2002). Six studies examined albumin levels, but only two studies found significant alterations due to PFOS treatment (Conley et al., 2022; NTP, 2019; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). In male mice dosed with 1.25 or 5 mg/kg/day of PFOS for 28 days, albumin was significantly increased above control levels at both doses (Yan et al., 2014). In rats dosed with PFOS for 28 days, albumin was significantly increased in females dosed with 1.25–5 mg/kg/day and in males dosed with 5 mg/kg/day (NTP, 2019). Bile salt/acids were significantly increased in male rats exposed to 5 mg/kg/day PFOS and in female rats exposed to 2.5 and 5 mg/kg/day PFOS (NTP, 2019). In monkeys, serum bile acids were significantly increased in males, but not in females, dosed with 0.75 mg/kg/day PFOS (Seacat et al., 2002).

3.4.1.2.3 Histopathology

Liver lesions were confirmed microscopically in male mice and male and female rats in several short-term and subchronic studies (Li et al., 2021c; NTP, 2019; Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Xing et al., 2016; Wan et al., 2012; Cui et al., 2009; Curran et al., 2008) and in two chronic studies of male and female rats and monkeys (Butenhoff et al., 2012; Seacat et al., 2002). Only three of these studies provided quantitative incidence data (NTP, 2019; Butenhoff et al., 2012; Curran et al., 2008).

Hepatocellular hypertrophy was shown to be significantly increased in male Sprague-Dawley rats dosed with 2.5 and 5 mg/kg/day PFOS and in females dosed with 5 mg/kg/day PFOS for 28 days (NTP, 2019) (Table 3-3). Cytoplasmic vacuolation and alterations were significantly increased in a dose-dependent manner in male and female rats, respectively, in the 2.5 (females only) and 5 mg/kg/day (males and females) exposure groups (NTP, 2019). Another 28-day study in Sprague-Dawley rats observed higher incidence of hepatocellular hypertrophy in zone 3 of the liver in males exposed to 3.21 and 6.24 mg/kg/day PFOS, the two highest concentrations; this lesion was not observed in females (Curran et al., 2008) (Table 3-4). A higher incidence of cytoplasmic homogeneity in zone 3 of the liver was also observed in both males and females exposed to 3.21 and 6.24 mg/kg/day PFOS (Curran et al., 2008). In the chronic study in Sprague-Dawley rats (Butenhoff et al., 2012; Thomford, 2002b), hepatocellular hypertrophy was significantly increased in males exposed to 0.098–0.984 mg/kg/day of PFOS and in females exposed to 0.299–1.251 mg/kg/day for 103 weeks; a positive dose-response relationship was observed (Table 3-5).

Table 3-3. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by NTP (2019)

	0 mg/kg/day	0.312 mg/kg/day	0.625 mg/kg/day	1.25 mg/kg/day	2.5 mg/kg/day	5 mg/kg/day
Males						
Hepatocyte, Hypertrophy	0/10	0/10	0/10	3/10	8/10**	10/10**
Hepatocyte, Vacuolization, Cytoplasmic	0/10	0/10	0/10	0/10	2/10	4/10*
Females						
Hepatocyte, Hypertrophy	0/10	0/10	0/10	2/10	3/10	10/10**
Hepatocyte, Cytoplasmic Alteration	0/10	0/10	0/10	3/10	5/10*	10/10**

Notes:
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

Table 3-4. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Curran et al. (2008)

Males					
	0 mg/kg/day	**0.14 mg/kg/day**	**1.33 mg/kg/day**	**3.21 mg/kg/day**	**6.34 mg/kg/day**
Hepatocyte, Hypertrophy in Zone 3	0/4	0/4	0/4	1/4	3/4
Cytoplasmic Homogeneity in Zone 3	0/4	0/4	0/4	1/4	3/4
Females					
	0 mg/kg/day	**0.15 mg/kg/day**	**1.43 mg/kg/day**	**3.73 mg/kg/day**	**7.58 mg/kg/day**
Hepatocyte, Hypertrophy in Zone 3	0/4	0/4	0/4	0/4	0/4
Cytoplasmic Homogeneity in Zone 3	0/4	0/4	0/4	1/4	3/4

Table 3-5. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Thomford (2002b)

Males					
	0 mg/kg/day	**0.024 mg/kg/day**	**0.098 mg/kg/day**	**0.242 mg/kg/day**	**0.984 mg/kg/day**
Hypertrophy, Hepatocellular, Centrilobular	0/50	2/50	4/50	17/50	29/50

Males					
Vacuolation, Hepatocellular Midzonal/Centrilobular	2/50	3/50	6/50	10/50	10/50
Hyperplasia, Bile Duct	19/50	20/50	25/50	24/50	25/50
Necrosis, Individual Hepatocyte	3/50	2/50	6/50	4/50	10/50
Altered Hepatocellular, Clear/Eosinophilic Cell	13/50	21/50	23/50	24/50	24/50
Degeneration, Cystic	5/50	15/50	19/50	17/50	22/50
Females					
	0 mg/kg/day	**0.029 mg/kg/day**	**0.120 mg/kg/day**	**0.299 mg/kg/day**	**1.251 mg/kg/day**
Hypertrophy, Hepatocellular, Centrilobular	2/50	1/50	4/50	15/50	39/50
Hyperplasia, Bile Duct	21/50	25/50	19/50	17/50	27/50
Necrosis, Individual Hepatocyte	3/50	4/50	4/50	5/50	9/50
Infiltrate, Lymphohistiocytic	33/50	37/50	33/50	36/50	42/50
Infiltrate, Macrophage, Pigmented	2/50	3/50	5/50	6/50	20/50
Degeneration, Cystic	0/50	1/50	1/50	2/50	4/50

Butenhoff et al. (2012) (peer-reviewed publication of data from a report by Thomford (2002b)) also observed a dose-dependent increase in cystic degeneration in male rats exposed to 0.024–0.984 mg/kg/day of PFOS (Table 3-5); this effect was observed at lower incidences in female rats, but also appeared to follow a dose-dependent positive trend. Lymphohistiocytic and macrophage infiltrate were increased in a dose-dependent manner in females exposed to 1.251 mg/kg/day. A dose-response relationship was also observed with hepatocellular single cell necrosis, which was increased in males and females exposed to 0.984 and 1.251 mg/kg/day PFOS, respectively (Butenhoff et al., 2012; Thomford, 2002b).

The most consistently observed liver lesions following short-term, subchronic, and chronic exposure to PFOS were hepatocellular hypertrophy and vacuolization. Other liver lesions commonly observed include single-cell and/or focal necrosis, hepatocytic or cystic degeneration, and inflammatory cell infiltration. However, in many instances these are qualitatively described as being observed by the study authors without quantitative data provided. A single study in male mice dosed with PFOS for 30 days observed hepatocellular hypertrophy and cytoplasmic vacuolation in all treatment groups (2.5, 5, and 10 mg/kg/day), but did not provide incidence data to evaluate a dose response (Xing et al., 2016). Cytoplasmic vacuolation was also observed in one study of female mice exposed to 0.1 mg/kg/day PFOS for 60 days (Li et al., 2021c). Male rats were used in multiple studies and this effect was observed at a range of exposures. Three studies from the same lab observed hepatocellular hypertrophy in male Sprague-Dawley rats dosed with 1 mg/kg/day of PFOS for 28 days (Han et al., 2018b; Han et al., 2018a; Wan et al., 2016); however, none of the studies provided incidence data. Hepatocellular hypertrophy and

centrilobular vacuolation were also observed in another 28-day rat study that was conducted with higher concentrations of PFOS (5 and 20 mg/kg/day) (Cui et al., 2009). Hepatocellular hypertrophy was also observed in male and female cynomolgus monkeys exposed to 0.75 mg/kg/day PFOS for 182 days (incidence data not provided) (Seacat et al., 2002).

Hepatocytic or cystic degeneration, inflammatory cell infiltration, and/or necrosis were observed in several short-term and subchronic studies (28–30 days) in male mice and rats (Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Xing et al., 2016; Cui et al., 2009). Livers of male C57BL/6J mice and Sprague-Dawley rats dosed with PFOS concentrations ranging from 2.5 to 20 mg/kg/day for approximately 4 weeks showed focal or flake-like necrosis, hepatocytic degeneration, and/or inflammatory cell infiltration (Xing et al., 2016; Cui et al., 2009). Three publications from the same lab described hepatocyte degeneration and inflammatory infiltration in male Sprague-Dawley rats dosed with lower concentrations of 1 mg/kg/day PFOS for 28 days (Han et al., 2018b; Han et al., 2018a; Wan et al., 2016). Hepatocytic degeneration and inflammatory cell infiltration were noted in a single study of female mice, with hepatocyte degeneration being observed in mice exposed to 0.1 mg/kg/day for 60 days and focal infiltration of inflammatory cells being observed in mice exposed to 1 mg/kg/day (Li et al., 2021c). However, no quantification or statistical analyses were performed in these studies.

3.4.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse hepatic outcomes is discussed in Sections 3.2.2, 3.2.3, 3.2.5, 3.3.4, 3.3.5, and 3.4.1.1 of the 2016 PFOS HESD (U.S. EPA, 2016b). There are 56 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to hepatic effects. A summary of these studies as organized by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-15.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Tot
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling				
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	9	0	6	15
Cell Growth, Differentiation, Proliferation, Or Viability	13	1	25	35
Cell Signaling Or Signal Transduction	13	1	15	25
Extracellular Matrix Or Molecules				
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	17	0	10	25
Hormone Function	3	1	0	4
Inflammation And Immune Response	5	1	2	7
Oxidative Stress	6	0	7	12
Renal Dysfunction	1	0	0	1
Xenobiotic Metabolism	3	1	6	10
Other	3	0	0	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	31	2	31	58

Figure 3-15. Summary of Mechanistic Studies of PFOS and Hepatic Effects

Interactive figure and additional study details available on HAWC.

3.4.1.3.1 Nuclear Receptor Activation

3.4.1.3.1.1 Introduction

The ability of PFOS to mediate hepatotoxicity via receptor activation has been investigated for several receptor-signaling pathways, including that of the peroxisome proliferator-activated receptor (PPAR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), liver X receptor (LXR), and retinoic acid receptor (RAR). Activation of PPARα has been cited as a mechanism of action for PFAS, including PFOS, because of the association between increased liver weight and peroxisome proliferation downstream of PPARα activation in rats. However, increased hepatic lipid content in the absence of a strong PPARα response (i.e., activation of downstream target genes) is a characteristic of exposure to PFOS, and many of the genes activated by PFOS are associated with nuclear receptors other than PPARα, namely CAR and LXR (U.S. EPA, 2016b). PPAR, PXR, CAR, LXR, and RAR are nuclear receptors that can form heterodimers with one another to induce transcription of linked genes, and therefore, the effects

of PFOS on one or multiple receptors may contribute to mechanisms underlying hepatotoxicity (U.S. EPA, 2016b). Additionally, hepatic effects observed with PFAS exposure including inflammation and necrosis cannot be fully explained by PPARα activation (Section 3.4.1.2.3). This updated assessment includes studies that have examined activation of PPARs (including PPARα, β/δ, and γ), CAR, PXR, LXR, and/or retinoid X receptor (RXR) activation, as well as the downregulation of hepatocyte nuclear factor 4-alpha (HNF4α) as potential mechanisms underlying the hepatic health effects induced by PFOS.

3.4.1.3.1.2 Receptor Binding and Activation

Receptor binding and activation assays have been conducted in vitro with the goal of examining the potential association between activation of PPARs, CAR, PXR, and LXR and PFOS-mediated hepatotoxicity. PPARs modulate gene expression in response to exogenous or endogenous ligands and play essential roles in lipid metabolism, energy homeostasis, development, and cell differentiation (U.S. EPA, 2016b).

Several studies used luciferase reporter assays to examine the activation of PPARα by PFOS in vitro with human and animal cell lines transfected with human or mouse PPARα with varying results (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Wolf et al., 2008; Takacs and Abbott, 2007). In COS-1 cells transfected with mouse PPARα, PPARα was activated in a concentration-dependent manner, with an approximate half maximal effective concentration (EC50) of 65 μM in one study (Wolf et al., 2014) and a lowest observed effect concentration (LOEC) of 90 μM for PPARα activation in another study (Wolf et al., 2008). However, a third study in transfected COS-1 cells found that PFOS activated mouse PPARα, with a significant increase in activity only at a concentration of 120 μM, but not at lower concentrations of 1–90 μM or at higher concentrations of 150 or 250 μM (Takacs and Abbott, 2007). In cell lines transfected with human PPARα, one study showed that PPARα was activated in COS-1 cells in a dose-dependent manner, with a LOEC of 30 μM (Wolf et al., 2008). A second study in HEK293T cells showed that human PPARα was only activated (i.e., upregulated by approximately 1.5-fold) at the highest concentration of 100 μM (Behr et al., 2020b). However, two additional studies reported that PFOS did not significantly increase the activity of human PPARα up to concentrations of 100 μM in HepG2 cells (Rosenmai et al., 2018) or 250 μM in COS-1 cells (Takacs and Abbott, 2007). In every study that compared the ability of PFOS to activate PPARα with that of PFOA, PFOS was a weaker PPARα activator (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Wolf et al., 2008; Takacs and Abbott, 2007).

In vitro luciferase reporter assays have also been used to examine the ability of PFOS to activate other PPAR receptors, namely PPARγ and PPARβ/δ (Behr et al., 2020b; Bagley et al., 2017; Zhang et al., 2014; Takacs and Abbott, 2007). One study showed that PFOS significantly activates human PPARγ by 1.5-fold at 10 μM and by threefold at 100 μM in a luciferase assay in HepG2 cells (Zhang et al., 2014). The authors also performed a cell-free binding assay to show that PFOS binds to human PPARγ with a half maximal inhibitory concentration (IC50) of 13.5 μM and dissociation constant of 93.7 μM. Mouse and rat PPARγ were also activated at 100 μM with a luciferase reporter assay conducted in Chinese hamster ovary (CHO) cells (Bagley et al., 2017). However, two other studies did not observe activation of PPARγ by PFOS (Behr et al., 2020b; Takacs and Abbott, 2007): PFOS did not activate human PPARγ or PPARδ in HEK29 cells at concentrations of up to 100 μM (Behr et al., 2020b), and neither human nor mouse PPARγ were activated by concentrations of up to 250 μM PFOS in COS-1 cells (Takacs

and Abbott, 2007). This study conducted in COS-1 cells also examined activation of human and mouse PPARβ/δ and observed activation of mouse PPARβ/δ only at concentrations of 20 and 30 μM, but not at a lower concentration of 10 μM or at higher concentrations of 40–80 μM. Human PPARβ/δ was not shown to be activated by PFOS in this study. Furthermore, this study demonstrated that the activities of mouse PPARα, γ, and β/δ were more responsive than their human counterparts to positive control agonists and antagonists, demonstrating species-specific differences in receptor activation (Takacs and Abbott, 2007). Given the discrepancies in the ability and magnitude of PFOS to activate either mouse or human PPAR receptors, the role of PPAR activation in mediating hepatotoxicity of PFOS is not fully understood.

Two studies examined the activation of CAR/PXR and/or LXR/RXR in vitro with luciferase reporter assays using HEK293 cells or CHO cells (Behr et al., 2020b; Bagley et al., 2017). No activation of human CAR, human PXR, rat PXR, rat LXRβ, human LXRα, or human RXRα was observed with concentrations of up to 100 μM PFOS. However, a luciferase reporter assay in HepG2 cells showed that PFOS activates human PXR with an EC_{50} of 7.87 μM (Zhang et al., 2017). Notably, these studies did not examine endogenous receptor activation, though other lines of evidence are available that evaluate endogenous receptor signaling in vivo and in vitro.

3.4.1.3.1.3 Receptor Signaling

3.4.1.3.1.4 In Vivo Models

PFOS can activate PPARα in rodents and humans. However, the extent to which activation of PPARα mediates hepatoxicity may be species-specific, and activation of other receptors may also contribute to toxicity (U.S. EPA, 2016b). Indeed, several studies in Sprague-Dawley rats have found evidence that PFOS may activate both PPARα and CAR/PXR in the liver (NTP, 2019; Dong et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a; Chang et al., 2009; Martin et al., 2007). In an acute/short-term study, male rats were exposed to 10 mg/kg/day PFOS for 1, 3, or 5 days, and gene expression changes were assessed in their livers with an expression microarray (Martin et al., 2007). Although PFOS exposure induced PPARα-regulated genes and pathway analysis revealed that PFOS clustered with PPARα agonists (e.g., bezafibrate, clofibric acid, and fenofibrate), the correlation between the gene response to PFOS and that of known peroxisome proliferators was weak (with a correlation coefficient of 0.26 for PFOS, in comparison to 0.76 for PFOA). Changes in cytochrome P450 3A (*Cyp3a*) genes were also observed, consistent with the activation of CAR/PXR.

Another transcriptomics study of the liver of rats exposed to 50 mg PFOS/kg diet for 28 days had similar results using an expression microarray (Dong et al., 2016). Upstream regulator analysis using Ingenuity Pathway Analysis (IPA, Qiagen) revealed that PFOS likely activated both PPARα and CAR/PXR, with alterations in 48 genes that have evidence of being regulated by PPARα in the IPA reference database (approximately 10% of all known genes in this pathway), and 29 genes from the reference database for the CAR/PXR pathway (approximately 14% of all known genes in this pathway). Two other studies support these results, reporting that genes regulated by either PPARα or CAR/PXR are altered by PFOS, according to qPCR analysis (NTP, 2019; Chang et al., 2009). In a developmental rat study, dams were dosed with 1 mg/kg/day PFOS from GD 0–19, and the expression of both PPARα- and CAR/PXR-regulated genes was found to be increased in liver samples from the dams on GD 20 and male offspring on PND 21; female offspring were not tested (Chang et al., 2009). A 28-day study in male and female rats found increases in the expression of both PPARα-regulated genes (*Cyp4a1, Acox1*)

and CAR-regulated genes (*Cyp2b1, Cyp2b2*) at all exposure concentrations tested (0.312–10 mg/kg/day) (NTP, 2019). However, there were apparent sex differences in this study; PPARα-regulated genes were increased by 2- to 31-fold in males and by 1.3- to 3-fold in females, while CAR-regulated genes were increased by 6- to 400-fold in males and 32- to 1,227-fold in females. Although *Acox1* was the least responsive gene in males, with increased expression in males exposed to 5 and 10 mg/kg/day and in females exposed to 0.312–10 mg/kg/day, the corresponding enzyme activity (acyl-CoA oxidase) was increased in males exposed to 5 and 10 mg/kg/day, but not in females.

Two studies in male rats provided additional evidence of PFOS activation of PPARα, CAR, and PXR through the use of enzymatic biomarkers (Elcombe et al., 2012b; Elcombe et al., 2012a). In one study, rats were fed diets containing either 20 or 100 ppm (approximately 2 and 10 mg/kg/day, respectively) PFOS for 7 days, and livers were collected on days 1, 28, 56, and 84 post-exposure (Elcombe et al., 2012b). In the second study, rats were fed the same dietary PFOS concentrations for up to 28 days, with livers collected on days 1, 7, and 28 of the exposure (Elcombe et al., 2012a). PPARα, CAR, and PXR activities (as measured by lauric acid 12-hydroxylation (CYP4A activity), pentoxyresorufin-O-depentylation (PROD; CYP2B activity), and testosterone 6B-hydroxylation (CYP3A activity), respectively) were found to be increased in the liver microsomes of rats exposed to PFOS at most time points and in both exposure concentrations tested. Liver palmitoyl-CoA oxidase (ACOX activity), another marker of PPARα activity, was not changed after 7 days of exposure to PFOS (Elcombe et al., 2012b), but was shown to be significantly increased at both concentrations after 28 days of exposure (Elcombe et al., 2012a). However, in another study in male rats exposed to 0.643–2.205 mg/kg/day PFOS for 28 days or 14 weeks, ACOX activity was unchanged (Seacat et al., 2003).

Studies in various strains of wild-type (WT) mice also examined PPARα activation as a mechanism of PFOS-induced liver toxicity (Huck et al., 2018; Lai et al., 2017b; Wang et al., 2014; Wan et al., 2012; Bijland et al., 2011; Rosen et al., 2009). Through genetic studies and pathway analysis, changes in PPARα signaling or expression of PPARα and/or downstream target genes were found to be associated with PFOS exposure in several studies (Lai et al., 2017b; Wang et al., 2014; Wan et al., 2012; Bijland et al., 2011; Rosen et al., 2009). However, these studies also found evidence of upregulation of other receptors such as PPARγ, CAR/PXR, or LXR/RXR. In one study, the authors concluded that the main mechanism of action of PFOS for observed changes in liver endpoints (increased absolute liver weight and histopathological changes including cytoplasmic vacuolization and steatosis) may be mitochondrial β-oxidation, which leads to the accumulation of free fatty acids and subsequent activation of PPARα (Wan et al., 2012). In another study, the authors did not report any changes in the expression of PPARα or a subset of the downstream target genes examined by qPCR (Acox1, Pdk4, Cpt1) in mice exposed to PFOS with or without high fat diet-induced hepatic steatosis (Huck et al., 2018). The authors suggested that alterations in PPARγ may be a mechanism of PFOS-induced liver hepatotoxicity, based on the fact that PPARγ gene expression was induced by PFOS in mice fed a normal diet. However, it should be noted that PPARγ gene expression was also upregulated in the livers of mice fed a high fat diet in the absence of PFOS, and PPARγ was unchanged in mice exposed to PFOS and fed a high fat diet.

Two additional studies comparing 129S1/SvlmJ WT mice to *Pparα*-null mice support PPARα activation as a mechanism of PFOS toxicity, but also support the hypothesis that other

mechanisms, including the activation of CAR/PXR, may play a role (Rosen et al., 2017; Rosen et al., 2010). The first study found that PPARα-regulated genes were altered in WT mice dosed with 10 mg/kg/day PFOS for 7 days (Rosen et al., 2010). However, other genes and pathways were affected in both WT and *Pparα*-null mice, including changes related to lipid metabolism, inflammation, xenobiotic metabolism, and CAR activation (as indicated by upregulation of *Cyp2b10*) (Rosen et al., 2010). In a connected study, the authors reanalyzed their data using different expression analysis software than the initial analysis (Rosen et al., 2017). They found that only approximately 15% of the PFOS-responsive gene changes in the liver were PPARα-independent, including CAR activation. In both WT and *Pparα*-null mice, there were significant similarities in gene expression changes induced by PFOS in comparison to the CAR biomarker gene set and the CAR agonist phenobarbital (Rosen et al., 2017). Two gene expression compendium studies further analyzed these data using gene expression biomarker signatures built using microarray profiles from livers of WT, *Car*-null mice (Oshida et al., 2015a), and *Pparα*-null mice (Oshida et al., 2015b). These analyses found that both CAR and PPAR were activated by PFOS, and that CAR activation was generally more significant in *Pparα*-null mice. The authors concluded that CAR likely plays a subordinate role to PPARα in mediating the adverse hepatic effects of PFOS (Oshida et al., 2015a).

Comparisons of 129S1/SvlmJ WT and *Pparα*-null mice also suggest that increases in liver weights may not be solely due to activation of PPARα. In the Rosen et al. (Rosen et al., 2010) study, absolute and relative liver weights were significantly increased in both WT and *Pparα*-null mice exposed to 10 mg/kg/day PFOS for 7 days. The absolute liver weights were increased by 63% in WT mice and by 42% in *Pparα*-null mice, while relative liver weights were increased by 44% in both strains. Similarly, in a study of male C57BL/6 (H-2^{b}) mice and *Pparα*-null 129/Sv mice exposed to 0.005% and 0.02% PFOS in diet for 10 days, absolute liver weight in WT mice was increased by 95% and 122% in the 0.005% and 0.02% groups, respectively (Qazi et al., 2009b). In *Pparα*-null mice, absolute liver weights were increased by 49% and 95% in the 0.005% and 0.02% groups, respectively. In a study by Abbott et al. (2009), WT mice were dosed with 4.5–10.5 mg/kg/day PFOS and *Pparα*-null mice were dosed with 8.5 or 10.5 mg/kg/day from GD 15–18. The authors reported that gestational exposure to 10.5 mg/kg/day resulted in increased relative liver weights in both WT (14%) and *Pparα*-null (29%) mouse pups. WT and *Pparα*-null mouse dams showed 11% and 14% increases, respectively, in relative liver weights, though these increases were not statistically significant.

A zebrafish study supports the involvement of CAR/PXR and LXR/RXR in PFOS-mediated hepatic steatosis (Cheng et al., 2016). Gene expression of liver X receptor alpha (*nr1h3*), retinoic acid receptor alpha (*rara*), retinoid X receptor gamma b *(rxrgb*), and pregnane X receptor (*nr1l2*) was elevated in WT male zebrafish livers after exposure to 0.5 µM PFOS for 5 months, which was accompanied by increased relative liver weight and lipid droplet accumulation. In female zebrafish, only a slight increase in *nr1l2* and mild lipid droplet accumulation was observed; there was no change in relative liver weight.

In comparison to the nuclear receptors mentioned above, the involvement of the nuclear receptor HNF4α, a regulator of hepatic differentiation and quiescence, has been less frequently studied in PFOS-induced liver toxicity. Only one in vivo study examined compared gene expression

changes in male WT mice exposed to 10 mg/kg/day PFOS for 7 days with genes regulated by HNF4α (Beggs et al., 2016). This study reported that 90 out of 681 genes (13%) altered by PFOS exposure were regulated by HNF4α. PFOS exposure was shown to decrease the protein expression of HNF4α in male WT mice. Increased relative liver weight in WT mice was also observed in this study, and the authors concluded that hepatomegaly, along with other liver effects such as steatosis and hepatocellular carcinoma (which were not observed in this short-term study) may be mediated by PFOS-induced dysregulation of HNF4α.

3.4.1.3.1.5 In Vitro Models

In vitro genetic studies corroborate the in vivo findings in rodents that suggest PPARα contributes to the mechanism of PFOS hepatotoxicity but is likely not the only contributor (Louisse et al., 2020; Song et al., 2016; Rosen et al., 2013; Bjork and Wallace, 2009). Two studies conducted in primary rodent and human hepatocytes had conflicting results, with one study finding no clear pattern of the differential expression of genes associated with PPARα activation in either mouse or human hepatocytes (Rosen et al., 2013), and the other study finding evidence of PPARα activation by altered expression of PPARα signaling pathway genes in rat hepatocytes, but not in human hepatocytes, neither primary nor HepG2 cells (Bjork and Wallace, 2009). In a third study in primary human hepatocytes, pathway analysis of gene expression changes induced by PFOS exposure were not significantly similar to those induced by known PPARα agonists, which is in contrast to changes following PFOA exposure (Beggs et al., 2016). However, transcripts associated with CAR/PXR activation were upregulated in human hepatocytes (Rosen et al., 2013). In contrast to the results from primary human hepatocytes, PFOS upregulated PPARα target genes in two human cell lines derived from the liver, HepaRG and HepG2 cells (Louisse et al., 2020; Song et al., 2016). Gene expression patterns in PFOS-exposed HepG2 cells were also consistent with activation of LXR (Louisse et al., 2020). Another study in HepG2 cells, however, reported reduced gene expression of *PXR* and *LXR* following treatment with 10–100 μM PFOS for 24 hours, with the reduction in *PXR* being attenuated by 48 hours (Behr et al., 2020a).

The involvement of HNF4α in PFOS-induced hepatotoxicity was examined in two in vitro studies, and the results support the findings of the in vivo study described above (Behr et al., 2020a; Beggs et al., 2016). In one study, protein levels of HNF4α were decreased in primary human hepatocytes after 48 and 98 hours of exposure to 10 μM PFOS (Beggs et al., 2016). A corresponding decrease in the expression of genes that are positively regulated by HNF4α (*CLDN1, CYP7A1, TAT,* and *ADH1B*) and increases in genes that are negatively regulated by HNF4α targets (*CCND1, AKR1B10,* and *PLIN2*) was observed. A study in HepaRG cells exposed to 1–100 μM PFOS for 24 or 48 hours corroborated these findings, as downregulations in both HNF4α and its target gene *CYP7A1* were observed (Behr et al., 2020a).

3.4.1.3.1.6 Conclusions

Although activation of PPARα is a widely cited mechanism of liver toxicity induced by PFAS exposure, PFOS has been shown to activate a number of other nuclear receptors, including PPARγ, PPARβ/δ, CAR/PXR, and LXR/RXR. Many of these nuclear receptors, including CAR and PPARγ, are also known to play important roles in liver homeostasis and have been implicated in liver dysfunction, including steatosis (Armstrong and Guo, 2019). Therefore, PFOS exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans.

3.4.1.3.2 Lipid Metabolism, Transport, and Storage

3.4.1.3.2.1 Introduction

The liver is the primary driver of lipid metabolism, transport, and storage. It is responsible for the absorption, packaging, and secretion of lipids and lipoproteins. Lipids are absorbed from digestion through biliary synthesis and secretion, where they are converted to fatty acids (Trefts et al., 2017). These fatty acids are then transported into hepatocytes, cells that make up roughly 80% of the liver mass, via a variety of transport proteins such as CD36, FATP2, and FATP5 (Lehner and Quiroga, 2016). Fatty acids can be converted to triglycerides, which can be packaged with high or very-low-density lipoproteins (HDL or VLDL, respectively) for secretion. Lipid handling for the liver is important for energy metabolism (e.g., fatty acid β-oxidation) in other organs and for the absorption of lipid-soluble vitamins. *De novo* cholesterol synthesis is another vital function of the liver (Huang et al., 2011). Cholesterol is important for the assembly and maintenance of plasma membranes. Dysregulation of any of these functions of the liver can have implications for metabolic and homeostatic processes within the liver itself and other organs and contribute to the development of diseases such as non-alcoholic fatty liver disease, steatosis, hepatomegaly, and obesity.

The liver is a major site of PFOS deposition and as such, not only influences hepatic lipid levels but can also alter gene expression for a variety of pathways involved in biological processes (U.S. EPA, 2016b). PFAS have been shown to induce steatosis and increase hepatic triglyceride levels in rodents via inducing changes in genes directly involved with fatty acid and triglyceride synthesis. These include genes such as fatty acid binding protein 1 (*Fabp1*), sterol regulatory element binding protein 1 (*Srebp1*), VLDL receptor (*Vldlr*), and lipoprotein lipase (*Lpl1*) (Armstrong and Guo, 2019). These genes can be altered through PPARα and PPARγ induction pathways due to regulation of HNF4α. PFOS upregulates hepatic nuclear receptor genes directly involved in lipid metabolism (e.g., *Pxr and Rar*) and the β-oxidation of fatty acids (e.g., *acyl-CoA oxidase 1* (*Acox1*) and carnitine palmitoyltransferase 1A (*Cpt1a*)) (Lee et al., 2020). The responses of lipids, bile acids, and associated genes and processes to PFOS exposure are dose-, model-, and, for some responses, sex-dependent.

3.4.1.3.2.2 In Vivo Models

While the sections below focus on hepatic-specific measurements of lipids from the available literature, measurements of lipids in the serum are also important indicators of lipid homeostasis and alterations in lipid metabolism, transport, and storage due to PFOS exposure. Serum lipid metrics from both animal and epidemiological studies are reported in Section 3.4.3.2 and Section 3.4.3.1, respectively.

3.4.1.3.2.2.1 Rats

Two studies conducted in both male and female Sprague-Dawley rats reported marked effects on lipid metabolism including sex-dependent effects of PFOS on hepatic outcomes (NTP, 2019; Bagley et al., 2017).

In a study by Bagley et al. (2017), male and female rats were exposed to 0 or 100 ppm of PFOS in their diet for 3 weeks. In males, the authors observed increased liver choline, an organic cation critical for the assembly/secretion of lipoproteins and the solubilization of cholesterol in bile; females fed PFOS diets had no change in liver choline levels. An increase in hepatic free fatty

acids, triglycerides, and liver lipid area percent was also observed in males fed PFOS, while a decrease was observed in females. This is indicative of hepatic steatosis occurring in males but not in females. Serum was collected from animals on days 2, 9, 16, and 23 during the 3 weeks of dietary PFOS exposure and subsequently analyzed for serum clinical chemistry. There were transient effects on the serum levels of enzymes related to lipid metabolism (e.g., lipase, lactate dehydrogenase) in the PFOS-fed groups. In comparison to controls, there was a reduction in lipase and lactate dehydrogenase in PFOS-fed males at all four of the timepoints tested. PFOS-fed females had similar reductions in lipase and lactate dehydrogenase concentrations at every timepoint except day 23. For days 2, 9, and 16, animals were not fasted prior to serum collection; on day 23, animals were instead fasted overnight, and serum was collected via exsanguination at necropsy. The gene expression of enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase (*Ehhadh*), one of the enzymes involved in peroxisomal β-oxidation, was upregulated to a larger degree in females than in males (4.1-fold vs. 3.7-fold). Similarly, stearoyl-CoA desaturase-1 (*Scd1*), involved in the conversion of oleic acid to stearate, was upregulated ninefold in females (compared with twofold in males, a change that was not significantly different from the control males). While nuclear receptors (such as CAR, PXR, LXR-α, LXR-β, and PPAR-γ) are involved in lipid accumulation, and an upregulation of the mRNA for enzymes involved in this process (such as *Scd1*) would indicate their activation, there was no lipid accumulation in females. *Ehhadh* was increased in both sexes compared with controls. Together, this may indicate that steatosis in rats is not induced by activation of these nuclear receptors or transcription levels of protein involved in key steatosis pathways. The authors also investigated the effect of choline supplementation along with PFOS administration and found that the steatosis phenotype persisted in males. The authors hypothesize that increased efficiency of female hepatic cytosolic fatty acid binding protein results in greater mobilization from lipid to VLDL causing faster excretion into serum and thus adipose tissue. However, the authors note that this apparent sex difference in lipid accumulation warrants further study (Bagley et al., 2017).

NTP (2019) used an oral dosing paradigm of 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day for 28 days and measured serum cholesterol and triglyceride concentrations (Section 3.4.3.2). Notably however, both males and females exhibited an increase in lipid metabolism/oxidation related genes (*Acox1, Cyp4a1, Cyp2b1*, and *Cyp2b2*). An increase in these genes indicates increases in PPARα and CAR activity.

In addition to the sex differences in liver lipid levels described Bagley et al. (2017), Luebker (2005b) reported that there may also be differences depending on the developmental stage. Female rats were exposed to 0, 0.4, 0.8, 1.0, 1.2, 1.6, or 2.0 mg/kg/day PFOS for 42 days (6 weeks) prior to mating through either GD 20 or LD 4. In the GD 20 group, dams were sacrificed and fetuses collected at GD 21, and liver cholesterol and triglycerides were measured in dams and fetuses exposed to 0, 1.6, or 2.0 mg/kg/day. In dams, liver cholesterol was significantly reduced at both doses of PFOS, whereas triglycerides were unchanged. No changes were observed in fetuses at this timepoint. In the LD 5 groups, dams and pups were sacrificed to measure liver cholesterol and triglycerides. In dams, liver cholesterol was unchanged at this time point, and liver triglycerides were significantly increased at 1.6 and 2.0 mg/kg/day. In pups, liver cholesterol was also unchanged; however, liver triglycerides were significantly decreased in pups exposed to 1.0–2.0 mg/kg/day in both sexes.

3.4.1.3.2.2.2 Mice

Several studies in a variety of mouse models were conducted to investigate the effects of PFOS on the transcription and translation of lipid metabolism and biliary pathways. The focus of these studies was to identify key regulators affected by PFOS exposure and the extent to which pathways were affected. To this end, the studies employed expression microarray, quantitative reverse transcription polymerase chain reaction (qRT-PCR), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analysis (IPA), and other biochemical measures such as Western Blot and enzyme-linked immunosorbent assay (ELISA).

3.4.1.3.2.2.2.1 Biochemical and Related Histological Changes

Many biochemical changes occurred with lipids and bile within the liver as well as lipid transport out of the liver (serum/plasma values). In several mouse studies, triglycerides, total cholesterol, and/or LDL levels were altered in liver (Liang et al., 2019; Huck et al., 2018; Lai et al., 2018; Xu et al., 2017). These changes often had potentially associated histopathological consequences, with steatosis and other lesions being observed in affected livers (Liang et al., 2019; Su et al., 2019; Huck et al., 2018).

In a 4-week study, decreased liver cholesterol was observed in male C57BL/6 mice dosed with 5 mg/kg/day PFOS (Xu et al., 2017); the mechanism of action was attributed to estrogen receptor ß (eRß) and is further described in Section 3.4.1.3.3. In a 7-week study, increased liver triglycerides were observed in female CD-1 mice exposed to 0.3 or 3 mg/kg/day PFOS (Lai et al., 2018). A yellowish appearance was also noted in the livers of the 3 mg/kg/day group, which the authors associated with lipid accumulation. The authors hypothesized that the increased hepatic triglycerides may be due to an impairment in lipid catabolism and/or lipid export.

A study in Kunming mice investigated lipid metabolism markers within pregnant mice and the offspring exposed prenatally (Liang et al., 2019). Lipid dysregulation was present in both mother and offspring. Specifically, the authors observed increased liver weight and triglyceride content at the 5 mg/kg/day dose of PFOS in both the mother and offspring. In maternal livers, hepatomegaly along with hepatic steatosis was observed. Further, the authors also found increased protein expression of CYP4A14 in offspring. This cytochrome P450 catalyzes the omega(ω)-hydroxylation of medium-chain fatty acids and arachidonic acid in mice and is a common indicator of PPARα activation. Authors also observed increases in CD36 protein levels, which has a direct effect on fatty acid uptake by hepatocytes, and decreased levels of the proteins apolipoprotein B (APOB), a cholesterol transporter, and FGF21 in the PND 1 mouse liver. Together, this evidence indicates that PFOS undergoes gestational transfer, impairing lipid homeostasis in the offspring.

In ICR mice exposed to 10 mg/kg/day PFOS for 21 days, lipid-based vacuolization was observed in the liver, which was accompanied by decreased fibroblast growth factor 21 (FGF21) protein concentration (Su et al., 2019). This hormone is produced by hepatocytes and regulates the metabolism of sugar and lipids through receptors in the hypothalamus. Interestingly, vitamin C showed a protective effect in the study, lowering the effect size of some of the increased parameters and reducing liver lesions. This indicates that nutritional status can mediate the hepatotoxicity of PFOS.

Beggs et al. (2016) observed a decrease in hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator of hepatic differentiation, in the livers of 10-week-old CD-1 mice exposed to 3

or 10 mg/kg/day PFOS by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcription of specific liver genes, and lipid metabolism. This decrease in HNF4α protein occurred without a subsequent reduction in messenger ribonucleic acid (mRNA) levels but appeared to cause a subsequent upregulation of genes that are negative targets of HNF4α. For example, downstream proteins such as CYP7a1 and perilipin 2 (PLIN2) were reduced. HNF4α is considered an orphan receptor with various fatty acids as its endogenous ligands. These fatty acids maintain the structure of the receptor homodimer. PFOA and PFOS are analogous in structure to fatty acids and may also provide stabilization of the homodimer. The authors investigated the role of PFOS interaction with this protein via in silico docking models, which showed a displacement of fatty acids by PFOS and PFOA, possibly tagging HNF4α for degradation. Although the authors, do not directly look at liver pathology, they hypothesize that steatosis, hepatomegaly, and carcinoma in rodents may be a consequence of the loss of this protein and also presents a potential mechanism for PFOS-induced hepatic effects in humans (Beggs et al., 2016).

3.4.1.3.2.2.2.2 Microarray Analyses and RT-PCR

Several studies observed perturbations in lipid transport, fatty acid synthesis, triglyceride synthesis, and cholesterol synthesis in PFOS-exposed mice (Liang et al., 2019; Su et al., 2019; Huck et al., 2018; Das et al., 2017; Rosen et al., 2017). Two of these studies, Das et al. (2017) and Rosen et al. (2017), investigated the effects of PFOS on lipid metabolism and homeostasis without the influence of PPARα using nullizygous models. After exposure to 3 or 10 mg/kg/day PFOS for 7 days, Das et al. (2017) observed that a smaller subset of genes related to lipid homeostasis was activated in *Pparα*-null mice compared with WT mice. In addition, there were three-to-fourfold reductions in the genes related to lipid homeostasis that were expressed in PFOS-exposed *Pparα*-null mice compared with WT mice, including carbohydrate response element binding protein (*Chrebp*), *Hnf4α*, Pparγ coactivator 1α (*Ppargc1a*), and sterol regulatory element binding transcription factor 2 (*Srebf2*). In *Pparα*-null mice, there was only a twofold decrease in *Hnf4α*, a fourfold decrease in *Ppargc1a*, and a threefold increase in *Srebf1*. *Srebf* genes encode transcription factors that bind to the sterol regulatory element-1 motif that is found in the promoter of genes involved in sterol biosynthesis. This indicates that some of the effects on lipid metabolism are independent of, or only partially dependent on, PPARα as an upstream regulator.

The results from Das et al. (2017) are concurrent with the findings in another study by the same authors (Rosen et al., 2017), which exposes WT and *Pparα*-null mice to 10 mg/kg/day PFOS for 7 days. PFOS exposure upregulated genes related to fatty acid β-oxidation, lipid catabolism, lipid synthesis, and lipid transport in both strains; however, the increase in expression was several-fold lower in *Pparα*-null mice than in WT mice. In fact, the authors suggest that the transcriptome of the mice resembled that of mice treated with PPARγ agonists, thus suggesting a role for other PPAR receptors in the dysregulation of lipid synthesis that occurs with PFOS exposure. Xu et al. (2017), in their investigations using *Erβ*-null mice (Section 3.4.1.3.3), found a difference in lipid metabolism and bile acid synthesis between *Erβ*-null and WT mice exposed to PFOS. In mice exposed to PFOS, mRNA levels of cholesterol-7a-hydroxylase (*Cyp7a1*), the rate limiting enzyme in the conversion of cholesterol to bile acid, was downregulated in WT but not in *Erβ*-null mice, supporting a role for pathways independent of PPARα in hepatic lipid responses to PFOS exposure.

Genes involved in lipid homeostasis and regulation were found to be differentially expressed in mice exposed to PFOS (Liang et al., 2019; Su et al., 2019; Huck et al., 2018). Key regulators of fatty acid oxidation including *Cyp4a14* and *Cd36* were upregulated in the livers of PND 1 mice exposed during gestation to PFOS (Liang et al., 2019). Interestingly, genes related to hepatic export of lipids, such as *Apob* and *Fgf21*, were downregulated. Downregulation of these genes may play a role in the hepatic steatosis, hepatomegaly, and hepatocyte hypertrophy observed across multiple studies. A study using C57BL/6 mice dosed at 1 mg/kg/day PFOS in the diet for 6 weeks, found that a high fat diet (HFD) protected against PFOS-induced steatosis and hepatomegaly by inducing *Apoa1, Apoa2, Apob*, and the microsomal triglyceride transfer protein (*Mttp*) gene expression (Huck et al., 2018). *Srebf1*, a regulator of hepatic lipogenesis, was significantly induced in PFOS-exposed mice in the HFD group compared with those fed normal diets. Similarly, gene expression of *Cd36*, a major lipid importer, was induced by PFOS in mice fed normal diet but was suppressed in HFD groups, suggesting that co-administration of PFOS and HFD mitigates steatosis and hepatomegaly. Together, these results suggest that diet could be a mediating factor in PFOS toxicity and warrants consideration for evaluation of human hepatic effects.

3.4.1.3.2.2.2.3 Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analyses (IPA)

KEGG and IPA tools (Qiagen) are useful for analysis and interpretation of large datasets generated from transcriptomic profiling. Two studies extensively utilized these tools to characterize the changes to liver lipid homeostasis. Much like in the studies described in the previous two subsections, many genes related to the synthesis of fatty acids, including lipid, fatty acid, triglyceride, linoleic acid and arachidonic acid metabolism, lipid transport, fatty acid biosynthesis, and triglyceride homeostasis were differentially expressed in mice administered PFOS (Lai et al., 2017b; Beggs et al., 2016).

Beggs et al. (2016) exposed CD-1 mice to 0 or 10 mg/kg/day PFOS for 7 days. The pathway for hydroxylation of lipids was significantly dysregulated in the PFOS-exposed group. Lai et al. (2017b) exposed pregnant CD-1 mice to 0 or 0.3 mg/kg/day PFOS before mating through to embryonic day 18.5. Pathway enrichment analysis using KEGG and IPA to understand the signaling pathways and biological processes that were affected, as evidenced by differentially expressed genes, highlighted changes in fatty acid metabolism including the deregulation of the PPAR signaling pathway (not specific to any isoform), fat digestion and absorption, the biosynthesis of unsaturated fatty acids, and bile secretion in both the maternal and offspring livers.

3.4.1.3.2.2.3 Zebrafish

Zebrafish have been increasingly used as a model to investigate the toxicity of PFAS. Several studies have evaluated the toxicity of PFOS in zebrafish, specifically in regard to effects on lipid metabolism. Similar to the results in rodent models, fatty acid oxidation enzymes and related gene expression, as well as lipidosis, was increased in PFOS-treated animals (Khazaee et al., 2019; Cui et al., 2017; Cheng et al., 2016; Du et al., 2014). The authors of these studies also reported increases in triglycerides, total cholesterol, and free fatty acid receptors in liver samples from PFOS-exposed zebrafish. Interestingly, as seen in rodent models, there can be a temporal shift in the levels of proteins or genes involved in lipid metabolism, with PFOS exposure. Khazaee et al. (2019) found that expression levels of the fatty acid binding protein 1-A gene

fabp1a, which binds free fatty acids and their coenzyme A derivatives and is involved in their intracellular transport into the liver, varied over a 30-day period of exposure to 0.1 or 1 mg/L PFOS. Expression in the liver peaked at day 14 of exposure but being below control levels at day 30 of exposure. This suggests that lipid metabolism is dynamic, and the authors concluded that more research is needed to understand if a key time point exists for evaluating such gene expression changes versus examining such changes over time.

Sex-dependent differences were also observed in a few studies in PFOS-treated zebrafish (Cui et al., 2017; Cheng et al., 2016). In one study in which zebrafish were exposed to 0.5 μM for 5 months beginning at 8 hours post-fertilization (hpf), males tended to have increased fatty accumulation and reduced hepatic glycogen storage compared with females (Cheng et al., 2016). In a 2-generation study, Cui et al. (2017) observed that the offspring of zebrafish exposed to PFOS from 8 hpf until 180 days post-fertilization (dpf) tended to have increased expression of the leptin α (*lepa*) and insulin receptor α (*insr*) genes. Diacylglycerol O-acyltransferase 1 (*dgat1b*), a metabolic enzyme in triglyceride biosynthesis, and *apoa1*, which regulates cholesterol transport, were downregulated by PFOS exposure. The authors also noted that along with indicators of lipid dysregulation, there were morphologically different mitochondria, potentially exacerbating lipid homeostasis.

3.4.1.3.2.3 In Vitro Models

Two studies reported genetic profiles and pathway analyses in mouse and human hepatocytes to determine the effect of PFOS treatment on lipid homeostasis and bile synthesis. Rosen et al. (2013) exposed mouse and human primary hepatocytes to 0–250 μM PFOS for 48 hours. Gene expression was evaluated using microarrays, IPA, and qRT-PCR. For PFOS-exposed murine hepatocytes, a much smaller group of genes was found to be altered compared with the whole liver (described in Section 3.4.1.3.4). These included genes associated with β-oxidation and fatty acid synthesis such as *Ehhadh* and *Fabp1*, which were both upregulated with PFOS exposure. In contrast to the transcriptome of primary mouse hepatocytes, in primary human hepatocytes, a relatively large group of genes related to lipid metabolism including *PLIN2* and *CYPT1A* were differentially expressed with PFOS exposure. The authors attribute some of these differences between mouse and human hepatocytes to a less robust activation of PPARα in humans. Further, many of the genes investigated were chosen to explore effects of PFOS exposure that are independent of PPARα activation but may include other nuclear receptors such as CAR, LXR, PXR and the aryl hydrocarbon receptor (AhR) (Section 3.4.1.3.1). Beggs et al. (2016) exposed human primary hepatocytes to 0.01–100 μM PFOS for 48 or 96 hours, to determine pathways affected by PFOS exposure. PFOS treatment altered genes primarily associated with liver necrosis and carcinogenesis. However, pathways associated with lipid metabolism and bile synthesis (hydroxylation of lipids), including several CYP450 enzymes associated with lipid homeostasis such as *CYP2B6, CYP2C8, CYP3A4, CYP3A5, CYP4A11, CYP4A22*, and *CYP7A1* were also altered. Notably, CYP7A1 was among the top 10 most downregulated genes with a fold change of −7.13 indicating potential limitations in the conversion of cholesterol to bile acid. Importantly, HNF4α, a master regulator of liver function, regulates many differentially expressed genes related to lipid metabolism which includes all the aforementioned CYP450s. Together these studies indicate PFOS-induced activation of CYP450 through a variety of PPARα-dependent and independent pathways. Interestingly, there may be crosstalk between some of these receptors. Beggs et al. (2016) notes that HNF4α can regulate PPARα in mice.

There are several studies that investigated the effect of PFOS on lipid homeostasis using human cells such as HepG2, HepaRG, and HL-7702 cells. Various endpoints were also investigated in these cell lines such as mRNA expression through microarray and qRT-PCR assays; lipid, triglyceride, cholesterol, and choline content; and protein levels via ELISA or Western Blot.

In human hepatic cell lines such as HepaRG or HepG2, PFOS treatment correlated with suppression of gene expression for genes regulating cholesterol homeostasis. Louisse et al. (2020) noted a concentration-dependent increase in triglycerides, a decrease of cholesterol, and downregulation of cholesterogenic genes, predominantly with the highest dose tested, in HepaRG cells exposed to 0–100 μM PFOS for 24 hours. Cellular cholesterol biosynthesis genes are regulated by SREBPs, which were also downregulated with PFOS exposure. In contrast, PPARα-responsive genes were upregulated with PFOS exposure, particularly at higher doses. Behr et al. (2020a) also exposed HepaRG cells to 0–100 μM PFOS for 24 or 48 hours. Similar to the results from Louisse et al. (2020), at 24 hours, genes related to cholesterol synthesis and transport were downregulated at the highest dose except for several genes that were upregulated, including bile and cholesterol efflux transporters (*UGT1A1* and *ABCG1*), and genes involved in bile acid detoxification (*CYP3A4*). The gene profiles after 48 hours of exposure were similar, except at the high dose, which saw some attenuation of the response in cholesterol synthesis and transport. Cholesterol content was significantly higher in the supernatant at the highest dose of 100 μM but there was no significant difference after 48 hours between treated cells and controls, in line with the genetic data of some response attenuation.

Franco et al. (2020a) exposed HepaRG cells to 0.0001–1 μM. Interestingly, lipid levels were elevated with the lower PFOS concentrations and reduced with the higher PFOS concentrations. PFOS increased diglyceride levels in a dose-dependent manner except for a decrease that was observed at the highest concentration. In contrast, triglyceride levels were not significantly different from controls. This study provides evidence of potential non-monotonic dose-responses that could result from low-dose PFOS exposures, a potential area that may require further consideration.

While alterations in lipid metabolism have been reported, Das et al. (2017) found that PFOS did not inhibit palmitate-supported respiration (i.e., mitochondrial metabolism) in HepaRG cells. There was no effect on oxidation or translocation of palmitoylcarnitine, an ester involved in the metabolism of fatty acids which plays a role in the tricarboxylic acid cycle.

3.4.1.3.2.4 Conclusions

As described in Section 3.4.3.2, serum lipid concentrations generally decrease with increasing PFOS doses in rodent bioassays. It is thought that the activation of PPARα, which is less robust in humans, mediates the effect seen in rodents. In the mechanistic evidence synthesized above, it appears that PFOS exposure in mammalian and non-mammalian species is associated with increased lipid accumulation within the liver. Interestingly, studies that measure both serum and liver lipid content generally follow this trend and report a decrease in serum lipids and an increase in liver lipid content; this effect may be contributing to the observed PFOS-induced hepatomegaly and steatosis. Additional data on human liver lipid accumulation would clarify whether the effects on liver lipid contents in animal bioassays are mechanistically relevant to humans.

Effects on hepatic lipid metabolism can be observed through the influence of PFOS on not only PPARα, but other key regulators of hepatic lipid homeostasis such as HNF4α. Gene ontology using receptor null mice has shown that lipid homeostasis is complex and PFOS is likely acting on more than one key regulator. Other PPAR isoforms and hormone receptors such as eRβ play a role in regulating lipid and bile metabolism/catabolism, transport, and storage. While minor conflicts exist between some cell line studies, the evidence supports that PFOS causes lipid dyshomeostasis and contributes to liver dysfunction and disease, likely through the modulation of multiple nuclear receptors.

3.4.1.3.3 Hormone Function and Response

While much of the literature relevant to hormone function and response is focused on reproductive outcomes (see Appendix, (U.S. EPA, 2024a)), recent literature has also shown a relationship between hepatic hormonal effects and PFOS exposure. For example, PFOS has been found to have estrogenic effects. Xu et al. (2017) reported an induction of eRβ, but not estrogen receptor alpha (eRα), when wild-type (C57BL/6) male mice were dosed with 5 mg/kg/day PFOS via oral gavage for 4 weeks. To further explore this relationship, the authors investigated PFOS administration in male wild-type (WT) and Erβ-null mice. They observed no significant changes in either WT or *Erβ*- null mice in genes related to lipid metabolism and bile synthesis (3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), scavenger receptor class B type I (*Srbi*), low-density lipoprotein (*Ldl*), ATP-binding cassette transporter (*Abca1*)) when following exposure to 5 mg/kg/day PFOS for 28 days by oral gavage. However, ATP-binding cassette subfamily G member 5 (*Abcg5*), a gene involved in sterol excretion, was increased due to PFOS exposure in WT mice but not in *Erβ*-null mice, while cholesterol 7α hydroxylase (*Cyp1a711*), the initiator of cholesterol catabolism, was reduced due to PFOS exposure in WT mice but not in *Erβ*-null mice. Further, liver cholesterol levels were significantly decreased in WT PFOS-treated animals but not in *Erβ*-null mice. This suggests that eRβ mediates PFOS hepatotoxicity via altered cholesterol and bile synthesis. To confirm induction of eRβ, the authors also investigated the response to PFOS exposure in HEPG2 cells. After exposing the cells to 0, 10, or 100 μmol/L of PFOS for 24 hours, the authors found that eRβ was induced at 10 μmol/L, but not at the highest dose, potentially indicating a non-monotonic dose response.

There is also in vitro evidence that in the liver, genes responsible for a response to hormone stimulus and hormone metabolism are altered with PFOS exposure (Song et al., 2016; Popovic et al., 2014). Differentially expressed genes due to PFOS treatment in these studies encode proteins such as serine peptidase inhibitor, clade A, proprotein convertase subtilisin/kexin type 9, activin A receptor type IC, and insulin-like growth factor binding protein 7, all of which are associated with hormone stimulus and/or metabolism. However, it should be noted that these genes were more significantly altered with PFOA exposure; the authors indicated that while PFOS was more cytotoxic, PFOA exposure induced more gene alterations, suggesting that PFOS may be a relatively weak agonist or activator for the transcription factors or nuclear response elements involved in regulating their transcription (Song et al., 2016).

3.4.1.3.3.1 Conclusions

While there is a small number of studies regarding hormone function and response specifically within the liver, there is evidence that PFOS has the potential to perturb hormonal balance and hormonal metabolism in hepatic cells. There is also some evidence from one in vivo study in mice that PFOS hepatotoxicity may be partially modulated by eRβ. This could have implications

for hormone function and responses in other organ systems and may also be important for mode of action considerations for hepatotoxicity.

3.4.1.3.4 Xenobiotic Metabolism

3.4.1.3.4.1 Introduction

Xenobiotic metabolism is the transformation and elimination of endogenous and exogenous chemicals via enzymes (i.e., cytochrome P450 (CYP) enzymes) and transporters (i.e., organic anion transporting peptides (OATPs)) (Lee et al., 2011). As described in Section 3.3.1.3, the available evidence demonstrates that PFOS is not metabolized in humans or other species. However, several studies have investigated how PFOS could alter activation of PXR/CAR as described in Section 3.4.1.3.1; subsequently, xenobiotic metabolism is altered via manipulation of the expression of key genes. For instance, the genes for OATP expression (i.e., *slco1d1* and *slco2b1*) in zebrafish or phase I and II biotransformation enzymes in human hepatocytes (i.e., *CYP3A4*), responsible for the transport or metabolism of xenobiotics, may be upregulated or downregulated following PFOS exposure.

Overall, results from both in vivo and in vitro model systems suggest that genes responsible for xenobiotic metabolism are upregulated as a result of PFOS exposure.

3.4.1.3.4.2 In Vivo Models

Four studies investigated xenobiotic metabolism endpoints with three studies using Sprague-Dawley rats (Elcombe et al., 2012a; Chang et al., 2009; Curran et al., 2008) and the remaining study using *Pparα*-null and WT mice (Rosen et al., 2010). In a gestational and lactational exposure study, Chang et al. (2009) reported increased *Cyp2b2* expression in dams and male pups (2.8-fold and 1.8-fold, respectively). Elcombe et al. (2012a) also reported the induction of CYP2B1/2, in addition to CYP2E1 and CYP3A1 proteins, following test diets of 20 ppm or 100 ppm PFOS. Additionally, Curran et al. (2008) and Rosen et al. (2010) reported upregulation of *Cyp4a22* and *Cyp2b10* expression.

Two studies examined xenobiotic metabolism endpoints, including CYP450 expression and CYP2B enzyme activity via the PROD biomarker response, in rats (NTP, 2019; Elcombe et al., 2012b). Sprague-Dawley rats were exposed to 0, 20, or 100 ppm PFOS for a 7-day dietary treatment and then were assessed for CYP450 protein expression in the liver at recovery days 28, 56, and 84 (Elcombe et al., 2012b). Total CYP450 concentration in liver microsomes was measured via carbon monoxide difference spectrum of ferrocytochrome P450. Across each dose group and recovery day, mean CYP450 concentrations were increased 123%–189% compared with the control group. However, there was a nonlinear PROD dose-response relationship; the 20 ppm group had decreased mean PROD activity across all recovery days, but the 100 ppm group had increased activity on recovery days 1 and 28, followed by similar activity on recovery day 56, then statistically significant decreased PROD activity by recovery day 84. NTP (2019) also assessed Sprague-Dawley rats following 28-day treatment of PFOS (0, 1.25, 2.5, or 5 mg/kg/day) by gavage. Across all treatments of PFOS, females and males both had increased hepatic expression of *Cyp2b1, Cyp2b2*, and *Cyp4a1*.

One study examined the expression of genes related to xenobiotic metabolism in zebrafish (Jantzen et al., 2016b). AB strain zebrafish embryos were exposed to PFOS from 3 to 120 hpf and evaluated at 180 dpf. Female zebrafish had significant reductions in *slco1d1* expression,

while males had significant reductions in both *slco1d1* and *slco2b1* expression (Jantzen et al., 2016b), which are the genes responsible for OATPs and significant in the transport of xenobiotics (Popovic et al., 2014). Jantzen et al. (2016b) noted that in their previous study, PFOS exposure from 5 to 14 dpf resulted in significantly reduced slco2b1 expression in zebrafish at 5 dpf but significantly increased expression at 14 dpf (Jantzen et al., 2016a). While their current study reported alterations in gene expression long-term, further studies with additional time points are needed to elucidate the effect of PFOS exposure on OATP expression.

3.4.1.3.4.3 In Vitro Models

Gene expression of CYP enzymes responsible for xenobiotic metabolism were assessed in one study using primary human (e.g., *CYP2B6* and *CYP3A4* genes) and mouse (e.g., *Cyp1a1* and *Cyp3a11* genes) hepatocytes (Rosen et al., 2013). Results varied between human and mouse hepatocytes, with *CYP2B6* and *CYP3A4* expression upregulated in human hepatocytes, but not in mouse hepatocytes. The authors noted that the reasons for the differences in gene expression in the human and mouse hepatocytes were unclear; however, cell density, collection methods, and time in culture were possible factors, as these were not consistent between models.

Xenobiotic metabolism endpoints were assessed in five studies using hepatic cell lines, including HepG2 (Song et al., 2016; Shan et al., 2013) and HepaRG (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020). Franco et al. (2020b) assessed several phase I biotransformation enzymes following exposure to PFOS concentrations (0.0001, 0.001, 0.01, 0.1, or 1.0 μM) for 24 or 48 hours. Gene expression of phase I enzymes varied across concentrations and between the 24- and 48-hour exposures. For *CYP1A2*, after 24 hours, the two lowest concentrations resulted in significant increases in expression; however, after 48 hours, the two highest concentrations resulted in significant decreases (~10-fold) in expression. For *CYP2C19*, after 24 hours, there were no clear trends; however, after 48 hours, expression was significantly reduced across all concentrations (Franco et al., 2020b).

Evidence varied for CYP3A4 induction, depending on the model and duration of exposure, as well as whether gene expression or enzyme activity was assessed (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; Shan et al., 2013). Franco et al. (2020b) reported that after 24 hours, there were no clear trends in *CYP3A4* expression. However, after 48 hours, *CYP3A4* expression was significantly reduced (up to fivefold) across all concentrations (Franco et al., 2020b). Conversely, Behr et al. (2020a) and Louisse et al. (2020) reported upregulation of CYP3A4 enzyme activity following 24- or 48-hour PFOS exposure (1, 10, 25, 50, and 100 μM) in HepaRG cells, while Shan et al. (2013) reported no significant changes in CYP3A4 enzyme activity following PFOS exposure (0, 100, 200, 300, and 400 μM) in HepG2 cells.

Franco et al. (2020b) also assessed gene expression of two phase II enzymes, glutathione S-transferase mu 1 (*GSTM1*) and UDP glucuronosyltransferase 1A1 (*UGT1A1*), which were not significantly affected in differentiated HepaRG cells by exposure to PFOS after 24 or 48 hours. The authors noted that it was unclear how PFOS alters gene expression of phase I enzymes but not phase II enzymes. Further research is needed to determine whether altered gene expression occurs by interference with cytoplasm receptors, inhibition of nuclear translocation, or inhibition of the interaction of nuclear translocator complexes with DNA sequences (Franco et al., 2020b).

Song et al. (2016) analyzed expression of over 1,000 genes via microarray and gene ontology analysis in HepG2 cells exposed to PFOS. HepG2 cells were first exposed to 0–1,000 μM PFOS

for 48 h to determine cell viability and cytotoxicity; an IC20 dose of 278 μM PFOS was determined from these results. HepG2 cells were then treated with 278 μM PFOS for 48 hours and used in microarray analysis. As a result of 278 μM PFOS treatment, 279 genes had ≥1.5-fold change in compared with the control group, including genes related to xenobiotic metabolism by cytochrome P450s such as flavin containing dimethylaniline monoxygenase 5 (*FMO5*), UDP glucuronosyltransferase family 1 member A6 (*UGT1A6*), glutathione S-transferase alpha 5 (*GSTA5*), alcohol dehydrogenase 6 (class V) (*ADH6*), and glutathione S-transferase alpha 2 (*GSTA2*).

3.4.1.3.4.4 Conclusions

Several studies are available that assessed xenobiotic metabolism endpoints as a response to PFOS exposure, including studies in rats (NTP, 2019; Elcombe et al., 2012b), zebrafish (Jantzen et al., 2016b), primary hepatocytes (Rosen et al., 2013), or hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; Song et al., 2016; Shan et al., 2013). Jantzen et al. (2016b) reported significant reductions in the expression of OATPs (*slco1d1* and *slco2b1*). While the majority of studies reported upregulation of gene expression of CYP enzymes (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; NTP, 2019; Song et al., 2016; Rosen et al., 2013; Elcombe et al., 2012b), direction and magnitude of change varied across doses and exposure times. Jantzen et al. (2016b) and Franco et al. (2020b) both noted the need for further studies to elucidate any potential relationships between PFOS exposure and xenobiotic metabolism.

3.4.1.3.5 Cell Viability, Growth and Fate

3.4.1.3.5.1 Cytotoxicity

Many in vitro studies have examined the potential for PFOS to cause cytotoxicity with various cell viability assays in both primary hepatic cell cultures (Xu et al., 2019b; Khansari et al., 2017) and in hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Franco et al., 2020a; Louisse et al., 2020; Ojo et al., 2020; Rosenmai et al., 2018; Sheng et al., 2018; Bagley et al., 2017; Oh et al., 2017; Song et al., 2016; Wan et al., 2016; Cui et al., 2015b; Wielsøe et al., 2015; Huang et al., 2014; Shan et al., 2013; Florentin et al., 2011), with varying results depending on the exposure time and culturing methods. In mouse primary hepatocytes, cell viability was reduced by approximately 10% as determined by the CCK-8 assay after 24 hours of exposure to 10 μM PFOS (Xu et al., 2019b) and by 64%, as determined by a trypan blue exclusion assay in rat primary hepatocytes exposed to 25 μM PFOS for 3 hours (Khansari et al., 2017). However, another study in mouse and human primary hepatocytes reported that 100 μM PFOS did not induce cytotoxicity after 48 hours, determined by a lack of treatment effect in genes related to cell damage such as heme oxygenase 1 (*HMOX1*), DNA damage inducible transcript 3 (*DDIT3*), and activating transcription factor 3 (*ATF3*) (Rosen et al., 2013).

Median lethal concentration (LC50) values in hepatic cell lines ranged from approximately 13 μM PFOS after for 24 or 48 hours of exposure in HepaRG cells (Franco et al., 2020b; Franco et al., 2020a), to 45–65 μM after 24 or 48 hours of exposure in HepG2 cells (Ojo et al., 2020; Wan et al., 2016), to 417 μM after 24 hours of exposure in HL-7702 cells (Sheng et al., 2018). However, two studies in HepG2 cells (Rosenmai et al., 2018) and HepaRG cells (Louisse et al., 2020) showed no effect on cell viability up to concentrations of 100 μM for 24 hours or 400 μM

for 72 hours, respectively. A subset of these studies looked further into the mechanisms of cytotoxicity, including the induction of apoptotic pathways (Section 3.4.1.3.5.2.2).

3.4.1.3.5.2 Apoptosis

3.4.1.3.5.2.1 In Vivo Models

Apoptosis induced by PFOS exposure was assessed in five studies in male rats (Han et al., 2018a; Eke et al., 2017; Wan et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a) and two studies in male mice (Lv et al., 2018; Xing et al., 2016), with varying results. Two short-term dietary studies exposed rats to 20 or 100 ppm PFOS (equivalent to approximately 2 and 10 mg/kg/day, respectively), and apoptosis was assessed through the TUNEL assay (Elcombe et al., 2012b; Elcombe et al., 2012a). In one of these studies, rats were exposed for 7 days and allowed to recover for 1, 28, 56, or 84 days (Elcombe et al., 2012b), while the other study exposed rats for 1, 7, or 28 days and collected liver directly after exposure (Elcombe et al., 2012a). In the recovery study, at both PFOS exposure concentrations, a decreased apoptotic index was observed at all timepoints tested. In the 28-day study, the apoptotic index was decreased with 100 ppm PFOS at days 7 and 28, and increased at 20 ppm on day 7; no changes were observed at other timepoints. It should be noted that cell proliferation was markedly increased, particularly with the higher dose (100 ppm), in both studies (Section 3.4.1.3.5.3); increases in the total number of cells due to cell proliferation may confound certain metrics of apoptosis that do not report comparisons of the absolute number of apoptotic cells along with cell percentages.

Contrary to the dietary studies, three short-term gavage studies in rats showed an increase in expression of apoptotic genes (caspase 3 (*Casp3*) and caspase 8 (*Casp8*)) and proteins (e.g., cleaved poly-ADP-ribose polymerases (PARP), CASP3, and BCL2 associated X, apoptosis regulator (Bax)) in livers collected after administrations of up to 10 mg/kg/day PFOS for 28 days (Han et al., 2018a; Eke et al., 2017; Wan et al., 2016). Similarly, two short-term gavage studies in male mice showed an increase in liver apoptosis (Lv et al., 2018; Xing et al., 2016). Increased apoptosis in the liver, as determined via the TUNEL assay, was observed in male mice administered 2.5–10 mg/kg/day PFOS for 30 days (Xing et al., 2016). Increased apoptosis was also observed in liver tissue of male mice dosed with 10 mg/kg/day PFOS for 21 days, as measured by an increased expression of apoptotic-related proteins (tumor suppressor p53 (p53) and BAX) and a corresponding decrease in B cell leukemia/lymphoma 2 (BCL2) and by an increase in CASP3 enzyme activity (Lv et al., 2018).

Several studies further examined the mechanisms by which PFOS exposure may lead to apoptosis in the liver (Xu et al., 2020b; Han et al., 2018a; Lv et al., 2018; Oh et al., 2017; Xing et al., 2016; Huang et al., 2014; Yao et al., 2014). One rat study suggested that hepatic apoptosis was induced through mitochondrial damage, as shown by an increased level of cytoplasmic cytochrome c and decreased level of mitochondrial cytochrome c (Han et al., 2018a). Two mouse studies concluded that hepatic apoptosis was induced by increases in oxidative stress, as evidenced by a decrease in antioxidant enzymes and a corresponding increase in lipid peroxidation (Lv et al., 2018; Xing et al., 2016). In a third mouse study that examined microRNA (miRNA) expression in the liver, an increase in the expression of *miR-34a-5p*, which has been shown to recapitulate p53-mediated apoptosis, was observed (Yan et al., 2014).

3.4.1.3.5.2.2 In Vitro Models

In vitro, apoptosis has been examined in primary mouse hepatocytes and mouse and human cell lines after exposure to various concentrations of PFOS (Xu et al., 2020b; Xu et al., 2019b; Oh et al., 2017; Song et al., 2016; Wan et al., 2016; Yao et al., 2016; Cui et al., 2015b; Huang et al., 2014). PFOS was shown to increase the percentage of apoptotic cells (Xu et al., 2019b; Oh et al., 2017; Yao et al., 2016; Cui et al., 2015b; Huang et al., 2014), to increase the expression of proteins and genes in apoptotic pathways (Song et al., 2016; Wan et al., 2016), or to increase CASP3 enzyme activity (Yao et al., 2016). Only one study in HL-7702 cells showed no change in the percentage of apoptotic cells (Cui et al., 2015a).

In mouse primary hepatocytes, PFOS induced apoptosis through activation of Caspase 3, which was mediated by PFOS-induced mitochondrial membrane damage and increased intracellular calcium levels (Xu et al., 2020b). One study in the Chang liver cell line suggested that apoptosis following exposure to PFOS may be caused by endoplasmic reticulum stress, mediated by the phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) (Oh et al., 2017). A study in human L-02 cells suggested that PFOS exposure may lead to apoptosis through the activation of p53 and myc proto-oncogene (myc) pathways (Huang et al., 2014). In two studies in HepG2 cells, PFOS exposure led to increases in apoptosis and alterations in autophagy, leading the authors to conclude that hepatotoxicity induced by PFOS exposure may be at least partially attributed to autophagy-dependent apoptosis (Yao et al., 2016; Yao et al., 2014).

No in vitro study directly evaluated cellular necrosis, although one RNA-sequencing study in primary human hepatocytes found that PFOS exposure was associated with changes in gene expression that aligned with cell death and hepatic system disease, including necrosis, cholestasis, liver failure, and cancer (Beggs et al., 2016). Another RNA-sequencing study showed that PFOS induced genetic changes in WT zebrafish that were comparable to those seen in a zebrafish model of fatty liver disease; pathways involved in apoptosis of hepatocytes and focal necrosis of liver were upregulated (Fai Tse et al., 2016).

3.4.1.3.5.3 Cell Cycle and Proliferation

3.4.1.3.5.3.1 In Vivo Models

Alterations in cell proliferation and the cell cycle were also seen in many in vivo and in vitro studies (Louisse et al., 2020; Han et al., 2018b; Huck et al., 2018; Lai et al., 2017b; Beggs et al., 2016; Song et al., 2016; Cui et al., 2015b; Cui et al., 2015a; Elcombe et al., 2012b; Elcombe et al., 2012a; Thomford, 2002b). Two short-term studies in male rats with PFOS doses of 20 or 100 ppm (approximately 2 and 10 mg/kg/day, respectively) found increased proliferation in the liver, as seen through increased BrdU staining, which was accompanied by increased liver weights (Elcombe et al., 2012b; Elcombe et al., 2012a). In a third study in male rats dosed with 1 or 10 mg/kg/day PFOS for 28 days, proliferation in the liver was also observed, via an increase in the percentage of cells staining for proliferating cell nuclear antigen (PCNA) and expression of proliferation-related proteins (PCNA, c-JUN, c-MYC, and CCND1) (Han et al., 2018b). Increased liver weight at 10 mg/kg/day was also observed. These results in short-term studies are in contrast to one chronic dietary study in male and female rats which did not identify significant increases in cell proliferation (as determined with PCNA or BrdU immunohistochemistry) after 4, 14, or 52 weeks of dietary PFOS administration (Thomford, 2002b). However, the study

authors noted that a biologically significant and test-compound related mild increase in proliferation was observed at week 4 in two out of five females in both of the highest dose groups. The biological significance was defined as having twice the mean of the controls and being greater than that of the highest control. Notably, this study did not use concentrations of PFOS greater than approximately 1 mg/kg/day.

Similarly, in mice exposed to 10 mg/kg/day PFOS for 7 days, proliferation in the liver, as seen through PCNA staining, was increased (Beggs et al., 2016); increased relative liver weights were also observed. However, no changes in PCNA positive cells or PCNA protein expression was observed in a second study in mice exposed to 1 mg/kg PFOS in their diet for 6 weeks (Huck et al., 2018). Using RNAseq, one study examined the fetal livers of mice exposed gestationally to 0.3 mg/kg/day PFOS and showed a positive association between PFOS exposure and pathways involved in the alteration of liver cell and hepatocyte proliferation (Lai et al., 2017b).

3.4.1.3.5.3.2 In Vitro Models

In one study in primary rat hepatocytes, increased proliferation, as seen by an increased percentage of EdU-positive cells, was observed with PFOS exposures of 50 µg/mL for 24 hours (Han et al., 2018b). A study in human HL-7702 cells found increased proliferation with 50–200 µM PFOS exposures for 48 or 96 hours using the MTT assay; they also reported an association between PFOS exposure and proteomic changes that correlated with increased proliferation (Cui et al., 2015a). This same study found that approximately half of the proteins changed with PFOS exposure were involved in the cell cycle. Using flow cytometry, Cui et al. (2015a) further found that in HL-7702 cells, 50–200 µM PFOS for 48 or 96 hours decreased the percentage of cells at the G1/G0 (non-dividing) phases of the cell cycle while increasing the percentage of cells at the S phase (DNA synthesis); the percentage of cells at G2/M phase (interphase growth/mitosis) was increased at the 100 µM exposure after 48 hours of exposure but was decreased at the 200 µM exposure after 48 and 96 hours. Another study in a zebrafish liver cell line (ZFL) also used flow cytometry to examine changes in the cell cycle after PFOS exposure (Cui et al., 2015b). In corroboration with the study in HL-7702 cells, PFOS concentrations of 27.9 and 56.8 µg/mL for 48 hours were shown to decrease the percentage of cells at the G1/G0 phases while increasing the percentage of cells at G2/M and S phases. In addition, two microarray studies in hepatic cell lines found that PFOS exposures ranging from 100 to 278 µM for 24 or 48 hours were associated with pathways involved in the regulation of cellular proliferation or the cell cycle (Louisse et al., 2020; Song et al., 2016).

Several in vitro and in vivo studies mention pathways through which PFOS may be inducing proliferation. The RNAseq study of fetal livers of mice exposed gestationally to 0.3 mg/kg/day PFOS described above suggested that proliferation may be induced by PFOS activating RAC and Wnt/β-catenin signaling pathways (Lai et al., 2017b). Additionally, in two studies, PFOS has been shown to decrease the expression of HNF4α (Behr et al., 2020a; Beggs et al., 2016), a regulator of hepatic differentiation and quiescence that has been suggested as a mediator of steatosis following PFOS exposure (Armstrong and Guo, 2019). In one study by Beggs et al. (2016) (as described in Section 3.4.1.3.1.3), the authors concluded that PFOS may be causing cellular proliferation by down-regulating positive targets of HNF4α, including differentiation genes, and by inducing the expression of negative targets of HNF4α, including pro-mitogenic genes such as CCND1 and protein levels of stem cell markers such as NANOG, leading to hepatocyte de-differentiation.

3.4.1.3.5.4 Conclusions

Although some results were conflicting, there is generally strong evidence that PFOS exposure can disrupt the balance between cell proliferation and cell death/apoptosis. Out of the multitude of studies examining cell proliferation both in vivo and in vitro, only a single in vivo study showed that PFOS did not alter hepatic cellular proliferation, with increased cell proliferation observed in all other studies. Although most in vitro studies suggested that PFOS could induce apoptosis, several in vivo studies showed that PFOS either did not alter or decreased apoptosis.

Disruption in cell cycle and the reduction of HNF4α were the most frequently cited mechanisms of proliferation induced by PFOS. This increase in proliferation in the liver could be linked to increased liver weights, steatosis, and cancer. Similarly, many pathways were implicated in PFOS-mediated apoptosis, including mitochondrial dysfunction, endoplasmic reticulum stress, and alterations in autophagy.

3.4.1.3.6 Inflammation and Immune Response

The liver is an important buffer between the digestive system and systemic circulation and is thus exposed to compounds that are potentially immunogenic that result in protective immune and inflammatory responses. Kupffer cells constitute the majority of the liver-resident macrophages and make up one third of the non-parenchymal cells in the liver. Kupffer cells phagocytose particles, dead erythrocytes, and other cells from the liver sinusoids and play a key role in preventing immunoreactive substances from portal circulation from entering systemic circulation (Dixon et al., 2013). While Kupffer cells can be protective in drug- and toxin-induced liver toxicity, dysregulation of Kupffer cell-mediated inflammatory responses is associated with a range of liver diseases, including steatosis. Other liver-resident immune cells include natural killer (NK) cells, invariant NKT cells, mucosal associated invariant T (MAIT) cells, γδT cells, and memory CD8 + T cells (Wang and Zhang, 2019). The non-immune cells of the liver, liver sinusoidal endothelial cells (LSECs), hepatocytes, and stellate cells, also participate in immunity. They can express pattern recognition receptors and present antigens to T cells (Robinson et al., 2016). However, the impact of PFOS on the immune function of these cell types has not been thoroughly investigated.

3.4.1.3.6.1 In Vivo and In Vitro Models

Investigations into the liver immune response has been reported in an epidemiological study in the C8 Health Project cohort (Bassler et al., 2019), rat models (Han et al., 2018b; Han et al., 2018a), mouse models (Su et al., 2019; Lai et al., 2017b), and in vitro models (Han et al., 2018b; Song et al., 2016). Bassler et al. (2019) collected 200 serum samples from participants of the C8 Health Project to analyze mechanistic biomarkers of non-alcoholic fatty liver disease (NAFLD) and test the hypothesis that PFAS exposures are associated with increased hepatocyte apoptosis and decreased pro-inflammatory cytokines. PFOS levels were significantly correlated with decreases in serum levels of two pro-inflammatory cytokines, tumor necrosis factor α (TNFα) and IL-8. The authors state that these results are consistent with other findings that PFAS are immunotoxic and downregulate some aspects of the immune responses, but paradoxically result in increased apoptosis, which may subsequently result in progression of liver diseases including NAFLD.

In 6-week-old male Sprague-Dawley rats gavaged with 0, 1, or 10 mg/kg/day PFOS for 28 days, changes in immune-related end points in the liver were measured through western blot, qRT-

PCR, histopathology, and ELISA (Han et al., 2018b; Han et al., 2018a). In contrast to the C8 Panel study in humans (Bassler et al., 2019), the authors reported dose-dependent increases in both serum TNFα and hepatic *Tnfα* mRNA levels, indicating an increased pro-inflammatory response to PFOS exposure. Likewise, in a histopathological analysis of the liver of these PFOS-exposed animals, the authors noted intense inflammatory infiltrates in the periportal area and an increase in inflammatory foci. Han et al. (2018b) also reported increased TNFα in the free supernatant and *Tnfα* mRNA in primary Kupffer cells treated with 100 μM PFOS for up to 48 hours. These increases were not linear over time; supernatant levels and hepatic mRNA levels appeared to peak at 24 hours and 1 hour, respectively. Altered supernatant TNFα concentrations were not observed in similarly treated primary hepatocytes. Similar effects were also reported by Han et al. (2018b) for interleukin-6 (IL-6), which is a contributor to inflammatory responses in cells. Dose-dependent increases in IL-6 levels were observed in rat serum and increases in IL-6 mRNA were observed in rat liver tissue after the 28-day in vivo exposure. The authors also reported increased IL-6 free supernatant concentrations and mRNA levels in primary Kupffer cells treated with 100 μM PFOS for up to 48 hours. In the primary Kupffer cells, supernatant IL-6 levels and mRNA levels peaked at 1 and 6 hours of treatment, respectively. No changes in IL-6 concentrations were observed in supernatant from primary hepatocytes treated with 100 μM PFOS for up to 48 hours. In activation/inhibition assays targeting the c-JUN amino-terminal kinase (JNK), IκB, and nuclear factor-κB (NF-κB) signaling pathways in Kupffer cells (all of which are associated with cellular stress and/or immune/inflammatory responses) PFOS exposure induced JNK and IκB phosphorylation and NF-κB activity. Han et al. (2018b) further reported partial mediation of the TNF-α and IL-6 response in Kupffer cells co-treated with PFOS and either a NF-κB or JNK inhibitor, indicating that these two pathways are at least partially responsible for hepatic inflammatory responses to PFOS. In addition to cytokine levels, Han et al. (2018b) used the F4/80 antibody as a macrophage marker and found dose-dependent increases in F4/80+ cells of the livers of rats treated with either 1 or 10 mg/kg/day PFOS for 28 days. The authors suggest that the increase in hepatic macrophages may be a result of Kupffer cell activation.

In mice, the observed changes were similar to the rat data in that inflammatory markers and pathways were upregulated with PFOS exposure. In one study conducted in male ICR mice, TNFα and IL-6 were significantly increased in serum of mice treated with 10 mg/kg/day PFOS for 21 days (Su et al., 2019). The authors also observed increased TNFα positive liver cells. In prenatally exposed CD-1 mouse offspring whose dams were treated with 0 or 0.3 mg/kg/day PFOS the day after mating until embryonic day 18.5, there was an upregulation of inflammatory pathways in the PFOS-exposed fetuses (Lai et al., 2017b). Using IPA, the authors identified numerous inflammatory genes that were upregulated in the fetal liver tissue. KEGG pathway analysis highlighted the deregulation of adipocytokines, pro-inflammatory cytokines produced by adipocytes, and TGFβ signaling. Interestingly, activation of TGFβ is associated with anti-inflammatory responses, immunosuppression, and tumor promoting pathways.

In another study investigating the hepatic effects of PFOS in vitro, Song et al. (2016) saw much of the same effects using human liver hepatocellular carcinoma line, HepG2. After exposing these cells to 278 μM PFOS (the IC_{20} dose) for 48 hours, through KEGG pathway analyses, the authors reported that genes related to immune response were the fifth most differentially expressed biological process out of the 189 processes with altered genetic profiles. Within the immune response, 17 genes were differentially expressed, including those related to the TNF

signaling pathway, as well as genes involved in the KEGG pathways of nucleotide-binding and oligomerization domain (NOD)-like receptor signaling, cytokine-cytokine receptor interactions, and the complement and coagulation cascade system.

3.4.1.3.6.2 Conclusions

While there are not many studies investigating the immunotoxicity of PFOS specifically related to the liver, evidence presented from various methods and biomarkers strongly indicate that PFOS can disrupt normal hepatic immunological function. However, the immune response to PFOS exposure in humans does not appear to be consistent with rodent and in vitro models. While a single study in the C8 Health Project cohort suggests that immunosuppression may be involved in the progression of NAFLD and potentially other types of liver disease, studies in rats, mice, primary hepatic (Kupffer) cells, and immortalized cell lines suggest that pro-inflammatory immune responses generally result from PFOS exposure. Specifically, there is evidence that activation through the JNK/NF-κB pathways may stimulate the production of pro-inflammatory cytokines such as TNFα and IL-6. Although further assessment of human populations and in human cell lines may be needed to understand the differences in responses between humans and laboratory models, both lines of evidence suggest PFOS exposure can alter the hepatic immune and inflammatory responses.

3.4.1.3.7 Oxidative Stress and Antioxidant Activity

3.4.1.3.7.1 Introduction

Oxidative stress, caused by an imbalance of reactive oxygen species (ROS) production and detoxification processes, is a key part of several pathways, including inflammation, apoptosis, mitochondrial function, and other cellular functions and responses. In the liver, oxidative stress contributes to the progression and damage associated with chronic diseases, such as alcoholic liver disease, non-alcoholic fatty liver disease, hepatic encephalopathy, and Hepatitis C viral infection (Cichoz-Lach and Michalak, 2014). Indicators of oxidative stress include but are not limited to increased oxidative damage (e.g., malondialdehyde (MDA) formation); increased reactive oxygen species (ROS) production (e.g., hydrogen peroxide and superoxide anion); altered antioxidant enzyme levels or activity (e.g., superoxide dismutase (SOD) and catalase (CAT) activity); changes in total antioxidant capacity (T-AOC); changes in antioxidant levels (e.g., glutathione (GSH) and glutathione disulfide (GSSG) ratios); and changes in gene or protein expression (e.g., nuclear factor erythroid factor 2-related factor 2 (Nrf2) protein levels). PFOS has been demonstrated to induce these indicators of oxidative stress, inflammation, and cell damage.

3.4.1.3.7.2 In Vivo Models

Several studies in rats and mice assessed hepatic oxidative stress in response to PFOS exposure. In male Sprague-Dawley rats, a positive association between markers of oxidative stress, potentially due to decreased antioxidant capacity, and oral PFOS exposure (1 or 10 mg/kg/day of for 28 days) was reported (Han et al., 2018a; Wan et al., 2016). In hepatocytes extracted from dosed rats, Wan et al. (2016) found decreased Nrf2 total protein levels and decreased activated Nrf2 in the nuclei at 10 mg/kg/day PFOS. Nrf2 is known for its role as a regulator of antioxidant response elements and is generally activated upon oxidant exposure. Additionally, liver lysates from rats at the highest PFOS dose showed decreases in expression of both heme oxygenase-1 (*Hmox1*) and NAD(P)H quinone dehydrogenase 1 (*Nqo1*) genes, both of which are associated

with antioxidant, anti-inflammatory, and/or stress responses, revealing an inhibition of the Nrf2 signaling pathway following PFOS exposure. Results from Han et al. (2018a) also provide evidence of increased hepatic oxidative stress following PFOS exposure. PFOS-exposed rats had significant dose-dependent increases in ROS, as measured by the 2,7-dichlorofluorescein diacetate (DCFDA) fluorescent probe, and significant increases in hepatic inducible nitric oxide synthase (*iNos*) and *Cyp2e1* mRNA expression, key producers of oxidants in the cell. MDA levels, an indicator of lipid peroxidation, were also significantly increased at both 1 and 10 mg/kg/day. Simultaneously, significant decreases were observed in CAT and SOD activities in liver tissues. Antioxidants typically responsible for returning cells to their homeostatic state were altered in the liver following PFOS exposure, including decreases in GSH levels, increases in GSSG levels, and a decrease in the GSH/GSSG ratio. A decrease in this ratio generally indicates an imbalance of the oxidation-reduction (redox) state of the cell.

Four additional studies examined indicators of oxidative stress in male mice (Lv et al., 2018; Xing et al., 2016; Rosen et al., 2010; Liu et al., 2009). Rosen et al. (2010) found exposure to PFOS in mice downregulated genes associated with oxidative phosphorylation. In their assessment of Kunming (KM) mice that were administered PFOS via subcutaneous injection, Liu et al. (2009) found evidence of oxidative damage that included decreased SOD activity in the male brain and female liver and decreased T-AOC in male and female livers. Overall, oxidative damage was observed in younger offspring and was slightly more evident among males. In a subchronic exposure study, evidence of increased oxidative stress was observed among male C57BL/6 mice dosed once with 0, 2.5, 5, or 10 mg/kg/day PFOS via oral gavage for 30 days (Xing et al., 2016). Dose-dependent reductions were observed for levels of the antioxidant enzymes SOD, CAT, and glutathione peroxidase (GSH-Px) in the liver; the T-AOC (i.e., free radical scavenging capacity) was also reduced in hepatic tissues, with the lowest capacity observed at the highest dose. Lipid peroxidation reported as MDA levels were significantly increased in hepatic tissues of rats exposed to PFOS. The highest MDA levels were observed in the highest dose group. Results from the Lv et al. (2018) subchronic exposure study also showed evidence of increased oxidative stress and decreased mechanisms of defense against oxidative stress following PFOS exposure (Lv et al., 2018). In an unspecified species of male mice, intragastric administration of 10 mg/kg/day PFOS for 3 weeks resulted in significant increases in MDA and hydrogen peroxide production and significant decreases in SOD activity and GSH levels in the liver. Nrf2 protein expression was significantly decreased following PFOS exposure compared with unexposed controls. Additionally, transcriptional levels of *Sod, Cat*, and *Ho-1* mRNA were significantly decreased in the liver.

One gene expression compendium study aimed to examine the relationship between activation of xenobiotic receptors, Nrf2, and oxidative stress by comparing the microarray profiles in mouse livers (strain and species not specified) (Rooney et al., 2019). The study authors compiled gene expression data from 163 chemical exposures found within Illumina's BaseSpace Correlation Engine. Gene expression data for PFOS exposure was obtained from a previously published paper by Rosen, et al., (2010). In WT (129S1/SvlmJ) male mice, Nrf2 activation was observed (as seen by increases in gene expression biomarkers) after a 7-day exposure to 10 mg/kg/day PFOS via gavage. In *Pppar*α-null mice, this activation was observed at both the 3 and 10 mg/kg/day doses. CAR was similarly activated in these two strains of mice. The authors proposed that CAR activation by chemical exposure (PFOS or otherwise) leads to Nrf2 activation and that oxidative stress may be a mediator.

3.4.1.3.7.3 In Vitro Models

Several studies examined oxidative stress endpoints in hepatic primary cells (Xu et al., 2020b; Xu et al., 2019b; Khansari et al., 2017; Rosen et al., 2013). Khansari et al. (2017) dosed rat hepatocytes with 25 μM PFOS for three hours and demonstrated significantly increased production of ROS, measured with the DCFDA probe, and lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS) content, compared with controls. Additionally, PFOS treatment resulted in increased damage of lysosomal membranes, likely caused by lipid peroxidation and increased levels of ROS. The authors also noted that PFOS treatment resulted in mitochondrial membrane potential collapse; disruptions in mitochondrial membrane potential in itself may result in increased ROS production, which could then create a positive feedback loop of further mitochondrial dysfunction and increased ROS. The authors suggest that these results demonstrate a potential oxidative stress-related mechanism underlying PFOS hepatoxicity.

Rosen et al. (2013) assessed oxidative stress-related gene expression changes using TaqMan low-density arrays (TLDA) in both mouse and human primary hepatocytes exposed to PFOS ranging from 0 to 250 μM. PFOS exposure led to increases in the expression of the nitric oxide synthase 2 (*Nos2* or *iNos*) and *Hmox1* genes in mouse primary hepatocytes. In human primary hepatocytes exposed to 100 μM PFOS, *NOS2* expression decreased while *HMOX1* expression increased.

Xu et al. (2019b) exposed primary hepatocytes from C57Bl/6J male mice to 10, 100, 500, or 1,000 μM PFOS for 24 hours. ROS levels, measured by a CM-H2DCFA fluorescent probe, were significantly increased in cells exposed to the highest level of PFOS. Interestingly, SOD activity was significantly increased in cells exposed to 500 and 1,000 μM PFOS, up to 117% with 1,000 μM, while CAT activity was reduced by 59% in cells at the highest dose level. PFOS exposure also led to alterations in the structure of SOD, with PFOS exposure resulting in an increased percentage of α-helix structures (26.9%) and a decreased percentage of β-sheet structures (21.9%), providing evidence of polypeptide chain shortening. These structural changes suggest that PFOS interacts directly with SOD. Alterations in the resonance light scattering (RLS) measures further revealed the impact of PFOS exposure on SOD protein structures in that protein aggregations were observed at low doses of PFOS, but the aggregations were destroyed at higher doses of PFOS, leading to increased SOD activity. The authors suggest that this may result from agglomerate dispersion following the destruction of the solvent shell on the surface of SOD at high doses of PFOS or from protein collapse following PFOS binding. Additionally, GSH content was increased by 199% in cells exposed to the highest dose level; the authors suggest that increases in GSH may reflect cellular adaptations to oxidative stress and can lead to detoxification of oxidized GSSG to GSH.

In a third study using primary mouse hepatocytes, Xu et al. (2020b) exposed cultured cells to 10, 100, 500, or 1,000 μM of PFOS for 24 hours to examine oxidative stress-related cell apoptosis. The authors examined the impact of PFOS exposure on endogenous levels of lysozyme (LYZ), an enzyme that inhibits oxidative stress-induced damage, and demonstrated that PFOS exposure impacted LYZ molecular structure, subsequently decreasing activity levels, leading to oxidative stress-induced apoptosis. Decreases in peak intensity at 206 nm during ultraviolet-visible (UV-vis) absorption spectrometry represented an unfolding of the LYZ molecule following exposure to PFOS, which inhibited enzyme activity. At exposure levels of 100 μM and above, LYZ

enzyme activity decreased to 761% of control levels. Such an impact on LYZ activity was deemed to be related to the high affinity of PFOS for key central binding sites on the LYZ molecule.

Four additional studies examined oxidative stress endpoints following PFOS exposure in HepG2 cell lines (Wan et al., 2016; Wielsøe et al., 2015; Shan et al., 2013; Florentin et al., 2011). Two studies reported increases in ROS levels following PFOS exposure (Wan et al., 2016; Wielsøe et al., 2015), while two studies did not observe statistical differences in ROS levels following 1- or 24-hour PFOS exposures up to 400 μM (Florentin et al., 2011) or following 3-hour PFOS exposures up to 400 μM (Shan et al., 2013). Wan et al. (2016) dosed HepG2 cells with either 0, 10, 20, 30, 40, or 50 μM PFOS for 24 hours or with 50 μM PFOS for 1, 3, 6, 12, or 24 hours. ROS generation, analyzed using DCFH-DA, was increased in a dose-dependent manner in cells dosed with 50 μM across multiple time points, with a peak in levels observed at 12 hours of exposure and a decrease in levels at 24 hours of exposure; ROS production was significantly increased compared with control levels at 24 hours. Significant decreases were observed in GSH and protein expression of total-Nrf2, HO-1, and NQO-1 in a dose- and time-dependent manner. Expression of *miR-155*, a microRNA suspected to play a key role in oxidative stress via the Nrf2 antioxidant pathway, increased nearly 12-fold following 24-hour 50 μM PFOS exposure. When cells were pre-treated with CAT prior to PFOS exposure, ROS production was decreased along with *miR-155* expression. SOD pre-treatment did not lead to significant effects. Wan et al. (2016) concluded that *miR-155* plays a key role in the inhibition of the Nrf2 signaling pathway and can be upregulated with PFOS exposure.

Wielsoe et al. (2015) incubated HepG2 cells with up to 200 μM PFOS to detect changes in ROS, T-AOC, and DNA damage. PFOS exposure significantly increased ROS production, as measured with the carboxy-H2DCFDA probe, as well as DNA damage, as indicated by increased mean percent tail intensity in a comet assay, which is an indicator of DNA strand breaks. Shan et al., 2013 exposed HepG2 cells to 100, 200, 300, or 400 μM PFOS for 3 hours and found an increase in ROS generation with only 100 μM PFOS, though the effect was not statistically significant. Additionally, no changes were observed in the GSH/GSSG ratio.

3.4.1.3.7.4 Conclusions

Results from new studies published since the 2016 PFOS HESD (U.S. EPA, 2016b) further support the conclusions that implicate PFOS in inducing oxidative stress leading to hepatocytic damage. Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in T-AOC, were observed following both in vivo and in vitro exposures to PFOS. PFOS exposure was also associated with increased levels of markers of oxidative damage and decreased activity or levels of protective antioxidants that play a role in the reduction of oxidative damage. Interestingly, PFOS exposure appeared to result in inhibition of the Nrf2 signaling pathway, with evidence of decreased Nrf2 protein levels and reductions of the expression and activity of genes and proteins downstream of this transcription factor. There was also evidence that PFOS can disrupt the structure and subsequent function of crucial enzymes that mitigate ROS production and oxidative damage, SOD and LYZ. While further research is needed to fully understand the mechanisms by which PFOS disrupts oxidative stress responses, it is clear that PFOS induces oxidative stress in hepatic tissues.

3.4.1.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and hepatic effects in humans based on associations with liver biomarkers, especially ALT, in several *medium* confidence studies. Across studies in the 2016 PFOS HESD (U.S. EPA, 2016b) and this updated systematic review, there is generally consistent evidence of a positive association between exposure to PFOS and ALT. The positive associations with ALT are also supported by the recent meta-analysis of 25 studies in adolescents and adults (Costello et al., 2022). However, in several studies, the associations were not large in magnitude.

One source of uncertainty in epidemiology studies of PFAS is confounding across the PFAS as individuals are exposed to a mixture of PFAS and it is difficult to disentangle the effects. This cannot be ruled out in this body of evidence given the attenuation of the association in Lin et al. (2010), the only general population study that performed multi-pollutant modeling. Among the studies of ALT in adults, two presented correlations across PFAS (Nian et al., 2019; Salihovic et al., 2018); PFOA and PFOS were moderately correlated in both studies (r = 0.4–0.5). Jin et al. (2020), which reported positive associations with histology, reported fairly low correlations between PFOS/PFOA (r = 0.14), which reduces the concern for confounding in that population. It is not possible to rule out potential confounding across PFAS with this evidence, but there is also no evidence that confounding can entirely explain the observed associations.

Evidence for other liver enzymes and in children and adolescents is less consistent. Results for functional measures of liver toxicity from epidemiological studies, specifically histology results, are mixed. There is some indication of higher risk of liver disease with higher exposure, coherent with the liver enzyme findings, but there is inconsistency for lobular inflammation among the two available studies, which decreases certainty. Associations for functional hepatic outcomes such as liver disease were also less consistent than the associations between PFOS and ALT.

The animal evidence for an association between PFOS exposure and hepatic toxicity is *robust* based on 20 *high* or *medium* confidence studies that show hepatic alterations. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those that indicate functional impairment or lesions (Hall et al., 2012; EMEA, 2010; FDA, 2009; U.S. EPA, 2002a). EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant increases in enzymes indicative of liver toxicity (U.S. EPA, 2002a).

Multiple studies in mice and rats report increases in relative liver weights accompanied by statistically significant increases in serum enzymes, though the increases in serum enzymes were generally under twofold (100% change relative to control) as compared with controls (NTP, 2019; Han et al., 2018b; Xing et al., 2016; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). However, across the animal toxicological database, these changes in serum enzyme levels were accompanied by histopathological evidence of damage. Of the four available animal toxicological studies with quantitative histopathological data, a chronic study in rats (Butenhoff et al., 2012) was the only study that identified dose-dependent increases in hepatocellular hypertrophy, hepatocellular vacuolation, hepatocytic necrosis, and inflammatory cell infiltration, though these effects were qualitatively reported in other studies (Han et al., 2018b; Xing et al., 2016; Cui et al., 2009). A 28-day study in male and female rats also reported

dose-dependent increases in hepatocellular hypertrophy and cytoplasmic alterations (NTP, 2019). A second short-term study in rats (Curran et al., 2008) only had a limited simple size of 4 rats/sex/treatment group, though there were apparent dose-dependent increases in hypertrophy and cytoplasmic alterations in PFOS-exposed rats. These two studies are supportive of the results observed by Butenhoff et al. (2012).

Mechanistic data can contribute to the understanding toxicity in the context of relevance of data collected from laboratory models in relation to observed human effects and the application of such data in human hazard. There are several studies that have proposed potential underlying mechanisms of the hepatotoxicity observed in rodents exposed to PFOS, some of which have also been tested in human cells in vitro. Mechanistic evidence supports a role of nuclear receptors, including the activation of PPARα and CAR and a decrease in HNF4α, in PFOS-induced hepatotoxicity based on data collected in vivo in rodents and in vitro in both human and rodent models. Findings support a role of these nuclear receptors in steatosis and hepatomegaly observed in rodents in laboratory studies. However, it should be noted that although substantial evidence exists demonstrating expression changes in gene targets of the nuclear receptor PPARα, conflicting results have been reported for activation of the PPARα signaling pathway in vitro between human and rodent cells, as well as across studies in different cells/cells lines from the same species. Nonetheless, cells transfected with human PPARα demonstrated that PFOS can increase PPAR activation. Gene expression signatures for CAR and PPAR activation has been observed in mice exposed to PFOS, with CAR activation generally more significant in *PPARα*-null mice, leading authors to conclude that CAR likely plays a subsequent role to PPARα in mediating the adverse hepatic effects of PFOS. PPARα and CAR are known to play important roles in liver homeostasis and have been implicated in liver dysfunction, including steatosis. Therefore, PFOS exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans.

HNF4α appears to play an important role in hepatotoxic effects related to PFOS exposure. PFOS exposure led to a decrease in the protein expression of HNF4α in mice, which was associated with an increase in relative liver weight. The in vivo alterations to HNF4α have been confirmed by in vitro studies conducted in primary human hepatocytes and HepaRG cells, in which HNF4α protein and gene expression was decreased. Importantly, increased cell proliferation in the liver is related to reduction in HNF4α, both of which are reported effects of PFOS.

Regarding the cytotoxic potential of PFOS, results from in vitro exposure of both human and rodent cells are variable and inconsistent in the concentrations at which PFOS causes cytotoxicity, as well as whether or not PFOS is cytotoxic at any concentration tested in vitro. Some studies evaluated mechanisms of the cell death, such as induction of apoptotic pathways, with inconsistent results. In vivo, increases and decreases in apoptosis were observed in the livers of mice, with variations related to duration of exposure, type of exposure (dietary or gavage), and whether or not a recovery period was included in the study design. Oxidative stress, alterations to p53 signaling, and mitochondrial damage have been reported in vivo in rodent studies as well as in vitro in rodent cells; however, additional research is necessary to fully characterize the involvement of such events in alterations to apoptotic signaling. While necrosis was not directly evaluated, two transcriptomic analyses (one in primary human hepatocytes and one in zebrafish) reported that PFOS induced changes in the expression of genes involved in liver necrosis and damage. Increased hepatic cell proliferation has been more consistently

reported in in vivo and in vitro models, and is associated with increased liver weights and steatosis, which have also been observed in rodents exposed to PFOS.

Inflammation and immunomodulation have also been reported in relation to PFOS, and molecular-level alterations in inflammatory and immune response pathways can be linked to inflammation observed in the livers of rodents exposed to PFOS. In rats, PFOS resulted in increased serum TNFα and hepatic *Tnfα* gene expression, indicating an increased pro-inflammatory response, which was accompanied by intense inflammatory infiltrates in the periportal area and an increase in inflammatory foci. Decreased serum TNFα has been observed in humans in relation to PFOS exposure, indicating that alterations to TNFα may have species differences and/or be dependent upon exposure duration and dose. Alterations to inflammatory response pathway genes have been reported in human cells in vitro (HepG2 cells), supporting the observation in rodents that PFOS exposure leads to inflammatory response. Although further assessment of human populations and human cell lines is needed to clarify the ability of PFOS to induce inflammatory and immune responses in humans, the currently available evidence suggest PFOS exposure can alter the hepatic immune and inflammatory responses.

3.4.1.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, ***evidence indicates*** that PFOS exposure is likely to cause hepatotoxicity in humans under relevant exposure circumstances (Table 3-6). This conclusion is based primarily on coherent liver effects in animal models following exposure to doses as low as 0.02 mg/kg/day PFOS. The available mechanistic information overall provide support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings. In human studies, there is generally consistent evidence of a positive association with ALT, at median plasma PFOS levels as low as 0.57 ng/mL. Although a few associations between other liver serum biomarkers and PFOS exposure were identified in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies.

Table 3-6. Evidence Profile Table for PFOS Exposure and Hepatic Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Evidence From Studies of Exposed Humans (Section 3.4.1.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Serum biomarkers of hepatic injury 12 *Medium* confidence studies 3 *Low* confidence studies	In adults, significant increases in ALT were observed in *medium* confidence studies (6/8). Findings for AST and GGT were similar to ALT, indicating increased levels of these enzymes, however, some analyses stratified by sex or weight status (i.e., obesity) were less consistent. Findings for liver enzymes in occupational populations and children were mixed. However, significant increases in ALT were observed in one occupational study in men (1/2), and significant increases in AST and GGT were observed in female children (1/3).	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for ALT • *Coherence* of findings across biomarkers	• *Inconsistent direction* of effect in children.	⊕⊕⊙ *Moderate* Evidence for hepatic effects is based on increases in ALT in adults. Other supporting evidence includes increases in other liver enzymes such as AST and GGT, and histological changes in children, such as non-alcoholic steatosis. Minor uncertainties remain regarding mixed liver enzyme findings in children and limited availability of high-quality studies on liver disease.	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of hepatoxicity as noted by increased serum biomarkers of hepatic injury (primarily ALT) with coherent results for increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury in animal models. Although associations between PFOS exposure and other serum biomarkers of hepatic injury were identified in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies. *Human relevance and other inferences:* The available mechanistic information overall provide support for the biological plausibility of the phenotypic effects observed
Liver disease or injury 3 *Medium* confidence studies 2 *Low* confidence studies	Findings for markers of liver inflammation were mixed in *medium* confidence studies (1/2). In adults, one study reported nonsignificant decreased odds of lobular inflammation (1/1). The only study in children reported significantly	• *Medium* confidence studies	• *Low* confidence studies • *Limited number* of studies examining the outcome • *Imprecision* of findings		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	increased odds of non-alcoholic steatosis while associations with other histological markers of liver injury were generally positive but less precise. Both *low* confidence occupational studies reported nonsignificant increases in liver disease (2/2), but findings were generally imprecise.				in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.
Serum protein 2 *Medium* confidence studies 1 *Low* confidence study	Three studies in adults reported significantly increased albumin (3/3). For one study, significance varied by glomerular filtration rate status. No studies were conducted in children.	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for albumin	• *Low* confidence study • *Limited number* of studies examining the outcome		
Serum iron 1 *Medium* confidence study	Only one large cross-sectional study examined serum iron concentrations and reported a significant positive association.	• *Medium* confidence study	• *Limited number* of studies examining the outcome		
Evidence From In Vivo Animal Studies (Section 3.4.1.2)					
Liver histopathology 2 *High* confidence studies 5 *Medium* confidence studies	Histopathological alterations in the liver were reported in rodents or non-human primates exposed to PFOS for varying durations (6/7). Hepatocellular hypertrophy was most	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species • *Dose-dependent* response	• No factors noted	⊕⊕⊕ *Robust* Evidence is based on 20 *high* or *medium* confidence animal toxicological studies indicating increased	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	consistently observed across sex, species, and duration of exposure and in a dose-responsive manner (5/7). Other observed lesions included: cystic or hepatocyte degeneration (2/7), focal or flake-like necrosis (2/7), steatosis (1/7), centrilobular or cytoplasmic vacuolation (6/7) and inflammatory cellular infiltration into liver tissue (4/7).	• *Coherence* of findings in other endpoints indicating liver damage (i.e., increased serum biomarkers and liver weight) • *Large magnitude* of effect, with some responses reaching 100% incidence in some dose groups (i.e., hypertrophy) or are considered severe (i.e., cell or necrosis and cystic degeneration)		incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those that indicate functional impairment or lesions. EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis and inflammation. Many of the studies discussed in this section reported dose-dependent increases in liver weight and hepatocellular hypertrophy in rodents of both sexes. However, a limited number of these studies additionally examined functional or histopathological hepatic impairment to provide evidence that the enlargement of hepatic	
Liver weight 2 *High* confidence studies 14 *Medium* confidence studies	Liver weights were increased in male and female mice, rats, and non-human primates at higher doses across a variety of study designs including developmental, short-term, subchronic, and chronic (11/14). Liver weight increases in pups exposed *in utero* were also observed (2/5).	• *High* and *medium* confidence studies • *Consistent direction* of effects across study design, sex, and species • *Coherence* of effects with other responses indicating increased liver size (e.g., hepatocellular hypertrophy)	• *Confounding variables* such as decreases in body weights		
Serum biomarkers of hepatic injury 3 *High* confidence studies 7 *Medium* confidence studies	ALT (7/7), AST (4/7), ALP (3/4), and GGT (1/1) levels were increased in male adult rodents. Measurements of ALT (1/5), AST (0/5), and ALP (1/2) in females found little evidence that PFOS	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species	• *Limited number of studies* examining specific endpoints • *Inconsistent* direction of effects between sexes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	exposure increased enzyme levels. Several studies found increased bilirubin (3/3), albumin (2/2), and albumin/globulin ratio (2/2) in male and female animals, with an increase in total protein in females only (1/2), occurring predominantly in high-dose groups only. Increased concentrations of bile salts/acids were found in males (2/3) and females (1/2).	• *Dose-dependent* response • *Coherence* of findings with other responses indicating hepatobiliary damage (i.e., histopathological lesions) • *Large magnitude* of effect, with evidence of biologically significant increases (i.e., ≥100% control responses) in serum liver enzymes indicating adversity		tissue was an adverse, and not adaptive, response.	

Mechanistic Evidence and Supplemental Information (Section 3.4.1.3)		
Biological Events or Pathways	**Summary of Key Findings, Interpretation, and Limitations**	**Evidence Stream Judgment**
Molecular initiating events — PPARα	**Key findings and interpretation:** • Activation of PPARα in vivo in rodents and in vitro in human and rodent cells. • Increased expression of PPARα-target genes in vitro in rat and human hepatocytes, and cells transfected with human PPARα. • Altered expression of genes involved in lipid metabolism and lipid homeostasis. • Gene expression changes related to lipid metabolism were observed in both wild-type and PPARα-null mice. **Limitations:** • Conflicting results have been reported for activation of the PPARα signaling pathway in vitro between human and rodent cells.	Overall, studies in rodent and human in vitro models and in vivo in rodent studies suggest that PFOS induces hepatic effects, at least in part, through PPARα. The evidence also suggests a role for PPARα-independent pathways in the MOA for noncancer liver effects of PFOS, particularly CAR activation and decreased expression of HNF4α.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
Molecular or cellular initiating events — other pathways	**Key findings and interpretation:** • Activation of CAR in vivo in rodents and in vitro in both human and rodent models. • Gene expression signatures for CAR activation observed in mice; more significant in *Pparα*-null mice than in wild-type mice. • Decrease in HNF4α protein expression, and changes in the expression of genes regulated by HNF4α in vivo in mice. • Decrease in HNF4α gene and protein expression in vitro in human hepatocytes. • Reduction in HNF4α is associated with increased cell proliferation, which was observed separately in PFOS-exposed animals. • Upregulation of PPARγ, CAR/PXR, or LXR/RXR in mice. **Limitations:** Evidence is limited for some receptors, such as PPARγ and LXR/RXR.				

Notes: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CAR = constitutive androstane receptor; GGT = gamma-glutamyl transpeptidase; HNF4α = hepatocyte nuclear factor 4-alpha; LXR = liver X receptor; PPARα = peroxisome proliferator-activated receptor alpha; MOA = mode of action; PPARγ = peroxisome proliferator-activated receptor gamma; PXR = pregnane X receptor; RXR = retinoid X receptor.

3.4.2 Immune

EPA identified 47 epidemiological and 13 animal toxicological studies that investigated the association between PFOS and immune effects. Of the epidemiological studies, 2 were classified as *high* confidence, 29 as *medium* confidence, 10 as *low* confidence, 5 as *mixed* (5 *medium/low*) confidence, and 1 was considered *uninformative* (Section 3.4.2.1). Of the animal toxicological studies, one was classified as *high* confidence, nine as *medium* confidence, one as *low* confidence, and two were considered *mixed* (*high/low* and *medium/low*) (Section 3.4.2.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.2.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.2.1.1 Immunosuppression

Immune function—specifically immune system suppression—can affect numerous health outcomes, including risk of common infectious diseases (e.g., colds, influenza, otitis media) and some types of cancer. The WHO guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications (WHO, 2012).

There are 10 studies (11 publications[8]) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and immune effects. Study quality evaluations for these 11 studies are shown in Figure 3-16. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-7 and below.

In the 2016 PFOS HESD, there was consistent evidence of an association between PFOS exposure and immunosuppression in children. Two studies reported decreases in response to one or more vaccines in relation to higher exposure to PFOS in children (Granum et al., 2013; Grandjean et al., 2012). In one study of adults, no association was observed (Looker et al., 2014). Antibody responses for diphtheria and tetanus in children (n = 587) were examined at multiple timepoints in a study on a Faroese birth cohort (Grandjean et al., 2012). Prenatal and age five serum PFOS concentrations were inversely associated with childhood diphtheria antibody response at all measured timepoints, and the association was significant for anti-diphtheria antibody concentrations pre-booster at age five and at age seven, modeled using prenatal and age five serum PFOS concentrations, respectively. The antibody response for tetanus was inversely associated with prenatal and age five serum PFOS concentrations but was only significant for the association between age five serum PFOS concentrations and post-booster anti-tetanus antibody concentrations. Another study on Faroese children conducted a pilot investigation on the association between elevated PFOS exposure and autoantibodies to antigens indicating tissue damage, but the results were unclear (Osuna et al., 2014). Prenatal PFOS exposure was associated with diminished vaccine response in a different birth cohort study (Granum et al., 2013). Decreases in the anti-rubella antibody response were significantly associated with elevated prenatal PFOS concentrations among 3-year-old children. Stein et al. (Stein et al., 2016b) reported significant inverse associations between PFOS exposure and mumps and rubella

[8] Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407.

antibody concentrations in seropositive adolescents (12–19 years old) from multiple NHANES cycles (1999–2000, 2003–2004), but no association was observed for measles. No association was observed for the only study (Looker et al., 2014) in adults, examining influenza vaccine responses in a high-exposure community (C8 Health Project).

Evidence based on studies of infectious disease in children from the 2016 PFOS HESD was limited. In the Danish National Birth Cohort (DNBC) study, Fei et al. (2010b) reported nonsignificant increases in risk of hospitalizations for infectious diseases in children 4 years and older, but no association was observed at younger ages. In sex-stratified analyses the risk for hospitalization for infectious disease was significantly increased in girls (IRR = 1.18, 95% CI: 1.03, 1.36), while findings for boys were null. No association was observed for gastroenteritis or common cold in children from the Norwegian Mother and Child Cohort study (MoBa) (Granum et al., 2013).

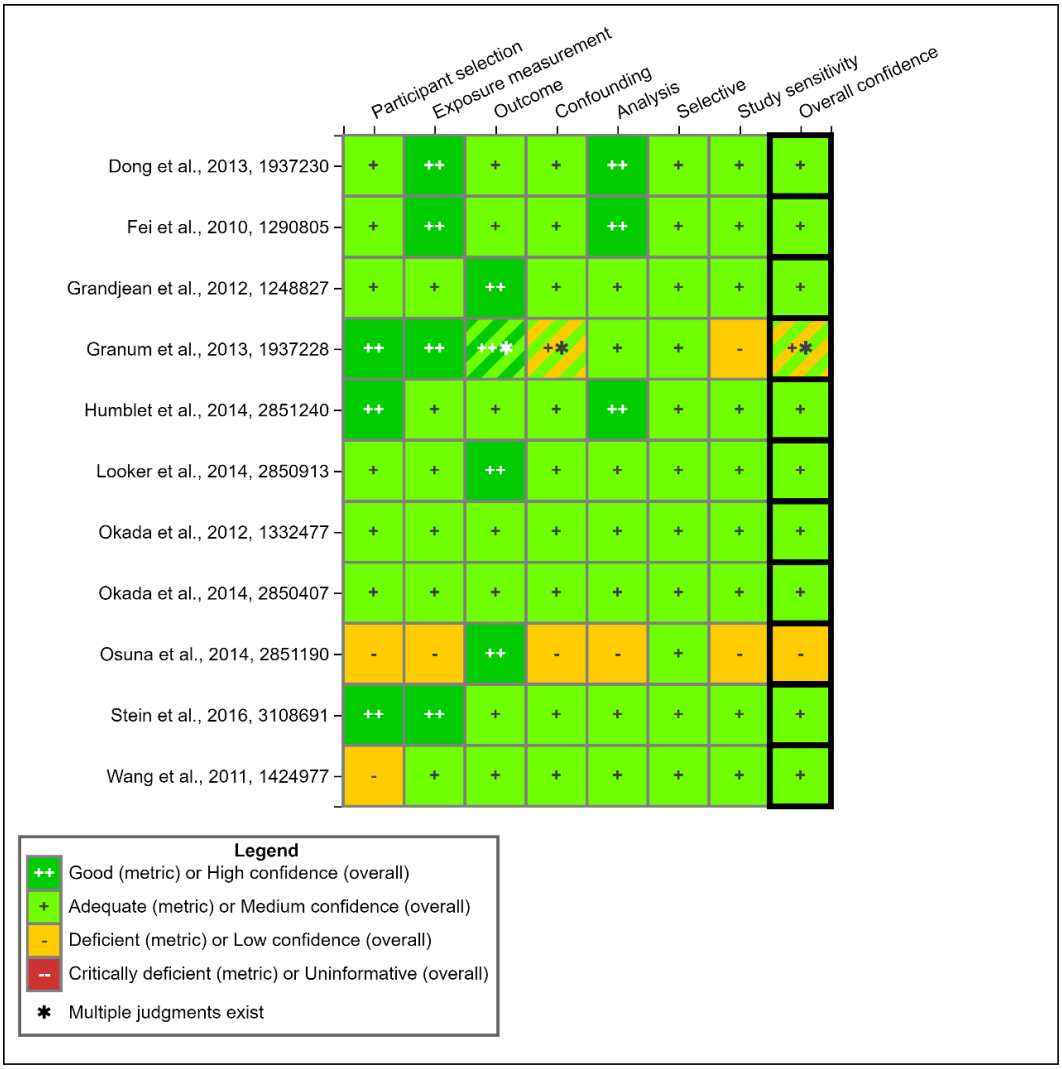


Figure 3-16. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immune Effects Published Before 2016 (References in 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-7. Associations Between Elevated Exposure to PFOS and Immune Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	Tetanus Ab[a]	Diphtheria Ab[a]	Rubella Ab[a]	Influenza Ab[a]	Infectious Disease[b]	Asthma[b]	Eczema[b]	Autoimmune Disease[b]
Dong, 2013, 1937230 *Medium*	Case-control	Children	NA	NA	NA	NA	NA	↑↑	NA	NA
Fei, 2010, 1290805 *Medium*	Cohort	Children	NA	NA	NA	NA	↑	NA	NA	NA
Grandjean, 2012, 1248827 *Medium*	Cohort	Children	↓↓	↓↓	NA	NA	NA	NA	NA	NA
Granum, 2013, 1937228 *Mixed*	Cohort	Children	–	NA	↓↓	NA	–	NA	NA	NA
Humblet, 2014, 2851240 *Medium*	Cross-sectional	Adolescents	NA	NA	NA	NA	NA	–	NA	NA
Looker, 2014, 2850913 *Medium*	Cohort	Children	NA	NA	NA	–	NA	NA	NA	NA
Stein, 2016, 3108691 *Medium*	Cross-sectional	Children	NA	NA	↓↓	NA	NA	↑	NA	NA
Okada, 2014, 2850407 *Medium*	Cohort	Children	NA	NA	NA	NA	NA	NA	–	NA
Wang, 2011, 1424977 *Medium*	Cohort	Children	NA	NA	NA	NA	NA	NA	↑	NA

Notes: Ab = antibody; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Osuna, 2014, 2851190 analyzed autoantibody response to indicators of tissue damage and was not included in the table.

Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407, which was considered the most updated data.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

Granum, 2013, 1937228 was rated *medium* confidence for antibody response, common cold, and gastroenteritis, and *low* confidence for all other outcomes.

There are 28 new studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and immunosuppression effects. Study quality evaluations for these 27 studies are shown in Figure 3-17 and Figure 3-18. One study from the 2016 PFOS HESD (Grandjean et al., 2012) was updated during this period, and the update was included in the systematic review (Grandjean et al., 2017a).

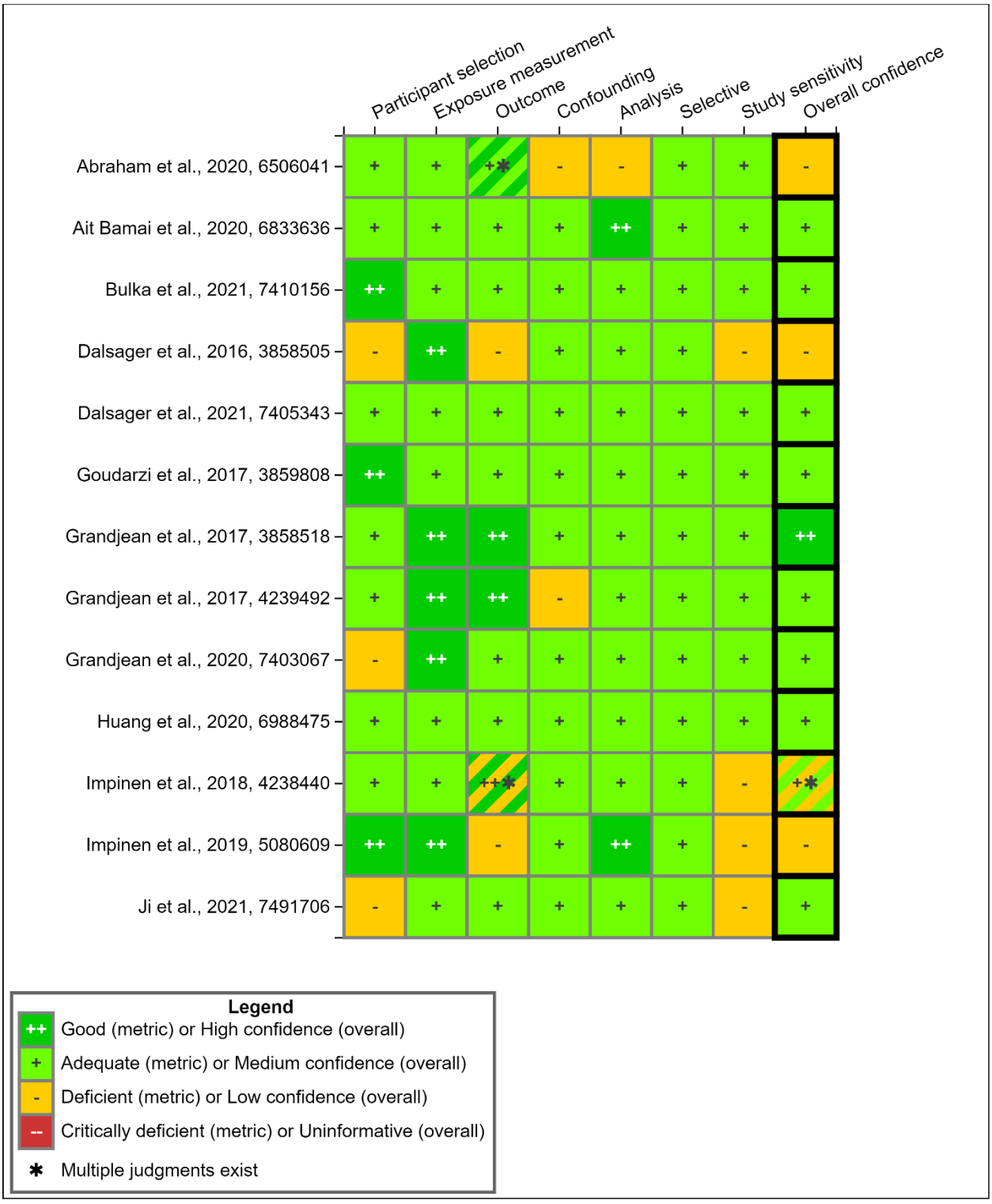


Figure 3-17. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immunosuppression Effects

Interactive figure and additional study details available on HAWC.

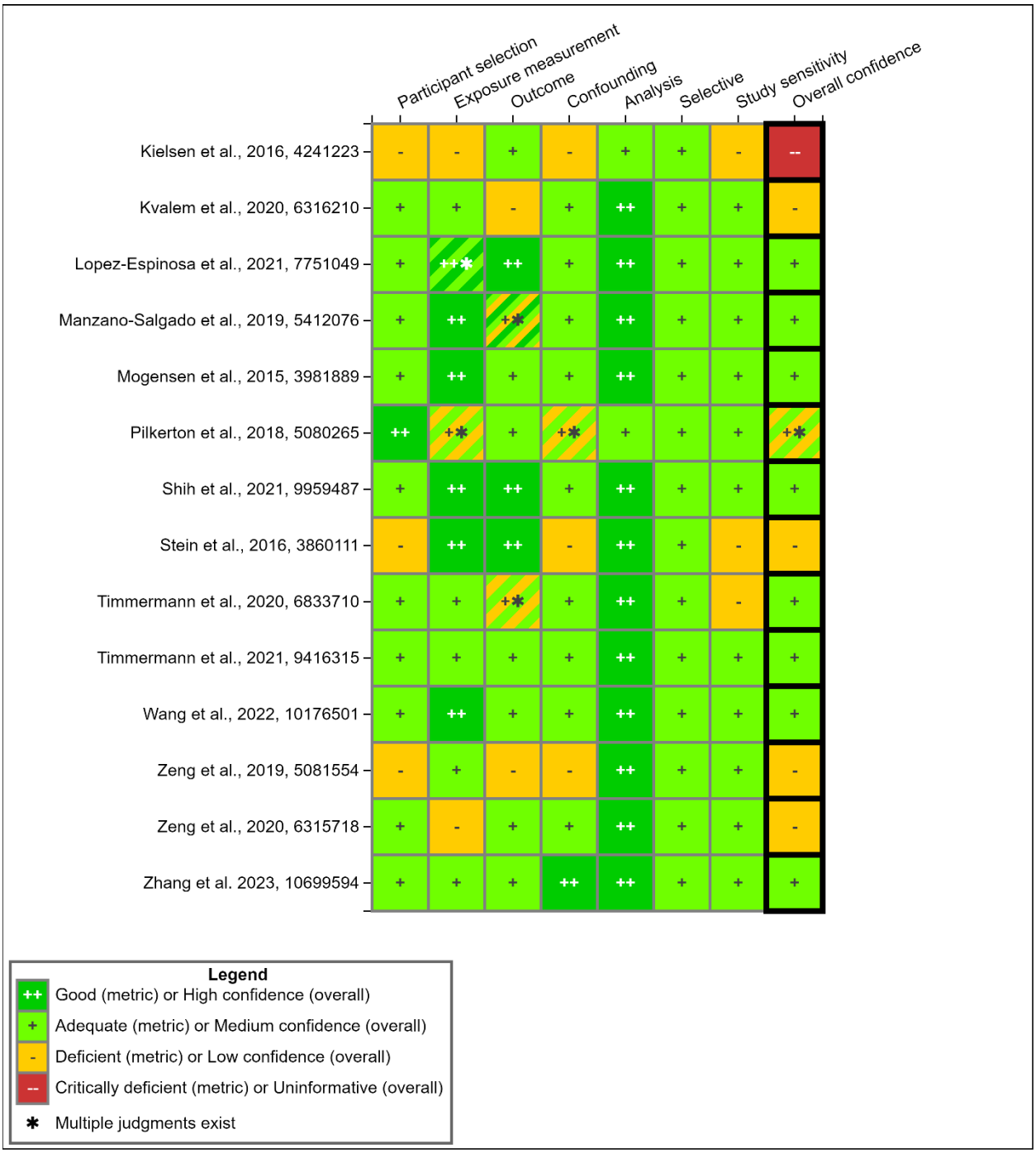


Figure 3-18. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immunosuppression Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.2.1.1.1 Vaccine Response

Thirteen studies (14 publications[9,10]) studied the relationship between antibody response to vaccination and PFOS exposure. Six of these studies investigated antibody response to vaccination in children (Zhang et al., 2023; Timmermann et al., 2021; Abraham et al., 2020; Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a). In adults, two studies investigated antibody response to diphtheria and tetanus (Shih et al., 2021; Kielsen et al., 2016), one study investigated hepatitis A and B vaccine response (Shih et al., 2021), one study investigated adult flu vaccine response (Stein et al., 2016a), one study measured rubella antibodies in both adolescents (aged 12 and older) and adults (Pilkerton et al., 2018), and one study measured rubella, measles, and mumps antibodies in adolescents (Zhang et al., 2023). In addition, one study (Zeng et al., 2019b) measured natural antibody exposure to hand, foot, and mouth disease (HFMD), and one study (Zeng et al., 2020) measured hepatitis B antibodies in adults. Overall, one study was *high* confidence (Grandjean et al., 2017a), six studies were *medium* confidence (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Timmermann et al., 2020; Grandjean et al., 2017b; Mogensen et al., 2015a), four were *low* confidence (Abraham et al., 2020; Zeng et al., 2020; Zeng et al., 2019b; Stein et al., 2016a), one was *mixed* (*medium/low* confidence) (Pilkerton et al., 2018), and one was *uninformative* (Kielsen et al., 2016).

Of the studies that measured antibody response to vaccination in children and adolescents, four studies were cohorts (Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a), and four were cross-sectional (Zhang et al., 2023; Timmermann et al., 2021; Abraham et al., 2020; Pilkerton et al., 2018) (maternal serum was available for a subset of participants in Timmermann et al. (2021)). These included multiple prospective birth cohorts in the Faroe Islands, one with enrollment in 1997–2000 and subsequent follow-up to age 13 (Grandjean et al., 2017a) and one with enrollment in 2007–2009 and follow-up to age 5 (Grandjean et al., 2017b) (one additional cohort in the Faroe Islands examined outcomes in adults with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021)). Five of these studies measured antibody response to tetanus vaccination (Timmermann et al., 2021; Abraham et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a); the same studies also measured antibody response to diphtheria vaccination. In addition, two studies measured antibody response to measles vaccination (Zhang et al., 2023; Timmermann et al., 2020), two studies measured antibody response to rubella vaccination (Zhang et al., 2023; Pilkerton et al., 2018), one study measured antibody response to mumps vaccination (Zhang et al., 2023), and one study to *Haemophilus influenzae* type b (Hib) vaccination (Abraham et al., 2020).

The results for this set of studies in children are shown in Table 3-8 and Appendix D (U.S. EPA, 2024a). The Faroe Islands studies (Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a) observed associations between higher levels of PFOS and lower antibody levels against tetanus and diphtheria in children at 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. These studies measured exposure levels in maternal blood during the perinatal period and at later time periods from children at age 5, 7, and

[9] Multiple publications of the same study: the study populations are the same in Grandjean et al. (2017a) and Mogensen et al. (2015a).
[10] Zhang (2023) analyzes NHANES cycles 2003–2004 and 2009–2010 partially overlapping with Pilkerton (2018) and Stein (2016b) which both analyze cycles 1999–2000 and 2003–2004.

13 years (Table 3-8). No biological rationale has been identified as to whether one particular time period or duration of exposure or outcome measurement is more sensitive to an overall immune response to PFOS exposure. Results from all *medium* and *high* confidence studies on tetanus and diphtheria antibody response in children are provided in Figure 3-19 and Figure 3-20.

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOS at 7 years median (25th-75th percentile)=15.3 ng/mL (12.4-19.0 ng/mL)	percent change (per doubling of PFOS)	--	Age 7	Age 13	30
	PFOS at 13 years median (25th-75th percentile)=6.7 ng/mL (5.2-8.5 ng/mL)	percent change (per doubling of PFOS)	--	Age 13	Age 13	22.2
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOS: Geometric mean=16.7 ng/mL (25th-75th percentile=13.5-21.1 ng/mL)	Percent difference (per doubling in age 5 PFOS)	--	Age 5	Age 7	-23.8
			Adj for Age 5 Ab	Age 5	Age 7	-11.4
			Pre-booster	Age 5	Age 5	-11.9
			Post-booster	Age 5	Age 5	-28.5
	Maternal PFOS: Geometric mean=27.3 ng/mL (25th-75th percentile=23.2-33.1 ng/mL)	Percent difference (per doubling in maternal PFOS)	--	Prenatal	Age 7	35.3
			Adj for Age 5 Ab	Prenatal	Age 7	33.1
					Age 5	-2.3
			Pre-booster	Prenatal	Age 5	-10.1
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOS)	Cohort 3	Age 1.5	Age 5	-8.05
				Age 5	Age 5	-11.86
				Cord blood	Age 5	-10.09
			Cohort 3 and 5	Age 1.5	Age 5	-7.08
				Age 5	Age 5	-10.52
				Cord blood	Age 5	-10.55
			Cohort 5	Cord blood	Age 5	-10.84
	Median = 4.7 ng/mL (25th - 75th percentile: 3.5 - 6.3 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 5	Age 5	-9.08
	Median = 7.1 ng/mL (25th - 75th percentile: 4.5 - 10.0 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 1.5	Age 5	-7.03
Mogensen et al. (2015, 3981889), Medium	median=15.5 ng/ml (25th-75th percentile=12.8-19.2 ng/ml)	Percent change per doubling of PFOS	Age 7	Age 7	Age 7	-9.1
Timmermann et al. (2022, 9416315), Medium	median=8.68 ng/mL (25th - 75th percentiles: 6.52 - 12.23 ng/mL)	percent difference (per unit increase in child PFOS concentration)	Ages 7-12	Age 7-12	Age 7-12	-3
	median=19.16 ng/mL (25th - 75th percentiles: 15.20 - 24.06 ng/mL)	percent difference (per unit increase in maternal PFOS concentration)	Ages 7-12	Prenatal	Age 7-12	2

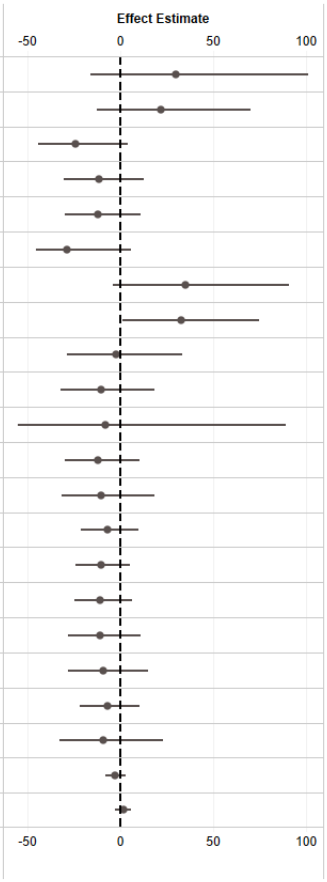


Figure 3-19. Overall Tetanus Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOS HESD.

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOS at 7 years median (25th-75th percentile)=15.3 ng/mL (12.4-19.0 ng/mL)	percent change (per doubling of PFOS)	--	Age 7	Age 13	-23.8
	PFOS at 13 years median (25th-75th percentile)=6.7 ng/mL (5.2-8.5 ng/mL)	percent change (per doubling of PFOS)	--	Age 13	Age 13	-8.6
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOS: Geometric mean=16.7 ng/mL (25th-75th percentile=13.5-21.1 ng/mL)	Percent difference (per doubling in age 5 PFOS)	--	Age 5	Age 7	-27.6
			Adj for Age 5 Ab	Age 5	Age 7	-20.6
			Pre-booster	Age 5	Age 5	-16
			Post-booster	Age 5	Age 5	-15.5
	Maternal PFOS: Geometric mean=27.3 ng/mL (25th-75th percentile=23.2-33.1 ng/mL)	Percent difference (per doubling in maternal PFOS)	--	Prenatal	Age 7	-19.7
			Adj for Age 5 Ab	Prenatal	Age 7	-10
			Pre-booster	Prenatal	Age 5	-38.6
			Post-booster	Prenatal	Age 5	-20.6
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOS)	Cohort 3	Age 1.5	Age 5	-21.21
				Age 5	Age 5	-16.02
				Cord blood	Age 5	-38.64
			Cohort 3 and 5	Age 1.5	Age 5	15.07
				Age 5	Age 5	-1.34
				Cord blood	Age 5	-24.47
			Cohort 5	Cord blood	Age 5	-14
	Median = 4.7 ng/mL (25th - 75th percentile: 3.5 - 6.3 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 5	Age 5	17.17
	Median = 7.1 ng/mL (25th - 75th percentile: 4.5 - 10.0 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 1.5	Age 5	17.55
Mogensen et al. (2015, 3981889), Medium	median=15.5 ng/ml (25th-75th percentile=12.8-19.2 ng/ml)	Percent change per doubling of PFOS	Age 7	Age 7	Age 7	-30.3
Timmermann et al. (2022, 9416315), Medium	median=8.68 ng/mL (25th - 75th percentiles: 6.52 - 12.23 ng/mL)	percent difference (per unit increase in child PFOS concentration)	Ages 7-12	Age 7-12	Age 7-12	-9
	median=19.16 ng/mL (25th - 75th percentiles: 15.20 - 24.06 ng/mL)	percent difference (per unit increase in maternal PFOS concentration)	Ages 7-12	Prenatal	Age 7-12	1

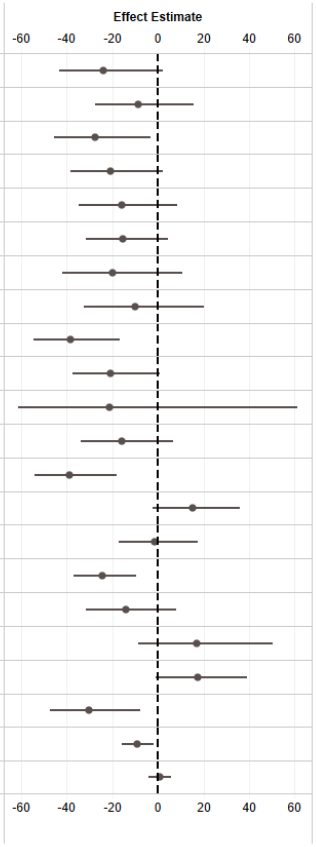


Figure 3-20. Overall Diphtheria Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOS HESD.

It is plausible that the observed associations with PFOS exposure could be explained by confounding across the PFAS, however, exposure levels to PFOS were higher than PFOA (PFOS 17 ng/mL, PFOA 4 ng/mL) in the Faroe Island studies. Though there was a moderately high correlation between PFOS and PFOA, PFHxS, and PFNA (0.50, 0.57, 0.48, respectively), the study authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018) where PFOS estimates were adjusted for PFOA and there was no notable attenuation of the observed effects. The other available studies did not perform multipollutant modeling. Overall, the available evidence does not show that confounding across PFAS is likely to completely explain the observed effects.

Table 3-8. Associations between PFOS Exposure and Vaccine Response in Faroe Island Studies

Exposure measurement timing, levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOS by Age at Assessment			Tetanus Antibody Associations with PFOS by Age at Assessment		
	5 Years (Pre-Booster) (C3 and/or C5)	7 Years (C3 Only)	13 Years (C3 Only)	5 Years (Pre-Booster) (C3 and/or C5)	7 Years (C3 Only)	13 Years (C3 Only)
Maternal C3: GM: 27.3 (23.2–33.1)	↓ (C3; age, sex)[b] **BMD/BMDL** (C3&5; sex, birth cohort, logPFOS)[c]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b]	–	↓ (C3; age, sex)[b] **BMD/BMDL** (C3&5; sex, birth cohort, logPFOS)[c]	↑↑ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b]	–
Birth (modeled)	↓↓ (C3; age, sex)[d] ↓↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–
18 months C3: NR C5: 7.1 (4.5–10.0)	↓ (C3; age, sex)[d] ↑ (C3&5; age, sex)[d] ↑ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–
5 years C3: GM: 16.7 (13.5–21.1) C5: 4.7 (3.5–6.3)	↓↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↑ (C5; age, sex)[d]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b] **BMD/BMDL** (C3; sex, age, and booster type at age 5)[e] **BMD/BMDL** (C3; sex, booster type at age 5, logPFOS)[c]	–	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b] **BMD/BMDL** (C3; sex, age, and booster type at age 5)[e] **BMD/BMDL** (C3; sex, booster type at age 5, logPFOS)[c]	–

Exposure measurement timing, levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOS by Age at Assessment			Tetanus Antibody Associations with PFOS by Age at Assessment		
	5 Years (Pre-Booster) (C3 and/or C5)	7 Years (C3 Only)	13 Years (C3 Only)	5 Years (Pre-Booster) (C3 and/or C5)	7 Years (C3 Only)	13 Years (C3 Only)
7 years C3: 15.3 (12.4–19.0)	–	↓↓ (C3; age, sex, booster type)[f] ↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	↓↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	–	↓ (C3; age, sex, booster type)[f] ↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]	↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]
13 years C3: 6.7 (5.2–8.5)	–	–	↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	–	–	↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]

Notes: C3 = cohort 3, born 1997–2000; C5 = cohort 5, born 2007–2009; GM = geometric mean; NR = not reported.
Arrows indicate direction of association with PFOS levels; double arrows indicate statistical significance ($p < 0.05$) where reported. Arrows are followed by parenthetical information denoting the cohort(s) studied and confounders (factors the models presented adjusted for).
[a] Exposure levels reported from serum as median (25th–75th percentile) unless otherwise noted.
[b] Grandjean et al. (2012); *medium* confidence.
[c] Budtz-Jørgensen and Grandjean (2018); *medium* confidence.
[d] Grandjean et al. (2017b); *medium* confidence.
[e] Grandjean and Budtz-Jørgensen (2013); *medium* confidence.
[f] Mogensen et al. (2015a); *medium* confidence.
[g] Grandjean et al. (2017a); *medium* confidence.

The cross-sectional study of these antibodies in Greenlandic children (Timmermann et al., 2021) reported results that differed in direction of association based on the covariate set selected. The exposure measurement in these analyses may not have represented an etiologically relevant window; cross-sectional analyses in the Faroe Islands studies at similar ages also found weaker associations than analyses for some other exposure windows. However, a subset of the study population did have maternal samples available, and those results were null. On the other hand, this study was the only one to examine the odds ratio for not being protected against diphtheria (antibody concentrations, which has clear clinical significance, and they reported elevated odds of not being protected (based on antibody concentrations <0.1 IU/mL, OR (95% CI) per unit increase in exposure: 1.14 (1.04, 1.26)). Looking at other vaccines, Timmermann et al. (2020) also observed inverse associations between elevated levels of PFOS and lower adjusted antibody levels against measles (statistically significant only in group with fewer measles vaccinations).

Two *medium* cross-sectional studies of adolescents examined associations between elevated levels of PFOS and vaccine response (Zhang et al., 2023; Pilkerton et al., 2018). Inverse associations were observed in cross-sectional analyses in adolescents from NHANES (2003–2004; 2009–2010) for rubella, mumps, and measles (Zhang et al., 2023), including a significant reduction in the antibody response to rubella per 2.7-fold increase in serum PFOS. No association was observed for rubella vaccine response in the other cross-sectional study of adolescents (Pilkerton et al., 2018), however, an overlapping study (Stein et al., 2016b) on adolescents from the same NHANES cycles (i.e., 1999–2000 and 2003–2004) reported a significant inverse association for rubella antibody response in seropositive adolescents.

Lastly, the *low* confidence cross-sectional study at age one, Abraham et al. (2020), did not observe associations between adjusted tetanus, Hib, and diphtheria antibody levels and PFOS concentrations.

Of the three studies that measured vaccine response in adults, two were cohorts (Shih et al., 2021; Stein et al., 2016a), and one was a cross-sectional analysis (Pilkerton et al., 2018). Shih et al. (2021) measured exposure in cord blood and at multiple points through childhood to early adulthood, with outcome measurement at age 28 years; this study was *medium* confidence. Stein et al. (2016a) utilized a convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding, so was *low* confidence. The study of the adult population in Pilkerton et al. (2018) was considered *low* confidence as the analysis suffered from potential exposure misclassification due to concurrent exposure and outcome measurements, considering the amount of time since rubella vaccination in childhood. This was less of a concern for the study of adolescent participants, which was rated as *medium* confidence for adolescence antibody response to vaccinations. Shih et al. (2021) reported inconsistent direction of associations across exposure windows and vaccines (diphtheria, tetanus, Hepatitis A, Hepatitis B). Results also differed by sex, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes men). Similar to the results in 13-year-olds in the other Faroe Island cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Neither of the other studies reported associations with immunosuppression.

In addition to these studies of antibody response to vaccination, there are two studies that examined antibody response to HFMD (Zeng et al., 2019b) and hepatitis B infection (Zeng et al., 2020). This birth cohort in China (Zeng et al., 2019b) measured antibody levels in infants at birth

and age 3 months, which represent passive immunity from maternal antibodies. This study (Zeng et al., 2019b) was rated *low* confidence because the clinical significance of the outcome is difficult to interpret in infants and there are concerns for confounding by timing of HFMD infection as well as other limitations. Statistically significant increased odds of HFMD antibody concentration below clinically protective levels per doubling of PFOS were observed. This is coherent with the vaccine antibody results, but there is uncertainty due to study deficiencies. Zeng et al. (2020) observed negative associations between serum n-PFOS concentration and hepatitis B surface antibody; however, there are study limitations due to concurrent measurement of exposure and outcome and potential for reverse causality.

In a C8 Health project study, Lopez- Espinoza et al. (2021) measured serum PFAS and white blood cell types in 42,782 (2005–2006) and 526 (2010) adults from an area with PFOA drinking water contamination in the Mid-Ohio Valley (USA). Generally positive monotonic associations between total lymphocytes and PFOS were found in both surveys (difference range: 1.95–3.39% for count and 0.61–0.77 for percentage, per PFOS IQR increment). Significant decreasing associations were observed for neutrophils across the surveys and total white blood cell count percent difference in the 2005–2006 survey. Findings were inconsistent for lymphocyte subtypes.

3.4.2.1.1.2 Infectious Disease

Overall, 10 studies (11 publications[11]) measured associations between PFOS exposure and infectious diseases (or disease symptoms) in children with follow-ups between one and 16 years. Infectious diseases measured included: common cold, lower respiratory tract infections, respiratory syncytial virus (RSV), otitis media, pneumonia, chickenpox, varicella, bronchitis, bronchiolitis, ear infections, gastric flu, urinary tract infections, and streptococcus. Of the studies measuring associations between infectious disease and PFOS exposure, eight (nine publications) were cohorts (Wang et al., 2022; Dalsager et al., 2021; Ait Bamai et al., 2020; Huang et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016), one was a case-control study nested in a cohort (Impinen et al., 2018), and one was a cross-sectional study (Abraham et al., 2020). Five studies measured PFOS concentrations from mothers during pregnancy (Ait Bamai et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016). Impinen et al. (2018) measured PFOS concentrations from cord blood at delivery. Two studies measured PFOS concentrations in children's serum at age 1 year (Abraham et al., 2020) and at age 10 years (Kvalem et al., 2020).

Several of the studies measured infectious disease incidences as parental self-report, which may have led to outcome misclassification (Abraham et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Impinen et al., 2018). Four studies measured infections as the doctor-diagnosed incidence of disease over a particular period (Ait Bamai et al., 2020; Huang et al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017), and Wang et al. (2022) used a combination of parental report and medical records. One study used hospitalizations as an outcome, with events identified based on medical records (Dalsager et al., 2021). Overall, seven studies were *medium* confidence (Wang et al., 2022; Dalsager et al., 2021; Abraham et al., 2020; Ait Bamai et al., 2020; Huang et

[11] Multiple publications of the same study: both Dalsager et al. (2016) and Dalsager et al. (2021) use data from the Odense cohort in Denmark and thus have overlapping, though not identical populations. They received different ratings due to outcome ascertainment methods.

al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017) and four were *low* confidence (Kvalem et al., 2020; Impinen et al., 2019; Impinen et al., 2018; Dalsager et al., 2016).

Increased incidence of some infectious diseases in relation to PFOS exposure was observed, although results were not consistent across studies. Results from these studies are available in Appendix D (U.S. EPA, 2024a). The most commonly examined type of infections was respiratory, including pneumonia/bronchitis, upper and lower respiratory tract, throat infections, and common colds. Dalsager et al. (2021), a *medium* confidence study, reported higher rates of hospitalization for upper and lower respiratory tract infections with higher PFOS exposure (statistically significant for lower respiratory tract). Among studies that examined incidence, two studies (one *medium* and one *low* confidence) examining pneumonia/bronchitis observed statistically significant associations between elevated PFOS concentration and increased risk of developing pneumonia in 0- to 3-year-old children (Impinen et al., 2019) and 7-year-old children (Ait Bamai et al., 2020); however, two other *medium* confidence studies did not report an increase in infections (Wang et al., 2022; Abraham et al., 2020). Huang et al. (2020) examined recurrent respiratory infections and found a positive association with recurrent respiratory infections but not total infections. Two *low* and one *medium* confidence studies found positive associations with lower respiratory infection (Dalsager et al., 2021; Kvalem et al., 2020; Impinen et al., 2018), while another *medium* confidence study reported no association (Manzano-Salgado et al., 2019). There were also non-statistically significant positive associations seen for PFOS in relation to chickenpox (Ait Bamai et al., 2020), common cold (Wang et al., 2022), and cough (Dalsager et al., 2016), but statistically significant inverse associations were observed for RSV (Ait Bamai et al., 2020) and common cold (Impinen et al., 2018). Outside of respiratory infections, two *medium* confidence studies examined total infectious diseases. Dalsager et al. (2021) reported higher rates of hospitalization for any infections with higher PFOS exposure (not statistically significant), while (Goudarzi et al., 2017) reported higher odds of total infectious diseases. Results for other infection types, including gastrointestinal, generally did not indicate a positive association.

In addition to the studies in children, three studies examined infectious disease in adults, (Bulka et al., 2021; Ji et al., 2021; Grandjean et al., 2020). Results from these studies are available in Appendix D (U.S. EPA, 2024a). All three studies were *medium* confidence. Ji et al. (2021) was a case-control study of COVID-19 infection. They reported higher odds of infection with higher exposure (OR (95% CI) per $\log_2$ SD increase in PFOS: 1.94 (1.39, 2.96)). In contrast, a cross-sectional study examining severity of COVID-19 illness in Denmark using biobank samples and national registry data Grandjean et al. (2020) reported no association between PFOS exposure and increased COVID-19 severity. Bulka et al. (2021) used NHANES data from 1999 to 2016 in adolescents and adults and examined immunoglobulin G (IgG) antibody levels to several persistent infections, including cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, human immunodeficiency virus (HIV), *Toxoplasma gondii* and *Toxocara* species. High levels of these antibodies were interpreted as presence of a persistent infection. They found higher prevalence of Herpes simplex viruses 1 and 2, *Toxoplasma gondii* and *Toxocara* species and total pathogen burden with higher PFOS exposure in adults (not statistically significant for HSV-2 and *Toxoplasma gondii*) but no association with other individual pathogens.

3.4.2.1.2 Immune Hypersensitivity

Another major category of immune response is the evaluation of sensitization-related or allergic responses resulting from exaggerated immune reactions (e.g., allergies or allergic asthma) to foreign agents (IPCS, 2012). A chemical may be either a direct sensitizer (i.e., promote a specific immunoglobulin E (IgE)-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. For example, chemical exposure could promote a physiological response resulting in a propensity for sensitization to other allergens (pet fur, dust, pollen, etc.). Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. Although these responses are mediated by circulating factors such as T cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of health effects such as allergies or asthma and skin prick tests.

In the 2016 PFOS HESD, one of two studies reported higher odds of asthma with higher PFOS exposure in children. A case-control study (Dong et al., 2013) of children in Taiwan reported an increased odds of asthma with increasing childhood PFOS exposure. The magnitude of association was particularly large comparing each of the highest quartiles of exposure to the lowest. In cross-sectional analyses of asthmatic children, the study authors reported monotonic increases by quartile of exposure for IgE in serum, absolute eosinophil counts, eosinophilic cationic protein, and asthma severity score. No association for current or ever asthma was observed among NHANES (1999–2000, 2003–2008) adolescents (Humblet et al., 2014). No association was observed for eczema in a Hokkaido birth cohort study (Okada et al., 2014).

There are 23 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and immune hypersensitivity (i.e., asthma, allergy, and eczema) effects. Study quality evaluations for these 23 studies are shown in Figure 3-21.

Figure 3-21. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immune Hypersensitivity Effects

Interactive figure and additional study details available on HAWC.

Thirteen studies (15 publications)[12] examined asthma (or asthma symptoms) and PFOS exposure. Ten of these studies were cohorts (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Gaylord et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Workman et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017; Smit et al., 2015); three studies (five publications) were case-control investigations (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016), including one nested case-control, (Impinen et al., 2018); and one was a cross-sectional analysis (Jackson-Browne et al., 2020). Seven studies measured the prevalence of "current" asthma for at least one time point (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Zeng et al., 2019a; Impinen et al., 2018). Eight studies measured "ever" asthma for at least one time point (Jackson-Browne et al., 2020; Averina et al., 2019; Gaylord et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018; Timmermann et al., 2017; Smit et al., 2015). Incident or recurrent wheeze was examined in one study (Workman et al., 2019). Overall, nine studies were rated *medium* confidence, and six studies were *low* confidence for asthma (Figure 3-21). Timmermann et al. (2017) was *low* confidence for asthma because the questionnaire used to ascertain status was not validated. Averina et al. (2019) was considered *low* confidence because results were not provided quantitatively. Studies from the Genetic and Biomarkers study for Childhood Asthma (GBCA) (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) were considered *low* confidence based on participant selection. Cases and controls were recruited from different catchment areas, and the resulting differences between cases and controls indicated potential for residual confounding by age. Additionally, the timing of exposure assessment in relation to outcome assessment was unclear, and it was not reported whether outcome status was confirmed in controls.

Results across these studies were inconsistent (see Appendix D, (U.S. EPA, 2024a)). Several studies observed positive associations with ORs greater than 1.2 between PFOS concentration levels and increased "current" or "ever" asthma (Jackson-Browne et al., 2020; Averina et al., 2019; Beck et al., 2019; Zeng et al., 2019a; Impinen et al., 2018; Timmermann et al., 2017), but often only within population subgroups. Averina et al. (2019) observed statistically significant increased odds of self-reported doctor diagnosed asthma among adolescents in their first year of high school. Jackson-Browne et al. (2020) reported statistically significant increased odds of "ever" asthma from increased PFOS concentrations in children aged 3 to 5 years. No association was observed at ages 6–11 years, and the overall association was small (OR: 1.1). Beck et al. (2019) observed increased odds of self-reported asthma per PFOS increase in boys ($p > 0.05$), but this was not observed in girls. For doctor diagnosed asthma in the same study, an inverse association ($p > 0.05$) was observed in boys and a positive association ($p > 0.05$) was observed in girls. Zeng et al. (2019a) observed a positive association in boys and an inverse association in girls (both $p > 0.05$). Impinen et al. (2018) reported higher odds of ever asthma. The *low* confidence study, Timmermann et al. (2017), observed positive associations ($p > 0.05$) between increased asthma odds and elevated PFOS concentrations in small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella (MMR) vaccination before age 5. However, in children of the same ages who had received their MMR vaccination before age 5, no association was observed. *Low* confidence studies from the GBCA study (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) observed elevated PFOS levels ($p = 0.002$) in children with asthma compared with those without (Zhou et al., 2017b), and the odds of current asthma was

[12] Three publications (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) reported on the same cohort (Genetic and Biomarker study for Childhood Asthma) and outcome and are considered one study.

also found to be elevated among boys and girls with increasing PFOS exposure (Zhu et al., 2016). One other study (Impinen et al., 2019) observed a small positive association (OR: 1.1) with current asthma in boys only. Two studies reported nonsignificant inverse associations with asthma (Manzano-Salgado et al., 2019; Smit et al., 2015), and in one study, all results were nonsignificant (Gaylord et al., 2019). One *low* confidence study did not observe a significant effect for recurrent wheeze (Workman et al., 2019).

In addition to the studies of asthma in children, one *medium* confidence study (Xu et al., 2020a) using data from NHANES examined fractional exhaled nitric oxide (FeNO), a measure of airway inflammation, in adults. Among participants without current asthma, this study found higher FeNO levels with higher PFOS exposure, indicating greater inflammation (percent change (95% CI) for tertiles versus T1, T2: 1.80 (−1.53, 5.25); T3: 5.02 (1.40, 8.77)).

Seven studies observed associations between PFOS exposure and allergies, specifically allergic rhinitis or rhinoconjunctivitis, skin prick test, and food or inhaled allergies. Five of these studies were cohorts (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Timmermann et al., 2017; Goudarzi et al., 2016), one study was a case-control analysis (Impinen et al., 2018), and one study was a cross-sectional study using data from NHANES 2005–2006 and 2007–2010 (Buser and Scinicariello, 2016). All studies were considered *medium* confidence for allergy outcomes. Results for these outcomes are presented in Appendix D (U.S. EPA, 2024a).

Three studies conducted skin prick tests on participants to determine allergy sensitization at age 10 years (Kvalem et al., 2020; Impinen et al., 2018), at age 13 years (Timmermann et al., 2017), and at age 16 years (Kvalem et al., 2020). Skin prick tests were conducted to test sensitization to dust mites, pets, grass, trees and mugwort pollens and molds, cow's milk, wheat, peanuts, and cod. Results were inconsistent across studies. Kvalem et al. (2020) reported a statistically significant but small association (OR: 1.09) with a positive skin prick test at age 16 years (results were similar at age 10 years but $p > 0.05$). Timmermann et al. (2017) also reported a positive association ($p > 0.05$) in children who had received an MMR before age 5 years, but an inverse association in those who had not received an MMR, and Impinen et al. (2018) reported an inverse association ($p > 0.05$). Five studies measured symptoms of "current" or "ever" allergic rhinitis or rhinoconjunctivitis (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2018; Timmermann et al., 2017; Goudarzi et al., 2016), and one study measured symptoms at 16 years old (Kvalem et al., 2020). Rhinitis was defined as at least one symptom of runny or blocked nose or sneezing. Rhinoconjunctivitis was defined as having symptoms of rhinitis, in addition to itchy and watery eyes. Results were null for these outcomes in all five studies. Impinen et al. (2019) measured parent-reported, doctor-diagnosed "current" or "ever" allergy symptoms at 7 years old, in addition to known food and inhaled allergies and reported higher odds of "ever" inhaled allergies ($p > 0.05$) but no associations with food allergies or "current" inhaled allergies. Buser et al. (2016) measured food sensitization (defined as having at least 1 food-specific serum IgE ≥ 0.35 kU/L) and self-reported food allergies and reported statistically significant positive associations with self-reported food allergies in NHANES 2007–2010 but not in in NHANES 2005–2006.

Seven studies measured the association between PFOS concentration and eczema (described by some authors as atopic dermatitis). Six of these studies were cohorts (Manzano-Salgado et al., 2019; Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Timmermann et al., 2017; Goudarzi et al., 2016), and one was a case-control analysis (Impinen et al., 2018). Four studies

measured PFOS concentrations in cord blood at delivery (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Impinen et al., 2018), three studies measured PFOS concentrations in pregnancy (Manzano-Salgado et al., 2019; Timmermann et al., 2017; Goudarzi et al., 2016), and one study measured child blood at age 5 and 13 years (Timmermann et al., 2017). All the studies were considered *medium* confidence for eczema. Results are presented in Appendix D (U.S. EPA, 2024a).

Positive associations ($p > 0.05$) with eczema were observed in two studies (three publications) (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018), as well as a small positive association at age 0–2 years in Impinen et al. (2018). However, inverse associations ($p > 0.05$) were reported in Manzano-Salgado et al. (2019), Timmermann et al. (2017), Goudarzi et al. (2016), and at age 10 years in Impinen et al. (2018).

One *medium* confidence nested case-control study examined chronic spontaneous urticaria (Shen et al., 2022). They found no association between PFOS exposure and case status.

3.4.2.1.3 Autoimmune Disease

Autoimmunity and autoimmune disease arise from immune responses against endogenously produced molecules. The mechanisms of autoimmune response rely on the same innate and adaptive immune functions responding to foreign antigens: inflammatory mediators, activation of T lymphocytes, or the production of antibodies for self-antigens (IPCS, 2012). Chemical exposures that induce immune response or immunosuppression may initiate or exacerbate autoimmune conditions through the same functions. Autoimmune conditions can affect specific systems in the body, such as the nervous system (e.g., multiple sclerosis (MS)), or the effects can be diffuse, resulting in inflammatory responses throughout the body (e.g., lupus).

The 2016 PFOS HESD did not identify epidemiological evidence examining the association between PFOS exposure and autoimmune conditions. There are 4 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and autoimmune disease effects. Study quality evaluations for these 4 studies are shown in Figure 3-22.

Four case-control studies examined PFOS exposure and autoimmune diseases (Figure 3-22). Two studies examined MS (Ammitzbøll et al., 2019) and ulcerative colitis (Steenland et al., 2018b) in adults, and two studies examined celiac disease in children (Sinisalu et al., 2020) and young adults (Gaylord et al., 2020). PFOS was measured in blood components (i.e., blood, plasma, or serum) for all studies (see Appendix D, (U.S. EPA, 2024a)). One study was *medium* confidence (Gaylord et al., 2020) with minimal deficiencies, and three studies were considered *low* confidence (Sinisalu et al., 2020; Ammitzbøll et al., 2019; Steenland et al., 2018b). Information on participant selection, particularly control selection, was not reported in Ammitzbøll et al. (2019). Additionally, PFOS was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOS exposure and risk of MS, and contributed to a *low* confidence rating. Steenland et al. (2018b) examined exposure concentrations 1 to 2 years after diagnosis of celiac disease, resulting in some concern for reverse causation. Additionally, there was potential for residual confounding by SES which was not considered in the analysis. These factors together contributed to a *low* confidence rating.

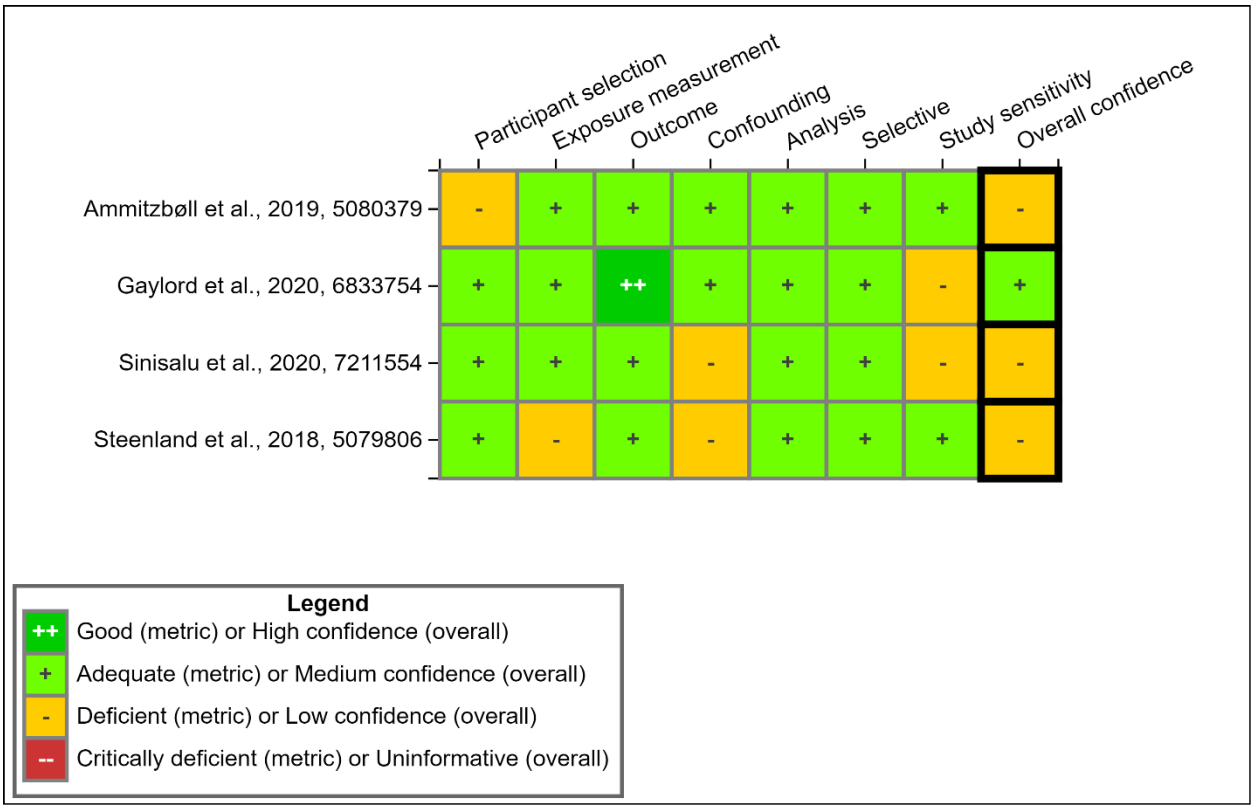


Figure 3-22. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Autoimmune Effects

Interactive figure and additional study details available on HAWC.

Ammitzbøll et al. (2019) observed lower PFOS concentrations among healthy controls compared with those with MS. Serum PFOS concentrations were 17% lower (95% CI: −27%, −6%; p = 0.004) in healthy controls compared with cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOS levels were 28% lower (95% CI: −32%, −3%; p = 0.023) in healthy controls compared with cases. The result was similar among women but did not reach significance (p = 0.093).

In children and young adults, the odds of celiac disease were elevated but not significantly (Gaylord et al., 2020). However, the effect was much stronger in females only (OR: 12.8; 95% CI: 1.17, 141; p < 0.05). A marginally significant (p = 0.06) decrease in serum PFOS was observed among adult cases of ulcerative colitis compared with healthy controls (Steenland et al., 2018b).

In the prospective observational Finnish Diabetes Prediction and Prevention (DIPP) study in which children genetically at risk to develop type 1 diabetes (T1D) and celiac disease (CD) were followed from birth, with blood samples taken at birth and 3 months of age (Sinisalu et al., 2020), there was no significant difference in the levels of PFOS exposure in those children that later developed CD, which may be due to the small sample size, but age at diagnosis of CD was strongly associated with the PFOS exposure.

Overall, the associations between PFOS exposure and autoimmune disease were very limited and mostly null, with one study with evidence of elevated odds of celiac disease. Two studies observed that PFOS levels in healthy controls were either higher than UC cases (Steenland et al., 2018b) or lower than in MS cases (Ammitzbøll et al., 2019).

3.4.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 3 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and 10 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 13 studies are shown in Figure 3-23.

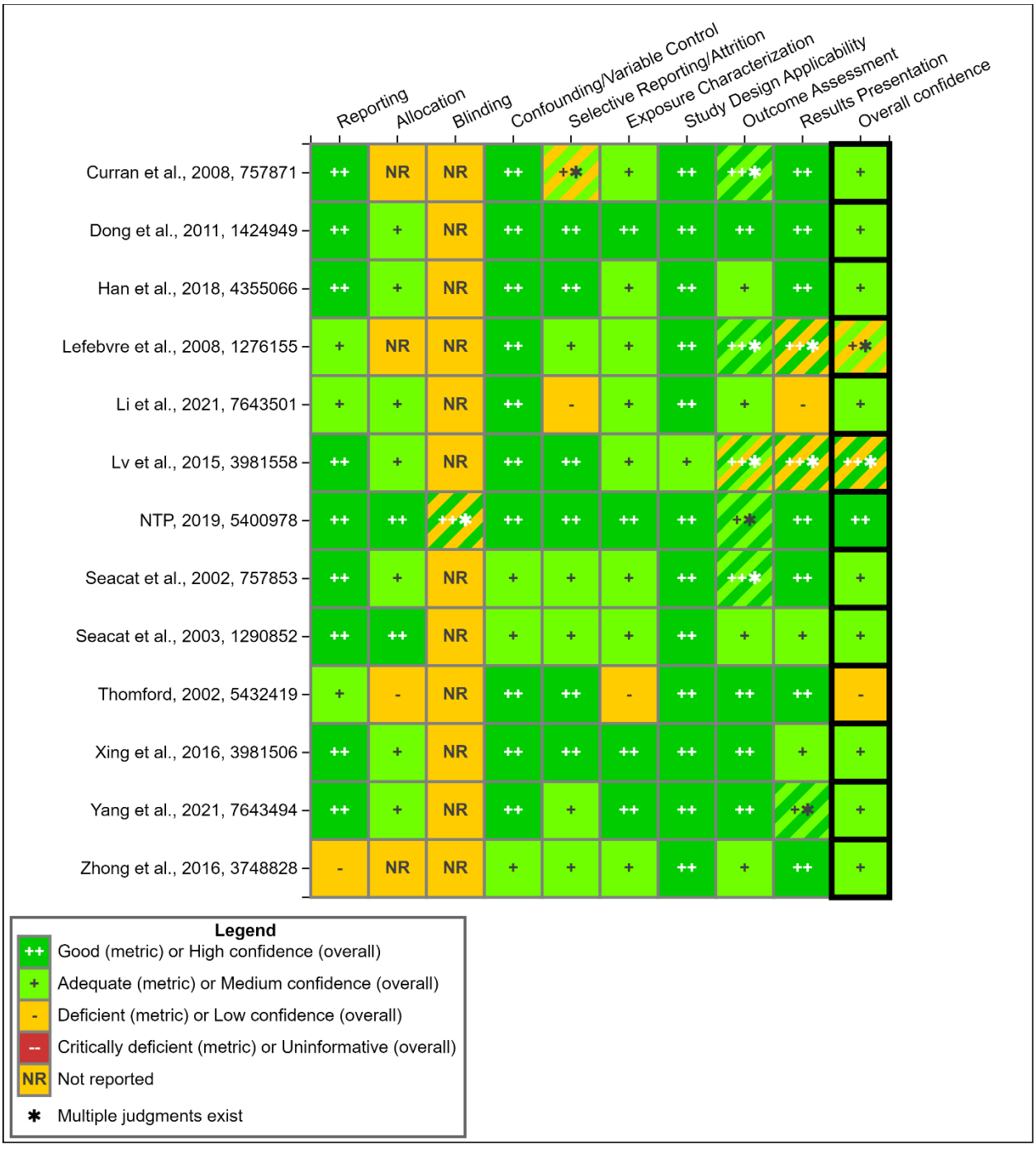


Figure 3-23. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Immune Effects[a]

Interactive figure and additional study details available on HAWC.
[a] Lefebvre et al. (2008) reported on the same animals as Curran et al. (2008).

The immune system could be a target of PFOS toxicity as effects have been observed across animal toxicological studies of varying durations of oral exposure to PFOS. Effects include changes in spleen and/or thymus weights, extramedullary hematopoiesis, perturbations in activity

level or composition of various immune cell populations, and diminished ability to generate an immune response. Studies indicate that PFOS exposure may result in dose- and sex-specific immunomodulatory effects.

3.4.2.2.1 Organ Weight

Several rodent studies have reported changes in thymus and/or spleen weights following oral exposure to PFOS.

3.4.2.2.1.1 Spleen

Two separate 28-day studies reported absolute and relative spleen weights in male and female rats exposed to PFOS. Lefebvre et al. (2008) observed reduced absolute spleen weights in male rats of the highest exposure group in Sprague-Dawley rats given PFOS in diet (0.14–6.34 mg/kg/day in males and 0.15–7.58 mg/kg/day in females). When expressed as percent body weight, these changes were not significant and were within 5% of control for any given exposed group. In contrast, absolute spleen weights were not affected by PFOS exposure in females, but relative spleen weights were significantly higher (18% higher than controls) in the highest exposure group. The increased relative spleen weights in females may be explained by lower body weights of the two highest exposure groups. Another 28-day study by NTP (2019) administered PFOS (0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day) to Sprague-Dawley rats for 28 days and observed dose-dependent reductions in absolute spleen weights at 1.25 mg/kg/day and higher in males only; no effects were observed in females. Spleen weights relative to body weight were not significantly reduced in either sex. While body weights were not significantly different throughout treatment, the high-dose group tended to have lower body weight with a significant, but <10%, difference from the control. Therefore, differences in body weight cannot explain the decreased absolute weight.

In four separate studies, male C57BL/6 mice were administered 5, 20, or 40 mg/kg/day PFOS for 7 days (Zheng et al., 2009), fed chow with 0.001, 0.005, or 0.02% PFOS (equivalent to ~40 mg/kg/day) for 10 days (Qazi et al., 2009b), 0.008–2.083 mg/kg/day PFOS for 60 days (Dong et al., 2009), or administered 0.008–0.833 mg/kg/day PFOS for 60 days via gavage (Dong et al., 2011). Decreased absolute and relative splenic weights tended to be observed only at the highest doses for each study. Female mice were not assessed. These findings are complimented by Xing et al. (2016), where a reduction in relative spleen weight was observed in male C57BL/6J mice following exposure to 10 mg/kg/day PFOS for 30 days via gavage. No effects were observed at other doses (2.5 and 5 mg/kg/day) (Xing et al., 2016).

In a developmental study, spleens were weighed in 4- and 8-week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–17 via gavage. Relative spleen weights were reduced in male pups from the 5 mg/kg/day exposure group at 4 weeks. No significant effects were observed in lower dose groups, at the 8-week time point, or in females (Zhong et al., 2016).

In three separate mouse studies, spleen weights were not significantly altered following short-term exposure to PFOS, including a study of male and female B6C3F1 mice administered 0.00017–0.166 mg/kg/day PFOS for 28 days (Peden-Adams et al., 2008), male C57BL/6 mice exposed to 0.25 or 2.5 mg/kg/day PFOS for 28 days (Yang et al., 2021), and male C57BL/6 (H-2^b) mice administered 0.005% PFOS in the diet for 10 days (Qazi et al., 2010). Similarly, relative

spleen weight in male BALB/c mice was not affected at the end of a 3-week exposure to 2.5–5 mg/kg/day PFOS (Lv et al., 2015). Although Qazi et al. (2010), observed that relative spleen weight was slightly reduced in C57BL/6 mice following 10-day exposure to 0.005% PFOS, the effects did not reach significance.

3.4.2.2.1.2 Thymus

Reductions in thymus weight have been reported across studies of varying durations (7–60 days) and species (mice or rats). It is unclear whether sex has an influence on toxicity, as a number of studies did not include females in their investigations.

The aforementioned 28-day studies by NTP (2019) and Lefebvre et al. (2008) reported reductions in absolute and/or relative thymus weights in male Sprague-Dawley rats administered oral PFOS, at the highest doses of 5–7.58 mg/kg/day (Figure 3-24). Reductions in absolute thymus weight were also observed in females of the highest dose in Lefebvre et al. (2008). In contrast, females in the NTP study exhibited reduced absolute thymus weights at doses as low as 1.25 mg/kg/day, suggesting a higher sensitivity in females (NTP, 2019) (Figure 3-24).

Similarly, reduced thymic weights were observed in male C57BL/6 mice administered 20 or 40 mg/kg/day PFOS via gavage for 7 days (Zheng et al., 2009), 0.02% PFOS for 10 days in diet (Qazi et al., 2009b), or 0.417–2.083 mg/kg/day PFOS for 60 days (Dong et al., 2009). A follow-up from the latter study (Dong et al., 2009) by Dong et al. (2011) also exposed adult male C57BL/6 to 0.008–0.833 mg/kg/day PFOS for 60 days via gavage, but reductions in relative thymus weight were only observed in the highest dose. Female mice were not assessed in these studies. Yang et al. (2021) exposed male C57BL/6 mice to 0.25 or 2.5 mg/kg/day PFOS for 28 days and observed an 18% and 24%, respectively, reduction in relative thymus weight although these changes were not statistically significant.

In a developmental exposure study, the thymus was weighed in 4- and 8-week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–17 via gavage. In male pups from the 5 mg/kg/day exposure group, relative thymus weights were reduced at 4 and 8 weeks of age. However, no effects were observed in lower dose groups or in females (Zhong et al., 2016) (Figure 3-24).

In contrast to the several studies that reported reductions in thymus weight, Qazi et al. (2010) and Peden-Adams et al. (2008) did not observe any changes in thymus weight. Qazi et al. (2010) exposed male C57BL/6 (H-2^b) mice to 0.005% PFOS in the diet for 10 days, while Peden-Adams et al. (2008) exposed male and female B6C3F1 mice to 0.00017–0.166 mg/kg/day PFOS for 28 days. The contrasting results of the 28-day study by Peden-Adams et al. (2008) and NTP (2019) may underscore species differences, however, the dose levels used in the mouse study were generally below the LOEL of the NTP study (5 mg/kg/day).

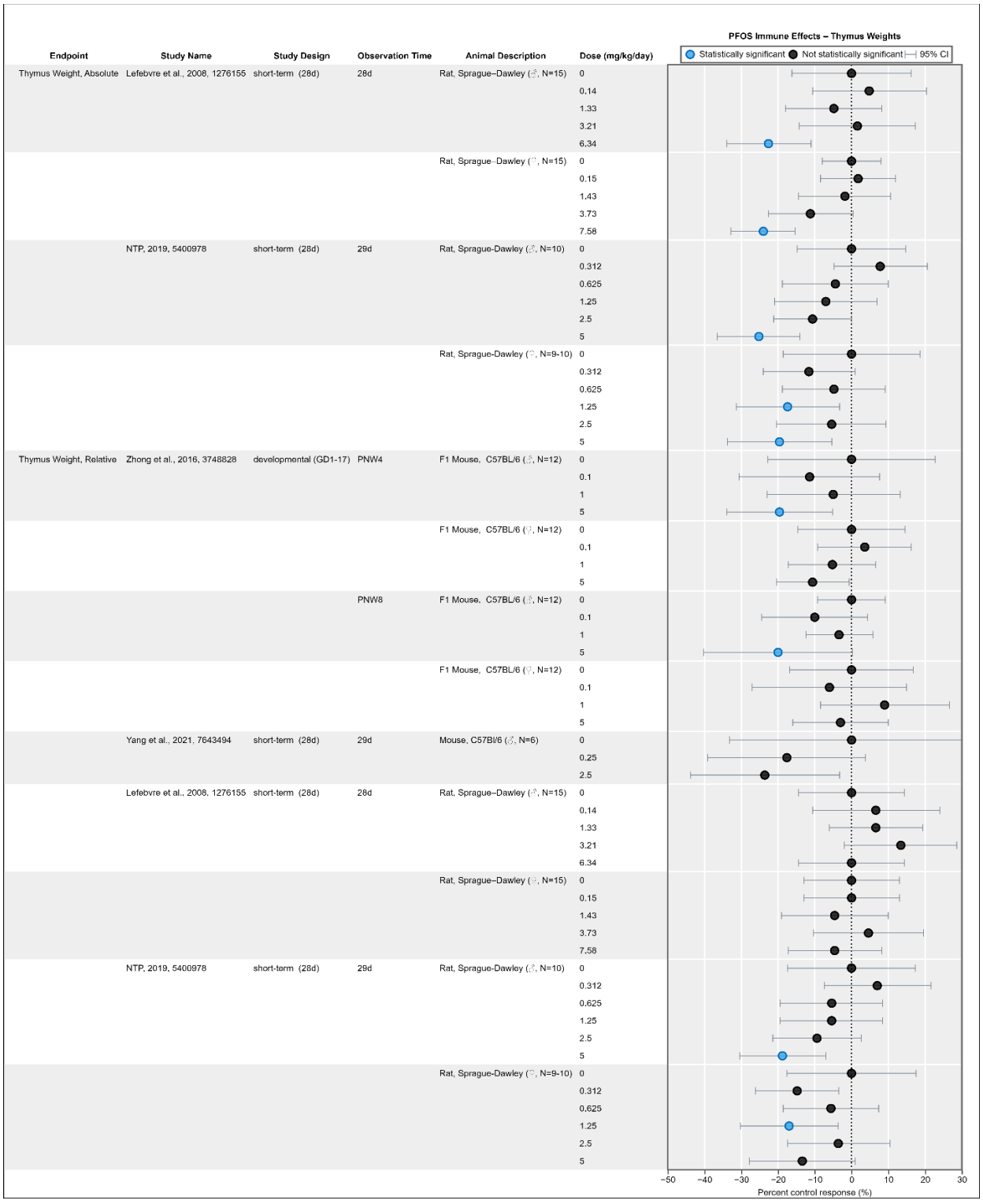


Figure 3-24. Percent Change in Thymus Weights Relative to Controls in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; F_1 = first generation

3.4.2.2.2 Histopathology

Histopathology of the spleen, thymus, and/or lymph nodes has been evaluated following oral exposure to PFOS across studies of varying durations in rodents (Figure 3-25). In general, short-term and subchronic studies have observed histopathology such as extramedullary hematopoiesis (NTP, 2019), bone marrow hypocellularity (NTP, 2019), and other aberrations in the immune organs (Lv et al., 2015; Qazi et al., 2009b).

One study included in the 2016 PFOS HESD (U.S. EPA, 2016b) by Qazi et al. (2009b) described perturbations in the thymus of male C57BL/6 (H-2^b) mice exposed to 0.02% (equivalent to ~40 mg/kg/day) PFOS in feed for 10 days; the thymic cortex was smaller and devoid of cells and the cortical/medullary junction was indistinguishable. These observations may coincide with the reduction in thymus weight described above (NTP, 2019; Qazi et al., 2009b). However, the 28-day study in rats by NTP did not observe histopathologic effects in the thymus of males or females following exposure to 0.312–5 mg/kg/day PFOS (NTP, 2019), and this finding was complemented by a chronic non-human primate study by Seacat et al. (2002), which also found no effects in the thymus of males or females following PFOS exposure (0, 0.03, or 0.15 mg/kg/day).

In spleens of male BALB/c mice, no significant increases in nonneoplastic lesions were observed following exposure to 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks, though quantitative results were not reported (Lv et al., 2015). However, the authors (Lv et al., 2015) state that alterations in spleen architecture were observed at the end of the exposure in the 5 and 10 mg/kg/day groups. Moreover, splenic sinusoids, which drain into pulp veins, were dilated and hyperemic. Peripheral splenic pulp structure and splenic cords (also known as red pulp cords or cords of Billroth) were destroyed, the marginal zone disappeared, and megakaryocytes (myeloid cell precursors) were abundant.

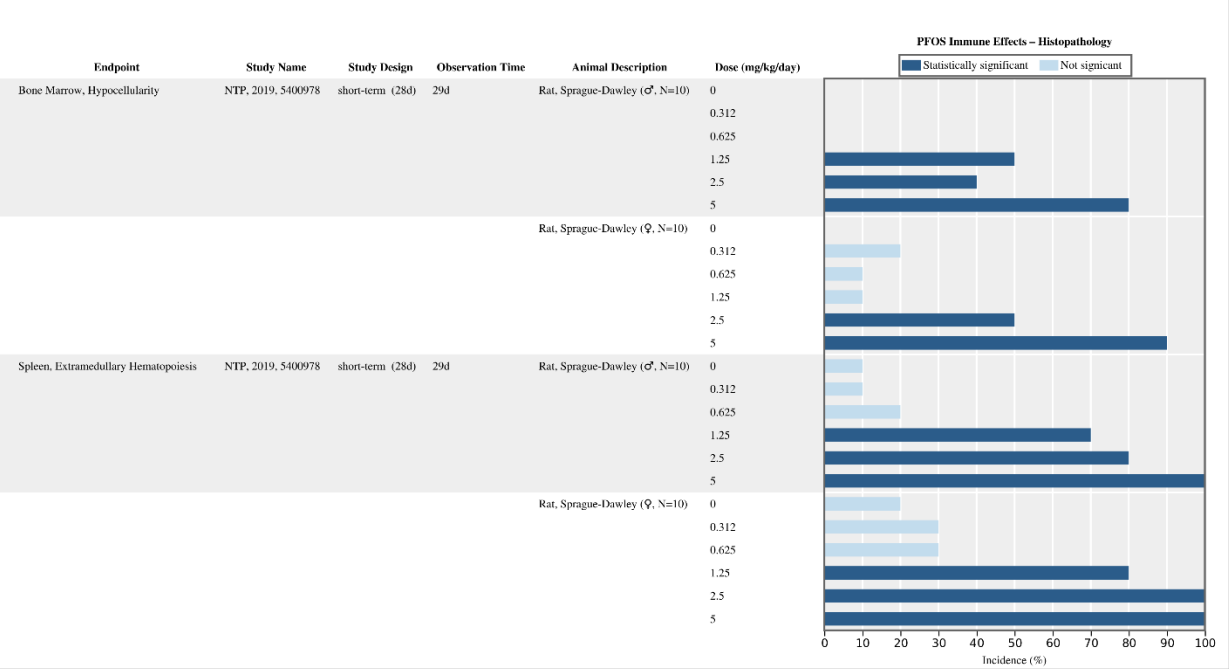


Figure 3-25. Incidences of Immune Cell Histopathology in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Xing et al. (2016) examined spleens of male C57BL/6J mice for histopathology; no distinguishable morphological differences were observed between any exposure group (2.5, 5, or 10 mg/kg/day for 30 days) and control. Similarly, Li et al (2021c) reported that there were no significant lesions observed in the spleen among female BALB/c mice exposed via gavage to 0.1 or 1 mg/kg/day PFOS for 60 days.

One study reported histology for the lymphatic system, but no histopathology was observed in the lymph nodes (mandibular and mesenteric) following PFOS exposure (NTP, 2019).

3.4.2.2.3 Circulating Immune Cells

Effects of PFOS exposure on circulating immune cells have been reported in rodents and non-human primates. Alterations in neutrophil and white blood cell (WBC) populations in the circulation have been observed in rodents, but the directionality of the effect is often inconsistent, possibly reflecting differences in the timing of exposure.

Qazi et al. (2009a) performed a study to see if exposure to PFOS influenced circulating immune cells. Male C57BL/6 mice were fed chow containing 0.02% PFOS for 10 consecutive days, after which levels of WBCs were evaluated in blood collected from retroorbital puncture. The absolute WBC count was significantly reduced and was mainly a reflection of decreased lymphocytes, as no change in neutrophils was seen. A significant reduction of the relative proportion and absolute number of macrophages in the bone marrow was also reported (Qazi et al., 2009a). In a study by Seacat et al. (2003), male and female Sprague-Dawley rats were exposed to 0, 0.5, 2, 5, or 20 ppm PFOS for 14 weeks and WBC counts were determined. The only statistically significant change was an increase in neutrophils in the 20 ppm exposure group

(1.33 mg/kg/day dose equivalent) in the males only. No effects were observed at lower exposure groups (0.5, 2.0, 5.0 ppm) nor in females (Seacat et al., 2003). A shorter (28-day) study in male and female Sprague-Dawley rats exposed to 0.14–7.58 mg/kg/day PFOS did not observe any statistically significant effects on circulating white blood cell populations (Lefebvre et al., 2008). The authors examined a myriad of circulating immune cell endpoints, including WBC, total lymphocytes, as well as the number and percentages of CD3+ (all T cells), CD3+/CD8+ (Cytotoxic T cells), CD3+/CD4+ (Helper T cells), CD45RA+ (B cells). Although not significant, Helper T cell counts in males and females were elevated from control by 35% or 42%, respectively, which coincided with a 29% or 41% increase in total T cell counts, suggesting that there may be a specific effect of PFOS on helper T cell populations. Similarly, Yang et al. (2021) found that exposure of male C57BL/6 mice to 2.5 mg/kg/day PFOS for 28 days did not significantly alter WBC counts, nor percent or number of neutrophils, total lymphocytes, eosinophils, monocytes, and basophils in the serum.

Evidence from one paper (Seacat et al., 2002) suggests that the effects of PFOS on WBCs that have been noted in some rodent studies do not extend to non-human primates. Male and female cynomolgus monkeys, orally administered 0.3–0.75 mg/kg/day PFOS for 26 weeks, exhibited no significant change in WBC counts, including neutrophils and total lymphocytes (Seacat et al., 2002). In contrast, reduced numbers of neutrophils were observed in male rats, but not females, in an NTP (2019) study. In that report, NTP also reported that male rats, and not females, exhibited significantly reduced WBC counts (NTP, 2019).

3.4.2.2.4 Natural Killer Cell Activity

The available data on the effect of PFOS exposure on natural killer (NK) cell activity indicate that there may be different effects in NK cell activity based on dose, but there are too few studies to make any determination and no single study assesses the continuum of doses to see if there is an opposing effect at different areas of the dose-response curve. Oral administration of 0.00017–0.166 mg/kg/day PFOS to male and female B6C3F1 mice for 28 days resulted in increased NK cell activity in males only exposed to 0.017, 0.033, and 0.166 mg/kg/day (Peden-Adams et al., 2008). Male C57BL/6 mice exposed to 0.083 mg/kg/day PFOS daily for 60 days displayed significantly increased NK cell activity by 38%, but treatment with 0.833 and 2.083 mg/kg/day resulted in decreased NK cell activity (Dong et al., 2009). Female mice were not assessed in this study. In another assessment of male C57BL/6 mice administered 0–40 mg/kg/day for 7 days, NK cell activity was reduced following exposure to 20 and 40 mg/kg/day (Zheng et al., 2009). Similarly, Zhong et al. (2016) reported that NK cell activity was decreased in 4-week-old male offspring from the 5 mg/kg/day group and also reduced in 8-week-old offspring from the 1 or 5 mg/kg/day group. The latter result was recapitulated in the study by Keil et al. (2008) where the female C57BL/6 mice were mated with C3H to derive B6C3F1 offspring. Female offspring from both studies were less sensitive to the PFOS-induced reduction in NK cell activity (Zhong et al., 2016; Keil et al., 2008) as indicated by the lack of statistically significant changes in females exposed to 1 mg/kg/day in each study. Moreover, at 8 weeks, NK cell activity was suppressed by 42.5% and 32.1% in males at the 1 and 5 mg/kg/day treatments, respectively, and was suppressed by 35.1% in females at the 5 mg/kg/day treatment (Keil et al., 2008). These studies indicate that male mice may be more susceptible to PFOS-induced altered NK cell activity, and that NK cell activity can be increased or decreased following low or high PFOS exposure, respectively (Table 3-9).

Table 3-9. Associations Between PFOS Exposure and Natural Killer Cell Activity in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008)	28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, 0.166	M	↓ 0.017–0.166 mg/kg/day
			F	n.s.
Dong et al. (2009)	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↑ (0.083 mg/kg/day) ↓ (0.833–2.083 mg/kg/day)
Zheng et al. (2009)	7 days	0, 5, 20, 40	M	↓ (20–40 mg/kg/day)
Zhong et al. (2016)	GD 1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 5 mg/kg/day
			F	n.s.
	GD 1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			F	↓ 5 mg/kg/day
Keil et al. (2008)	GD 1–17 4-week assessment	0, 0.1, 1, 5	M	n.s.
			F	n.s
	GD 1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
		0, 0.1, 1, 5	F	↓ 5 mg/kg/day

Notes: F = female; M = male; n.s. = nonsignificant.

3.4.2.2.5 Spleen Cellularity

Splenocyte sub-classes were quantified in several rodent studies (Figure 3-26). Splenic T cell immunophenotypes were slightly affected in male and female B6C3F1 mice exposed to oral administration of 0.00017–0.166 mg/kg/day PFOS for 28 days (Peden-Adams et al., 2008). In males, $CD4^-/CD8^+$ and $CD4^-/CD8^-$ cells were increased, whereas numbers of $CD4^+/CD8^-$ and $CD4^+/CD8^+$ cells were decreased beginning at 0.0033 mg/kg/day. In females, splenic $CD4^-/CD8^+$ and $CD4^+$/CD8- cells were decreased beginning at 0.0033 mg/kg/day. Significantly decreased splenocyte populations were also observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days (Qazi et al., 2009b), 20 or 40 mg/kg/day PFOS for 7 days (Zheng et al., 2009), and 0.417–2.083 mg/kg/day for 60 days (Dong et al., 2009). Female mice were not evaluated in these studies.

Altered splenic cellular composition was observed in a study by Lv et al. (2015) where male BALB/c mice were exposed to 0, 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks (Lv et al., 2015), and spleens harvested for lymphocyte counting and phenotyping. Fluctuations in lymphocyte counts and T cell proliferation were apparent at the 3-week timepoint. A dose-dependent increase in the number of splenic T cells ($CD3^+$) relative to controls was observed at the end of 3 weeks, reaching significance in the 2.5 and 10 mg/kg/day exposure groups. This coincided with a nonsignificant increase in T-helper (CD3 + $CD4^+$) and T-cytotoxic (CD3 + $CD8^+$) lymphocytes in the 5 and 10 mg/kg/day groups, all relative to controls. The percentages of T-helper

(CD3 + $CD4^+$) and T-cytotoxic (CD3 + $CD8^+$) lymphocytes were increased in the 10 mg/kg/day groups (Lv et al., 2015).

Further effects of PFOS on immune cell composition in the spleen have also been reported following developmental exposure by Keil et al. (2008) and Zhong et al. (2016). Zhong et al. (2016) exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD 1–17, and then quantified various immune cell populations in male and female pups. Decreased splenic cell subpopulations ($CD4^+$ and $CD8^+$ cell counts) were observed in the 4-week-old male pups from the 5 mg/kg/day exposure group. At 8-weeks, reductions in $CD8^+$ cells in the spleen were observed in the 5 mg/kg/day exposure group (Zhong et al., 2016).

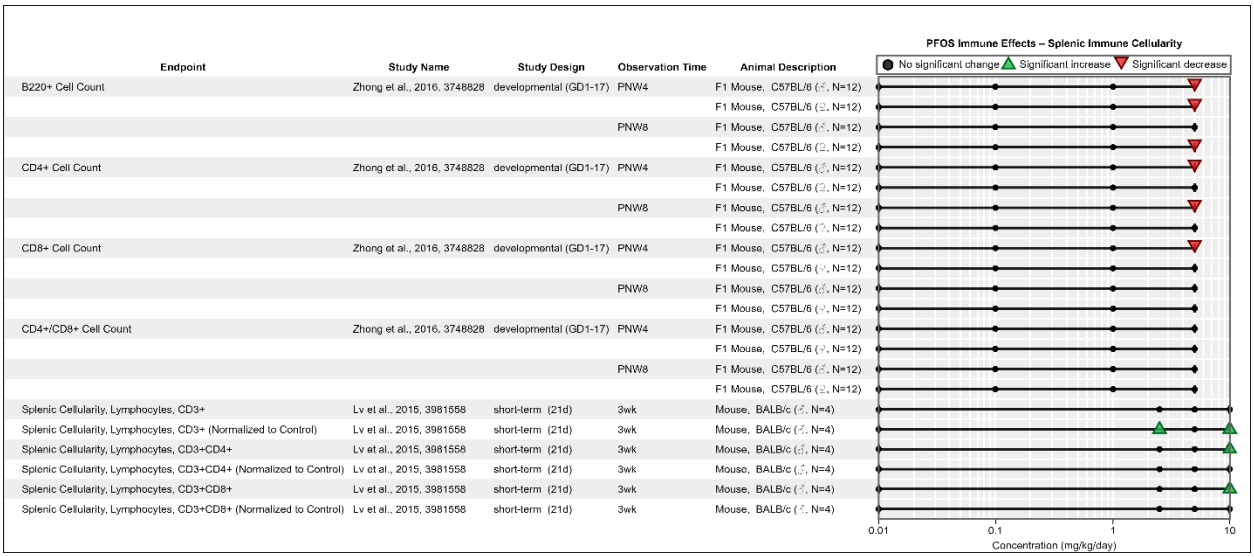


Figure 3-26. Splenocyte Cellularity in Rodents Following Exposure to PFOS (Logarithmic Scale)[a]

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; F_1 = first generation.
[a] Zhong et al. (2016) reported data on both splenic and thymic lymphocyte populations for the same experimental animals. Results are shown in separate figures.

3.4.2.2.6 Thymus Cellularity

Thymus cell populations were less sensitive to the effects of PFOS compared with the effects observed in the spleen, as determined by the dose where the change occurred and the number of endpoints that changed following PFOS exposure (Figure 3-27). Indeed, while all splenic T cell CD4/CD8 subpopulations were altered in one study of male B6C3F1 mice beginning at 0.1 mg/kg/day exposures, none of the thymic T cell subpopulations were affected. Furthermore, the effects appeared to also have a female-bias; although thymic $CD4^-/CD8^+$ cells were increased in female B6C3F1 mice exposed to 0.033 or 0.166 mg/kg/day, no effects were observed in males (Peden-Adams et al., 2008). In contrast, significantly decreased thymocyte populations were observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days (Qazi et al., 2009b), 20 or 40 mg/kg/day PFOS for 7 days (Zheng et al., 2009), and 0.417–2.083 mg/kg/day for 60 days (Dong et al., 2009). Female mice were not evaluated in these studies.

Effects of PFOS on immune cell composition in the thymus have also been reported following developmental exposure. Pregnant female C57BL/6 mice were dosed with 0.1–5 mg/kg/day PFOS from GD 1–17, and immune cell populations were quantified in male and female pups at 4 and 8 weeks after birth. Decreased thymic lymphocyte subpopulations ($CD4^+$, and $CD4^-/CD8^-$ cell counts) and decreased thymic cellularity were observed in the 4-week-old male pups from the 5 mg/kg/day exposure group, and no effects were observed in females (Zhong et al., 2016). At 8-weeks, no effects were observed in females and reductions in thymic $CD4^+$ cells were observed in males from the 5 mg/kg/day exposure group. These findings were complimented by Keil et al. (2008), who observed a reduction in $CD3^+$ and $CD4^+$ thymocytes in 8-week C57BL/6N male mice following exposure to 0.1–5 mg/kg/day from GD 1–17 (Keil et al., 2008).

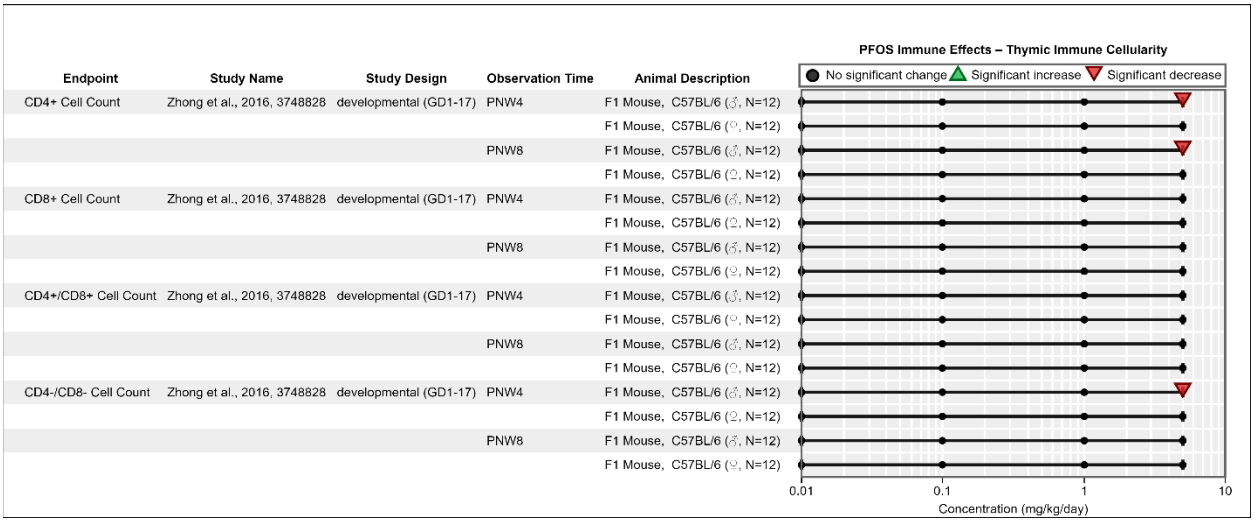


Figure 3-27. Thymocyte Cellularity in Rodents Following Exposure to PFOS (Logarithmic Scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; F_1 = first generation.
[a] Zhong et al. (2016) reported data on both splenic and thymic lymphocyte populations for the same experimental animals. Results are shown in separate figures.

3.4.2.2.7 Ability to Generate an Immune Response

Many studies have investigated the effect of PFOS on the ability of rodents to generate an immune response to various antigens. Several mouse studies of varying durations and exposure levels have provided consistent evidence that PFOS can reduce the immune response as determined by reductions in sheep red blood cell-specific immunoglobulin M (IgM) production. Two rodent studies (Yang et al., 2021; Lee et al., 2018a) provide consistent evidence that PFOS can exacerbate the allergic immune response.

Several animal toxicological studies have found evidence indicative of immunosuppression, including reduced IgM titers. Peden-Adams et al. (2008) found that the sheep red blood cell (SRBC) plaque forming cell (PFC) response, which measures IgM-producing cells, was reduced in male and female B6C3F1 mice administered 0.0017–0.166 mg/kg/day PFOS for 28 days. The response was suppressed at lower PFOS doses in male mice (effect first observed at 0.0017 mg/kg/day) than female mice (effect first observed at 0.017 mg/kg). Because IgM

suppression can result from effects on both T and B cells, antibody production was also measured in response to a bacteria-like challenge, trinitrophenyl (TNP)-lipopolysaccharide (LPS), which would induce a T-independent response. Following the TNP-LPS challenge, a decrease in IgM titers was observed in female B6C3F1 mice that had been exposed to 0.334 mg/kg/day PFOS for 21 days. Male animals were not assessed in this study (Peden-Adams et al., 2008). Similarly, Dong et al. (2009) observed a dose-dependent reduction in the SRBC-specific IgM PFC response in male C57BL/6 mice exposed to PFOS daily for 60 days. These results are consistent with a similar study by the same authors in 2011, including a dose-dependent reduction in IgM levels in serum (Dong et al., 2011). The authors also examined the delayed-type hypersensitivity response (DTH) to SRBC. Although IgM levels were reduced in groups exposed to 0.0833 mg/kg/day PFOS or higher, IgG, IgG1, and IgE levels were elevated only in the highest exposure group (0.833 mg/kg/day), and no change was observed in IgG2a levels (Dong et al., 2011). To further assess the DTH response, footpad thickness was measured using digital calipers on the foot used to sensitize the mice to SRBC relative to the non-sensitized foot; no significant increase in footpad swelling was observed. Female mice were not assessed in either of these studies. The DTH response was also assessed by Lefebvre et al. (2008) in male and female rats sensitized with the T-dependent antigen, keyhole limpet hemocyanin (KLH), during a 28-day exposure to 0.14–7.58 mg/kg/day PFOS (on days 14 and 21) and challenged at the end of study with KLH. There were no significant changes in anti-KLH IgG titers in males or females compared with control, and there were no changes in footpad swelling. Zheng et al. (2009) also found that the PFC response to a SRBC challenge was suppressed in male C57BL/6 mice given 5, 20, or 40 mg/kg/day PFOS for 7 days. These rodent studies provide evidence of a PFOS-induced suppression of the immune response to a SRBC challenge that may be more sensitive in male mice (Table 3-10).

Table 3-10. Associations Between PFOS Exposure and Immune Response in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008)[a]	28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, 0.166	M	↓ 0.0017–0.166 mg/kg/day
			F	↓ 0.017–0.166 mg/kg/day
Lefebvre et al. (2008)[b]	28 days	0, 0.14, 1.33, 3.21, 6.34 (males) or 0, 0.15, 1.43, 3.73, 7.58 (females)	M	n.s.
			F	n.s.
Dong et al. (2009)[a]	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↓ 0.083–2.083
Dong et al. (2011)[a]	60 days	0, 0.008, 0.0167, 0.083, 0.417, 0.833	M	↓ 0.083–0.833
Zheng et al. (2009)[a]	7 days	0, 5, 20, 40	M	↓ 5–40 mg/kg/day
Zhong et al. (2016)[a]	GD 1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			F	↓ 5 mg/kg/day
	GD 1–17 8-week assessment	0, 0.1, 1, 5	M	n.s.
			F	n.s.
Keil et al. (2008)[a]	GD 1–17	0, 0.1, 1, 5	M	↓

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
	8-week assessment			5 mg/kg/day
			F	n.s.

Notes: F = female; M = male; n.s = nonsignificant.
[a] Sheep red blood cell-specific IgM production.
[b] Keyhole limpet hemocyanin-specific IgG production.

Similar observations were reported in two developmental PFOS exposure studies. Keil et al. (2008) and Zhong et al. (2016), each exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD 1–17 and then tested the immune responses in offspring at 4 and 8 weeks of age. Four days before sacrifice, mice were injected with SRBC to induce an immune response. Keil et al. (2008) reported that the primary IgM response to SRBC was significantly suppressed by 53% at 8-weeks in males from the 5 mg/kg/day exposure group. In females, the primary IgM response was not altered (Keil et al., 2008). Similarly, Zhong et al. (2016) observed that SRBC-specific IgM production by B-lymphocytes in the spleens of 4-week-old mouse pups exposed to 1 or 5 mg/kg/day PFOS in utero was reduced by 15% or 28%, respectively. In females, the SRBC-specific IgM response was significantly suppressed by 24% in the 5 mg/kg/day group only. However, no significant changes were observed at 8 weeks.

Alterations in the serum levels of globulin can be associated with decreases in antibody production (FDA, 2002). Two 28-day studies (NTP, 2019; Curran et al., 2008) in male and female Sprague-Dawley rats reported effects on serum globulin levels. In the first study, rats were orally administered 0.312–5 mg/kg/day PFOS. Male rats exhibited significantly decreased globulin while globulin in females did not significantly differ from control values (NTP, 2019). These findings are complemented by a study by Curran et al. (2008), in which male and female rats fed diets containing 2–100 mg/kg PFOS (equivalent to 0.14–6.34 mg/kg/day in males and 0.15–7.58 mg/kg/day in females) for 28 days. In male rats, serum albumin/globulin ratios were elevated in the highest exposure group in conjunction with a significant dose-related negative trend in globulin levels. In female rats, no changes were observed in albumin/globulin ratio or globulin levels. In a separate study (Lefebvre et al., 2008) the same authors also reported total levels of IgM, IgG, IgG1, IgG2a, IgG2b, and IgG2c in serum of male and female rats exposed to 0, 2, 20, 50, or 100 mg/kg/day PFOS for 28 days. In males, significant reductions in IgG1 levels were observed at the two lowest doses and a significant positive trend was observed for trend for IgG, IgG2a, and IgG2c. In females, both IgM and IgG2c levels were significantly elevated in the highest dose group.

Two studies by Lee et al. (2018a) and Yang et al. (2021) found evidence that PFOS exposure can exacerbate an allergic immune response in mice. Lee et al. sensitized male ICR mice with ovalbumin (OVA) on day 0 and day 7 and exposed them to 50–150 mg/kg/day PFOS on study day 9, 11, and 13. Serum histamine, TNF-α, IgE, and IgG levels were increased following exposure, suggesting that PFOS exacerbates mast cell-mediated allergic inflammation. These findings are complemented by studies in male C57BL/6 mice by Yang et al. (2021). In that study, mice were exposed to PFOS for 28 days via gavage, sensitized to OVA and adjuvant via subcutaneous injection on days 4 and 11, and challenged with an aerosol of 1% OVA on days 26 to 28. In the serum, exposure to OVA alone or to OVA + PFOS did not lead to elevations in WBC counts, nor percent or number of neutrophils, total lymphocytes, eosinophils, monocytes,

and basophils. Serum IgE levels and anti-OVA IgE antibodies were elevated in groups exposed to 0.25 or 2.5 mg/kg/day PFOS + OVA compared with OVA alone or untreated controls. Mice exposed to 0.25 or 2.5 mg/kg/day PFOS alone showed a low level of serum IgE, similar to the control group.

3.4.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse immune outcomes is discussed in Sections 3.1.1.6, 3.3.2, 3.3.4, and 3.3.6 of the 2016 PFOS HESD (U.S. EPA, 2016b). There are 24 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to immune effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-28.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	6	0	9	13
Cell Signaling Or Signal Transduction	2	0	4	6
Extracellular Matrix Or Molecules	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Hormone Function	1	0	0	1
Inflammation And Immune Response	6	5	12	19
Oxidative Stress	1	0	3	4
Other	1	0	0	1
Grand Total	8	5	15	24

Figure 3-28. Summary of Mechanistic Studies of PFOS and Immune Effects

Interactive figure and additional study details available on HAWC.

3.4.2.3.1 Mechanistic Evidence for PFOS-Mediated Effects on the Immune System

Since the 2016 PFOS HESD advisory was released, 26 studies were identified that inform the mechanism by which PFOS may alter or perturb immune system function or immune system development and physiology. Recent studies provide mechanistic insights into PFOS effects on immune system development and physiology (5 studies), adaptive immune responses (6 studies), innate immune responses (4 studies), intrinsic cellular defense (1 study), and disruption of inflammatory responses (9 studies). Mechanistic pathways associated with the immune system identified in the recent PFOS literature included inflammation, immune responses, cell viability, cell signaling, oxidative stress, and hormone function.

3.4.2.3.1.1 Mechanistic Evidence for PFOS-Mediated Effects on Immune System Development and Physiology

Alterations in immune and allergic responses in exposed children may suggest PFOS-mediated effects in immune system development. In addition, changes in white blood cell count (Oulhote et al., 2017) and alterations in gene expression related to immune and inflammation responses in

human cord blood (Pennings et al., 2016) present potential mechanisms of immunotoxicity in children. In animals, PFOS-related health effects related to immune system development and physiology are described in Sections 3.4.2.2.1 to 3.4.2.2.7. Briefly, effects in mice and rats included reduced spleen and thymus weights, alterations in spleen and thymus morphology, and changes in the cellularity and immunophenotypes of lymphocytes. Effects varied by sex and strain.

Three mechanistic studies in mice suggest that changes in immune physiology and development following exposure to PFOS can be sex-dependent. Zhong et al. (2016) demonstrated sex-specific impacts of PFOS on immune organ development and physiology in C57BL/6 mice exposed during development. Pups were evaluated after maternal oral exposure to PFOS (0.1, 1.0, or 5.0 mg PFOS/kg/day) from gestational day (GD) 1–17. Sex-dependent alterations in spleen and thymus organ weights, cellularity, and cellular immunophenotypes are discussed in Section 3.4.2.2. These may be linked to sex hormones during development as there was a significant interaction between sex and PFOS concentrations for serum testosterone at 4 and 8 weeks of age, and estradiol at 4 weeks of age. The authors suggest that sex-dependent differences in PFOS excretion, the endocrine-disrupting properties of PFOS, or male or female sex hormone-differences may influence the sex-specific impact on spleen and thymus physiology.

Lv et al. (2015) reported disrupted splenic architecture and reduced absolute numbers (albeit increased percentages) of T-helper (CD3 + CD4+) and cytotoxic T (CD3 + CD8+) cells in the spleen of male BALB/c mice administered 10 mg/kg/day PFOS via gastric gavage for 3 weeks followed by a 1-week recovery. Gene expression profiling identified differential regulation of genes involved in mitogen-activated protein kinase (MAPK) signal transduction pathways and in cellular responses to oxidative stress. The effects on gene expression paralleled a dose-dependent increase in intracellular free calcium ($[Ca^{2+}]$, which plays an important role in immune cell proliferation in response to foreign antigens) concentration in splenocytes of exposed animals, suggesting that activation of MAPK signaling pathway and/or oxidative stress genes in response to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes.

Qazi et al. (2012) also observed decreased spleen and thymus weights and cellularity as well as reduced numbers of myeloid, pro/pre-B, and immature B cells in bone marrow (BM). In male C57BL/6 (H-2b) mice fed diets containing PFOS compounds (0.001–0.02%, w/w) for 10 days, atrophy of the thymus and spleen as well as hypocellularity of BM was observed at the higher dose of 0.02%. PFOS exposure caused reduced feed consumption and atrophy of the thymus and spleen and hypocellularity of bone marrow cells. Histopathological and flow cytometric analysis of BM showed significant reductions in the total numbers of bone marrow cells as well as the numbers of pro/pre-B (CD19 + CD138 + IgM+) and immature B (CD19+ CD138+ IgM+) cells. Myeloid (Gr1+ CD11b+) cells and B-lymphoid (CD19+) cells were also reduced in mice administered the high dose of PFOS. After 10 days of withdrawal of PFOS from feed, the effects in bone marrow partially or completely reversed. Interestingly, food restriction alone in the absence of PFOS exposure also led to reduced cell numbers in the thymus and spleen and resulted in reductions of the total numbers of B-lymphoid cells, pro/pre-B, and immature B cells. These findings indicate that immunotoxicity of PFOS may, at least in part, be a consequence of reduced food consumption. Additionally, perturbation of the bone marrow may contribute to

reduced numbers of splenic B cells, atrophy of the spleen, and impaired humoral immune responses caused by exposure to PFOS.

3.4.2.3.2 Mechanistic Evidence for PFOS-Mediated Effects on Adaptive Immune Responses

3.4.2.3.2.1 Mechanistic Data Informing Suppression of Immune Responses to Vaccines and Infectious Diseases

The effects of prenatal, childhood, or adult PFOS exposure on responses to vaccines and infectious diseases are described in Section 3.4.2.1. Briefly, studies observed an inverse association between PFOS exposure and vaccine-induced antibody levels to tetanus and to pathogens including human foot and mouth disease (HFMD) and hepatitis B infection. Other studies identified associations between PFOS exposure and increased incidence of infections including those caused by pneumonia and chickenpox, though PFOS was associated with a decrease in the incidence of respiratory syncytial virus (RSV), common cold, ear infection, and urinary tract infection. Six new mechanistic studies were identified that inform PFOS-mediated effects on adaptive immunity (3 in humans and 3 in mice). One mechanistic study directly evaluated PFOS-mediated effects on adaptive immune responses specific to vaccines and infectious disease (Pennings et al., 2016), and 5 mechanistic studies evaluated non-allergic adaptive immune responses.

As described in Section 3.4.2.1.1, in children exposed to PFOS in utero, Granum et al. (2013) previously reported an inverse association between maternal serum concentrations of PFOS and anti-rubella antibody levels in serum of 3-year-old children, as well as an increased incidence of the common cold, using samples and data from the Norwegian BraMat cohort. In a follow-up study of early-life immunosuppression again using Norwegian BraMat cohort data, Pennings et al. (2016) conducted a whole genome transcriptomic microarray analysis of neonatal cord blood samples and compared the results to maternal levels of PFOS (as well as PFOA, perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS)) in the blood. Dose-response relationships between PFOS and expression of individual genes, rubella antibody levels, and episodes of the common cold were analyzed. Expression of 636 genes was positively associated with PFOS exposure, and 671 were negatively correlated. A set of 27 genes were correlated between all four of the PFAS evaluated and the number of common cold episodes. Of these, three genes were related to immunological and/or hematopoietic functions, including peroxisome proliferator-activated receptor delta (PPARD), SHC adaptor protein 4 (SHC4), and cytokine like 1 (CYTL1), expressed in CD34+ in bone marrow and cord blood mononuclear cells. Of the six genes related to development and/or morphogenesis, two overlapped with immune and hematopoietic functions (PPARD and CYTL1). Interestingly, another gene associated with development and morphogenesis, sphingosine-1-phosphate lyase 1 (SGPL1), has been recently associated with immune responses to viral infections including inhibition of influenza virus replication by promoting antiviral type I interferon innate immune responses (Wolf et al., 2019). A set of 26 genes overlapped between PFAS and rubella titers, including two genes also identified in pathway analysis as relevant to regulation of T cell activation (interleukin 27 (IL27) and the adenosine A2a receptor (ADORA2A)). Only one gene (CYTL1) was in common between the sets of genes that overlapped with PFAS exposure and common cold episodes, and PFAS exposure and rubella titers. However, a clear understanding of the function of CYTL1 in hematopoiesis and immune function is lacking. While the correlation between gene

expression changes and changes in protein expression or function in cord blood was not investigated in this study, these represent potential candidate genes that mediate the mechanism(s) of early childhood immunotoxicity associated with prenatal exposure to PFOS and other PFAS chemicals.

Lv et al. (2015) examined T cells in male BALB/c mice administered 10 mg/kg/day PFOS via gavage for 3 weeks followed by 1-week recovery. Gene expression profiling in spleens was performed using GeneChip® Mouse Genome 430 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA) and quantitative real time PCR (qRT-PCR). The authors identified 1,327 differentially expressed genes (4% of all analyzed genes) in response to PFOS exposure. Biological processes associated with differentially expressed genes included cell cycle, DNA metabolism, mitosis, and DNA replication. Pathway analysis identified significantly upregulated pathways related to the T cell receptor (TCR) and to immune signaling (primary immunodeficiency signaling, inducible co-stimulator (iCOS)–iCOS ligand (iCOSL) signaling in T-helper cells, OX40 signaling pathway, and calcium-induced T lymphocyte apoptosis). However, the transducer of ErbB-2.1 (TOB) T cell signaling pathway was significantly downregulated, as were genes associated with nuclear factor erythroid derived 2 like 2 (Nrf2)-mediated oxidative stress response (such as GSTM3 and MGST3). During the recovery period following 4 weeks of PFOS exposure, immunoblotting confirmed a dose-dependent upregulation of protein levels in spleens for several genes involved in TCR signaling and calcium signaling, including thymocyte selection associated (THEMIS), the CD3 gamma subunit of T-cell receptor complex (CD3G), and calcium/calmodulin dependent protein kinase IV (CAMK4). Additionally, in splenocytes of exposed animals, [Ca2+]i increased in a concentration-dependent manner, and T-cell proliferation in response to Concanavalin A (Con A) stimulation was inhibited by PFOS. The authors suggest that activation of MAPK signaling pathway and/or oxidative stress genes in response to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes. These findings also suggest that altered expression of cell cycle genes, upregulation of genes involved in TCR signaling, and altered calcium homeostasis impact T cell function through inhibition of T cell proliferation and induction of T cell anergy (intrinsic functional inactivation of lymphocytes following an antigen encounter).

Li et al. (2020c) used an integrative ‘omics approach to evaluate perturbations in the transcriptome and lipidome in human lymphocytes that may impact adaptive immune responses to vaccines or infectious diseases. Lymphocytes were isolated from human donors and cultured before treatment with 50 mM PFOS for 72 hours. PFOS treatment led to a significant induction of the cytokines IL-1, IL-4, IL-6, and IL-8 cytokines relative to controls, as measured by ELISA. Subsequent deep sequencing of RNA for PFOS-treated lymphocytes revealed that numerous differentially expressed genes were related to lymphocyte function and biological processes related to immunity, including immune responses, innate immune responses, and inflammatory responses. Enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database linked PFOS treatment to stimulation of cytokine-cytokine receptor interactions, extracellular matrix (ECM)-receptor interactions, the PI3K-Akt signaling pathway, the peroxisome proliferator-activated receptor (PPAR) signaling pathway, cholesterol metabolism, and phagosome and lysosome regulation at the gene expression level. The analysis identified differentially expressed genes associated with cytokines, growth factors, and differentiation and migration of antigen-presenting cells. Additionally, the authors conducted a lipidomic analysis of treated cells using liquid chromatography–mass spectrometry (LC-MS). Lipid metabolites (40

upregulated and 56 downregulated) were identified in PFOS-exposed lymphocytes relative to control lymphocytes. Clusters of lipids associated with immune function were dysregulated, including lipids involved in glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism. Taken together with the transcriptomic and functional analyses reported by Lv et al. (2015) and Pennings et al. (2016), these findings suggest that PFOS exposure may disrupt adaptive immunity through dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and anergy.

The potential for PFOS to suppress immune responses to vaccines and infection are also informed by studies investigating PFOS-mediated effects on TH1/TH2-type cytokines in mice (Zhong et al., 2016), glycosylation of immunoglobulins in humans (Liu et al., 2020c), and lymphocyte toxicity in vitro (Zarei et al., 2018). Zhong et al. (2016) exposed pregnant female C57BL/6 mice to PFOS (0.1, 1.0, or 5.0 mg/kg/day) from GD 1–17 and cultured splenocytes of male pups at 4 and 8 weeks of age. Spontaneous IL-4 formation was increased and spontaneous production of TH1 cytokines (i.e., IL-2) was decreased in the 5 mg/kg/day group at 8 weeks. Functionally, lymphocyte proliferation was significantly decreased in splenocytes from both males and females exposed to the highest dose at 4 weeks, and natural killer (NK) cell activity exhibited a decreasing trend with dose (males only at 4 weeks, males and females at 8 weeks). Given the reductions in serum testosterone at 4 and 8 weeks of age, and increased estradiol levels in male pups at 4 weeks of age (discussed in Section 3.4.2.2), these findings suggest that in utero exposure may elicit sex-specific alterations in TH1 and TH2 cytokine profiles in immune cells as well as diminished lymphocyte and NK functions.

A recent study suggests that PFOS may also alter antibody glycosylation patterns (Liu et al., 2020c). Altered IgG glycosylation patterns are associated with disease states and immune functions including cancer immunosurveillance and anti-inflammatory reactions (Cobb, 2020). The N-glycome profiles of immunoglobulins from serum samples of adults and children were analyzed by subjecting the IgG fraction to glycan release, derivatization, and matrix-assisted laser desorption/ionization-MS (MALDI-MS) analysis. Specifically, increasing PFOS exposure was associated with decreased galactosylation, increased fucosylation and sialylation in adults, and increased agalactosylation, bisecting GlcNAcylation, sialylation and decreased galactosylation in children. The authors suggested several mechanisms by which altered IgG glycosylation impacts immunity including antibody-dependent cellular cytotoxicity (ADCC). While no functional studies were conducted, these preliminary findings provide a potential mechanism for altered antibody-dependent immune responses in PFOS-exposed persons.

Zarei et al. (2018) isolated lymphocytes from the blood of healthy humans and analyzed cytotoxicity in vitro in response to exposure to 100–500 μM PFOS for 12 hours. The IC50 for cytotoxicity was calculated to be 163.5 μM. Exposure to 75, 150, and 300 μM PFOS for 2, 4, 6, 8, 10, or 12 hours was associated with increased reactive oxygen species (ROS) formation, lipid peroxidation, and glutathione depletion. PFOS also damaged mitochondrial and lysosomal membranes and was associated with significantly increased levels of cellular proteolysis and caspase 3 activity. These findings suggest that PFOS could mediate immunosuppressive effects through direct cytotoxicity of lymphocytes.

3.4.2.3.2.2 Mechanistic Data Informing Autoimmune Diseases

As described in Section 3.4.2.1, two studies reported that PFOS levels in healthy controls were either higher than in ulcerative colitis (UC) cases (Steenland et al., 2018b) or lower than in multiple sclerosis (MS) cases (Ammitzbøll et al., 2019). While no mechanistic studies directly investigated the mechanism by which PFOS could promote the development of autoimmunity, one study evaluated PFOS effects on TH17 cells, implicated in the pathophysiology of both MS and UC (Chen et al., 2020; Fu et al., 2020). Suo et al. (2017) examined the effects of 2 mg/kg PFOS in a mouse model of *Citrobacter rodentium* infection. PFOS was administered for 7 days by oral gavage before mice were infected with C. rodentium and throughout the early and late phases of infection. Large intestinal lamina proprial lymphocytes were isolated 5 days after infection and analyzed by flow cytometry after treatment with immune stimulators. Levels of IL-17 and IL-22 produced by Th17 cells were significantly elevated in PFOS-treated mice compared with the control group. These findings support that PFOS-mediated effects on pathogenic TH17 cells may impact development of autoimmune diseases as well as bacterial infections of the gut.

3.4.2.3.2.3 Mechanistic Data Informing Allergic Responses

Several studies were identified that evaluated associations between PFOS exposure and immune hypersensitivity, including asthma, allergy, and eczema as described in Section 3.4.2.1.2. Five new mechanistic studies informed allergy and asthma. Oulhote et al. (2017) observed a significant association between PFAS exposures and increased basophil counts between birth and age 5 in human children. Although PFAS exposure was analyzed collectively (included PFOA, PFOS, PFHxS, PFNA, and perfluorodecanoic acid (PFDA)), PFOS showed the highest serum concentrations at all ages. The authors suggested that enhanced basophil levels could be associated with dysregulated allergic and asthma-related responses, possibly by promoting TH2-type responses.

Zhu et al. (2016) evaluated 231 asthmatic children and 225 non-asthmatic control children from Northern Taiwan. A significant positive association was identified for PFOS blood levels and TH2 cytokines while a nonsignificant inverse association was found for TH1 cytokines among asthmatic children. Male asthmatics exhibited elevated IgE levels with increasing PFOS levels. Also, in males only, significant positive associations between PFOS levels in blood and TH2:TH1 cytokine ratios were observed for both the IL-4/IFN-γ ratio and IL-5/IFN-γ ratio. This finding suggests that PFOS may exacerbate asthma by altering availability of key TH1 and TH2 cytokines. However, the effects of PFOS on TH1- and TH2-type cytokine profiles may be dependent on disease context or the cell types under study. For example, in earlier studies of human peripheral blood leukocytes (PBLs) treated with phytohemagglutinin (PHA), PFOS exposure led to diminished IL-4, IL10, and IFN-γ (NTP, 2016a; Corsini et al., 2012; Corsini et al., 2011).

Lee et al. (2018a) used an albumin-induced active systemic anaphylaxis model to evaluate type I hypersensitivity in mice. After sensitization with ovalbumin (OVA), PFOS (50–150 mg/kg) was orally administered on days 9, 11, and 13. On day 14, OVA was administered by intraperitoneal (IP) injection, and mice were evaluated for signs of allergy. PFOS significantly aggravated allergic symptoms such as hypothermia and significantly increased serum histamine, TNF-α, IgE, and IgG1 relative to controls. Further findings suggest the mechanism of aggravated allergic responses mediated by PFOS is through release of histamine and β hexosaminidase associated

with upregulation of intracellular calcium in IgE-stimulated mast cells. Elevated levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) were also observed in PFOS-exposed non-sensitized rat basophilic leukemia cells, which were linked to NF-kB activation. Together, these findings provide a plausible pathway for PFOS-mediated exacerbation of allergic responses.

3.4.2.3.2.4 Mechanistic Evidence for PFOS-Mediated Effects on Innate Immune Responses

As described in Sections 3.4.2.2.3 and 3.4.2.2.4, several studies in animals suggest PFOS may negatively impact NK cells and macrophage function, indicating innate immune effector cells are susceptible to perturbations by PFOS. Very few studies were identified that evaluated the mechanisms by which PFOS may alter innate immunity and no studies evaluated the mechanisms by which PFOS alters NK cell activity. Among the studies reporting NK activity in Table 3-9 in Section 3.4.2.2.4, most studies observed decreased NK activity, though at least one study observed enhanced NK responses at low doses of exposure (Dong et al., 2009). In all of these studies, NK cells were obtained from animals exposed in vivo and analyzed in vitro using target cells that were not exposed to PFOS, suggesting PFOS directly alters NK maturation or activity. Whether PFOS alters the spectrum of activating and inhibiting receptors on NK cells or some other aspect of NK activity is not known. At least one study treated NK and target YAC-1 cells in vitro, though neither NK receptor nor ligand expression were evaluated (Wirth et al., 2014). Thus, an important outstanding mechanistic question that may directly impact observations of dose- and sex-dependent effects is whether PFOS alters expression of NK cell receptors or target cell ligands for NK receptors.

Two studies were identified that evaluated mechanisms of PFOS activity on innate immune responses mediated by macrophages, and one evaluated PFOS effects on gut immunity and innate lymphoid cells (ILC3). Rainieri et al. (2017) measured PFOS effects in TREM-like transcript (TLT) cells, a human macrophage-derived cell line. Treatment of cells with 15.6–500 mg/L PFOS for 24 hours increased cell viability relative to controls, which was associated with a significant decrease in the number of apoptotic cells. Using non-confluent cell cultures, 500 mg/L PFOS treatment significantly decreased the number of cells in the G2/M phase. PFOS treatment significantly increased ROS production. However, Berntsen et al. (2018) found no PFOS-specific effects on macrophage phagocytosis in primary cells including peritoneal macrophages (PCM) from adult Wistar rats and C57Bl/6 mice, non-obese diabetic mice, IL-1 knockout (KO) mice, and newly born rats. In addition, PFOS did not alter phagocytosis in human or rat monocyte-derived macrophages (MDM). Taken together, these limited findings suggest that while PFOS does not alter macrophage function, it may affect viability and induce ROS and lipid peroxidation in macrophage cell lines.

Suo et al. (2017) examined effects of PFOS in a mouse model of *C. rodentium* infection. PFOS at 2 mg/kg or vehicle control was administered for 7 days before infecting mice with *C. rodentium* and throughout the observation period of infection. Part of this study evaluated effects on ILC3s, which have been suggested to be important in controlling *C. rodentium* at the early phase of infection prior to induction of adaptive immune responses. ILC3s secrete IL-17 and IL-22 that act to stimulate epithelial cells to secrete anti-microbial peptides or through recruitment of neutrophils (Ishigame et al., 2009; Takatori et al., 2009; Zheng et al., 2008). PFOS inhibited the expansion of *C. rodentium* by promoting IL-22 production in ILC3 cells in an aryl

hydrocarbon receptor (AhR)-dependent manner. However, PFOS also led to decreased mucin production from goblet cells, which may contribute to the observation that PFOS altered the gut microbiome. Specifically, PFOS-exposed mice at late stages of infection exhibited decreased levels of *Lactobacillus casei* and *Lactobacillus johnsonii,* and increased levels of *E. coli*. The authors crossed Ahrf/f mice (in which the Ahr gene is flanked by loxP sites) to mice in which the cre recombinase gene is driven by the RAR-related orphan receptor gamma promoter (RORc-cre) to delete Ahr in ILC3 and T cells (Ahrf/f RORc-cre). Cells isolated from either Ahrf/f RORc-cre or Ahrf/f mice were exposed to PFOS, and cytokines were analyzed using flow cytometry. PFOS-exposed mice exhibited increased IFN-γ production from CD3− non-T cells compared with control mice, indicating a pro-inflammatory role of PFOS. Taken together, PFOS-associated dysbiosis and persistent inflammation in the intestine ultimately led to a failure to clear *C. rodentium* at the late phase of infection. These findings suggest PFOS may impact gastrointestinal health in animals (see Appendix, (U.S. EPA, 2024a)) and raises the possibility that immune mechanisms associated with AhR activation are disrupted by PFOS.

3.4.2.3.2.5 Mechanistic Evidence for PFOS-Mediated Effects on Intrinsic Cellular Defense Pathways

There is limited evidence of PFOS exposure related to the disruption of intrinsic cellular defense pathways. Sørli et al. (2020) used HBEC3-KT human bronchial epithelial cells to study inflammatory changes in response to PFOS, including modulation of the inflammatory response induced by polyinosinic:polycytidylic acid (Poly I:C), a toll-like receptor 3 (TLR3) ligand. In cells exposed to 30 or 60 μM PFOS for 48 hours, IL-1α/β release was elevated, indicative of a pro-inflammatory response. In cells treated with 5 μg/mL poly I:C for 3 hours followed by exposure to 10 μM PFOS for 48 hours, release of the chemokines CXCL8 and CXCL10 was suppressed, but IL-1 α/β release was enhanced. The authors hypothesized that IL- β release may be related to the fact that it requires only proteolytic cleavage of preformed IL-1 in the cytosol, and thus may not be dependent on TLR3-dependent gene expression. The authors also hypothesized that PFOS may inhibit NF-κB activation in a cell type-dependent manner in the lung. TLR3 stability and/or function, other double-stranded RNA sensors in these cells, or associated signal transduction pathways were not evaluated. These results indicate that PFOS can exert divergent effects on chemokine and cytokine release in a dose-dependent manner in human bronchial epithelial cells and modulates the activity of intrinsic cellular defense responses mediated by toll receptors and/or other double-stranded RNA sensors.

3.4.2.3.2.6 Mechanistic Evidence for PFOS-Mediated Effects on Inflammation

PFOS-mediated effects on inflammation may impact a wide range of diseases given that chronic inflammation can be a key driver of many diseases such as cancer, cardiovascular, metabolic, and neurological diseases (Hunter, 2012). Earlier studies suggest that PFOS differentially impacts pro-inflammatory cytokine release in a cell type and tissue-specific manner. For example, as described in 2016 PFOS HESD (U.S. EPA, 2016b), cells isolated from the peritoneal cavity and bone marrow, but not spleen, of mice exposed to high levels of PFOS had enhanced levels of the pro-inflammatory cytokines, TNF-α and IL-6, in response to stimulation by lipopolysaccharide (LPS). The levels of these cytokines in the serum were not elevated (Qazi et al., 2009a). Since the 2016 document, 9 additional mechanistic studies reported correlations between PFOS exposure and modulation of pro-inflammatory cytokines or serum markers of inflammation. Consequences of PFOS exposure are not consistent across species and are

summarized in Table 3-11. Pro-inflammatory cytokines were elevated in PFOS-exposed rodents and in human and animal cells in culture. In both studies evaluating human subjects (Mitro et al., 2020; Bassler et al., 2019), either no significant changes were observed in serum cytokine or marker levels (IL-6, IFN-γ, C-reactive protein (CRP), or C3a) or levels were reduced (TNF-α, IL-8) relative to subjects with lower PFOS exposures.

Table 3-11. Effects of PFOS Exposure on Pro-Inflammatory Cytokines and Markers of Inflammation

Study	Species or Cell Type	Cytokine or Inflammatory Marker	Matrix and Measurement	Direction of Change Following PFOS Exposure
Mitro et al. (2020)	Human females 3 years postpartum, Project Viva	IL-6	blood protein (ELISA)	None
		CRP	blood protein (immunoturbidimetric high-sensitivity assay)	None
Bassler et al. (2019)	Human males and females, C8 Health Project	IL-6	serum protein (Multispot Immunoassay)	None
		TNF-α	serum protein (Multispot Immunoassay)	↓
		IL-8	serum protein (Multispot Immunoassay)	↓
		IFN-γ	serum protein (Multispot Immunoassay)	None
		C3a	serum protein (ELISA)	↓
Li et al. (2020c)	Human lymphocytes	IL-1	culture supernatant protein (ELISA)	↑
		IL-6	culture supernatant protein (ELISA)	↑
Sørli et al. (2020)	Human bronchial epithelial cell line	IL-1α	culture supernatant protein (ELISA)	↑
		IL-1β	culture supernatant protein (ELISA)	↑
Liao et al. (2013)	Human umbilical vein endothelial cells (HUVECs)	IL-6	cellular mRNA (qRT-PCR)	↑
		IL-1β	cellular mRNA (qRT-PCR)	↑
Han et al. (2018b)	Sprague-Dawley male rats	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑
Su et al. (2019)	ICR male mice	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑
Han et al. (2018b)	Primary rat hepatocytes and Kupffer cells	IL-6	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑
		TNF-α	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑

Study	Species or Cell Type	Cytokine or Inflammatory Marker	Matrix and Measurement	Direction of Change Following PFOS Exposure
Zhu et al. (2015)	Murine microglial cell line	IL-6	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑
		TNF-α	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑

Notes: C3a = cohort 3a; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbent assay; IL-1α = interleukin 1 alpha; IL-1β = interleukin 1 beta; IL-6 = interleukin 6; IL-8 = interleukin 8; PCR = polymerase chain reaction; TNF-α = tumor necrosis factor alpha; qRT-PCR = quantitative reverse transcription polymerase chain reaction.

3.4.2.3.2.6.1 Animal Toxicological Studies

Han et al. (2018b) investigated PFOS effects on hepatic inflammation in male Sprague-Dawley (SD) rats exposed to 1 or 10 mg/kg body weight PFOS by gavage and in isolated primary rat Kupffer cells cultured in vitro. In vivo, PFOS induced Kupffer cell activation and elevated serum TNF-α and IL-6 and stimulated release of these cytokines from cultured primary Kupffer cells in vitro. Studies with a Kupffer cell-blocking and depleting agent, gandolinium chloride (GdCL3), demonstrated that PFOS exposure stimulated Kupffer cell release of TNF-α and IL-6 in vivo (measured by ELISA) and in vitro (increased mRNA expression measured by PCR and protein expression measured by ELISA). Furthermore, Kupffer cell activation was mitigated by treatment with anti-TNF-α or anti-IL-6 antibodies. In vivo, PFOS exposure upregulated the protein expression of proliferating cell nuclear antigen (PCNA), c-Jun, c-MYC, and Cyclin D1 (CyD1) in liver, a finding mirrored in Kupffer cells cultured in vitro. Treatment with a drug inhibitor of NF-κB (pyrrolidine dithiocarbamate (PDTC)) and a c-Jun N-terminal kinase (JNK) inhibitor (SP600125) significantly inhibited production of PFOS-induced TNF-α and IL-6. Together, these findings suggest that PFOS induces Kupffer cell activation, leading to NF-κB/TNF- α/IL-6-dependent hepatocyte proliferation.

Su et al. (2019) also examined liver-specific immunotoxicity. Male ICR mice were dosed with 10 mg/kg/day for 21 days. TNF-α and IL-6 were significantly elevated, whereas fibroblast growth factor 21 (FGF21) was significantly reduced in sera from these mice. Co-treatment with 200 mg/kg per day of vitamin C led to a significant reversal in PFOS-induced changes in serum TNF-α, IL-6, and FGF21, consistent with results of immunostaining for TNF-α and FGF21 in liver cells. The mechanism by which vitamin C exerts protection from inflammatory responses in this model was not elucidated.

3.4.2.3.2.6.2 In Vitro Studies

Four studies demonstrated increased inflammatory cytokine expression in human cells cultured in vitro. PFOS exposure at concentrations of ≥30 μM led to increased IL-1α/β release in HBEC3-KT human bronchial epithelial cells (Sørli et al., 2020). Li et al. (2020c) demonstrated induction of IL-1 and IL-6 in human lymphocytes that were isolated from human donors and exposed in culture to 50 mM PFOS for 72 hours. Giménez-Bastida and Surma (2015) investigated inflammatory cytokine responses in human CCD-18 Co myofibroblasts as a model of colonic subepithelial myofibroblasts in the intestinal lamina propria. Cells were exposed to PFOS at concentrations ranging from 0.6 to 100 μM in combination with IL-1β (1 ng/mL). Exposure to PFOS reduced IL-1β-induced IL-6 production at all doses except 100 μM, but this reduction only

reached significance at 6 μM. Liao et al. (2013) pretreated human umbilical cord endothelial cells (HUVECs) with 100 mg/L PFOS for 5 hours and then co-treated with polyphenols (Flos Lonicerae extract and chlorogenic acid) for 24 or 48 hours. PFOS exposure resulted in increased levels of mRNA transcripts for inflammatory cytokines (IL-1β, IL-6) as well as COX-2 (cyclooxygenase 2) and NOS3 (nitric oxide synthase 3), the protein products of which function in cellular defense and prostaglandin synthesis. PFOS exposure also led to upregulation of transcripts for adhesion molecules P-Selectin (SELP) and ICAM1 (intercellular adhesion molecule 1). Functionally, PFOS treatment for 48 h increased adhesion of THP-1 monocytes to HUVECs. These PFOS-mediated changes in HUVECs were mitigated by co-treatment of cells with polyphenols.

In immortalized murine BV2 microglial cells, which are brain resident macrophage-like cells that are considered central to inflammatory responses in the brain, PFOS exposure increased inflammatory cytokine expression (Zhu et al., 2015) via similar pathways observed in primary rat hepatocytes and Kupffer cells exposed to 100 μM PFOS (Han et al., 2018b). Zhu et al. (2015) reported that treatment with 10 μM PFOS for 6 hours resulted in increased levels of Tnfα and Il6 gene expression. Time-course studies were performed using 1 μM PFOS and indicated that elevated Tnf-α and IL-6 mRNA expression occurs within 1 hour, peaks at 3 hours, and begins to diminish by 6 hours of PFOS exposure. Protein levels of these cytokines in culture supernatant continually increased with 6, 12, and 24 hours of 1 μM PFOS treatment. Transcriptional activation of TNF-α and IL-6 correlated with activation of NF-κB (measured by immunoblot of the phosphorylated form) and was mitigated by targeting JNK and the extracellular regulate kinase (ERK1/2) with a drug inhibitor (SP600125) or blocker (PD98059). Together, the data support a role for MAPK signaling pathways and NF-κB activation in PFOS-mediated inflammatory gene expression in cultured microglial cells and primary Kupffer cells.

In addition to activation of MAPK signal transduction pathways, epigenetic mechanisms may impact inflammatory gene expression mediated by PFOS. Park et al. (2019b) found increased gene expression of sirtuin (SIRT) genes in RAW 264.7 macrophage cells (cell line derived from BALB/c mice). The SIRT family of proteins act to deacetylate the lysine residues of histone proteins, but they also can deacetylate nonhistone substrates, such as inflammation-related transcription factors including NF-κB (Frescas et al., 2005; Yeung et al., 2004). PFOS exposure increased expression of Sirt2, Sirt3, Sirt5, and Sirt6. The authors did not investigate the effect of increased expression of Sirt genes observed after PFOS on the acetylation status or expression of inflammatory proteins.

3.4.2.3.2.6.3 Human Studies

Bassler et al. (2019) examined 200 adult participants of the C8 Health Project to test the hypothesis that environmental perfluoroalkyl acids (PFAAs) are associated with increased hepatocyte apoptosis and decreased pro-inflammatory cytokines in serum. In support of this hypothesis, PFOS levels were associated with significantly reduced serum TNF-α and IL-8 serum levels. However, there was no correlation between PFOS serum levels and other cytokines (IL-6, IFN-γ), inflammatory markers (cleaved complement C3a) or markers of hepatocyte cell death (caspase 3 cleaved cytokeratin 18). The authors hypothesized that under certain circumstances such as with non-alcoholic fatty liver disease (NAFLD), PFAAs are associated with immunotoxic suppressive effects on innate immunity and inflammation.

Mitro et al. (2020) set out to evaluate PFAS exposures and cardiometabolic health in pregnant women and in the years postpartum as part of Project Viva. The study obtained 3-year postpartum anthropometry measurements and blood biomarker measurements of inflammation including IL-6 and CRP. While exposure to some PFAS was associated with elevated IL-6 levels 3 years postpartum, no significant associations were observed for PFOS. None of the PFAS chemicals examined other than 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA) showed a strong association with CRP levels in this study.

3.4.2.3.2.7 Summary

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), new mechanistic information has emerged informing immune system physiology, innate and adaptive immune functions, intrinsic cellular defense, and inflammation. Earlier studies summarized in the 2016 PFOS HESD (U.S. EPA, 2016b) linked PFOS-mediated PPARγ activation to decreased spleen and thymus weight and reduced spleen and thymus cellularity (NTP, 2016b; Yang et al., 2002). Recent studies such as Zhong et al. (2016) suggest a role for PFOS in disrupting spleen and thymic weights and cellularity through sex hormones, activation of MAPK signaling pathway and/or oxidative stress genes associated with apoptosis in lymphocytes (Lv et al., 2015), and reduced numbers of myeloid, pro/pre-B, immature B, and early mature B cells in bone marrow (Qazi et al., 2012).

New mechanistic insights into PFOS-mediated suppression of adaptive immune responses include PFOS-mediated effects on TH1/TH2-type cytokines and IgE titers in response to allergens in mice and humans (Zhong et al., 2016; Zhu et al., 2016), glycosylation of immunoglobulins in humans (Liu et al., 2020c), and lymphocyte toxicity in vitro (Zarei et al., 2018). Effects of PFOS exposure on allergy (Lee et al., 2018a) included release of histamine and β hexosaminidase associated with upregulation of intracellular calcium in IgE-stimulated mast cells and release of inflammatory cytokines linked to NF-kB activation. PFOS was also found to stimulate release of IL-17 and IL-22 from TH17 cells in an animal model of intestinal infection (Suo et al., 2017). Additional insights were provided by transcriptomic and lipidomic studies (Li et al., 2020c; Pennings et al., 2016; Lv et al., 2015). Transcriptomic studies identified candidate genes that may mediate immunotoxicity in children exposed in utero to PFOS including SHC4, PPARD, CYTL1, IL-27, and ADORA2A (Pennings et al., 2016). In mice, PFOS exposure upregulated THEMIS and CD3G and altered calcium homeostasis, cell cycle genes that may impact T cell immunophenotypes observed in spleen, and T cell function through inhibition of T cell proliferation and induction of T cell anergy (Lv et al., 2015).

With respect to innate immune responses, PFOS is associated with a depression of NK cell activity. An important outstanding mechanistic question that may directly impact observations of dose- and sex-dependent effects is whether PFOS alters NK cells directly or influences NK cell receptor ligand expression on potential target cells. Two new studies evaluated mechanisms of PFOS activity on innate immune responses mediated by macrophages and ILC3 (Berntsen et al., 2018; Rainieri et al., 2017). Together, these findings suggest that while PFOS does not alter macrophage function, it may induce ROS and lipid peroxidation in macrophage cell lines. Also, Suo et al. (2017) examined effects of PFOS in a mouse model of *C. rodentium* infection. PFOS inhibited the expansion of *C. rodentium* by promoting IL-22 production in ILC3 cells in an AhR-dependent manner and increased IFN-γ production from CD3− non-T cells compared with control mice.

Very little information is available regarding whether PFOS impacts intrinsic cellular defenses. One recent study, Sørli et al. (2020), demonstrated that PFOS exerts divergent effects on chemokine and cytokine release in a dose-dependent manner in human bronchial epithelial cells. This study also proposed that PFOS can modulate the activity of intrinsic cellular defense responses mediated by toll receptors and/or other double-stranded RNA sensors.

Nine recent studies reported correlations between PFOS exposure and modulation of pro-inflammatory cytokines or serum markers of inflammation; however, the inflammatory responses to PFOS exposure are not consistent across species. Pro-inflammatory cytokines were elevated in PFOS-exposed rodents and in human and animal cells in culture through activation of MAPK signaling pathways and activation of NF-κB (Han et al., 2018b; Zhu et al., 2015). In contrast, the available studies evaluating human subjects observed either no changes in serum cytokine or marker levels (IL-6, IFN-γ, or CRP) or reduced levels (TNF-α, IL-8, or C3a) relative to subjects with lower PFOS exposures.

Despite recent research informing a range of immunotoxicity endpoints, a comprehensive understanding of the mechanisms by which PFOS alters immune system development, physiology, and function is lacking. Data from transcriptomic studies have advanced the understanding regarding the potential of PFOS to disrupt lymphocyte signaling and function. A particularly promising area of research relates to the observation that PFOS exposure in human lymphocytes is associated with dysregulated lipid profiles that encompass glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism (Li et al., 2020c). However, further studies are needed to determine if these gene expression changes result in altered protein accumulation and if gene expression and lipid profile changes mediate functional changes in immunity.

3.4.2.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and immunosuppressive effects in human studies based on largely consistent decrease in antibody response following vaccinations (against three different infectious agents) in multiple *medium* confidence studies in children. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease. Changes in antibody levels of 10%–20% per doubling of PFOS exposure were observed in the Faroe Islands cohorts, and a change in antibody levels of approximately 11% per 2.7-fold increase of PFOS exposure was observed in adolescents from NHANES. The variability in the results, including null and positive associations, could be related to differences in sample sizes, individual variation, vaccine type, and differences in timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available data. Overall, the evidence indicates an association between increased serum PFOS levels and decreased antibody production following routine vaccinations in children. Evidence in adults does not indicate an association with immunosuppression, but *high* confidence studies are not available in these populations.

There is *slight* evidence for sensitization and allergic responses from studies in humans, but notable limitations and uncertainties in the evidence base remain. Associations in epidemiological studies measuring PFOS exposure and hypersensitivity outcomes were mixed.

There is some evidence from epidemiological studies of an association between PFOS exposure and asthma, but there is considerable uncertainty due to inconsistency across studies and subgroups. Sex-specific differences were reported in multiple studies, but there was inconsistency in the direction of association within each sex. There is not an obvious pattern of results by analysis of "ever" versus "current" asthma, and no studies beyond the Dong et al. (2013) described in the 2016 PFOS HESD examined asthma incidence. For allergy and eczema outcomes, results were inconsistent across studies.

There is limited evidence of an association between PFOS exposure and infectious diseases. While one *medium* confidence study reported higher odds of total infectious diseases, results from studies examining individual diseases including respiratory infections, chickenpox, cough, RSV, common cold, ear infections, and urinary tract infections were inconsistent.

Epidemiological evidence on autoimmune effects was limited to three studies reporting on different autoimmune conditions. Similar to the findings from the 2016 PFOS HESD, there was insufficient information to draw conclusions on the effect of PFOS exposure on autoimmune disease.

The animal evidence for an association between PFOS exposure and immunosuppressive responses is *moderate* based on decreased PFC responses and NK cell activities observed in 12 *high* or *medium* confidence rodent studies. Additionally, fluctuations in splenic and thymic cell populations and increased bone marrow hypocellularity in conjunction with extramedullary hematopoiesis were observed. Extramedullary hematopoiesis, blood cell production outside of the bone marrow, occurs when normal cell production is impaired. Bone marrow hypocellularity in parallel with extramedullary hematopoiesis suggest that PFOS impedes hematopoiesis in the bone marrow. As such, EPA concluded that elevated extramedullary hematopoiesis and bone marrow hypocellularity, as well as reduced ability to generate an immune response to a bacteria-like challenge and reduced PFC response indicate toxicity of relevance to humans exposed to PFOS.

It is clear that PFOS can alter immune cells and signaling in experimental systems. However, the connection between various alterations to immune and inflammation signaling and immunologic effects reported in humans is not clear. Transcriptomics data represent some of the most informative findings in regard to potential underlying mechanisms of immunotoxicity of PFOS. Together, the findings from transcriptomic and functional analyses reported in human lymphocytes exposed to PFOS, in human cord blood samples from gestational exposure to PFOS, and in mice treated with PFOS suggest that PFOS exposure may disrupt adaptive immunity through the dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and inactivation. PFOS effects on gene expression paralleled a dose-dependent increase in intracellular free calcium (which plays an important role in immune cell proliferation in response to foreign antigens) concentration in splenocytes of mice treated with PFOS, suggesting that activation of MAPK signaling pathway and/or oxidative stress genes in response to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes. Relatedly, additional in vitro transcriptomic data collected from mouse microglial cells and rat hepatocytes and Kuppfer cells demonstrate activation of TNF-α and IL-6, correlated with activation of NF-κB. These data support a role for MAPK signaling pathways and NF-κB activation in PFOS-mediated inflammatory gene expression in vitro. TNF-α, IL-6, and NF-κB are all related to inflammation, allergy, and other immune responses.

Despite recent research informing a range of immunotoxicity endpoints, a comprehensive understanding of the mechanisms by which PFOS alters immune system development, physiology, and function is lacking. A particularly promising area of research relates to the observation that PFOS exposure in human lymphocytes is associated with dysregulated lipid profiles that encompass glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism. Additional research is needed to determine if these gene expression changes result in altered protein accumulation and if gene expression and lipid profile changes mediate functional changes in immunity; specifically, alterations to antibody response and susceptibility to infection, as reported in humans.

3.4.2.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the ***evidence indicates*** that PFOS exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans under relevant exposure circumstances (Table 3-12). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from epidemiological studies at levels of 0.8 ng/mL PFOS (median exposure in studies observing an adverse effect). The evidence in animals showed coherent immunomodulatory responses at doses as low as 0.0017 mg/kg/day that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOS exposure might also have the potential to affect sensitization and allergic responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and with limited support from animal or mechanistic studies. Given the antibody response data in humans, children, and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOS. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental lifestages represents a major source of uncertainty in the immunotoxicity database of PFOS.

Table 3-12. Evidence Profile Table for PFOS Exposure and Immune Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.2.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Immunosuppression 1 *High* confidence study 20 *Medium* confidence studies 8 *Low* confidence studies 2 *Mixed*[a] confidence studies	Studies conducted in the Faroe Islands examined antibody levels among children at various timepoints compared with exposure measured prenatally and throughout childhood. Lower antibody levels against tetanus and diphtheria were observed in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years. Similarly, antibody levels against rubella (2/2) were significantly decreased in *medium* confidence studies of children. Findings in the four studies examining adults were less consistent than children. Infectious disease was examined in 14 studies of children. Studies examining infections of the respiratory system observed some positive associations (5/12), although many findings from other studies were	• *High* and *medium* confidence studies that reported effects • *Consistent direction* of effect • *Coherence* of findings across antibody response and increased infectious disease	• *Imprecision* of findings	⊕⊕⊙ *Moderate* Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus, and some effect for rubella. Reductions in antibody response were observed at multiple timepoints in childhood, using both prenatal and childhood exposure levels. An increased risk of upper and lower respiratory tract infections was observed among children, coherent with findings of reduced antibody response. There was also supporting evidence of increased risk of asthma, and autoimmune disease, however, the number of studies examining the same type of autoimmune disease was limited.	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of reduced antibody response. Evidence in animals showed coherent immunomodulatory responses that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOS exposure might also have the potential to affect sensitization and allergic responses in humans under relevant exposure circumstances, the human evidence underlying this possibility is uncertain and with limited support from animal or mechanistic studies.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	not precise. Findings for infectious disease in adults were mixed, with two studies reporting inconsistent results for COVID-19 infections.				*Human relevance and other inferences:* Given the antibody response data in humans, children, and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOS. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental lifestages represents a major source of uncertainty in the immunotoxicity database of PFOS.
Immune hypersensitivity 1 *High* confidence study 20 *Medium* confidence studies 4 *Low* confidence studies 3 *Mixed*[a] confidence studies	Examination of immune hypersensitivity includes outcomes such as asthma, allergies, and eczema. Increased odds of asthma were reported in multiple *medium* confidence studies (7/12), although associations were often inconsistent by subgroups. *Low* confidence studies supported the findings of increased odds of asthma or higher exposure levels among asthmatics, although results were not always consistent or precise. Nine studies examined allergies, rhinitis, or rhinoconjunctivitis. Some positive associations (3/9) were observed, although this varied by outcome timing and were at times inconsistent. Ten studies examined eczema, and	• *High* and *medium* confidence studies • *Consistent direction* of effect for asthma across *medium* confidence studies	• *Inconsistent direction* of effect between subpopulations		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	results were generally mixed.				
Autoimmune disease 1 *Medium* confidence study 3 *Low* confidence studies	Lower exposure levels were observed in healthy controls compared with multiple sclerosis cases in one study of adults. An increased risk of celiac disease was also observed in a study of children and young adults. Another study observed lower exposure levels among ulcerative colitis cases compared with healthy controls.	• *No factors* identified	• *Low* confidence studies • *Limited number* of studies examining outcome		
Evidence from *In Vivo* Animal Toxicological Studies (Section 3.4.2.2)					
Immune response 4 *Medium* confidence studies	In response to a SRBC challenge, decreased IgM response in the PFC assay was reported (2/2) in a subchronic and developmental study in mice and was dose-dependent in males. In the developmental study, NK cell activity was reduced up to 8 weeks after a gestational exposure (1/1). One short-term study in rats examined the effect of PFOS on a delayed-type hypersensitivity response to a KLH challenge (1/1)	• *Medium* confidence studies • *Dose-response* relationship seen within multiple studies	• *Limited number* of studies examining specific outcomes	⊕⊕⊙ *Moderate* Evidence is based on decreased immune responses and NK cell activities observed in several *high* or *medium* confidence rodent studies. Additionally, fluctuations in splenic and thymic cell populations and increased bone marrow hypocellularity in conjunction with extramedullary hematopoiesis were	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	and observed no changes in IgG levels (1/1) or footpad swelling (1/1). Another short-term study observed no changes in circulating white blood cells but an increase in IgE after an OVA challenge (1/1).			observed. Extramedullary hematopoiesis, blood cell production outside of the bone marrow, occurs when normal cell production is impaired. Bone marrow hypocellularity in parallel with extramedullary hematopoiesis suggest that PFOS impedes hematopoiesis in the bone marrow.	
Immune cellularity 2 *High* confidence studies 6 *Medium* confidence studies	Of the studies that measured circulating WBCs and differentials (5/8), one short-term rat study found decreases in WBCs and segmented neutrophils in males only, while a chronic rat study found increases in segmented neutrophils in males only. In another short-term study in rats, a negative trend for subsets of T cells and a positive trend for B cells were observed in males. In females a positive trend was observed for WBCs, lymphocytes, and subsets of T cells; a negative trend was observed for B cells. No effects on WBCs or differentials were seen in a short-term study of male mice and in a chronic study in	• *High* and *medium* confidence studies • *Coherence* of findings across circulating immune cells, splenic cellularity, and thymic cellularity and with histopathological changes	• *Inconsistent direction* of effects across studies and sex		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	monkeys. Decreases in total spleen cellularity and/or subsets of splenic cells were observed in 2 short-term studies in male and female rats and mice. Similar decreases were seen in the thymus in these studies; however, no changes were observed in females.				
Histopathology 1 *High* confidence study 5 *Medium* confidence studies	In 1 *high* confidence short-term study, a dose-dependent increase in both extramedullary hematopoiesis in the spleen and hypocellularity in the bone marrow was observed in male and female rats. No changes were observed in the thymus or lymph nodes. None of the *medium* confidence studies (5) reported histopathologic changes in the spleen (4), thymus (2), or lymph nodes (2).	• *High* and *medium* confidence studies • *Dose-response* relationship observed • *Coherent* changes with those observed in circulating immune cells, splenic cellularity, and thymic cellularity	• *Inconsistent direction* of effects across studies		
Organ weights 2 *High* confidence studies 5 *Medium* confidence studies	Mixed results were reported for absolute and relative spleen (7) and thymus (5) weights. Both studies in male and female rats reported	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across species and sex • *Confounding* variables such as decreases in body weights		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	decreases in absolute spleen (2/2) (males only) and thymus weights (2/2) (males and females), which generally coincided with decreases in body weights. Relative spleen weights were unchanged (2/2) or increased (1/2) in rats, while relative thymus weights were unchanged (1/2) or decreased (1/2). In mouse studies, absolute spleen and thymus weights were not reported. Decreased relative spleen weights were observed in mice (4/5); however, this result was not always consistent between sex and timepoint. Relative thymus weights were decreased in male mice (2/2) and unchanged in female mice (1/1).		• Lack of dose-response relationship		
Globulins and immunoglobulins 1 *High* confidence studies 4 *Medium* confidence studies	Two short-term studies found decreased globulin levels (2/3) in male rats and no changes in female rats. One short-term study found increases in subsets of immunoglobulins (1/1) in both male and female	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *Inconsistent direction* of effects across sex		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	rats, and one short-term study found no changes in IgE (1/1) in male mice				

Mechanistic Evidence and Supplemental Information (Section 3.4.2.3)		
Biological events or pathways	**Summary of Key Findings, Interpretation, and Limitations**	**Evidence Stream Judgment**
Immune system development and physiology	**Key findings and interpretation:** • Changes in WBC and alterations in expression of immune and inflammation-related genes in human cord blood have been reported. • Reduction in immune organ weight, cellularity, and morphology (spleen and thymus) in mice and rats. • Disrupted splenic architecture and reduction in T-helper and cytotoxic T cells in the spleen in mice. **Limitations:** • No direct effects related to immune system development or physiology in humans to anchor mechanistic findings.	PFOS can alter immune cells and signaling in experimental systems. However, the connection between various alterations to immune and inflammation signaling and immunologic effects reported in humans is not clear.
Effects on adaptive immune responses	**Key findings and interpretation:** • Inverse association between PFOS exposure and vaccine-induced antibody levels in human studies (in utero exposure to PFOS). • Dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and anergy in vitro in human lymphocytes. • Alterations to the expression of genes involved in adaptive immune responses (i.e., immunological and/or hematopoietic functions) in cord blood of samples from cases of maternal exposure to PFOS, as well as in spleens of PFOS-exposed mice, and in human lymphocytes exposed to PFOS in vitro. **Limitations:** • Association between gene expression changes and apical endpoints need further confirmation.	
Autoimmune diseases	**Key findings and interpretation:** • PFOS-mediated effects on pro-inflammatory T-helper cells, specifically increased IL-17 and IL-22 production, in mice. **Limitations:**	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	• Only a single study; no studies directly evaluated the mechanism by which PFOS could promote autoimmunity.				
Allergic responses	**Key findings and interpretation:** • Serum levels PFAS, including PFOS, was positively associated with basophil counts in children between birth and age 5. • PFOS blood levels were associated with alterations in cytokines in asthmatic and non-asthmatic children, with some effects being specific to asthmatic children. **Limitations:** • Human data include exposure to other PFAS in addition to PFOS.				
Innate Immunity	**Key findings and interpretation:** • Conflicting results for NK cell activity across studies of cells from animals exposed to PFOS in vivo. • Alterations to apoptosis and cell cycle stage in a human macrophage-derived cell line. **Limitations:** • Limited database, no human studies of innate immunity endpoints.				
Effects on Intrinsic Cellular Defense Pathways	**Key findings and interpretation:** • PFOS-induced elevation of IL-1α/β release, indicative of a pro-inflammatory response, in human bronchial epithelial cells exposed in vitro. **Limitations:** • Only a single study.				
Effects on Inflammation	**Key findings and interpretation:** • Altered levels of pro-inflammatory cytokines or serum markers of inflammation have been reported in humans, mice, and rats both in vivo as well as in vitro. • No association between PFOS exposure and increased acute or chronic inflammatory responses in humans in vivo. **Limitations:** • Limited database.				

Notes: HFMD = hand, foot, and mouth disease; COVID-19 = coronavirus disease 2019; SRBC = sheep red blood cells; IgM = immunoglobulin M; PFC = plaque forming cell; NK = natural killer; KLH = keyhole limpet hemocyanin; IgG = immunoglobulin G; IgE = immunoglobulin E; OVA = ovalbumin; WBC = white blood cells; IL-17 = interleukin 17; IL-22 = interleukin 22; IL-1α/β = interleukin 1 alpha/beta.

[a] Studies may be of mixed confidence due to differences in how individual outcomes within the same study were assessed (e.g., clinical test vs self-reported data).

3.4.3 Cardiovascular

EPA identified 106 epidemiological and 13 animal toxicological studies that investigated the association between PFOS and cardiovascular effects. Of the 46 epidemiological studies addressing cardiovascular endpoints, 4 were classified as *high* confidence, 24 as *medium* confidence, 11 as *low* confidence, 3 as *mixed* (1 *high/medium* and 2 *medium/low*) confidence, and 4 were considered *uninformative* (Section 3.4.3.1). Of the 80 epidemiological studies addressing serum lipid endpoints, 2 were classified as *high* confidence, 29 as *medium* confidence, 26 as *low* confidence, 16 as *mixed* (1 *high/medium* and 15 *medium/low*) confidence, and 7 were considered *uninformative* (Section 3.4.3.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 7 as *medium* confidence, 2 as *low* confidence, 2 and were considered *mixed* (*medium/low*) (Section 3.4.3.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.3.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.3.1.1 Cardiovascular Endpoints

3.4.3.1.1.1 Introduction

Cardiovascular disease (CVD) is the primary cause of death in the United States with approximately 12% of adults reporting a diagnosis of heart disease (Schiller et al., 2012). Studied health effects include ischemic heart diseases (IHD), coronary artery disease (CAD), coronary heart disease (CHD), hypertension, cerebrovascular disease, atherosclerosis (plaque buildup inside arteries and hardening and narrowing of their walls), microvascular disease, markers of inflammation (e.g., C-reactive protein), and mortality. These health outcomes are interrelated – IHD is caused by decreased blood flow through coronary arteries due to atherosclerosis resulting in myocardial ischemia. Cardiovascular outcomes were synthesized separately by population (i.e., adults, children, occupational populations), and definitions of certain conditions may vary by age. For example, high blood pressure and/or hypertension is generally defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in adults and SBP ≥ 130 mmHg and DBP ≥ 80 mmHg in children and adolescents, although consistent blood pressure measurements in youth can be challenging (Falkner et al., 2023).

The 2016 PFOS HESD (U.S. EPA, 2016b) did not assess evidence for associations between CVD diseases and PFOS, besides the review of its effects on serum lipids which are further described in subsequent sections. There are 2 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 2 studies are shown in Figure 3-29. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-13 and below.

The developmental section in the 2016 PFOS HESD describes results from Geiger et al. (2014b) which reported no association with hypertension in 1,655 children aged 12–18 years from the NHANES (1999–2000 and 2003–2008 cycles). An occupational study (Alexander et al., 2003) reported an inverse association for mortality from heart disease among all cohort members. The decreased SMR was consistent in sensitivity analyses of cohort members ever employed in a high-exposure job and those only working in non-exposed jobs. The study was considered *low*

confidence due to concerns about healthy work effect and potential residual confounding by smoking status and race/ethnicity.

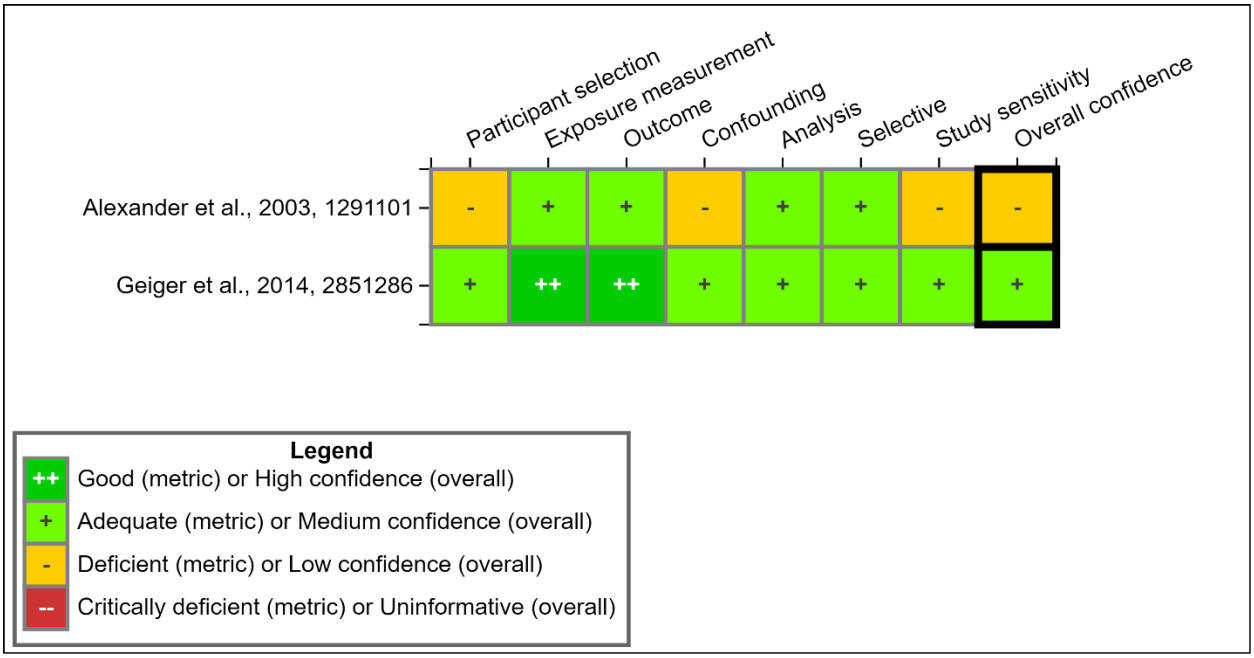


Figure 3-29. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cardiovascular Effects Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-13. Associations Between Elevated Exposure to PFOS and Cardiovascular Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	Hypertension[b]	Heart Disease Mortality[b]	Cerebrovascular Disease Mortality[b]
Alexander, 2003, 1291101 *Low*	Cohort	Occupational	NA	↓	↓
Geiger, 2014, 2851286 *Medium*	Cross-sectional	Children	–	NA	NA

Notes: NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 44 new epidemiological studies report on the association between PFOS and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 19 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed

in the synthesis on female reproductive effects (see Appendix D, (U.S. EPA, 2024a)). All studies were conducted on the general population with six (Ye et al., 2021; Yu et al., 2021; Hutcheson et al., 2020; Mi et al., 2020; Honda-Kohmo et al., 2019; Bao et al., 2017) conducted in a high-exposure community in China (i.e., C8 Health Project and "Isomers of C8 Health Project" populations), and three studies (Canova et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020) were conducted in a high-exposure community in Italy (i.e., Vento Region). Different study designs were also used including three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b), 11 cohort studies (Li et al., 2021b; Papadopoulou et al., 2021; Lin et al., 2020c; Mitro et al., 2020; Donat-Vargas et al., 2019; Warembourg et al., 2019; Fry and Power, 2017; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017), one case-control study (Mattsson et al., 2015), and 33 cross-sectional studies (Koskela et al., 2022; Averina et al., 2021; Canova et al., 2021; Ye et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020d; Mi et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Mobacke et al., 2018; Yang et al., 2018; Bao et al., 2017; Koshy et al., 2017; Lind et al., 2017b; Christensen et al., 2016; Lin et al., 2016; Lin et al., 2013). The three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b) were not controlled trials of PFAS exposures, but rather health interventions: prevention of type 2 diabetes in Diabetes Prevention Program and Outcomes Study (DPPOS) (Osorio-Yáñez et al., 2021; Cardenas et al., 2019) and weight loss in the Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study (Liu et al., 2018b). Thus, these studies could be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The available studies were conducted in different study populations with the majority of studies conducted in the United States (Koskela et al., 2022; Li et al., 2021b; Osorio-Yáñez et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Cardenas et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Fry and Power, 2017; Koshy et al., 2017; Christensen et al., 2016). The remaining studies were conducted in China (Ye et al., 2021; Yu et al., 2021; Yang et al., 2018; Bao et al., 2017), Taiwan (Lin et al., 2016; Lin et al., 2013), Spain (Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017), Croatia (Chen et al., 2019), Sweden (Donat-Vargas et al., 2019; Mobacke et al., 2018; Lind et al., 2017b; Mattsson et al., 2015), Denmark (Jensen et al., 2020), Italy (Canova et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020), Norway (Averina et al., 2021), and two studies conducted in several European countries (Papadopoulou et al., 2021; Warembourg et al., 2019). All the studies measured PFOS in blood components (i.e., serum or plasma) with three studies measuring levels in maternal serum (Li et al., 2021b; Papadopoulou et al., 2021; Warembourg et al., 2019), and four studies measuring levels in maternal plasma (Papadopoulou et al., 2021; Mitro et al., 2020; Warembourg et al., 2019; Manzano-Salgado et al., 2017b).

3.4.3.1.1.2 Study Quality

There are 45 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 45 studies are shown in Figure 3-30 and Figure 3-31.

Of the 45 studies identified since the 2016 assessment, 4 studies were *high* confidence, 23 were *medium* confidence, 10 were *low* confidence, 4 studies were *mixed* (1 *high/medium* due to difference exposure estimates and 3 *medium/low* for different cardiovascular endpoints) confidence, and 4 studies included an outcome considered *uninformative* (Jain, 2020a, b; Leary et al., 2020; Seo et al., 2018). The main concerns with the *low* confidence studies included the possibility of outcome misclassification (e.g., reliance on self-reporting) in addition to the potential for residual confounding or selection bias (e.g., unequal recruitment and participation among subjects with outcome of interest, lack of consideration and potential exclusion due to medication usage). Residual confounding was possible due to socioeconomic status (SES), which can be associated with both exposure and the cardiovascular outcome. Although PFOS has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further, temporality of PFOS exposure could not be established for several *low* confidence studies due to their cross-sectional design. Several of the *low* confidence studies also had sensitivity issues due to limited sample sizes (Girardi and Merler, 2019; Graber et al., 2019; Khalil et al., 2018; Christensen et al., 2016). Two studies were rated *adequate* for all domains, indicating lower risk of bias; however, both studies treated PFOS as the dependent variable, resulting in both studies being considered *uninformative* (Jain, 2020a, b). Analyses treating PFOS as the dependent variable support inferences for characteristics (e.g., kidney function, disease status, race/ethnicity, etc.) that affect PFOS levels in the body, but it does not inform the association between exposure to PFOS and incidence of cardiovascular disease. Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study (Leary et al., 2020). Studies considered *uninformative* were not considered further.

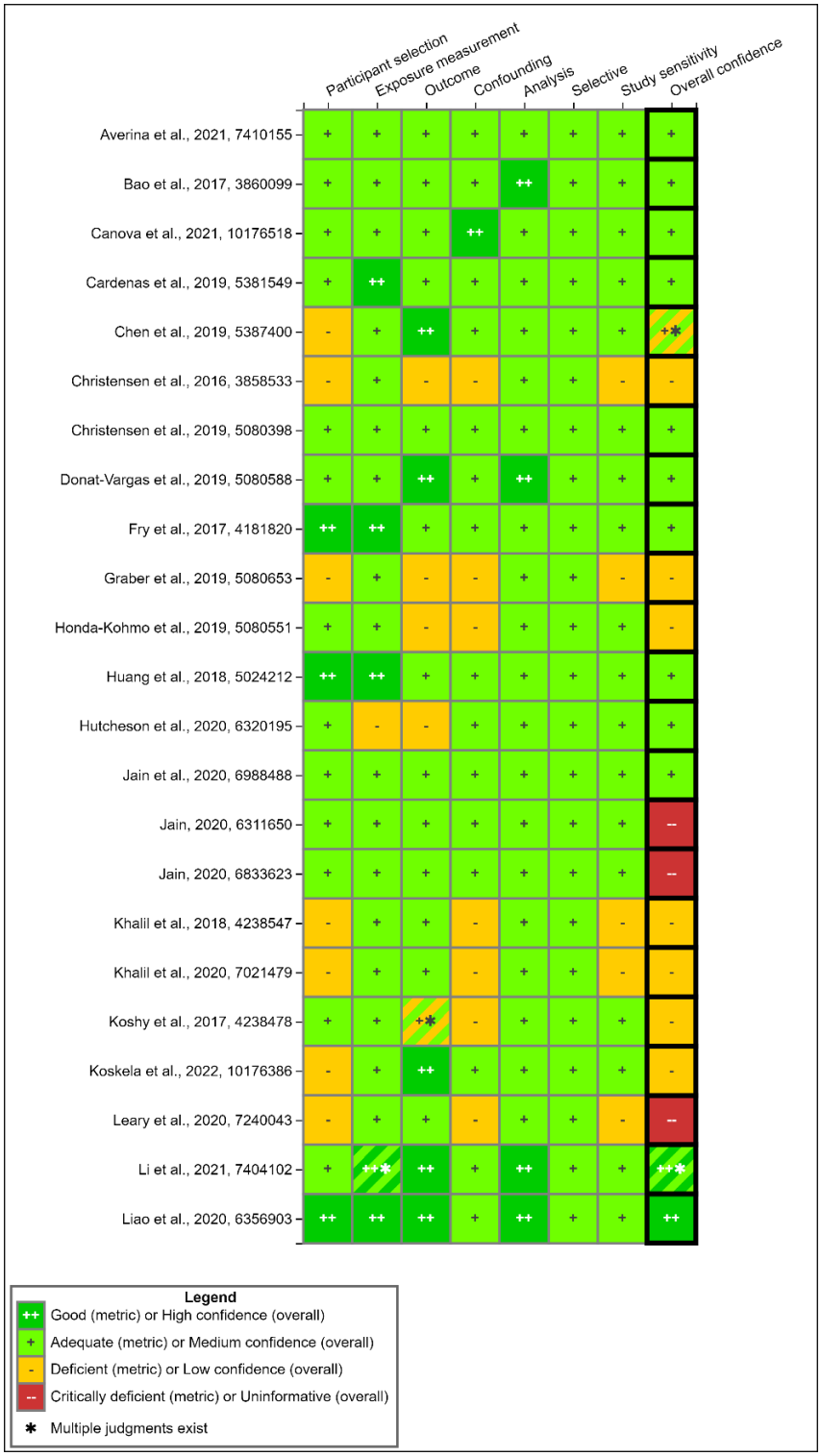


Figure 3-30. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

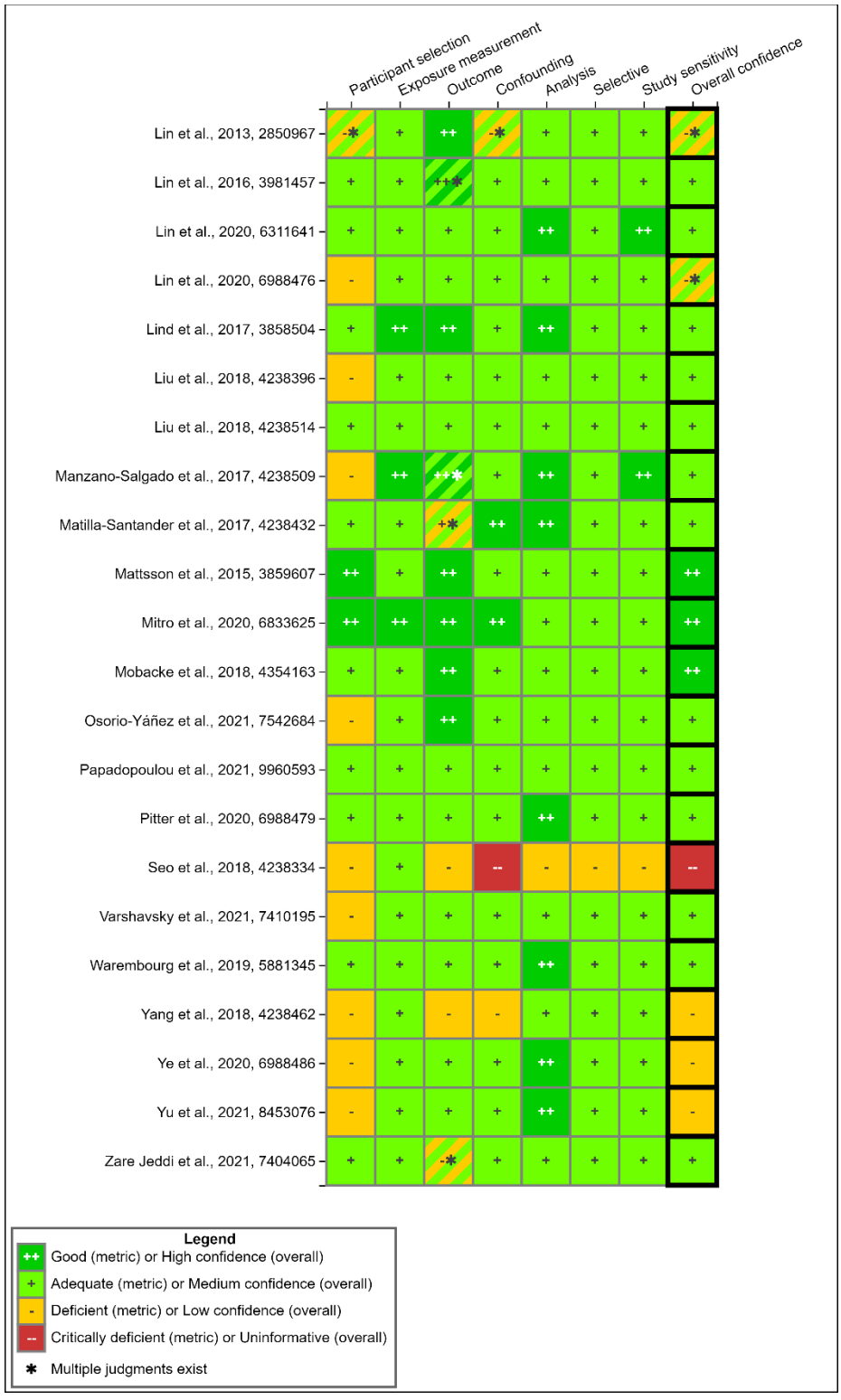


Figure 3-31. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.1.3 Findings From Children

The single *high* confidence study examined the association between PFOS at several ages (prenatal, cord blood, 3 years, 8 years, and 12 years) and blood pressure at age 12 and all observed associations were essentially null. Of the six *medium* confidence studies that examined blood pressure in children and adolescents, one reported positive association with diastolic blood pressure (DBP) only (Ma et al., 2019), one reported an inverse association with systolic blood pressure (SBP) and DBP in adolescents, and one reported an increased risk of hypertension among first-level high school students (Averina et al., 2021). Results from the remaining *medium* confidence studies were essentially null (see Appendix D, (U.S. EPA, 2024a)). Among 2,251 NHANES (2003–2012) adolescents (mean age 15.5 years) Ma et al. (2019) observed a positive association with DBP, which was significant only in boys (0.025; 95% CI: 0.001, 0.049). The study also reported that male adolescents with PFOS levels in the highest quintile (> 18 ng/mL) had mean DBP values that were 2.70% greater (95% CI: 0.32%, 5.02%) than the lowest quartile (< 6.2 ng/mL). Blood pressure also was examined in children (n = 2,693) and adolescents (n = 6,669) participating in a health surveillance program in a high-exposure community (Italy, Veneto Region). Inverse associations were observed for both SBP and DBP in adolescents which were significant for DBP in continuous analyses. Inverse associations for DBP were observed in quartile analyses of children, but none reached significance. No association was observed for SBP in children. In contrast, an increased risk of hypertension was observed among first-level high school students (n = 940) participating in the Fit Futures Study (Averina et al., 2021). In quartile analyses, the association was positive for the second to fourth quartiles compared with the first but was only significant for the fourth quartile comparison. No association was observed for DBP among female adolescents, or for SBP among all adolescents. Manzano-Salgado et al. (2017b) reported that maternal PFOS was not associated with blood pressure in combined or in gender-stratified analyses at age 4 and 7 years. In a cohort of 1,277 children (age 6–11 years), Warembourg et al. (2019) observed that PFOS measured in maternal blood during the pre-natal period, and in plasma during the postnatal period were not associated with blood pressure in single-pollutant models. Results from an overlapping study (Papadopoulou et al., 2021) on the same cohort were consistent with Warembourg et al. (2019)

Two *low* confidence studies did not observe associations between serum PFOS and blood pressure in children or adolescents (Khalil et al., 2018; Lin et al., 2013).

Other cardiovascular conditions reported in the recent literature include carotid artery intima-media thickness (CIMT) and brachial artery distensibility. Two *medium* confidence studies examined CIMT among 664 (Lin et al., 2013) and 848 (Lin et al., 2016) adolescents and young adults from the Young Taiwanese Cohort Study. Both studies observed a statistically significant increase in the mean CIMT with higher serum PFOS levels ($p < 0.001$ in test for trend). A *low* confidence study of children and adolescents from the World Trade Center Health Registry (WTCHR) reported that the association between PFOS and brachial artery distensibility was borderline significant ($p = 0.06$), with no association reported for pulse wave velocity (Koshy et al., 2017). However, concerns for residual confounding by age and SES contributed to the *low* confidence.

Overall, the limited evidence available among children and adolescents was inconsistent and indicates PFOS is not associated with blood pressure in these age groups. The evidence for an

association between PFOS and other CVD-related endpoints assessed in this study population was limited and inconsistent.

3.4.3.1.1.4 Findings From the General Adult Population

Most of the studies identified since the last assessment were conducted among general population adults (see Appendix D, (U.S. EPA, 2024a)). A total of 16 studies examined PFOS in association with SBP, DBP, hypertension, and elevated blood pressure (Ye et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017; Christensen et al., 2016).

Of the eight studies that examined blood pressure as a continuous measure, five observed statistically significant positive associations (Liao et al., 2020; Mi et al., 2020; Mitro et al., 2020; Liu et al., 2018b; Bao et al., 2017). However, the results were not always consistent between SBP and DBP. A *high* confidence study in 6,967 participants 20 years and older in NHANES (2003–2012) reported a statistically significant positive association with SBP (per 10-fold change in PFOS: 1.35; 95% CI: 0.18, 2.53) (Liao et al., 2020). Using a generalized additive model and restricted cubic splines, a nonlinear (J-shaped) relationship between PFOS and DBP was observed, with the inflection point of PFOS at 8.20 ng/mL. Each 10-fold increase in PFOS was inversely associated with DBP (OR: −2.62; 95% CI: −4.73, −0.51) on the left side of the inflection point and positively associated on the right side of the inflection point (OR: 1.23; 95% CI: −0.42, 2.88). A *high* confidence study (Mitro et al., 2020) conducted in 761 women that examined associations between PFOS concentrations measured during pregnancy and blood pressure assessed at 3 years postpartum reported significantly higher SBP levels among all women (beta per doubling of PFOS: 1.2; 95% CI: 0.3, 2.2) and among women 35 years or older (percent difference per doubling of PFOS: 2.3; 95% CI: 0.9, 3.6). No association was observed with DBP.

Two *medium* confidence cross-sectional studies with overlapping data from the "Isomers of C8 Health Project", a high-exposed population of Shenyang, China (Mi et al., 2020; Bao et al., 2017) also reported positive associations for blood pressure. In adults with very high PFOS levels (median 24.22 ng/mL), Bao et al. (2017) observed statistically significant increases in DBP (2.70; 95% CI: 1.98, 3.42) and SBP (4.84; 95% CI: 3.55, 6.12). A positive trend for the association between PFOS, linear (n-PFOS), and branched isomers, and blood pressure was highly significant ($p < 0.001$). In adults with high PFOS levels (median 10.33 ng/mL) Mi et al. (2020) reported statistically significant increases in SBP (2.23; 95% CI: 0.58, 3.89). After stratification by sex, significant positive associations were observed in women only for SBP, the estimate was 3.08 (95% CI: 1.53, 4.62; p-value for interaction by sex = 0.03). For DBP, the associations were positive but nonsignificant overall or among women. Another high-exposure community study (Pitter et al., 2020) examined risk of hypertension in a large population (n = 15,786) of young adults (20–39 years old) living in a PFAS-contaminated region of Italy (Veneto Region) and observed an increased risk of hypertension. The risk of hypertension was significantly increased in continuous analyses (OR per ln-ng/mL PFOS: 1.12; 95% CI: 1.02, 1.22), but quartile analyses indicated the association may have been driven by males in the highest two quartiles of exposure. An overlapping study (Zare Jeddi et al., 2021) on the same population examined blood pressure as a criterion for metabolic syndrome and results were consistent with an increased risk of hypertension among the whole population.

Lin et al. (2020c) using data from the Diabetes Prevention Program, a randomized controlled health intervention trial, reported that higher baseline PFOS concentrations were significantly associated with a decrease in SBP over time (year 2: −2.13 mmHg; 95% CI: −3.54, −0.71) among participants assigned to the lifestyle intervention arm, but no association was observed in participants in the placebo-medication arm. However, the study authors attribute the negative findings for BP trajectories (decreases over time) in the lifestyle group to regression toward the mean, a statistical phenomenon in which a more extreme value from the population mean can experience a greater change toward the mean; however, it is unclear why this phenomenon would apply only to the lifestyle arm.

In a weight loss-controlled trial population (POUNDS-Lost study) Liu et al. (2018b) observed that baseline PFOS was positively correlated with DBP ($p < 0.001$) but at 6- and 24-month follow-up assessments no associations were observed for SBP or DBP.

No association was observed for blood pressure in two *low* confidence studies (Chen et al., 2019; Yang et al., 2018).

Of the eight studies that examined risk of elevated blood pressure (hypertension), two reported statistically significant associations (Mi et al., 2020; Bao et al., 2017). Hypertension was defined as average SBP >140 mmHg and average DBP >90 mmHg, or self-reported use of prescribed anti-hypertensive medication. Mi et al. (2020) and Bao et al. (2017), which had overlapping data on high exposed Isomers of C8 Health Project participants, reported significant associations. Bao et al. (2017) reported significantly higher odds of hypertension (OR: 1.24; 95% CI: 1.08, 1.44) for PFOS, and for several PFOS isomers. The associations remained significant in women for PFOS (OR: 1.63; 95% CI: 1.24, 2.13; p-value for interaction by sex = 0.016), and some isomers. These results suggest branched PFOS isomers have a stronger association with increased risk of hypertension compared with linear isomers (n-PFOS). Mi et al. (2020) reported a significant positive association for hypertension (OR: 2.52; 95% CI: 1.91, 3.33) overall, and in women (OR 2.32; 95% CI: 1.38, 3.91; p-value for interaction by sex <0.01).

The *high* confidence study (Liao et al., 2020) reported in a fully adjusted analysis that the OR among adults exposed to PFOS levels in the highest tertile compared with the lowest tertile and the test of trend, respectively, were not significant. Additionally, a significant interaction was observed between gender and hypertension ($p = 0.016$), although the association between PFOS and hypertension was nonsignificant among males and females in stratified analysis. No association was observed for elevated blood pressure in two *medium* confidence studies (Christensen et al., 2019; Liu et al., 2018d) and for hypertension in one *medium* (Lin et al., 2020c) and one *low* confidence study (Christensen et al., 2016). One *medium* confidence study (Donat-Vargas et al., 2019) reported a significant protective effect for hypertension (OR: 0.71; 95% CI: 0.56, 0.89).

Increased risk of elevated blood pressure was also observed in both *low* confidence studies (Ye et al., 2021; Yu et al., 2021), both of which examined participants of the Isomers of C8 Health Project (overlapping with Mi et al. (2020) and Bao et al. (2017)). Yu et al. (2021) examined components of metabolic syndrome and reported significantly increased risk of elevated blood pressure. The association was significant in continuous analyses and the trend was significant in quartile analyses. When stratified by sex, the association was more pronounced in women and was not significant in men. Ye et al. (2021) reported a nonsignificant increased risk in elevated

blood pressure. The magnitude of association for total PFOS was similar to individual PFOS isomers.

Nine studies examined other CVD-related outcomes in adults, including CHD, stroke, carotid artery atherosclerosis, angina pectoris, C-reactive protein, CHF, microvascular disease, and mortality. Graber et al. (2019) reported a positive, borderline significant association with self-reported cardiovascular conditions (i.e., high blood pressure, CAD, stroke) (1.08; 95% CI: 0.98, 1.21). However, potential selection bias is a major concern for this study owing to the recruitment of volunteers who already knew their PFAS exposure levels and were motivated to participate in a lawsuit.

Among the four studies that examined CHD, the findings were mixed, with three studies reporting positive nonsignificant associations, and one study reporting negative associations. A *high* confidence study (Mattsson et al., 2015), a *medium* confidence NHANES study (Huang et al., 2018), and a *low* confidence study (Christensen et al., 2016) reported positive nonsignificant associations with CHD. A *low* confidence study from the C8 Health Project (Honda-Kohmo et al., 2019) reported a significant inverse association between PFOS and CHD among adults with and without diabetes. However, study limitations that may have influenced these findings include the reliance on self-reporting of a clinician-based diagnosis for CHD outcome classification and residual confounding by SES.

A *medium* confidence study of 10,850 NHANES participants (1999–2014) (Huang et al., 2018) reported significantly higher odds of heart attack for the third quartile (OR: 1.56; 95% CI: 1.01, 2.43) compared with the first quartile, and a very similar but not significant effect in the fourth quartile. No associations were observed with stroke, CHF, and angina pectoris. A *medium* confidence study (Hutcheson et al., 2020) of 3,921 adults with and 44,285 without diabetes participating in the C8 Health Project found a significant inverse association with history of stroke (OR: 0.90; 95% CI: 0.82, 0.98; p = 0.02). A significant inverse association with history of stroke (OR: 0.81; 0.70–0.90) was observed among people with diabetes. No association with stroke was observed among those without diabetes.

Cardenas et al. (2019) reported significant increases in risk of any microvascular disease, that were significant only in the lifestyle arm of a health interventions-controlled trial (OR: 1.37; 95% CI: 1.04, 1.84). No associations were observed for nephropathy, retinopathy, or neuropathy.

Two studies assessed potential PFOS-associated changes in heart structure (Mobacke et al., 2018) and carotid atherosclerosis (Lind et al., 2017b) in participants 70 years and older, with mixed results. Mobacke et al. (2018) evaluated alterations of left ventricular geometry, a risk factor for CVD and reported that serum PFOS (linear isomer) was significantly associated with higher left ventricular end-diastolic diameter (0.47; 95% CI: 0.08, 0.87; p = 0.02) and lower relative wall thickness (−0.01; 95% CI: −0.01, −0.001; p = 0.03). PFOS was not significantly associated with left ventricular mass. Lind et al. (2017b) reported that plasma PFOS was not associated with markers of carotid artery atherosclerosis, including atherosclerotic plaque, the intima-media complex, and the CIMT, a measure used to diagnose the extent of carotid atherosclerotic vascular disease. Aortic and coronary artery calcification was examined in a *medium* confidence study (Osorio-Yáñez et al., 2021) on prediabetic participants from the DPPOS. A significantly increased risk of ascending aortic calcification was reported along with increased risk of coronary artery calcification. Coronary artery calcification was represented as a

score of severity (Agatston score) indicating mild, moderate, or severe calcification. The odds of a moderate score (11–400) compared with a mild score (< 11) was increased with respect to PFOS exposure, and the odds of a severe score (> 400) compared with a mild score were significantly increased. Koskela et al. (2022), a *low* confidence study, examined abdominal aortic calcification among participants aged 40 years and older in NHANES (2013–2014) and did not observe an association.

No association between PFOS and C-reactive protein levels, a risk factor for CVD, was observed in two studies of pregnant and postpartum women (Mitro et al., 2020; Matilla-Santander et al., 2017).

Mortality due to heart/cerebrovascular diseases was examined in one *medium* confidence study (Fry and Power, 2017). Among a cohort of 1,043 NHANES participants 60 years and older, PFOS was not associated with mortality due to heart/cerebrovascular diseases.

Overall, the findings from a single *high* confidence study and several *medium* confidence studies conducted among the general population provided consistent evidence for an association between PFOS and blood pressure. The directionality of this association was mostly positive, although a single *medium* confidence study (Lin et al., 2020c) reported an inverse association. The limited evidence for an association between PFOS and increased risk of hypertension was inconsistent. There was evidence suggesting an increased risk of hypertension among women (Liao et al., 2020; Bao et al., 2017) in the general adult population, but additional studies are needed to confirm this finding. Evidence for other CVD-related endpoints also was limited and inconsistent. No occupational studies examining PFOS exposure and CVD were identified.

3.4.3.1.2 Serum Lipids

3.4.3.1.2.1 Introduction

Serum cholesterol and triglycerides are well-established risk factors for CVDs. Major cholesterol species in serum include LDL and HDL cholesterol. Elevated levels of total cholesterol (TC), LDL, and triglycerides are associated with increased cardiovascular risks, whereas higher levels of HDL are associated with reduced risks. Evidence for changes in serum lipids was synthesized by population (i.e., children, pregnant women, adults, occupational populations), and there may be differences in the interpretation of an effect depending on age. For example, while elevated levels of TC, LDL, and triglycerides are associated with increased cardiovascular risks in adults, serum lipid changes in children are age-dependent and fluctuate during puberty (Daniels et al., 2008).

There are 15 studies (17 publications)[13] from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 15 studies are shown in Figure 3-32. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-14 and below.

In the 2016 PFOS HESD (U.S. EPA, 2016b), the epidemiologic evidence overall supported an association between PFOS and increased TC. An association between PFOS and small increases in TC in the general population was observed in several studies (Geiger et al., 2014a; Eriksen et al., 2013; Frisbee et al., 2010; Nelson et al., 2010; Steenland et al., 2009). Steenland (Steenland

[13] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b).

et al., 2009) examined serum PFOS levels among over 46,000 C8 Health Project participants and reported significant positive associations for all serum lipids except HDL. A cross-sectional study (Frisbee et al., 2010) of children enrolled in the C8 Health Project also reported significantly increased TC and LDL, with increasing serum PFOS. Positive associations were seen in another general population study (Eriksen et al., 2013) conducted among Danish adults (50–65 years old). A positive association between PFOS and hypercholesterolemia also was observed in two separate cohorts (C8 Health Project and Canadian Health Measures Survey) (Fisher et al., 2013; Steenland et al., 2009). Cross-sectional occupational studies (Olsen et al., 2003; Olsen et al., 2001a) reported positive associations between PFOS and increased TC and triglycerides (TG), however, the association was not observed in longitudinal analyses. Evidence for associations between other serum lipids and PFOS was mixed including HDL, LDL, VLDL, non-HDL cholesterol, and triglycerides.

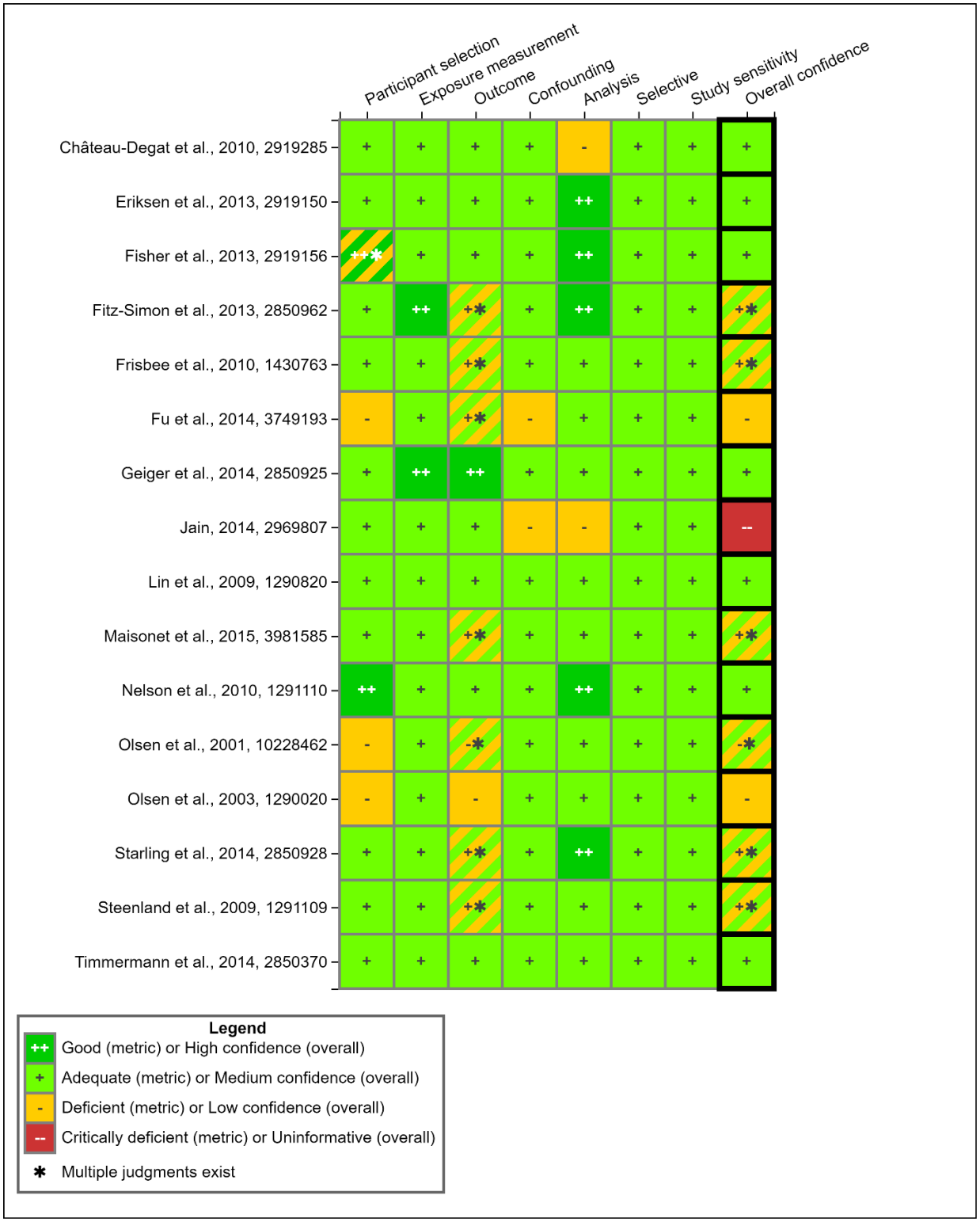


Figure 3-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Serum Lipids Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-14. Associations Between Elevated Exposure to PFOS and Serum Lipids From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]
Chateau-Degat, 2010, 2919285 *Medium*	Cross-sectional	Adults	↑	↑↑	↓	↓
Eriksen, 2013, 2919150 *Medium*	Cross-sectional	Adults	↑↑	NA	NA	NA
Fisher, 2013, 2919156 *Medium*	Cross-sectional	Adults	–	–	–	–
Fitz-Simon, 2013, 2850962 *Mixed*[b]	Cohort	Adults	↑	↓	↑	–
Frisbee, 2010, 1430763 *Mixed*[b]	Cross-sectional	Children	↑↑	–	↑↑	↑
Fu, 2014, 3749193 *Low*	Cross-sectional	Adults and children	↑	↓	↑	↑
Geiger, 2014, 2850925 *Medium*	Cross-sectional	Adolescents	↑↑	–	↑↑	↓
Lin, 2009, 1290820 *Medium*	Cross-sectional	Adults	NA	↑↑	NA	–
Maisonet, 2015, 3981585 *Mixed*[b]	Cohort	Children	–	–	–	↓
Nelson, 2010, 1291110 *Medium*	Cross-sectional	Adults	↑↑	↑	↑	NA
Olsen, 2001, 10228462 *Mixed*[b]	Cohort	Adults	↑	↓	NA	↑
Olsen, 2003, 1290020 *Low*	Cohort	Occupational	–	NA	NA	–

Reference, confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]
Starling, 2014, 2850928 *Mixed*[b]	Cohort	Children	↑↑	↑↑	↑	–
Steenland, 2009, 1291109 *Mixed*[b]	Cross-sectional	Occupational	↑	↑	↑	↑
Timmerman, 2014, 2850370 *Medium*	Cohort	Children	NA	NA	NA	↑

Notes: HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein; NA = no analysis was for this outcome was performed; TC = total cholesterol; TG = triglycerides; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] *Mixed* confidence studies were rated *medium* confidence for TC and HDL and *low* confidence for LDL and TG due to non-fasted blood samples.

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 66 new epidemiologic studies (65 publications)[14] were identified. These studies examined the associations between PFOS and serum lipids in children (n = 24), in pregnant women (n = 7), in the general adult population (n = 32), and in workers (n = 3). Except for 10 studies (Blomberg et al., 2021; Li et al., 2021b; Liu et al., 2020b; Sinisalu et al., 2020; Tian et al., 2020; Donat-Vargas et al., 2019; Lin et al., 2019; Liu et al., 2018b; Domazet et al., 2016; Olsen et al., 2012), all studies were cross-sectional. Some cohort studies provided additional cross-sectional analyses (Blomberg et al., 2021; Li et al., 2021b; Sinisalu et al., 2020). Most studies assessed exposure to PFOS using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but some studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on the clinical cut-points, medication use, doctor's diagnosis, or criteria for metabolic syndrome.

3.4.3.1.2.2 Study Quality

All studies were evaluated for risk of bias, selective reporting, and sensitivity following the methods in Appendix A (U.S. EPA, 2024a) and Section 2.1.3. Three considerations were specific to evaluating the quality of studies on serum lipids. First, because lipid-lowering medications strongly affect serum lipid levels, unless the prevalence of medication use is expected to be low in the study population (e.g., children), studies that did not account for the use of lipid-lowering medications by restriction, stratification, or adjustment were rated as *deficient* in the *participant selection* domain. Second, because triglycerides levels are sensitive to recent food intake (Mora, 2016), outcome measurement error is likely substantial when TG is measured without fasting. Thus, studies that did not measure triglycerides in fasting blood samples were rated *deficient* in the *outcome measures* domain for triglycerides. The *outcome measures* domain for LDL was also rated *deficient* if LDL was calculated based on triglycerides. Fasting status did not affect the *outcome measures* rating for TC, directly measured LDL, and HDL because the serum levels of these lipids change minimally after a meal (Mora, 2016). Third, measuring PFOS and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.4 years) (Li et al., 2018b), current blood concentrations are expected to correlate well with past exposures. Furthermore, although reverse causation due to hypothyroidism (Dzierlenga et al., 2020b) or enterohepatic cycling of bile acids (Fragki et al., 2021) has been suggested, there is yet clear evidence to support these reverse causal pathways.

There are 65 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 65 studies are shown in Figure 3-33, Figure 3-34, and Figure 3-35.

Consistent with the considerations mentioned, 2 studies were considered *high* confidence, 1 study was rated *high* for one exposure measurement and *medium* for the other, 22 studies were rated *medium* confidence for all lipid outcomes, 9 studies were rated *medium* confidence for TC or HDL, but *low* confidence for triglycerides or LDL, 24 studies were rated *low* confidence for all lipid outcomes, and 7 studies were rated *uninformative* for all lipid outcomes (Sinisalu et al., 2021; Abraham et al., 2020; Leary et al., 2020; Huang et al., 2018; Seo et al., 2018; Predieri et al., 2015). Notably, nine studies (Blomberg et al., 2021; Canova et al., 2021; Dalla Zuanna et al.,

[14] Dong et al. (2019) counted as two studies, one in adolescents and one in adults.

2021; Canova et al., 2020; Tian et al., 2020; Yang et al., 2020b; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015) were rated *low* confidence specifically for triglycerides and/or LDL because these studies measured triglycerides in non-fasting blood samples. The *low* confidence studies had deficiencies in participant selection (Cong et al., 2021; Kobayashi et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Chen et al., 2019; Graber et al., 2019; He et al., 2018; Khalil et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015; Lin et al., 2013; Wang et al., 2012), outcome measures (Kobayashi et al., 2021; Graber et al., 2019; Yang et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Kishi et al., 2015; Rotander et al., 2015), confounding (Liu et al., 2021; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; van den Dungen et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012), analysis (He et al., 2018; Liu et al., 2018b; Sun et al., 2018), sensitivity (Khalil et al., 2020; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012), or selective reporting (Dong et al., 2019) (adolescent portion only).

The most common reason for a *low* confidence rating was concerns for participant selection. These concerns include a lack of exclusion based on use of lipid-lowering medications (Cong et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Li et al., 2020d; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; van den Dungen et al., 2017; Wang et al., 2012), potential for self-selection (Li et al., 2020d; Graber et al., 2019; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015), highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOS and lipids (Lin et al., 2013), and missing key information on the recruitment process (Khalil et al., 2020; Khalil et al., 2018; Yang et al., 2018). Another common reason for *low* confidence was a serious risk for residual confounding by SES (Li et al., 2020d; Lin et al., 2020a; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; van den Dungen et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012). Frequently, deficiencies in multiple domains contributed to an overall *low* confidence rating. The *uninformative* studies had *critical deficiencies* in at least one domain or were *deficient* in several domains. These *critical deficiencies* include a lack of control for confounding (Abraham et al., 2020; Huang et al., 2018; Seo et al., 2018), convenience sampling (Sinisalu et al., 2021), and treating PFOS as an outcome of all lipids instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination (Predieri et al., 2015). Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study (Leary et al., 2020). Studies considered *uninformative* were not considered further. In the evidence synthesis below, *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

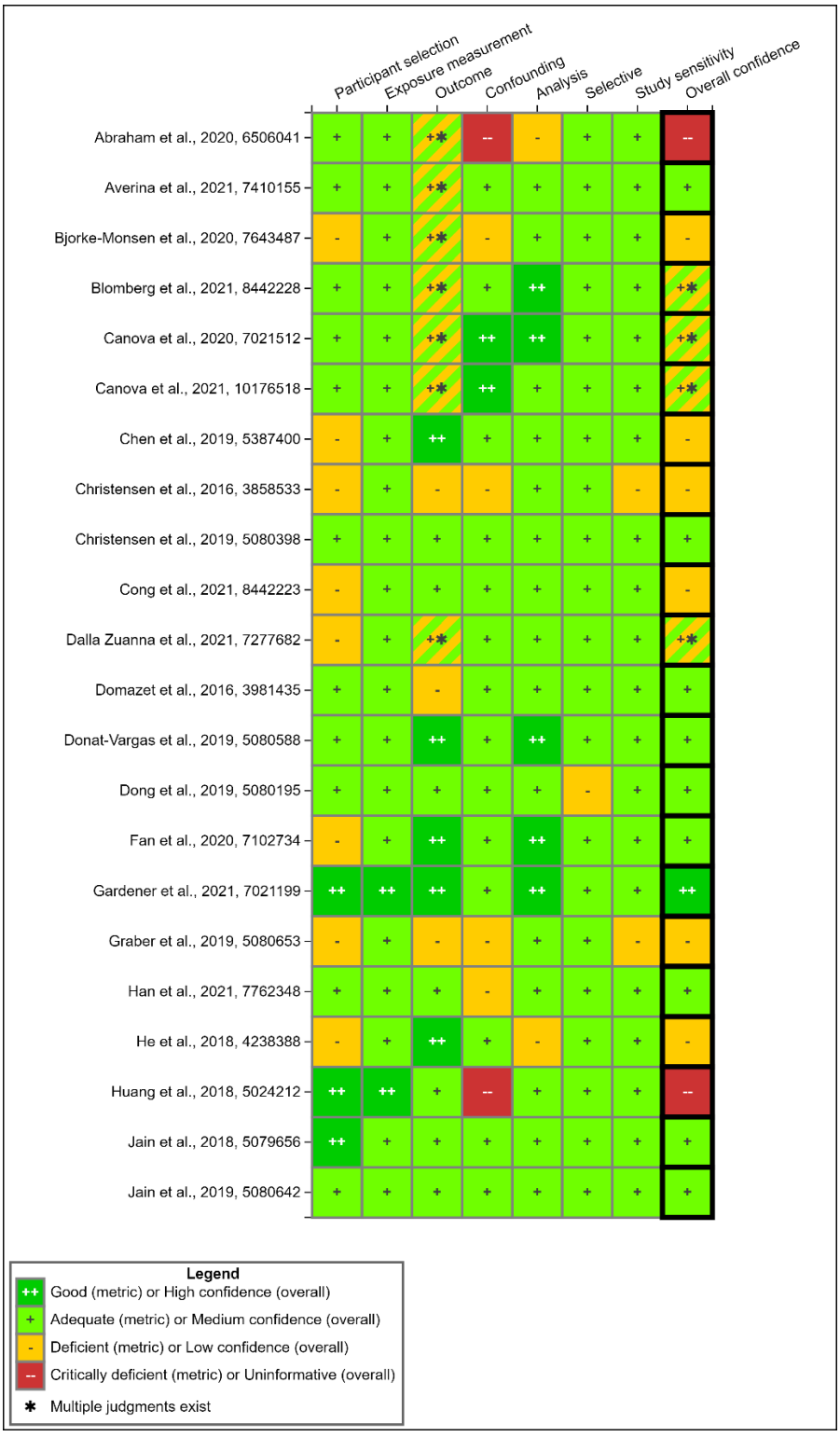


Figure 3-33. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids

Interactive figure and additional study details available on HAWC.

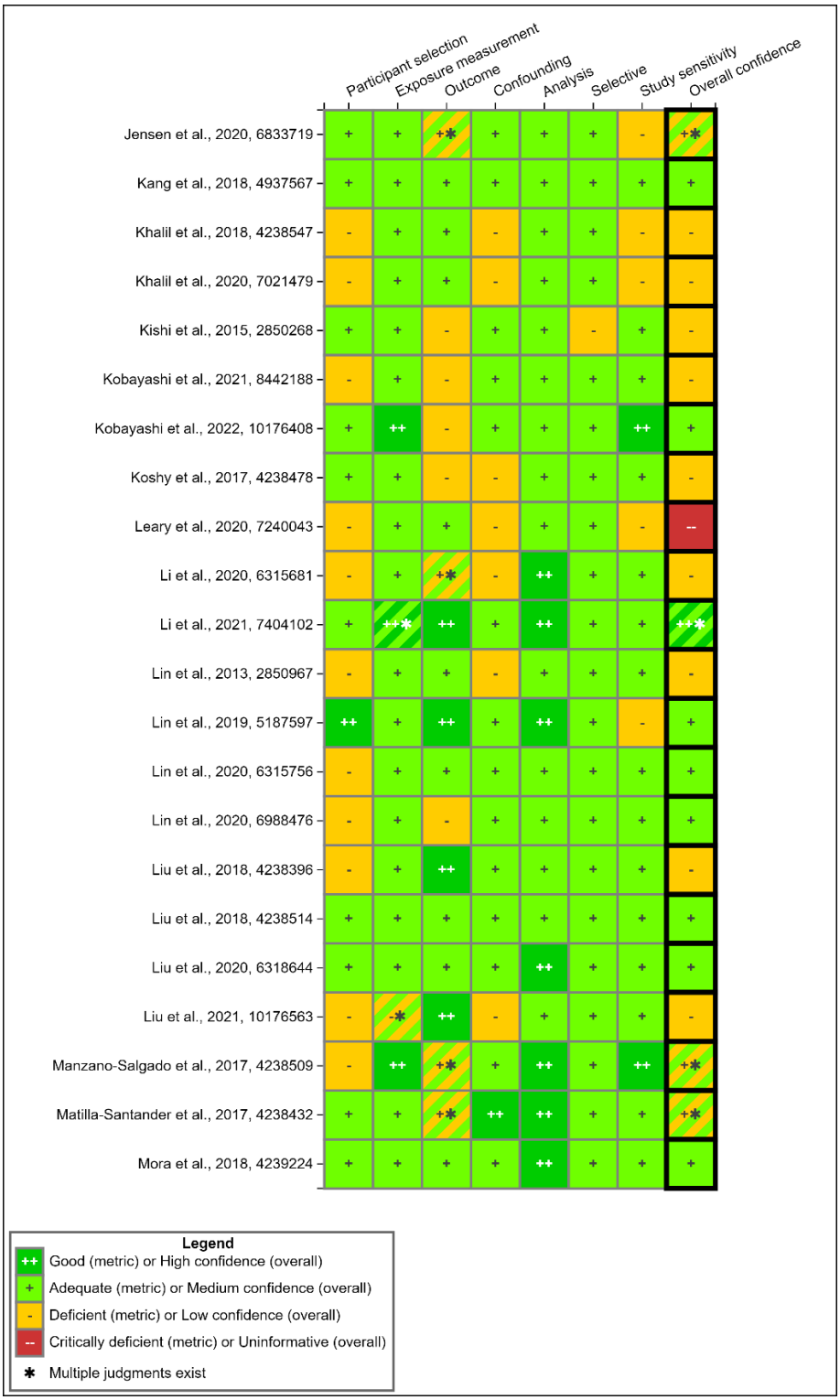


Figure 3-34. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

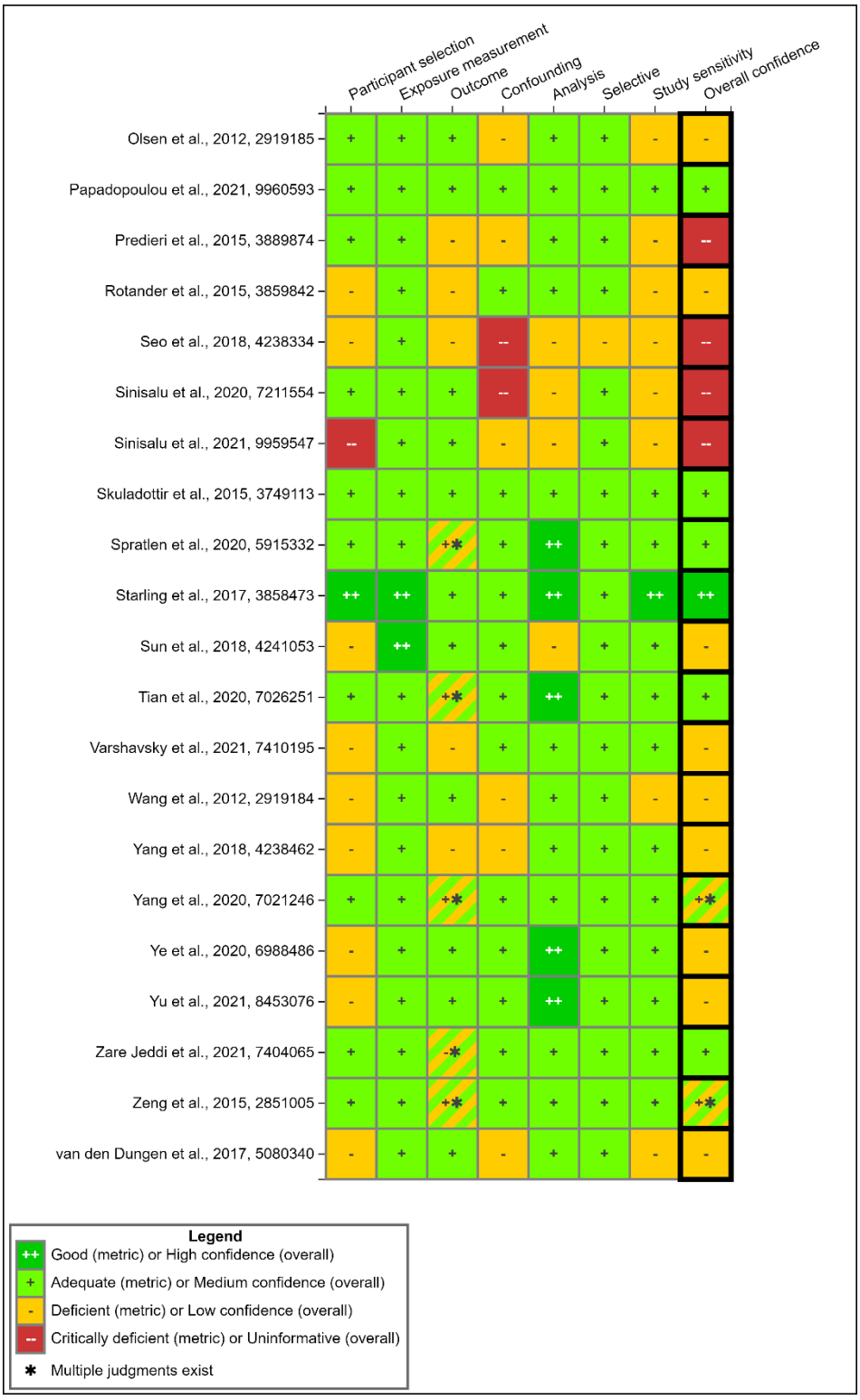


Figure 3-35. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.2.3 Findings From Children

Results for the studies that examined TC in children are presented in Appendix D (U.S. EPA, 2024a). Eleven *medium* confidence and three *low* confidence studies examined the association between PFOS and TC in children. Of these, four studies examined the association between prenatal PFOS exposure and TC in childhood (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined exposure and TC at multiple timepoints throughout childhood (Blomberg et al., 2021), and 10 examined the association between childhood PFOS exposure and concurrent TC (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Jain and Ducatman, 2018; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). Higher PFOS was significantly associated with higher TC in all children in five *medium* confidence studies (Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Jain and Ducatman, 2018; Zeng et al., 2015). Notably, significant positive associations were observed among children {n = 2,693} and adolescents (n = 6,669) of a high-exposure community in Italy (Veneto Region). The associations were significant in continuous and all quartile analyses and were more prominent in children compared with adolescents. Significant positive associations were observed in 9-year-old cross-sectional analyses and one prospective comparison (PFOS measured at 5 years, TC measured at 9 years of age) of children belonging to a Faroese cohort (Blomberg et al., 2021). Comparisons of PFOS and TC measured at other timepoints were less consistent. Positive associations were also found in four other *medium* confidence studies (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), but the associations were small and statistically not significant except for girls in mid-childhood (Mora et al., 2018). In contrast, one *medium* confidence study (Tian et al., 2020) reported inverse associations, however, this analysis was only conducted concurrently in cord blood. In two out of three *low* confidence studies, positive associations were reported, including a statistically significant finding in Koshy (Khalil et al., 2018; Koshy et al., 2017). However, residual confounding by SES may have positively biased the results of both studies. Taken together, these studies support a positive association between PFOS and TC in children, particularly for childhood exposure.

Five *medium* confidence and seven *low* confidence studies examined the association between PFOS and LDL in children. Of these, three examined prenatal exposure (Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined prenatal and childhood exposure (Papadopoulou et al., 2021) and nine examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). The *medium* studies generally found small, positive associations between PFOS and LDL, but only one study in first-level high school students reported a significant association (Averina et al., 2021). None of the associations were statistically significant in the remaining *medium* confidence studies (see Appendix D, (U.S. EPA, 2024a)) (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). Most *low* confidence studies found a positive association between PFOS and LDL (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), including statistically significant findings in three studies (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017). However, residual confounding by SES (Khalil et al., 2018; Koshy et al., 2017) and the use of non-fasting samples (Canova et al., 2021; Manzano-Salgado et al., 2017b; Zeng et al., 2015) were concerns in these studies. Overall, increases in LDL with increasing PFOS were observed in children, but the magnitudes were small.

One *high* confidence, 11 *medium* confidence, and 3 *low* confidence studies examined the association between PFOS and HDL in children. Of these, three examined prenatal exposure (Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined prenatal and postnatal exposure (Papadopoulou et al., 2021), two examined exposure and HDL at multiple timepoints throughout childhood (Blomberg et al., 2021; Li et al., 2021b), and six examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Jain and Ducatman, 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). The only *high* confidence study (Li et al., 2021b) reported significant positive associations for HDL at 12 years of age among child participants of the HOME study. PFOS measured at 8 years of age and concurrently at 12 years of age was significantly associated with increased HDL. The associations for PFOS measured prenatally, at birth, and at 3 years of age were all non-significantly positive. Higher PFOS was significantly associated with higher HDL in children in mid-childhood in two *medium* confidence studies (Canova et al., 2021; Mora et al., 2018). The positive association observed in Canova et al. (2021) was consistent when examining adolescent participants. In Faroese children (Blomberg et al., 2021), higher PFOS was significantly associated with higher HDL when measured concurrently at 9 years of age. Comparisons of other timepoints (18-month concurrent measurements, 18-month PFOS and 9-year HDL, and 5-year PFOS and 9-year HDL) were all positively associated with HDL with increasing PFOS concentrations. Other *medium* confidence studies found positive (Jain and Ducatman, 2018), inverse (HDL at 18 months in Jensen et al. (2020); Papadopoulou et al. (2021), prenatal PFOS; Manzano-Salgado et al. (2017b); Zeng et al. (2015); Tian et al. (2020)), or close to zero (HDL at 3 months in Jensen et al. (2020); Papadopoulou et al. (2021), postnatal PFOS) associations; none of these associations were statistically significant. Two of the three *low* confidence studies found positive associations between PFOS and HDL (Khalil et al., 2018; Koshy et al., 2017). In summary, mixed associations were found between PFOS and HDL in children.

Five *medium* confidence studies and four *low* confidence studies examined the association between PFOS and triglycerides in children. Of these, four examined prenatal exposure (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and six examined childhood exposure (Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Domazet et al., 2016; Zeng et al., 2015). Higher mid-childhood PFOS exposure was significantly associated with lower triglycerides in one *medium* confidence study (Mora et al., 2018). The other *medium* confidence studies reported positive (Spratlen et al., 2020; Kang et al., 2018), inverse (triglycerides at 3 months in Jensen et al. (2020); PFOS exposure at age 9 years in Domazet et al. (2016)), or close to zero associations (triglycerides at 18 months in Jensen et al. (2020); PFOS exposure at age 15 years in Domazet et al. (2016)); none of these associations were statistically significant. Of note, in Jensen et al. (2020) and Domazet et al. (2016), the direction of association changed depending on the timing of outcome or exposure assessment. One *medium* confidence study (Kobayashi et al., 2022) and one *low* confidence study (Kobayashi et al., 2021) conducted on mother-child pairs from the Hokkaido Study on Environment and Children's Health examined the association between prenatal PFOS exposure, maternal polymorphisms of nuclear receptor genes, and triglyceride levels in infants. Inverse associations for PFOS and TG were observed, but both studies reported no significant interaction between maternal nuclear gene polymorphisms and PFOS exposure on triglyceride levels. All other *low* confidence studies reported positive associations between PFOS and triglycerides, but all associations were small and not statistically significant (Sinisalu et al., 2020; Khalil et al.,

2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015). The use of non-fasting samples and residual confounding by SES may have biased these results upward. Overall, mixed associations were found between PFOS and triglycerides in children.

In summary, the available evidence supports positive associations between PFOS and TC and LDL in children. The associations with HDL and triglycerides were mixed.

3.4.3.1.2.4 Findings From Pregnant Women

One *high* confidence study (Gardener et al., 2021) and four *medium* confidence studies examined the association between PFOS and TC in pregnant women and four reported positive associations between PFOS and TC (see Appendix D, (U.S. EPA, 2024a)) (Dalla Zuanna et al., 2021; Matilla-Santander et al., 2017; Skuladottir et al., 2015). A significant positive trend across quartiles of PFOS exposure was observed for TC in a cohort study of pregnant women from the United States (Gardener et al., 2021). Skuladottir et al. (2015) reported a statistically significant linear trend of increasing TC with increasing PFOS. Positive associations also were observed in an Italian high-exposure community study (Dalla Zuanna et al., 2021) on pregnant women. The association from continuous analyses indicated non-significantly increased TC, which was supported by positive associations when analyzing the second and fourth quartile of exposure but not the second. No association between PFOS and TC was observed in a Chinese study of pregnant women (Yang et al., 2020b). No association was found in the single *low* confidence study (Varshavsky et al., 2021) on total serum lipids after adjustment for race/ethnicity, insurance type, and parity. These findings suggest a consistently positive association between PFOS and TC in pregnant women.

Two studies (Dalla Zuanna et al., 2021; Yang et al., 2020b) considered *low* confidence for LDL due to lack of fasting did not observe an association between PFOS exposure and LDL in pregnant women. Three *medium* confidence studies examined the association between PFOS and HDL, and two reported positive associations. In a high-exposure community study (Dalla Zuanna et al., 2021), serum HDL was significantly increased among pregnant Italian women (beta per ln-ng/mL PFOS: 4.84; 95% CI: 2.15, 7.54), and the association was consistent in quartile analyses. A study on pregnant women in the Healthy Start Study reported a positive, though statistically nonsignificant, association between PFOS and HDL (see Appendix D, (U.S. EPA, 2024a)) (Starling et al., 2017). No association between PFOS and HDL was observed in a Chinese study of pregnant women (Yang et al., 2020b).

One *high* confidence, one *medium* confidence and three *low* confidence studies examined the association between PFOS and triglycerides in pregnant women. A significant positive trend across quartiles of PFOS exposure was observed for triglycerides in a cohort study of pregnant women from the United States (Gardener et al., 2021). The *medium* confidence study reported no association between PFOS and triglycerides (see Appendix D, (U.S. EPA, 2024a)) (Starling et al., 2017). Two *low* confidence studies reported statistically significant, inverse associations between PFOS and triglycerides (Matilla-Santander et al., 2017; Kishi et al., 2015) while the remaining study (Yang et al., 2020b) reported a nonsignificant inverse association. All *low* confidence studies were limited by their use of non-fasting blood samples. Given that recent food intake is associated with increased triglycerides and may be a source of PFOS, using non-fasting blood samples is expected to positively bias the PFOS- triglycerides association. The inverse associations observed in the *low* confidence studies provides support for an inverse association

between PFOS and triglycerides. These inverse associations are inconsistent with the finding in the only *medium* confidence study. In summary, the available evidence suggests an inverse association between PFOS and triglycerides in pregnant women. However, additional *high* or *medium* confidence evidence is needed to confirm this association.

Kishi et al. (2015) additionally examined the association between PFOS and select fatty acids in serum. Except for stearic acid and eicosapentaenoic acid, PFOS was inversely associated with serum fatty acids; most of these associations were statistically significant (Kishi et al., 2015). This study suggests PFOS may disrupt fatty acid metabolism in pregnant women, but additional studies are needed to confirm this finding.

In summary, the available evidence supports a positive association between PFOS and TC in pregnancy. The available evidence does not support a consistent, positive association between PFOS and triglycerides and HDL. Finally, the available evidence is too limited or non-existent to determine the association between PFOS and LDL in pregnant women.

3.4.3.1.2.5 Findings From the General Adult Population

Ten *medium* confidence and 12 *low* confidence studies examined PFOS and TC or hypercholesterolemia in adults. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020d; Liu et al., 2020b; Chen et al., 2019; Donat-Vargas et al., 2019; Dong et al., 2019; Graber et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016; Wang et al., 2012); two studies additionally examined the association between baseline PFOS and changes in TC or incident hypercholesterolemia (Liu et al., 2020b; Lin et al., 2019).

Of the 10 *medium* confidence studies, nine reported positive associations (Figure 3-36, Figure 3-37, Figure 3-38, and Figure 3-39). In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020) reported statistically positive associations with TC. Canova et al. (2020) also reported a concentration-response curve for risk of high TC when PFOS was categorized in quartiles or deciles, with a higher slope at higher PFOS concentrations (Figure 3-40). Another high-exposure community study (Lin et al., 2020d) conducted in Taiwan provided a sensitivity analysis of older adults (age 55–75 years), restricting to those participants not taking lipid-lowering or anti-hypertensive medications. In quartile analyses of TC, the association was significantly positive for the second (beta for Q2 vs. Q1: 15.06; 95% CI: 4.66, 25.46) and third quartile (beta for Q3 vs. Q1: 11.47; 95% CI: 1.03, 21.91) of exposure. The magnitude of association was similar for the fourth quartile of exposure but did not reach significance.

Four *medium* studies using overlapping data from NHANES 2003–2014 reported positive associations between PFOS and TC in adults (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) (see Appendix D, (U.S. EPA, 2024a)). The association was statistically significant when data from all cycles were pooled in analyses (Dong et al., 2019). A cross-sectional analysis (Han et al., 2021) of type 2 diabetes cases and healthy controls in China reported a positive association for TC, but it did not reach significance. PFOS also was associated with slightly higher TC at baseline in the POUNDS-Lost cohort (Liu et al., 2020b) and the DPPOS (Lin et al., 2019), but neither association was statistically significant. The

DPPOS also reported that PFOS was associated with a slightly higher prevalence of hypercholesterolemia at baseline (OR = 1.02, 95% CI: 0.85, 1.21) and a slightly higher incidence of hypercholesterolemia prospectively (HR = 1.01, 95% CI: 0.91, 1.12). In contrast to these findings, Donat-Vargas et al. (2019) reported inverse associations between PFOS and concurrently measured TC. Further, it reported positive associations between PFOS averaged between baseline and follow-up and TC at follow-up (Donat-Vargas et al., 2019). All associations in Donat-Vargas et al. (2019) were small and few were statistically significant. It is noteworthy that all participants in Lin et al. (2019) were prediabetic, approximately half of all participants in Han et al. (2021) were diabetic, all participants in Liu et al. (2020b) were obese and enrolled in a weight loss trial, and all participants in Donat-Vargas et al. (2019) were free of diabetes for at least 10 years of follow-up. It is unclear whether differences in participants' health status explained the studies' conflicting findings.

In *low* confidence studies, positive associations between PFOS and TC or hypercholesterolemia were reported in 10 of 12 studies (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Li et al., 2020d; Chen et al., 2019; Graber et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016). However, oversampling of persons with potentially high PFOS exposure and health problems was a concern in three of these studies (Li et al., 2020d; Graber et al., 2019; Christensen et al., 2016). Medication status and potential residual confounding by SES was a concern in three other studies (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020). Further, He et al. (2018) used similar data as the four *medium* NHANES studies and thus added little unique information. Considering *medium* and *low* confidence studies together, small increases in TC with increased PFOS were observed, though less consistently.

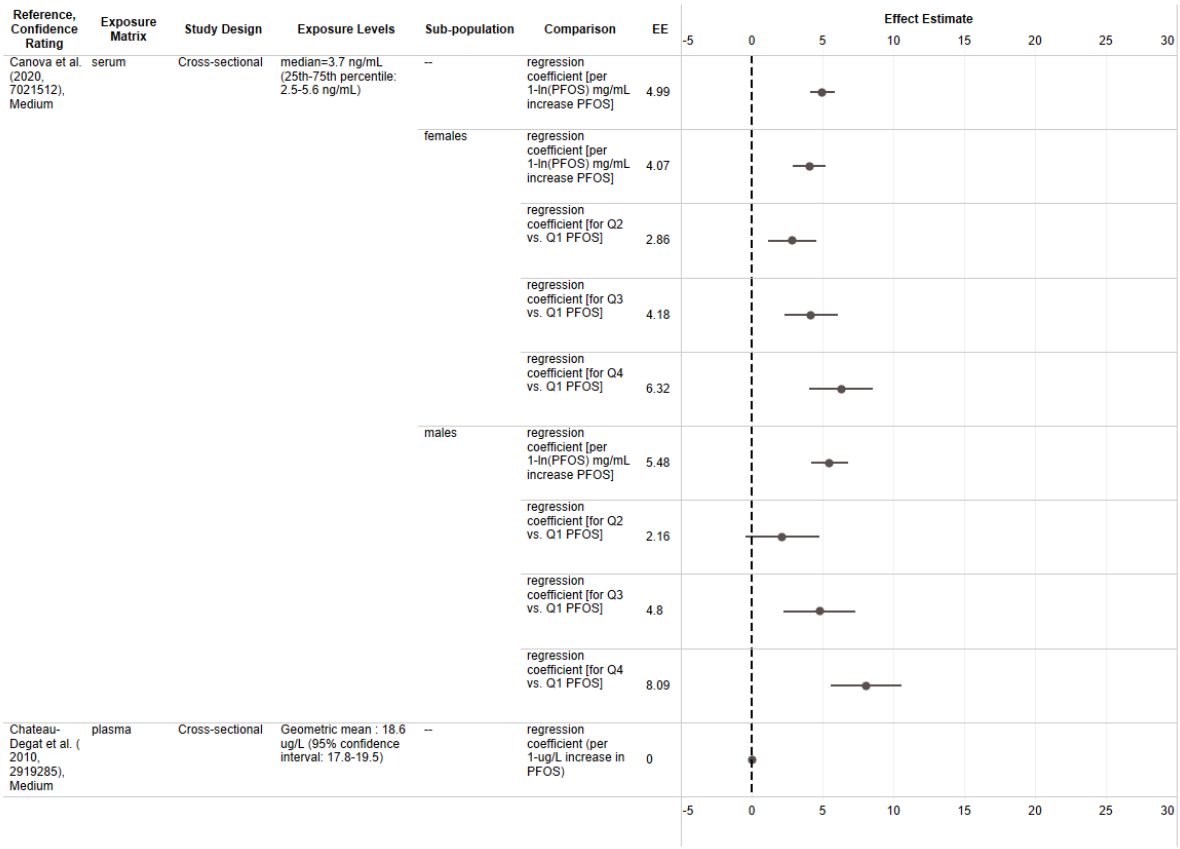


Figure 3-36. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

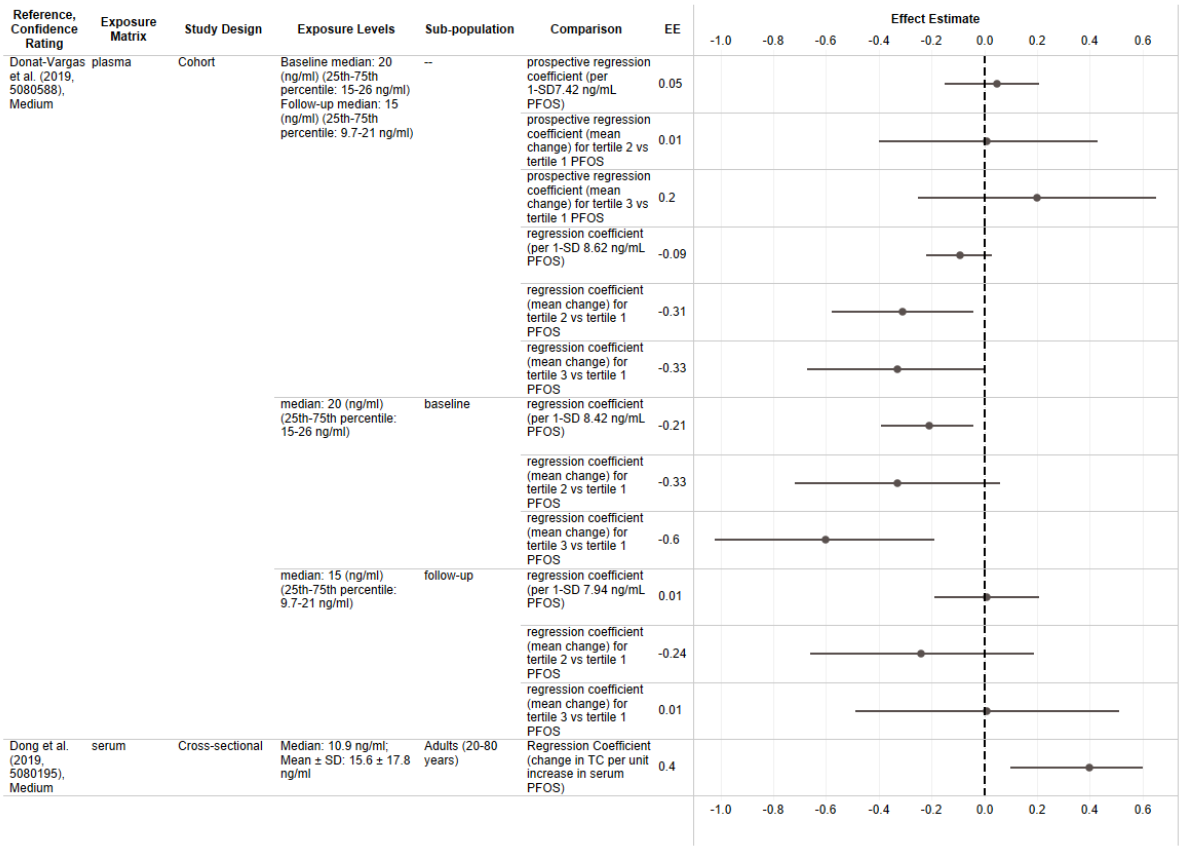


Figure 3-37. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

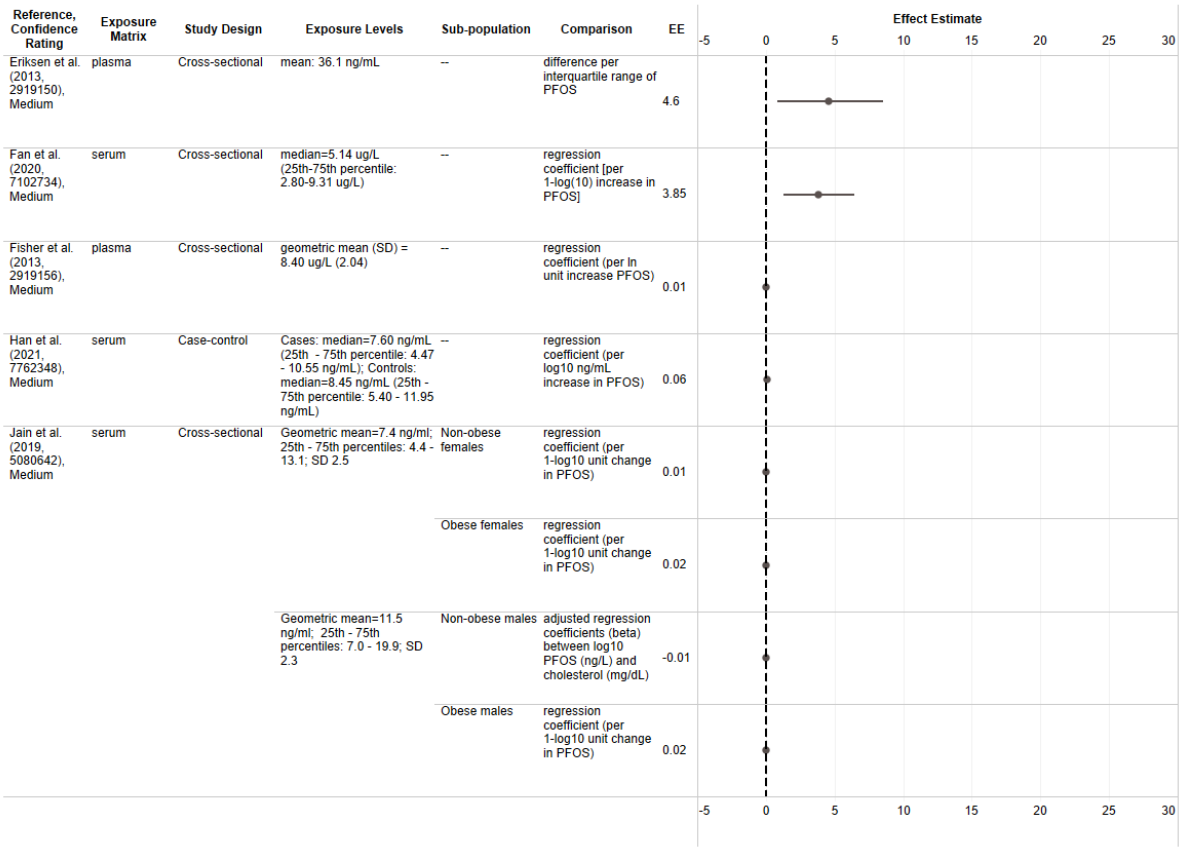


Figure 3-38. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

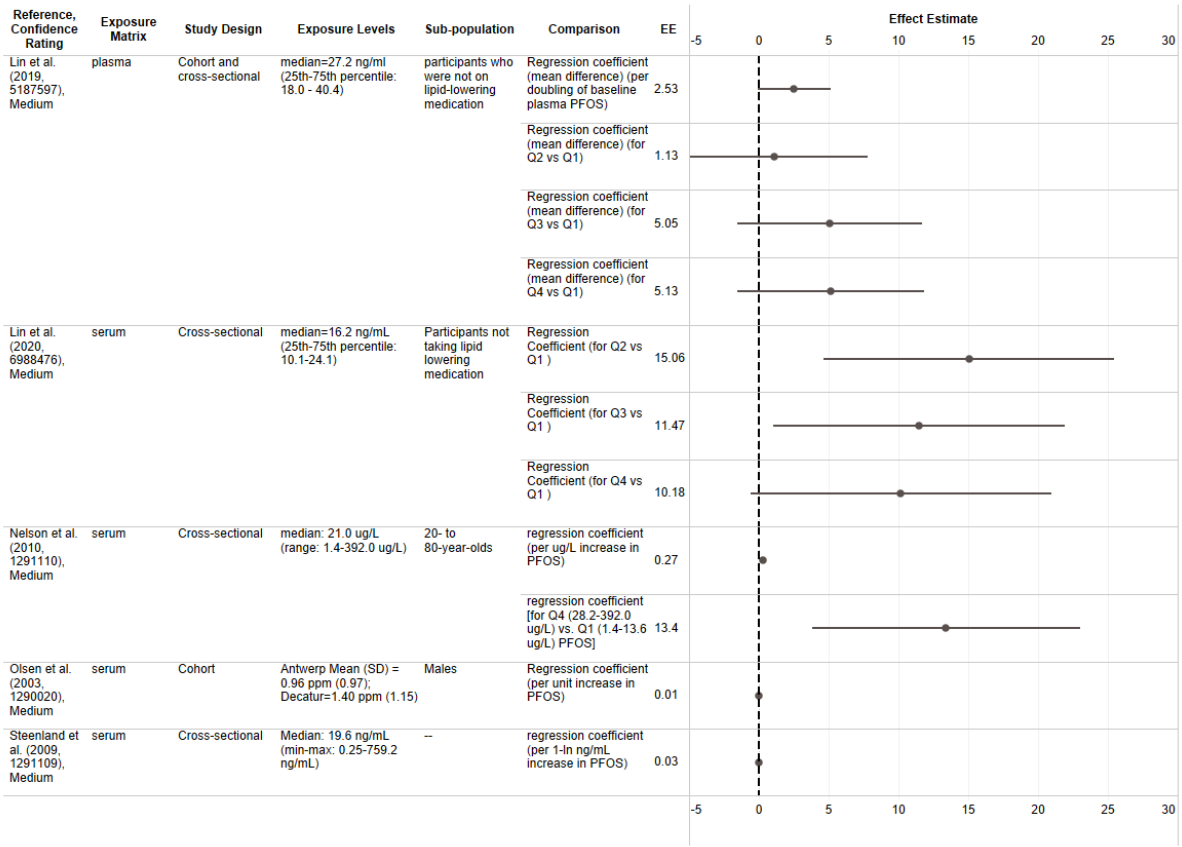


Figure 3-39. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

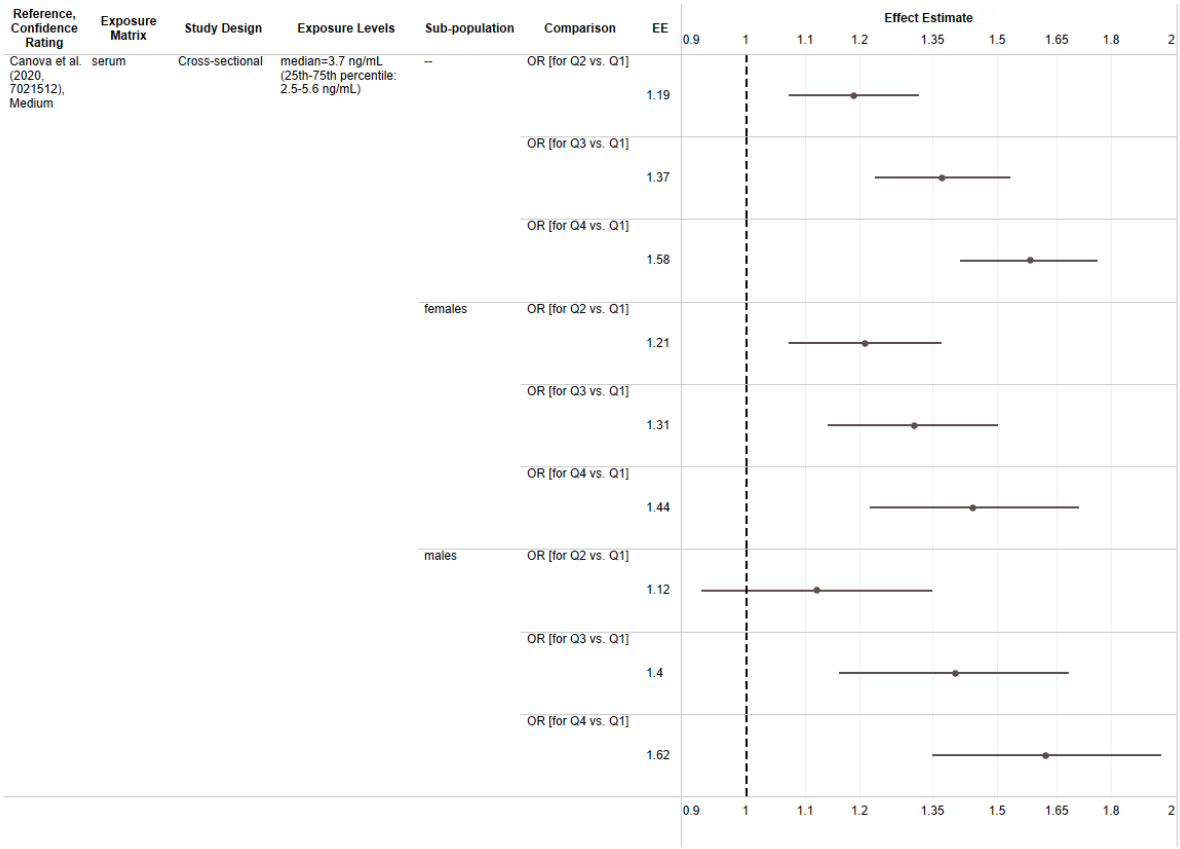


Figure 3-40. Odds of High Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Six *medium* confidence studies examined PFOS and LDL in adults and all reported positive associations. The four studies using overlapping data from NHANES 2003–2014 reported positive associations between PFOS and LDL (Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d), but the association was statistically significant in obese women only (Jain and Ducatman, 2019b) (see Appendix D, (U.S. EPA, 2024a)). The association was inverse, but not statistically significant, in non-obese persons (Jain and Ducatman, 2019b). A cross-sectional analysis (Han et al., 2021) of a case-control study conducted in China reported a significant positive association among 55–75-year-olds. This analysis combined cases of type 2 diabetes and healthy controls, and it is unclear whether the health status of cases explained some of the association. Positive association between PFOS and LDL also was reported at baseline in the DPPOS, but this association was not statistically significant (Lin et al., 2019). This study additionally reported that PFOS was significantly associated with higher VLDL and non-HDL (Lin et al., 2019), which are cholesterol species related to LDL and known to increase cardiovascular risks. Liu et al. (2020b) reported that PFOS was associated with slightly higher cholesterol in combined fractions of intermediate-density (IDL) and LDL that contained apolipoprotein C-III (ApoC-III), but this association was not statistically significant. ApoC-III-containing IDL and LDL are strongly associated with increased cardiovascular risks. Thus, the

positive associations with cholesterol in ApoC-III-containing fractions of IDL and LDL were coherent with the positive associations found for LDL in the other *medium* confidence studies. APOB was also examined in a single *medium* confidence NHANES study (Jain and Ducatman, 2020) that reported a significantly positive association among non-diabetic, non-lipid-lowering medication users. Consistent with these findings, 9 of the 10 *low* confidence studies reported positive associations between PFOS and LDL (Cong et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Lin et al., 2013). However, residual confounding by SES (Cong et al., 2021; Bjorke-Monsen et al., 2020; Lin et al., 2020a; Lin et al., 2013) and oversampling of persons with potentially high PFOS exposure and health problems (Li et al., 2020d) were major concerns in these studies. In addition, He et al. (2018) provided little new information because it used similar data as the four *medium* confidence NHANES studies. Altogether, the available evidence supports a positive association between PFOS and LDL. Few available findings were statistically significant however, suggesting that the association between PFOS and LDL may be relatively small.

Eleven *medium* confidence and 13 *low* confidence studies examined PFOS and HDL or clinically defined low HDL in adults. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020d; Lin et al., 2020a; Liu et al., 2020b; Chen et al., 2019; Christensen et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; van den Dungen et al., 2017; Wang et al., 2012) including Dong et al. (2019) in the adult portion of the study. Two studies additionally examined the association between baseline PFOS and changes in HDL (Liu et al., 2020b; Liu et al., 2018b). In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020) reported statistically positive associations with HDL. Canova et al. (2020) also reported a concentration-response curve when PFOS was categorized in deciles. An overlapping study (Zare Jeddi et al., 2021) in the same community was consistent with Canova et al. (2020), reporting significantly decreased odds of reduced HDL (< 40 mg/L, male; < 50 mg/L, female) in young adults (aged 20 to 39 years). PFOS was associated with lower HDL at baseline in the DPPOS, but this association was not statistically significant (Lin et al., 2019) (see Appendix D, (U.S. EPA, 2024a)). The POUNDS-Lost study (Liu et al., 2020b), most cycles of NHANES 2003–2014 (Dong et al., 2019), a study conducted in a Taiwanese high-exposure community (Lin et al., 2020d), and a cross-sectional analysis (Han et al., 2021) of type 2 diabetes cases and healthy controls reported no association between PFOS and HDL. In *low* confidence studies, PFOS was positively associated with HDL in 5 of 13 studies (Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Yang et al., 2018) (association with concurrent HDL). Of note, in Lin et al. (2020a), the positive association was limited to linear PFOS only; the association between branched PFOS and HDL was inverse and statistically significant (Lin et al., 2020a). The *low* confidence studies had limitations in participant selection, residual confounding by SES, and analysis. It is unclear to what extent these limitations explained the inconsistent findings between *medium* and *low* confidence studies. Overall, the available evidence does not support a consistently inverse association between PFOS and HDL in adults.

Nine *medium* confidence and 13 *low* confidence studies examined the association between PFOS and TG or hypertriglyceridemia. All studies examined the cross-sectional association (Cong et

al., 2021; Han et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Liu et al., 2020b; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Lin et al., 2013; Wang et al., 2012); three studies additionally examined the association between baseline PFOS and changes in TG or incident hypertriglyceridemia (Liu et al., 2020b; Lin et al., 2019; Liu et al., 2018b). Higher PFOS was significantly associated with higher levels of TG in the DPPOS (Lin et al., 2019) (see Appendix D, (U.S. EPA, 2024a)). This study also reported that PFOS was associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively; the prospective association was particularly strong in participants enrolled in the placebo arm of the DPPOS (Lin et al., 2019). In contrast, PFOS was not associated with triglycerides or changes in triglycerides in the POUNDS-Lost study (Liu et al., 2020b), a cross-sectional analysis (Han et al., 2021) of type 2 diabetes cases and healthy controls, and a high-exposure community study in Italian young adults (aged 20–39 years) (Zare Jeddi et al., 2021). Furthermore, PFOS was inversely associated with TG in the three studies using overlapping NHANES data (Christensen et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) and in Donat-Vargas et al. (2019). In this latter study, there was a statistically significant, linear trend of lower TG with increasing PFOS, regardless of whether PFOS was measured concurrently with TG or averaged between baseline and follow-up (Donat-Vargas et al., 2019). In *low* confidence studies, five reported inverse associations (Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Lin et al., 2013), six reported essentially null associations (Cong et al., 2021; Ye et al., 2021; Canova et al., 2020; Khalil et al., 2020; Chen et al., 2019; Sun et al., 2018), one reported a positive association (Yang et al., 2018), and one qualitatively stated the association was not statistically significant (Wang et al., 2012). Altogether, the association between PFOS and TG was inconsistent.

In summary, in the general adult population, the available evidence supports positive associations between PFOS and TC and LDL, although some inconsistency exists. The available evidence does not support a consistent association between PFOS and reduced HDL and elevated TG.

3.4.3.1.2.6 Findings From Occupational Studies

Workers are usually exposed to higher levels of PFOS, in a more regular manner, and potentially for a longer duration than adults in the general population. At the same time, according to the "healthy worker effect," workers tend to be healthier than non-workers, which may lead to reduced susceptibility to toxic agents (Shah, 2009). Because of these potential differences in exposure characteristics and host susceptibility, occupational studies are summarized separately from studies among adults in the general population.

Three *low* confidence studies examined the association between PFOS and TC in workers. Of these, two examined the cross-sectional association between PFOS and TC in fluorochemical plant workers or firefighters exposed to AFFF (Rotander et al., 2015; Wang et al., 2012); one investigated the association between baseline PFOS and changes in TC over the course of a fluorochemical plant demolition project (Olsen et al., 2012). PFOS was positively associated with TC in Rotander et al. (2015), but the association was not statistically significant. The other cross-sectional study simply reported no significant association (Wang et al., 2012). Olsen et al. (2012) reported an inverse or positive association between changes in PFOS and changes in TC,

depending on whether the outcome was log transformed (Olsen et al., 2012). This pattern is unusual and suggests different data subsets may have been used for analyses with and without log-transformed outcome. Taken together, the occupational studies are limited in both quantity and quality. On the basis of these studies, it is difficult to discern the pattern of association between PFOS and TC in workers.

Two studies examined PFOS and LDL in workers. One study examined PFOS and non-HDL, of which LDL is a major component. All studies were considered *low* confidence. PFOS was positively associated with LDL in Rotander et al. (2015), but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found (Wang et al., 2012). The study examining non-HDL found that changes in PFOS during the fluorochemical plant demolition project were inversely associated with changes in non-HDL, but the association was not statistically significant (Olsen et al., 2012). Overall, these studies suggest no consistent association between PFOS and elevated LDL in workers.

The studies that examined LDL or non-HDL also examined the association between PFOS and HDL (Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012). PFOS was positively associated with HDL in Rotander et al. (2015), but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found (Wang et al., 2012). In Olsen et al. (2012), changes in PFOS over the demolition project was positively associated with changes in HDL (Olsen et al., 2012). Together, the occupational studies suggest a positive association between PFOS and HDL in workers, although these findings were limited by potentially unmeasured confounding (Rotander et al., 2015; Olsen et al., 2012) and self-selection of subjects (Rotander et al., 2015).

Two *low* confidence cross-sectional studies examined PFOS and TG in workers and found that PFOS was inversely associated with TG in Rotander et al. (2015), but this association was not statistically significant. Wang et al. (2012) only reported that no significant association was found. Given these limited data, it is not possible to determine the pattern of association between PFOS and TG in workers.

In summary, the available studies examining associations between PFOS serum concentrations and serum lipids among workers was limited to 3 *low* confidence studies. A positive association between PFOS and HDL was observed in some studies. There was not a consistent positive association between PFOS and elevated LDL. The evidence is too limited to determine the association between PFOS and TC and TG in workers.

3.4.3.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 4 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and 9 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 13 studies are shown in Figure 3-41.

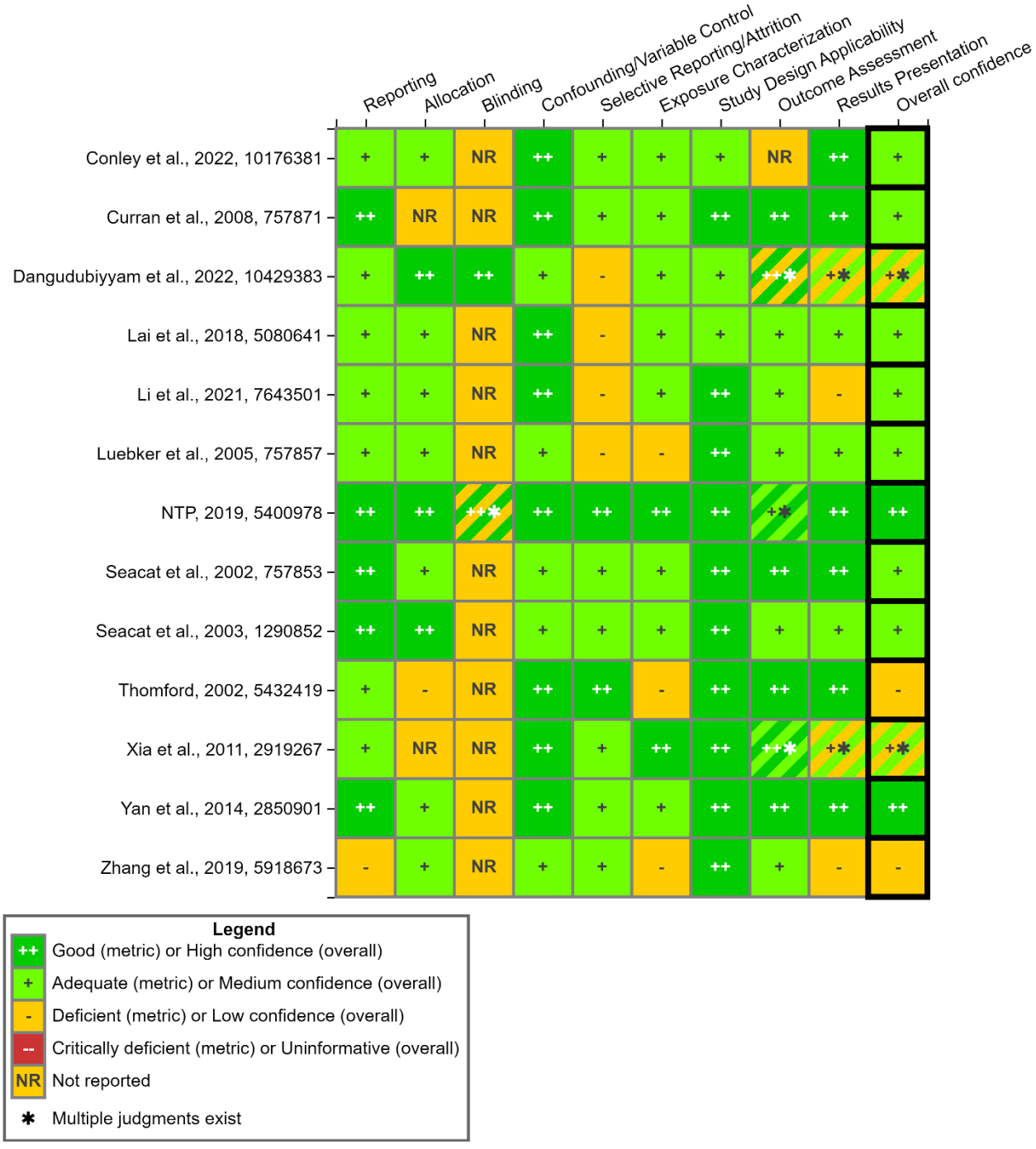


Figure 3-41. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

Cardiovascular effects, including blood pressure, heart weight, heart histopathology, and/or serum lipid levels, following exposure to PFOS were minimal (Dangudubiyyam et al., 2022; Li et al., 2021c; NTP, 2019; Rogers et al., 2014; Xia et al., 2011; Curran et al., 2008). In male and female mice (sexes combined), relative heart weight was increased at PND 21 after gestational

exposure (GD 2–21) to 2 mg/kg/day PFOS; however, this was confounded by decreased body weights. Absolute heart weights were unchanged (Xia et al., 2011). In 10–11-week-old Sprague-Dawley rats exposed daily by gavage for 28 days, a decrease in absolute (14% relative to control animals) and relative (9% relative to control animals) heart weight were reported in females exposed to 5 mg/kg/day while a decrease in absolute (9% relative to control animals) heart weight was reported in male rats exposed to 5 mg/kg/day (NTP, 2019). The authors note that the biological significance of this is not clear. No alterations were observed in the heart following histopathological analysis in either sex. It should be noted that this study design (e.g., 28-day duration) is not sufficient to address whether PFOS exposure leads to injuries in the cardiovascular system like plaque formation in atherosclerosis as this often requires 10–12 weeks for development to accurately be evaluated in a rodent model (Daugherty et al., 2017). H&E staining of tissues extracted from PFOS-exposed female BALB/c mice revealed that exposure (0.1 or 1 mg/kg/day for 2 months) accumulated in the epicardial area of the heart that correlated regionally with inflammatory cell infiltration (results reported qualitatively) (Li et al., 2021c). In female Sprague-Dawley rats exposed to 50 µg/mL PFOS in drinking water from GD 4–20, H&E and Trichrome-Masson staining of the heart revealed a significant increase in ventricular wall thickness as well as a slight increase in the percentage of fibrotic area (approximately 1% in the control animals and 2% in the exposed animals) (Dangudubiyyam et al., 2022).

Curran et al. (2008) measured blood pressure in 35–37-day old Sprague-Dawley rats exposed to PFOS in the diet (doses up to approximately 6.34 mg/kg/day for males and 7.58 mg/kg/day for females) for 28 days; no significant change in blood pressure measurements were observed across the groups, though results were not quantitatively reported. However, in female Sprague-Dawley rats exposed to 0.5–50 µg/mL PFOS in drinking water from GD 4–20, blood pressure was significantly increased at GD 20 (Dangudubiyyam et al., 2022). Adult Sprague-Dawley offspring of dams treated with PFOS (18.75 mg/kg/day) via oral gavage from GD 2–6 had increased blood pressure measurements (Rogers et al., 2014). Male offspring exhibited an 18% and 12% increase in systolic blood pressure at 7 and 52 weeks of age, respectively. Female offspring exhibited a 24% and 19% increase in systolic blood pressure at 37 and 65 weeks of age, respectively; no change in blood pressure was noted at the 7-week timepoint. In male offspring, increased systolic blood pressure was associated with a significantly decreased number of nephrons in the kidney (measurements were taken at PND 22; body weights and kidney weights were not significantly different compared with control animals). Rogers et al. (2014) discussed that the association is a consequence of a higher load on the available nephrons. The higher load results in a cycle of sclerosis and pressure natriuresis that can increase blood pressure. However, the exact mechanisms have yet to be elucidated. In contrast to the results of Rogers et al. (2014), no changes in blood pressure were observed at PND 21 in male and female mice gestationally exposed to 0.2–2 mg/kg/day PFOS (Xia et al., 2011). Heart rate was also unchanged in this study.

PFOS has been observed to cause perturbations in lipid homeostasis, which may have effects on the cardiovascular system. Alterations in serum lipid levels have been observed in non-human primates and rodent models in subchronic, chronic, and developmental studies of oral exposure to PFOS (Figure 3-42). Decreased serum TC, triglycerides, and/or HDL levels occurred in rhesus monkeys (Goldenthal et al., 1979), cynomolgus monkeys (Seacat et al., 2002), rats (Conley et al., 2022; NTP, 2019; Curran et al., 2008; Luebker et al., 2005b; Thibodeaux et al., 2004; Seacat et al., 2003), and mice (Lai et al., 2018; Wang et al., 2014; Yan et al., 2014; Wan et al., 2012;

Bijland et al., 2011) following PFOS exposure. In Sprague-Dawley rats exposed daily by gavage for 28 days, significant decreases in serum TC (males) and triglyceride (females) levels were reported following PFOS exposure as low as 0.312 and 2.5 mg/kg/day, respectively (NTP, 2019). Serum triglyceride levels were significantly decreased in female CD-1 mice exposed daily by gavage to 3 mg/kg/day PFOS for 7 weeks (Lai et al., 2018). One study reported decreased serum HDL levels but an approximate twofold increase in serum LDL levels in male BALB/c mice following exposure to 5 mg/kg/day PFOS by gavage for 28 days (Yan et al., 2014).

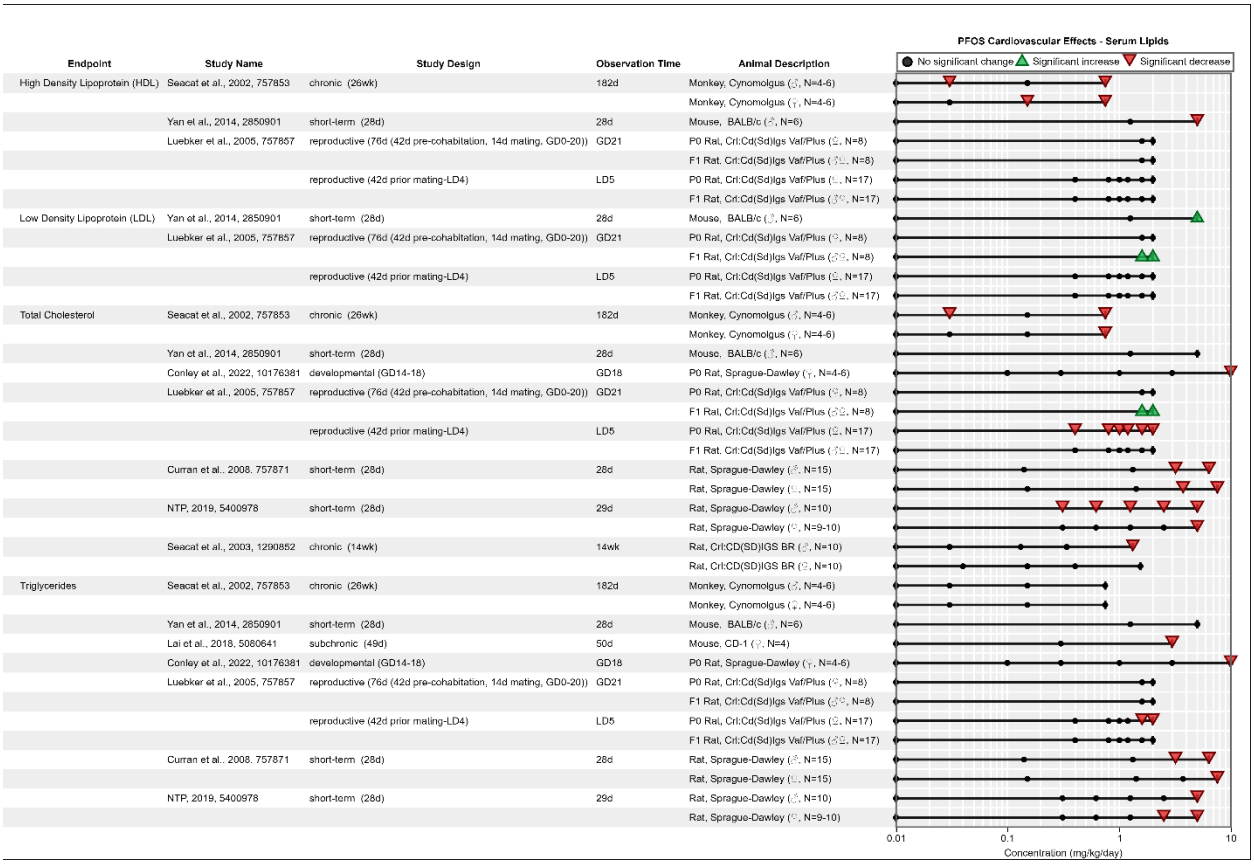


Figure 3-42. Serum Lipid Levels in Animal Models Following Exposure to PFOS

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; PND = postnatal day; PNW = postnatal week; F_1 = first generation.

Conclusions from these studies are limited by differences in serum lipid composition between humans and commonly used rodent models, which may impact the relevance of the results to human exposures (Oppi et al., 2019; Getz and Reardon, 2012). Some rodent studies (Yan et al., 2014) exhibit a biphasic dose response where low exposure concentrations lead to increased serum lipid levels while high-exposure concentrations lead to decreased serum lipid levels. This has called in the validity of using rodent models to predict human lipid outcomes. Additionally, food consumption and food type may confound these results (Cope et al., 2021; Fragki et al., 2021; Schlezinger et al., 2020), as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection and laboratory diets contain a lower fat content

compared with typical Westernized human diets. More research is needed to understand the influence of diet on the response of serum cholesterol levels in rodents treated with PFOS.

3.4.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cardiovascular outcomes is discussed in Section 3.2.6 of the 2016 PFOS HESD (U.S. EPA, 2016b). There are nine studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to cardiovascular effects. A summary of these studies organized by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-43.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	0	1	1	2
Atherogenesis And Clot Formation	1	1	2	4
Cell Growth, Differentiation, Proliferation, Or Viability	0	1	1	2
Cell Signaling Or Signal Transduction	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Inflammation And Immune Response	0	0	2	2
Oxidative Stress	0	2	2	4
Grand Total	2	3	4	9

Figure 3-43. Summary of Mechanistic Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

3.4.3.3.1 Fatty Acid Synthesis, Metabolism, Storage, Transport, and Binding

One study published in 2019 found that in vivo exposure to PFOS significantly upregulated the expression of genes associated with fatty acid metabolism in zebrafish heart tissue (Khazaee et al., 2019). Fatty acid binding proteins are highly expressed in tissues involved in active lipid metabolism, such as the heart and liver, and they act as intracellular lipid chaperones (Nguyen et al., 2020a). In this study, adult male and female zebrafish were exposed to 0.1 or 1 mg/L PFOS for 30 days, and the expression of genes that encode fatty acid binding proteins *fabp1a*, *fabp10a*, and *fabp2* was measured in several tissues (liver, heart, intestine, and ovary) at four timepoints. PFOS upregulated the expression of fatty acid binding proteins *fabp10a* and *fabp2* in the heart tissue of males and females at all timepoints, while *fabp1a* expression was not detected in heart tissue. The authors found that the heart had the most consistent results out of all tissues examined (Khazaee et al., 2019). For additional information on the disruption of fatty acid synthesis, metabolism, transport, and storage in the liver following PFOS exposure, please see Section 3.4.1.3.2.

3.4.3.3.2 Serum Lipid Homeostasis

Epidemiological studies (Section 3.4.3.1) provide consistent evidence that PFOS alters serum lipid levels, demonstrated by significant positive associations between PFOS and TC and LDL cholesterol. The mechanisms underlying these associations have not yet been determined. One study summarized in EPA's 2016 *Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)*(U.S. EPA, 2016b) provides mechanistic evidence related to these outcomes (Fletcher et al., 2013). The authors of this study evaluated a subset of 290 adults in the C8 Health Project for evidence that PFOS can influence the expression of genes involved in cholesterol metabolism, mobilization, or transport measured in whole blood. When both sexes were analyzed together, a positive association was found between PFOS and a gene involved in cholesterol mobilization (Neutral Cholesterol Ester Hydrolase 1 (*NCEH1*)), and a negative relationship was found between PFOS and a transcript involved in cholesterol transport (Nuclear Receptor Subfamily 1, Group H, Member 3 (*NR1H3*)). When males and females were analyzed separately, serum PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport in females (*NCEH1* and *PPARα*), but no associations were found in males. For additional information on the disruption of lipid metabolism, transport, and storage in the liver following PFOS exposure, please see Section 3.4.1.3.2.

3.4.3.3.3 Oxidative Stress, Apoptosis, Inflammation, and Vascular Permeability Leading to Atherogenesis

Epidemiological studies (Section 3.4.3.1) provide consistent evidence for an association between PFOS and blood pressure in some human populations, and limited evidence for an association between PFOS and increased risk of hypertension. The biological mechanisms underlying the association between PFOS and elevated blood pressure are still largely unknown, but pathways that have been proposed include PFOS-induced oxidative stress leading to endothelial dysfunction and impaired vasodilation, intra-uterine exposure leading to reduced number of nephrons at birth, interference with signaling pathways of thyroid hormones that regulate blood pressure, and transcriptional induction of aldosterone (Pitter et al., 2020).

Oxidative damage, inflammation, and increased vascular permeability are all pathways associated with the early stages of atherosclerosis. Atherosclerosis is an inflammatory disease of vessel walls characterized by plaque buildup inside arteries caused by high blood lipid levels and endothelial dysfunction. Atherosclerosis is an established risk factor for cardiovascular diseases including myocardial infarction and stroke (Nguyen et al., 2020a). One epidemiological study found no significant associations between PFOS and carotid artery atherosclerotic plaque or CIMT (Lind et al., 2017b), but two other studies found significant associations between PFOS and CIMT (Lin et al., 2016; Lin et al., 2013).

3.4.3.3.4 Endothelial Dysfunction

3.4.3.3.4.1 In Vivo Evidence

A cross-sectional study in adolescents and young adults in Taiwan (1992–2000) studied the associations between serum PFOS, CIMT, circulating endothelial and platelet microparticles, and urinary 8-hydroxydeoxyguanosine (8-OHdG) (Lin et al., 2016). CIMT is a measure used to diagnose the extent of carotid atherosclerotic vascular disease. Cluster of differentiation 31 (CD31), also known as platelet endothelial cell adhesion molecule (PECAM-1), is a protein involved in cell-to-cell adhesion. CD42 is a protein expressed on the surface of platelets that is

involved in platelet adhesion and plug formation at sites of vascular injury. This study evaluated serum CD31+/CD42a- as a marker of endothelial apoptosis and serum CD31+/CD42a+ as a marker of platelet apoptosis. The results showed that both markers of apoptosis increased significantly across quartiles of PFOS exposure. No significant associations were found between PFOS and CD62E, a marker of endothelial activation, or between PFOS and CD62P, a marker of platelet activation. In addition, no significant associations were found between serum PFOS and urinary 8-OhdG, a marker of DNA oxidative stress. The authors observed a positive association between PFOS and CIMT that was stronger when serum markers of endothelial and platelet apoptosis were higher. The adjusted odds ratio (OR) for CIMT with PFOS was 2.86 (95% CI: 1.69, 4.84), $p < 0.001$) when the levels of CD31+/CD42a- and CD31+/CD42a+ were both above 50%, compared with the OR of 1.72 (95% CI: 0.84, 3.53, $P = 0.138$) when both apoptosis markers were below 50%. The authors postulated that PFOS may play a role in atherosclerosis by inducing apoptosis of endothelial and platelet cells (Lin et al., 2016).

Another cross-sectional study in Taiwanese adults (2009–2011) evaluated the associations between serum PFOS and urinary 8-OhdG and 8-nitroguanine (8-NO2Gua) as biomarkers of DNA oxidative and nitrative stress (Lin et al., 2020a); however, unlike Lin et al. (2016), this study found significant associations between PFOS and biomarkers of oxidative DNA damage. Linear PFOS levels were positively associated with adjusted levels of 8-OhdG and 8-NO2Gua, while no association was found for branched PFOS levels. The authors also evaluated the associations between PFOS and serum lipid profiles (LDL, small dense LDL, HDL, triglycerides), and found that the adjusted OR for elevated LDL (>75th percentile) with linear PFOS was higher when each DNA stress marker was above 50% compared with below 50% (OR 3.15, 95% CI: 1.45, 6.64, $p = 0.003$ for both stress markers above 50% vs. OR 1.33, 95% CI: 0.78, 2.27, $p = 0.302$ for both stress markers below 50%). Linear PFOS levels were also positively correlated with HDL, but the relationship with stress markers was not studied.

3.4.3.3.4.2 In Vitro Evidence

Liao et al. (2013) found that expression of peroxisome proliferator-activated receptor gamma (*PPARγ)* and estrogen receptor alpha (*Erα*) were significantly upregulated in human umbilical vein endothelial cells (HUVECs) exposed to PFOS (100 mg/L) for 48 hours. PFOS exposure also significantly upregulated expression of six inflammatory response-related genes (interleukin-1-beta (*IL-1β*), interkeukin-6 (*IL-6*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) also known as COX2, nitric oxide synthase 3 (*NOS3*), *P-Selectin*, and intracellular adhesion molecule 1 (*ICAM1*)) and increased the generation of intracellular reactive oxygen species (ROS) in HUVECs. In addition, adhesion of monocytes onto HUVECs was increased 2.1-fold over the control when the cells were treated with PFOS (100 mg/L) for 48 hours. The authors postulated that the PFOS-induced inflammatory response in this in vitro system was mediated by *PPARγ*, *Erα*, and ROS, and that PFOS upregulation of *ICAM1* and *P-Selectin* may play an important role in adhesion of monocytes to vascular epithelium leading to vascular inflammation.

Similarly, Qian et al. (2010) found that PFOS-induced ROS production in human microvascular endothelial cells (HMVECs) even at low concentrations (2–5 μM) within one hour. These authors also studied permeability changes in HMVEC monolayers following PFOS exposure by measuring transendothelial electrical resistance. The results showed that PFOS induced endothelial permeability in a concentration-dependent manner. Confocal microscopy imaging

analysis revealed many gaps in the PFOS-treated HMVEC monolayers that increased in a concentration-dependent manner. PFOS also induced actin filament remodeling. Pretreating HMVEC monolayers with catalase, a ROS scavenger, prior to PFOS exposure substantially blocked the PFOS-induced gap formation and actin filament remodeling.

Two studies evaluated the potential for PFOS and other PFAS to activate the plasma kallikrein-kinin system (KKS) using in vitro and *ex vivo* activation assays and in silico molecular docking analysis (Liu et al., 2018e; Liu et al., 2017a). The plasma KKS plays important roles in regulating inflammation, blood pressure, coagulation, and vascular permeability. Activation of the plasma KKS can release the inflammatory peptide, bradykinin (BK), which can lead to dysfunction of vascular permeability (Liu et al., 2018e). The cascade activation of KKS involves autoactivation of Hageman factor XII (FXII), cleavage of plasma prekallikrein (PPK), and activation of high-molecular-weight kininogen (HK) (Liu et al., 2018e). These studies examined the potential for PFOS and other PFAS chemicals to act as FXII activators due to their structural similarities to natural long-chain fatty acids (Liu et al., 2017a). The addition of PFOS (1–5 mM) to mouse plasma *ex vivo* resulted in dose-dependent PPK activation measured by analysis of PPK and plasma kallikrein expression levels after 2 hours of incubation, and the approximate lowest-observed-effect concentration (LOEC) for PFOS was 3 mM (Liu et al., 2017a). This demonstrated the potential for PFOS to activate the plasma KKS, but at a relatively high concentration compared with typical human exposure levels in the general population. PFAS with longer carbon chain lengths activated the KKS at a much lower concentration compared with PFOS (e.g., PFHxDA activated the KKS at 30 μM). Time-course experiments showed that PPK activation occurred within 5 min after addition of PFOS or other PFAS to mouse plasma (Liu et al., 2017a).

The potential effects of PFOS on KKS activation in mouse plasma *ex vivo* were also evaluated using protease activity assays. Plasma samples were incubated with PFOS (100–5,000 μM) for 15 minutes and then analyzed for FXIIa activity and kallikrein-like activity. PFOS significantly increased FXIIa activity only at the highest concentration tested (5 mM) Liu et al. (2018e), and kallikrein-like activity was significantly increased only at 3 and 5 mM PFOS (Liu et al., 2018e; Liu et al., 2017a). Western blot analyses demonstrated that 5 mM PFOS could induce the KKS waterfall cascade activation both in vitro, utilizing human plasma zymogens FXII, PPK, and HK, and *ex vivo* utilizing plasma from human volunteers (Liu et al., 2017a).

Binding of PFOS with purified human FXII was further evaluated by Liu et al. (2017a) using native PAGE separation and FXII Western blot assay. Two hours of incubation of FXII with PFOS (1 or 3 mM) reduced the amount of free FXII in a concentration-related manner. The results from *ex vivo*, in vitro, and in silico experiments were compared for different PFAS, and the authors concluded that the degree of KKS activation was related to structural properties such as carbon chain length, terminal groups, and fluorine atom substitution. For example, PFAS terminated with sulfonic acid, including PFOS, demonstrated a stronger binding affinity for FXII and higher capability of inducing KKS activation than PFAS terminated with carboxylic acid or other terminal groups. (Liu et al., 2017a).

3.4.3.3.5 Coagulation and Fibrinolysis

The coagulation and fibrinolytic pathways can contribute to the progression of atherosclerosis. Two studies from the literature published after the 2016 PFOS HESD evaluated the potential of

PFOS to affect these pathways. Bassler et al. (2019) evaluated a subset of 200 individuals from the C8 Health Project for a variety of disease biomarkers including plasminogen activator inhibitor (PAI-1), a glycoprotein that inhibits the formation of plasmin from plasminogen and thus prevents clot lysis in vessel walls. Elevated PAI-1 levels are associated with thrombotic risk, but this study found no significant association between PFOS and PAI-1 levels. Likewise, Chang et al. (2017) saw no significant changes in coagulation parameters measured in male and female cynomolgus monkeys following acute oral exposure to PFOS with serum concentrations up to 165 μg/mL, including measures of prothrombin time, activated partial thromboplastin time, and fibrinogen.

3.4.3.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and cardiovascular effects in humans based on consistent positive associations with serum lipid levels, specifically TC and LDL. Additional evidence of positive associations with blood pressure and hypertension in adults supported this classification. The available data for CVD and atherosclerotic changes was limited and addressed a wider range of outcomes, resulting in some residual uncertainty for the association between PFOS exposure and these outcomes.

On the basis of this systematic review of epidemiologic studies, the available evidence supports a positive association between PFOS and TC in the general population, including children and pregnant women. The available evidence also generally supports a positive association between PFOS and LDL in children and adults in the general population. Although PFOS appeared not to be associated with elevated TC and LDL in workers, this conclusion is uncertain as the occupational studies included in this review are limited in both quantity and quality. Finally, for all populations, the association between PFOS and HDL and TG were mixed, suggesting no consistent associations between PFOS and reduced HDL and elevated TG. Overall, these findings are largely consistent with the 2016 PFOS HESD. The positive associations with TC are also supported by the recent meta-analysis restricted to general population studies in adults (U.S. EPA, 2024b). Similarly, a recent meta-analysis including data from 11 studies reported consistent associations between serum PFOS or a combination of several PFCs including PFOA and PFOS, and increased serum TC, LDL, triglyceride levels in children and adults (Abdullah Soheimi et al., 2021).

The human epidemiological studies identified since the 2016 PFOS HESDs provided additional clarity regarding the association between PFOS and CVD outcomes. Most of the CVD-related evidence identified focused on blood pressure in general adult populations (12 studies). The findings from one *high* confidence study and five *medium* confidence studies provide evidence for a positive association between PFOS and blood pressure, although the results were not always consistent between SBP and DBP, and one study reported an inverse association. The limited evidence for an association between PFOS and increased risk of hypertension was inconsistent. There was evidence suggesting an increased risk of hypertension among women, but additional studies are needed to confirm this finding. One *high* confidence study in women with PFOS measured during pregnancy reported a positive association with blood pressure assessed at 3 years postpartum. Evidence in children and adolescents is also less consistent. The six studies available among children and adolescents suggest PFOS was not associated with elevated blood pressure. Evidence for other CVD-related outcomes across all study populations

was more limited and inconsistent. The limited evidence for CVD outcomes discussed in the 2016 PFOS HESD also indicated association with blood pressure in children.

The animal evidence for an association between PFOS exposure and cardiovascular toxicity is *moderate* based on serum lipids effects observed in eight *high* or *medium* confidence studies. The most consistent results are for total cholesterol and triglycerides, although direction of effect can vary by dose. In animal toxicological studies, no effects or minimal alterations were noted for blood pressure, heart weight, and histopathology of the heart. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism.

The mechanisms underlying the positive associations between PFOS and serum TC, LDL, and blood pressure in humans have yet to be determined. Data from the C8 Health Project demonstrated that serum PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport (NCEH1 and PPARα) in samples from women, while there were no associations in men. The results for PFOS-induced changes in serum lipid levels contrast between rodents (generally decreased) and humans (generally increased). PFOS exposure led to upregulation of genes that encode fatty acid binding proteins in zebrafish, which play a role in lipid binding, particularly in the heart. Evidence is ultimately limited in regard to clear demonstration of mechanisms of alterations to serum lipid homeostasis caused by PFOS exposure.

Regarding the potential for PFOS to lead to atherosclerosis as evidenced by related mechanisms or mechanistic indicators, one epidemiologic study found no association between PFOS and carotid artery atherosclerotic plaque or CIMT, while two other epidemiologic studies found significant associations between PFOS and CIMT. The two studies that reported PFOS-associated CIMT demonstrated endothelial dysfunction via increases in markers of endothelial and platelet apoptosis in the serum: increased serum CD31+/CD42a-, which is a marker of endothelial apoptosis, and increased serum CD31+/CD42a+, which is a marker of platelet apoptosis. Markers of serum and platelet activation were not changed, nor was there evidence of DNA oxidative damage (no change in urinary 8-OhdG). The authors of the study postulated that PFOS-induced apoptosis of endothelial and platelet cells may play a role in the development of atherosclerosis. In contrast, another human study reported increased urinary 8-OhdG and 8-nitroguanine (8-NO2Gua) resulting in limited and inconsistent results for oxidative damaging potential of PFOS. In vitro, PFOS was shown to induce oxidative stress and upregulate inflammatory response genes in human umbilical vein endothelial cells. The authors concluded that oxidative stress and changes in the expression of genes involved in adhesion of monocytes to vascular epithelium may lead to vascular inflammation. Binding of PFOS to human FXII was demonstrated, which is the initial zymogen of plasma kallikrein-kinin system (KKS) activation, an important regulator of inflammation, blood pressure, coagulation, and vascular permeability. The authors attributed the degree of KKS activation to structural properties of PFOS (among other PFAS). There was no association between PFOS and disease biomarkers related to clotting and coagulation in both human and non-human primate data. While there is mechanistic evidence that PFOS exposure can lead to molecular and cellular changes that are related to atherosclerosis, human studies identified herein reported a lack of an association between PFOS

exposure and markers of atherosclerosis. Thus, the relevance of these mechanistic data is unclear.

3.4.3.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the ***evidence indicates*** that PFOS exposure is likely to cause adverse cardiovascular effects, specifically serum lipids effects, in humans under relevant exposure circumstances (Table 3-15). The hazard judgment is driven primarily by consistent evidence of serum lipids response from epidemiological studies at median PFOS levels between 3.7–36.1 ng/mL (range of median exposure in studies observing an adverse effect). The evidence in animals showed coherent results for perturbations in lipid homeostasis in non-human primates and rodent models in developmental, subchronic, and chronic studies following exposure to doses as low as 0.03 mg/kg/day PFOS. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence studies.

Table 3-15. Evidence Profile Table for PFOS Exposure and Cardiovascular Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.3.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Serum lipids 2 *High* confidence studies 28 *Medium* confidence studies 21 *Low* confidence studies 12 *Mixed*[a] studies	Examination of serum lipids included measures of TC, LDL, HDL, TG, and VLDL. In studies of serum lipids in adults from the general population (33), there is evidence of positive associations with TC (13/15) in the *medium* confidence studies. Positive associations were also observed for LDL (9/11) *medium* confidence studies. Results for HDL and TG were mixed, with some positive associations for HDL (8/14) and some inverse associations for TG (8/13) in *medium* confidence studies. Evidence from studies of children (21), reported significant increases in TC (7/16) and LDL (7/16), though others observed no association. While some studies observed significantly increased HDL (3/17), others reported significant decreases or no associations. Studies examining pregnant	• *High* and *medium* confidence studies • *Consistent* findings of positive associations for LDL and TC across study populations • *Coherence* of findings across serum lipids	• *Low* confidence occupational studies	⊕⊕⊙ *Moderate* Evidence for cardiovascular effects is based on numerous *medium* confidence studies reporting positive associations with serum lipids (LDL and TC) in adults from the general population. Studies of children reported mixed findings in most serum lipids, but results were largely consistent for LDL and TC, with some reaching significance. However, interpretations of changes in serum lipids for children are less clear. *High* and *medium* confidence studies reported positive associations with blood pressure and increased risk of hypertension. *Low* confidence studies reported nonsignificant associations, while most *mixed* confidence studies reported significant associations. Observed	*Primary basis and cross-stream coherence*: Human evidence indicated consistent evidence of serum lipids response and animal evidence showed coherent results for perturbations in lipid homeostasis in non-human primates and rodent models in developmental, subchronic, and chronic studies following exposure to PFOS. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence studies. *Human relevance and other inferences:* No specific factors are noted.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	women were of *medium* and *mixed* confidence and reported mixed results (6). While three studies reported evidence of increased HDL and TC levels, the others failed to reach significance or reported inverse associations. Most occupational studies (5) were considered *low* confidence (4/5), and no association was observed for TC or HDL-C in the single *medium* confidence occupational study.			effects were inconsistent for CVD and imprecise for atherosclerotic changes across all study populations.	
Blood pressure and hypertension 2 *High* confidence studies 17 *Medium* confidence studies 7 *Low* confidence studies	Studies examining changes in blood pressure, including DBP and SBP, and risk of hypertension in general population adults showed consistent positive associations with increased risk of hypertension (4/7), positive associations for SBP (7/9) and DBP (7/8), including four *medium* or *high* confidence studies reporting significant increases (4/6). Studies in children (10) reported mostly nonsignificant associations with blood pressure and/or	• *High* and *medium* confidence studies	• *Inconsistent findings* in children, likely due to variation in measured exposure windows		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	hypertension, though one study in adolescents reported significantly increased DBP (1/10) and another reported decreased (1/10) SBP. No studies examined blood pressure or hypertension in occupational populations.				
Cardiovascular disease 1 *High* confidence study 4 *Medium* confidence studies 5 *Low* confidence studies	In adults from the general population (8), significantly decreased odds of stroke (1/2) and significantly increased odds of MVD (1/1), heart attack (1/1), and CVD in the third exposure group (1/4), were observed. Other studies of stroke, CHD, and CVD reported nonsignificant associations, including one *high* confidence study that reported no associations with CHD among Swedish men and a *medium* confidence study that reported no association with mortality from CVD or other heart diseases. One *low* confidence occupational study reported a significant inverse relationship between employees in high-	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *Inconsistent findings* for CVD-related outcomes • *Imprecision* of findings, particularly for two studies with self-reported outcome measures		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	exposure jobs and all heart disease mortality (1/2).				
Atherosclerotic changes 1 *High* confidence study 4 *Medium* confidence studies 3 *Low* confidence studies	In studies of children and young adults (3), two studies observed significant associations with CIMT across exposure groups (2/3), among females, and among those ages 12–19. In studies of adults from the general population (5), two focused on adults older than 70 years of age. One study reported a significant increase in left ventricular end-diastolic diameter and a significant decrease in relative wall thickness (1/2). One *medium* confidence study in prediabetic adults aged over 25 also reported significantly increased odds of an Agatatson Scores over 400, a measure of arterial calcification (1/1).	• *High* and *medium* confidence studies	• *Imprecision* of findings across children and adult study populations • *Limited number* of studies examining specific outcomes		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.3.2)					
Serum lipids 2 *High* confidence studies 6 *Medium* confidence studies	Significant decreases in serum TG were observed in 5/7 studies that examined this endpoint, regardless of species, sex, or study design. No	• *High* and *medium* confidence studies • *Consistency* of findings across species, sex, or study design	• *Incoherence* of findings in other cardiovascular outcomes •	⊕⊕⊙ *Moderate* Evidence based on eight *high* or *medium* confidence studies	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	changes were observed in one monkey study and one short-term study in male mice. Similar decreases were observed in serum TC (6/7), with no changes being observed in one short-term study in male mice. In a developmental study, decreases were observed in dams, but no change was observed in pups. Fewer studies examined HDL and LDL, with decreases in HDL (2/3) and increases in LDL (2/2) being observed.	• *Dose-response* relationship observed within multiple studies	• *Biological significance* of the magnitude of effect is unclear	observed that PFOS affects serum lipids in animal models. The most consistent results are for total cholesterol and triglycerides, although direction of effect can vary by dose. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism. No effects or minimal alterations were noted for blood pressure, heart weight, and histopathology in the heart. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system.	
Histopathology 1 *High* confidence study 2 *Medium* confidence studies	No changes in heart histopathology were reported in 2 rat studies. One study in female mice qualitatively reported an increase in inflammatory cell infiltration.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome		
Organ weight 1 *High* confidence study, 2 *Medium* confidence studies	Mixed results were reported for absolute and relative heart weight. Two short-term studies reported decreases in absolute heart weights in male and female rats, but mixed results (no change or decreases) were reported for relative heart weights. A developmental study reported no change in	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Confounding* variables such as decreases in body weights may limit ability to interpret these responses		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	absolute heart weight and an increase in relative heart weight which was confounded by decreases in body weights.				
Blood pressure and heart rate 3 *Medium* confidence studies	A developmental study found increased blood pressure in dams. A short-term study found no effect on blood pressure in male and female rats. One developmental study found no effect on heart rate.	• *Medium* confidence studies	• *Limited number* of studies examining outcome		

Mechanistic Evidence and Supplemental Information (Section 3.4.3.3)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
Key findings and interpretation: • PFOS exposure was associated with changes in the expression of genes involved in cholesterol metabolism, mobilization, or transport in whole blood of adult humans. • PFOS induced oxidative stress and upregulated inflammatory response genes in human umbilical vein endothelial cells exposed in vitro, which can lead to vascular inflammation. • PFOS can bind to human FXII in vitro, which is the initial zymogen of plasma KKS activation, a regulator of inflammation, blood pressure, coagulation, and vascular permeability. **Limitations:** • Small database; the only in vivo evidence is reported in two human studies with conflicting results for markers of platelet activation. • Results regarding the association between PFOS exposure and carotid artery atherosclerotic plaques or CIMT, which are mechanisms of atherosclerosis, are inconsistent in human epidemiological studies.	Findings support the plausibility that PFOS exposure can lead to changes in the expression of genes involved in cholesterol regulation, as well as molecular and cellular changes that are related to atherosclerosis, although no association was observed between PFOS exposure and atherosclerosis in human epidemiological studies.

Notes: CHD = coronary heart disease; CIMT = carotid intima-media thickness; CVD = cardiovascular disease; DBP = diastolic blood pressure; FXII = Factor XII; HDL = high-density lipoprotein; KKS = kallikrein-kinin system; LDL = low-density lipoprotein; density lipoprotein; SBP = systolic blood pressure; MVD = microvascular disease; TC = total cholesterol; TG = triglycerides.

[a] *Mixed* confidence studies had split confidence determinations for different serum lipid measures with some measures rated *medium* confidence and others rated *low* confidence.

3.4.4 Developmental

EPA identified 96 epidemiological and 20 animal toxicological studies that investigated the association between PFOS and developmental effects. Of the epidemiological studies, 28 were classified as *high* confidence, 37 as *medium* confidence, 20 as *low* confidence, 3 as *mixed* (2 *high/medium* and 1 *medium/low*) confidence, and 8 were considered *uninformative* (Section 3.4.4.1). Of the animal toxicological studies, 15 were classified as *medium* confidence, 4 as *low* confidence, and 1 was considered *mixed* (*medium/uninformative*) (Section 3.4.4.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.4.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.4.1.1 Introduction

This section describes studies of PFOS exposure and potential in utero and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. Developmental endpoints include gestational age, measures of fetal growth (e.g., birth weight), and miscarriage, as well as infant/child development.

3.4.4.1.2 Study Evaluation Considerations

There were multiple outcome-specific considerations that informed domain-specific ratings and overall study confidence. For the Confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Some hemodynamic factors related to physiological changes during pregnancy were also considered in this domain as potential confounders (e.g., glomerular filtration rate and blood volume changes over the course of pregnancy), because these factors may be related to both PFOS levels and the developmental effects examined here. More confidence was placed in the epidemiologic studies that adjusted for glomerular filtration rate in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, this was considered in the study quality evaluations and as part of the overall weight of evidence determination. Further discussion of considerations for potential confounding by co-occurring PFAS can be found in Section 5.1.1.

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFOS in humans as described in Section 3.3, samples collected during all three trimesters, before birth or and shortly after birth) were considered adequately representative of the most critical in utero exposures for fetal growth and gestational duration measures. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where in utero perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in (De Boo and Harding, 2006) and (Perng et al., 2016)). There is some evidence that birth weight deficits can be followed by increased weight gain that may occur especially among those with rapid growth catchup periods during childhood (Perng et al., 2016).

Therefore, the primary critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero for study evaluation purposes, and studies of this outcome were downgraded in the exposure domain if exposure data were collected later during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (e.g., for sex-stratified results). In the Outcome domain, specific considerations address validation and accuracy of specific endpoints and adequacy of case ascertainment for some dichotomous (i.e., binary) outcomes. For example, birthweight measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias toward the null if ascertainment of fetal loss is not associated with PFOS exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null and can manifest as an apparent protective effect. Fetal and childhood growth restriction were examined using several endpoints including low birth weight, small for gestational age (SGA), ponderal index (i.e., birth weight grams/birth length (cm^3) × 100), abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher quality endpoints (i.e., classified as good in the Outcome domain) that were available in multiple studies to allow for an evaluation of consistency and other considerations across studies. However, even when databases were more limited, such as for spontaneous abortions, the evidence was evaluated for its ability to inform developmental toxicity more broadly, even if available in only one study.

Overall, mean birth weight and birth weight-related measures are considered very accurate and were collected predominately from medical records; therefore, more confidence was placed in these endpoints in the Outcome domain judgments. Some of the adverse endpoints of interest examined here included fetal growth restriction endpoints based on birth weight such as mean birth weight (or variations of this endpoint such as standardized birthweight z-scores), as well as binary measures such as SGA (e.g., lowest decile of birthweight stratified by gestational age and other covariates) and low birth weight (i.e., typically <2500 grams; 5 pounds, 8 ounces) births. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary to be classified as good for these endpoints in this domain. In contrast, other measures of fetal growth that are subject to more measurement error (e.g., head circumference and body length measures such as ponderal index) were given a rating of adequate (Shinwell and Shlomo, 2003). These sources of measurement error are expected to be non-differential with respect to PFOS exposure status and, therefore, would not typically be a major concern for risk of bias but could impact study sensitivity.

Gestational duration measures were presented as either continuous (i.e., per each gestational week) or binary endpoints such as preterm birth (typically defined as gestational age <37 weeks).

Although changes in mean gestational age may lack some sensitivity, especially given the potential for measurement error, many of the studies were based on ultrasound measures early in pregnancy, which should increase the accuracy of estimated gestational age and the ability to detect associations that may be present. Any sources of error in the classification of these endpoints would also be anticipated to be non-differential with respect to PFOS exposure. While they could impact precision and study sensitivity, they were not be considered a major concern for risk of bias.

3.4.4.1.3 Summary of Evidence From the 2016 PFOS HESD

The 2016 PFOS HESD (U.S. EPA, 2016b) summarized epidemiological studies of developmental effects in relation to PFOS exposure. There are 18 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and developmental effects. Study quality evaluations for these 18 studies are shown in Figure 3-44. Studies included those conducted both in the general population as well as in communities known to have experienced relatively high PFAS exposure (e.g., the C8 population in West Virginia and Ohio). Results from studies summarized in the 2016 PFOS HESD are described in Table 3-16 and below.

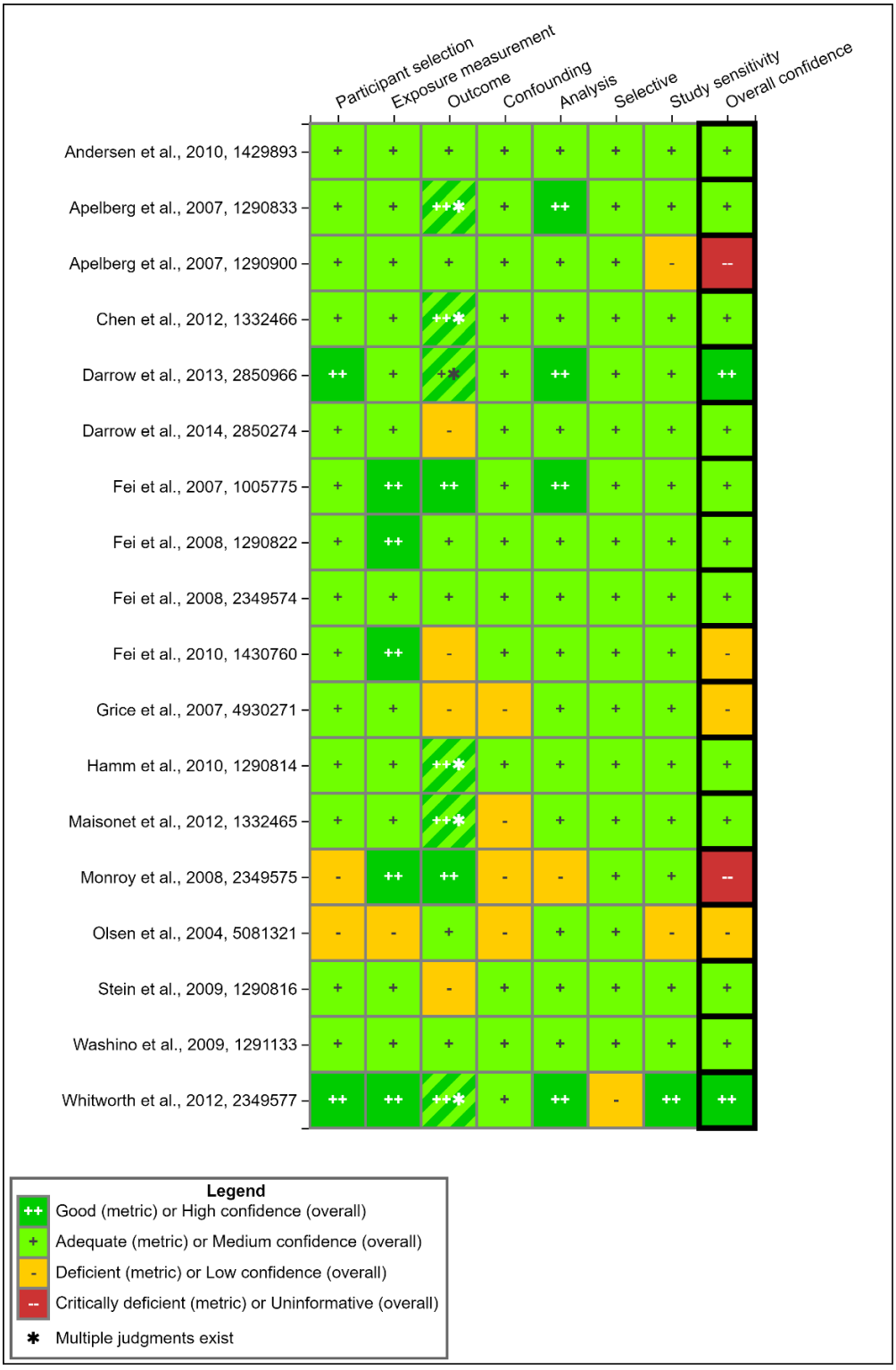


Figure 3-44. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Developmental Effects Published before 2016 (References from 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

As noted in the 2016 PFOS HESD, several available studies measured fetal growth outcomes. Apelberg et al. (2007b) found that birth weight, head circumference, and ponderal index were inversely associated with umbilical cord PFOS concentration in 293 infants born in Maryland in 2004–2005. In particular, large deficits in mean birth weight per one ln-unit increase in PFOS concentration were found (β = −69; 95% CI: −149, 10; PFOS was detected in >99% of samples at a mean concentration of 0.005 μg/mL). Maisonet et al. (2012) evaluated fetal growth outcomes in 395 singleton female births of participants in the Avon Longitudinal Study of Parents and Children (ALSPAC) and found that increased maternal PFOS concentration (median concentration of 0.0196 μg/mL) was associated with reduced birth weights, but not with lower 20-month body weights. A study of 252 pregnant women in Alberta, Canada found no statistically significant association between birth weight or gestation length and PFOS concentration measured in maternal blood during the second trimester (mean concentration of 0.009 μg/mL) (Hamm et al., 2010), although mean birth weight increased slightly by increasing PFOS tertiles (3,278 g for <0.006 μg/mL; 3,380 g for 0.006–0.010 μg/mL; 3,387 g for >0.010–0.035 μg/mL). In a prospective cohort study in Japan (2002–2005), Washino et al. (2009) found an inverse association between PFOS concentration in maternal blood during pregnancy (mean PFOS concentration of 0.006 μg/mL) and birth weight. As noted in the 2016 PFOS HESD, these researchers reported large reductions in mean birth weight (β = −149; 95% CI: −297.0, −0.5 g) for each log-10 change in maternal PFOS concentration, especially among female infants (β = −269.4; 95% CI: −465.7, −73.0 g). Chen et al. (2012a) examined 429 mother-infant pairs from the Taiwan Birth Panel Study and found that umbilical cord blood PFOS concentration (geometric mean of 5.94 ng/mL) was inversely associated with gestational age (β = −0.37, 95% CI: −0.60, −0.13, weeks), birth weight (β = −110.2, 95% CI: −176.0, −44.5, g), and head circumference (β = −0.25, 95% CI: −0.46, −0.05, cm). Additionally, ORs for preterm birth, low birth weight, and small for gestational age increased with PFOS exposure (adjusted OR (95% CI) = 2.45 (1.47, 4.08), 2.61 (0.85, 8.03) and 2.27 (1.25, 4.15), respectively).

Some studies evaluated fetal growth parameters in the prospective Danish National Birth Cohort (DNBC; 1996–2002) (Andersen et al., 2010; Fei et al., 2008b, 2007). Maternal blood samples were taken in the first and second trimester. The median maternal plasma PFOS concentration was 0.0334 μg/mL (range of 0.0064–0.1067 μg/mL). Fei et al. (2007) found no associations between maternal PFOS concentration (blood samples taken in the first and second trimester) and birth weight. Also, these researchers found that ORs for preterm birth (OR range: 1.43–2.94) were consistent in magnitude across the upper three PFOS quartiles, and that ORs for low birth weight (OR range: 3.39–6.00) were consistently elevated across the upper three quartiles. The 2016 PFOS HESD notes, however, that analyses in this study were limited by small cell sizes due to low incidence of these outcomes. Fei et al. (2008b) found an inverse association between maternal PFOS levels and birth length and ponderal index in the DNBC in a stratified analysis, but the associations were not statistically significant. Andersen et al. (2010) examined the association between maternal PFOS concentrations and birth weight, birth length, and infant body mass index (BMI) and body weight at 5 and 12 months of age in DNBC participants. They found an inverse association between PFOS concentration and birth weight in girls (β = −3.2; 95% CI: −6.0, −0.3), 12-month body weight in boys (β = −9; 95% CI: −15.9, −2.2), and 12-month BMI in boys (β = −0.017; 95% CI: −0.028, −0.005).

Some studies described in the 2016 PFOS HESD evaluated developmental outcomes in the C8 Health Project study population, which comprises a community known to have been subjected to

high PFAS exposure. The C8 Health Project included pregnancies within 5 years prior to exposure measurement, and many of the women may not have been pregnant at the time of exposure measurement. Stein et al. (2009) found an association between maternal PFOS concentration and increased risk of low birth weight (adjusted OR = 1.5; 95% CI: 1.1,1.9; dose-related relationship for the 50th–75th, 75th–90th and >90th percentile PFOS exposure concentrations), but not pre-term birth. Mean PFOS serum concentration was 0.014 µg/mL. Darrow et al. (2013) evaluated birth outcomes in 1,630 singleton live births from 1,330 women in this study population and found an inverse association between maternal PFOS concentration and birth weight (−29 g per log unit increase; 95% CI: –66, –7); they found no association with preterm birth or low birth weight. Darrow et al. (2014) and Stein et al. (2009) found no association between maternal serum PFOS and increased risk for miscarriage in this population.

Table 3-16. Associations Between Elevated Exposure to PFOS and Developmental Outcomes in Children From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Andersen, 2010, 1429893[c] *Medium*	Cohort	↓	NA	NA	NA	NA	NA	NA	↓↓
Apelberg, 2007, 1290833 *Medium*	Cross-sectional	↓	NA	NA	↑	NA	NA	NA	NA
Chen, 2012, 1332466[c] *Medium*	Cohort	↓↓	↑	↑↑	↓↓	↑↑	NA	NA	NA
Darrow, 2014, 2850274 *Medium*	Cohort	NA	NA	NA	NA	NA	NA	↑	NA
Darrow, 2013, 2850966 *High*	Cohort	↓	↑	NA	NA	–	NA	NA	NA
Fei, 2007, 1005775[d] *Medium*	Cohort	↓	↑	–	NA	↑	NA	NA	NA
Grice, 2007, 4930271[e] *Low*	Cohort	–	NA	NA	NA	NA	NA	–	NA
Hamm, 2010, 1290814 *Medium*	Cohort	–	NA	–	–	↑	NA	NA	NA
Maisonet, 2012, 1332465 *Medium*	Cohort	↓↓	NA	NA	–	NA	NA	NA	↑
Olsen, 2004, 5081321 *Low*	Cross-sectional	NA	NA	NA	NA	↑	–	NA	NA
Stein, 2009, 1290816 *Medium*	Cohort	NA	↑↑	NA	NA	↑	↑	–	NA
Washino, 2009, 1291133[f] *Medium*	Cohort	↓↓	NA	NA	NA	NA	NA	NA	NA

Reference, confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Whitworth, 2012, 2349577 *High*	Cohort	↓	NA	↑	NA	↓	NA	NA	NA

Notes: LBW = low birth weight; NA = no analysis was for this outcome was performed; PNG = postnatal growth; SGA = small-for-gestational age;↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Apelberg et al. (2007a) and Monroy et al. (2008) were not included in the table due to their *uninformative* overall study confidence ratings. Fei et al. (2008a), Fei et al. (2008b), and Fei et al. (2010a) were not included in the table because the studies only analyzed other developmental outcomes that were more prone to measurement error (see Study Evaluation Considerations in Section 3.4.4.1.2) or were not as heavily studied (i.e., other measures of fetal growth restriction such as birth length and head circumference and breastfeeding duration or developmental milestones, respectively).

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Chen, 2012, 1332466 reports results from a population overlapping with Chen et al. (2017b), which was considered the most updated data.

[d] Fei, 2007, 1005775 reports results from a population overlapping with Meng et al. (2018), which was considered the most updated data.

[e] Grice, 2007, 4930271 reported results from children born to women in an occupational cohort.

[f] Washino et al. (2009) reports results from a population overlapping with Kashino et al. (2020), which was considered the most updated data.

3.4.4.1.4 Study Inclusion For Updated Literature Searches

There are 78 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and developmental effects. Although every study is included in the study evaluation heat maps for comprehensiveness, eight developmental epidemiological studies identified in the literature search were excluded for consideration in this synthesis because other studies report results for the same health outcomes and from the same study cohorts (i.e., were considered duplicative). More specifically, the Rokoff et al. (2018) study overlapped with the Project Viva study by Sagiv et al. (2018). The Gennings et al. (2020) study is also not further considered here as it is a smaller subset of the Aarhus Birth Cohort described in Wikström et al. (2020). Similarly, the Li et al. (2017) Guangzhou Birth Cohort Study overlapped with a more recent study by Chu et al. (2020). Four studies (Kobayashi et al., 2022; Kobayashi et al., 2017; Minatoya et al., 2017; Kishi et al., 2015) were also not considered in this synthesis, because they provided overlapping data from the same Hokkaido Study on Environment and Children's Health birth cohort population as Kashino et al. (2020). For those Japanese studies with the same endpoints such as mean birthweight (BWT), the analysis with the largest sample size was used in forest plots and tables (e.g., Kashino et al., (2020)). Although the Kobayashi et al. (2017) study included a unique endpoint called ponderal index, this measure is more prone to measurement error and was not considered in any study given the wealth of other fetal growth restriction data. Similarly, the Costa et al. (2019) study that examined a less accurate in utero growth estimate was not considered in lieu of their more accurate birth outcomes measures reported in the same cohort (Manzano-Salgado et al., 2017a). One additional study by Bae et al. (2015) was the only study to examine sex ratio and was therefore not further considered here.

In general, to best gauge consistency and magnitude of reported associations, EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Studies with overlapping cohorts were included in the synthesis, as each study provided some unique data for different endpoints. Specifically, the Woods et al. (2017) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018) but has additional mean BWT data (received via communication with study author). The mean BWT results for singleton and twin births from Bell et al. (2018) are included in forest plots here as are the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019) as they target different developmental windows. The Bjerregaard-Olesen et al. (2019) study from the Aarhus birth cohort also overlaps with Bach et al. (2016). The main effect results are comparable for head circumference and birth length in both studies despite a smaller sample size in the Aarhus birth cohort subset examined in Bjerregaard-Olesen et al. (2019). Given that additional sex-specific data are available in the Bjerregaard-Olesen et al. (2019) study, the synthesis for head circumference and birth length are based on this subset alone. Chen et al. (2021) reported an implausibly large effect estimate for head circumference. After correspondence with study authors, an error was identified, and the study was not considered for head circumference.

Following exclusion of the nine studies noted above, 69 developmental epidemiological studies were included in the synthesis that were not included in the 2016 PFOS HESD. Six additional studies (Gundacker et al., 2021; Jin et al., 2020; Maekawa et al., 2017; Alkhalawi et al., 2016; Lee et al., 2016; Lee et al., 2013) were considered *uninformative* due to critical study deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies

and are not further examined here. Thus, 63 studies were included across various developmental endpoints for further examination and synthesis.

Forty-three of the 63 different studies examined PFOS in relation to fetal growth restriction measured by the following endpoints: small for gestational age (SGA), low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Twenty-two studies examined gestation duration, 12 examined postnatal growth, 5 each examined fetal loss, and birth defects.

3.4.4.1.5 Growth Restriction: Fetal Growth

3.4.4.1.5.1 Birth Weight

Of the 40 informative and non-overlapping studies that examined BWT measures in relation to PFOS exposures, 34 studies examined mean BWT differences. Fifteen studies examined standardized BWT measures (e.g., z-scores) with nine of these reporting results for mean and standardized BWT (Eick et al., 2020; Wikström et al., 2020; Wang et al., 2019; Workman et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016). Twenty-five of the 34 mean BWT studies shown in Figure 3-45,Figure 3-46, and Figure 3-47 provided results based on a prospective birth cohort study design, and the remaining nine were cross-sectional analyses defined here as if biomarker samples were collected at birth or postpartum (Gao et al., 2019; Wang et al., 2019; Xu et al., 2019a; Bell et al., 2018; Gyllenhammar et al., 2018b; Shi et al., 2017; Callan et al., 2016; de Cock et al., 2016; Kwon et al., 2016).

Overall, eight of the PFOS studies relied on umbilical cord measures (Wang et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016), and one collected blood samples in infants 3 weeks following delivery (Gyllenhammar et al., 2018b). Results from the Bell et al. (2018) study were based on infant whole blood taken from a heel stick and captured onto filter paper cards at 24 hours or more following delivery, and one study used both maternal serum samples collected 1–2 days before delivery and cord blood samples collected immediately after delivery (Gao et al., 2019). One study examined pre-conception maternal serum samples (Robledo et al., 2015). Twenty-one studies had maternal serum or plasma PFOS measures that were sampled during trimesters one (Sagiv et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), two (Lauritzen et al., 2017), or three (Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Kashino et al., 2020; Valvi et al., 2017; Callan et al., 2016), or across multiple trimesters (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Marks et al., 2019; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016). The study by Meng et al. (2018) pooled exposure data from two study populations, one which measured PFOS in umbilical cord blood and one which measured PFOS in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean BWT, only one biomarker measure was used here (e.g., preferably maternal samples when collected in conjunction with umbilical cord samples or maternal only when more than parent provided samples). In addition, other related publications (e.g., Gyllenhammar et al. (2017)) or additional information or data (e.g., Woods et al. (2017)) provided by study authors were used.

Fifteen of the 34 mean BWT studies included in the synthesis were rated *high* in overall study confidence (Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Wikström et

al., 2020; Bell et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016; Govarts et al., 2016), while 12 were rated *medium* (Chang et al., 2022; Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Woods et al., 2017; de Cock et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015), and seven were classified as *low* (Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016). Twenty-three of the 27 *high* or *medium* confidence studies detailed in this synthesis were classified as having *good* study sensitivity (Chen et al., 2021; Kashino et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Lenters et al., 2016; Robledo et al., 2015) or *adequate* study sensitivity (Chang et al., 2022; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Govarts et al., 2016), while four had *deficient* study sensitivity (Wang et al., 2019; Bell et al., 2018; de Cock et al., 2016; Kwon et al.,

2016) as shown in

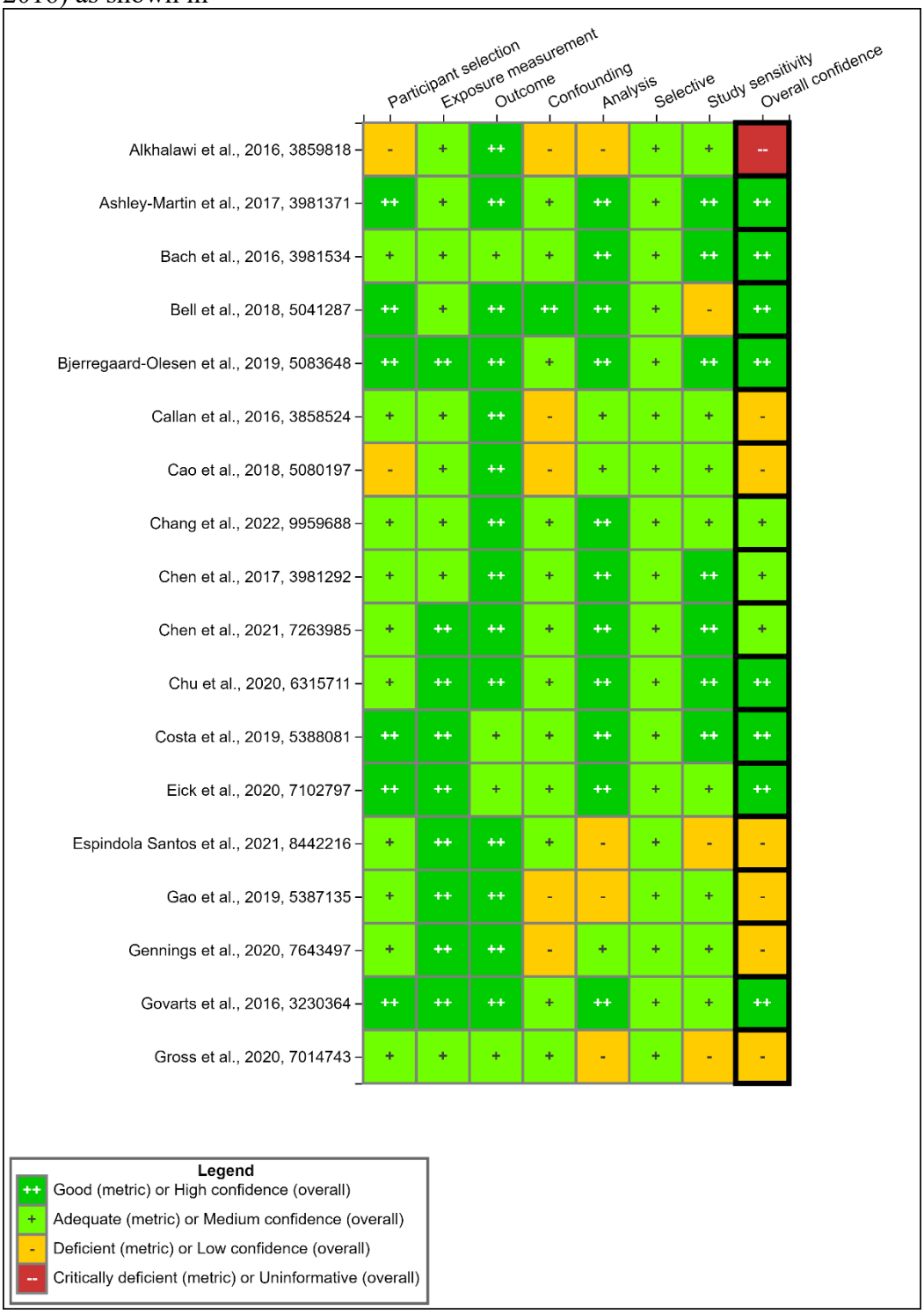


Figure 3-45, Figure 3-46, and Figure 3-47. The median PFOS exposure values across all of the studies were quite variable and ranged from 0.38 ng/mL (Kwon et al., 2016) to 30.1 ng/mL (Meng et al., 2018).

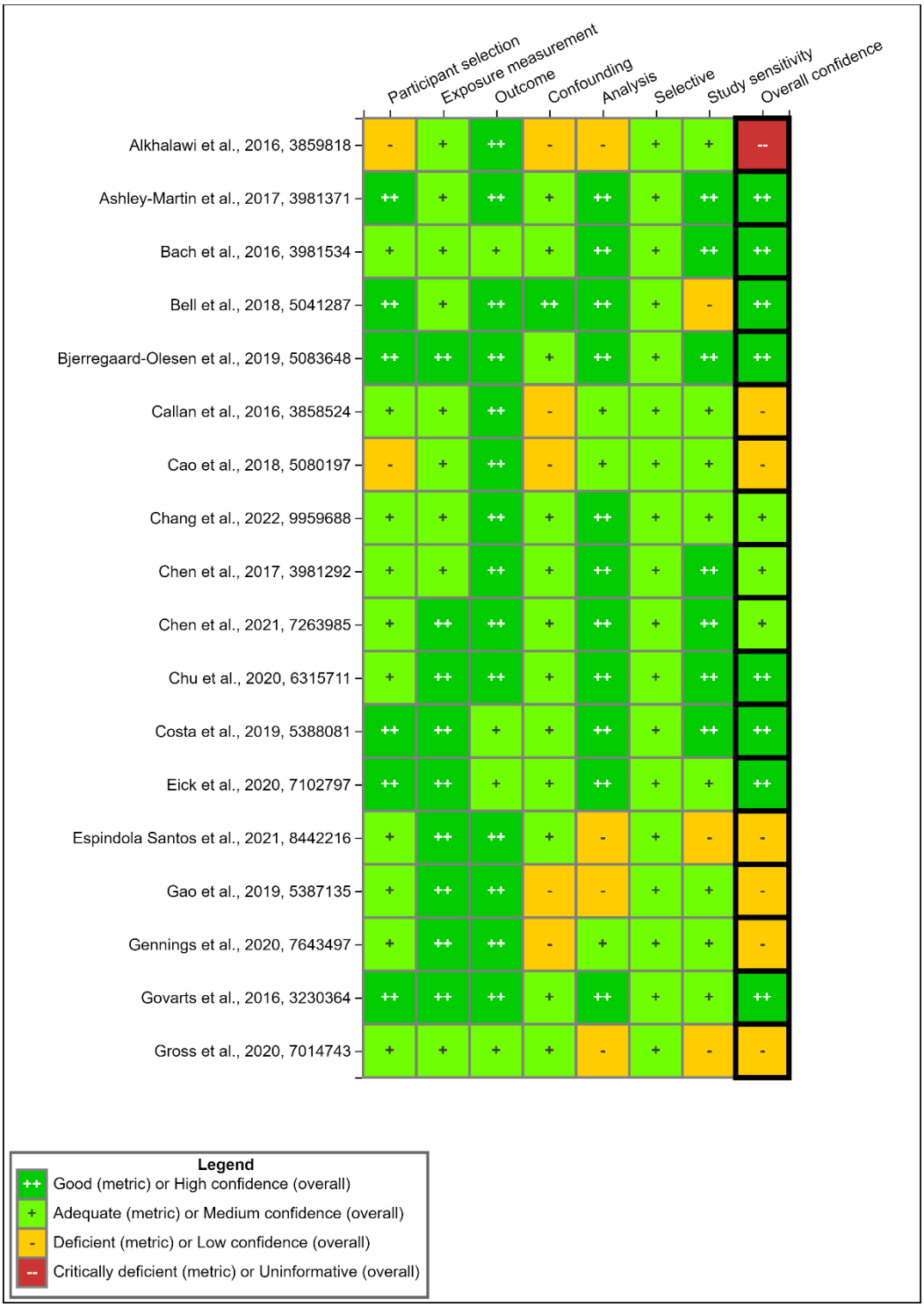


Figure 3-45. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Weight Effects [a]

Interactive figure and additional study details available on HAWC.

[a] Includes six overlapping studies (Bjerregaard-Olesen et al., 2019; Rokoff et al., 2018; Kobayashi et al., 2017; Li et al., 2017; Minatoya et al., 2017; Kishi et al., 2015).

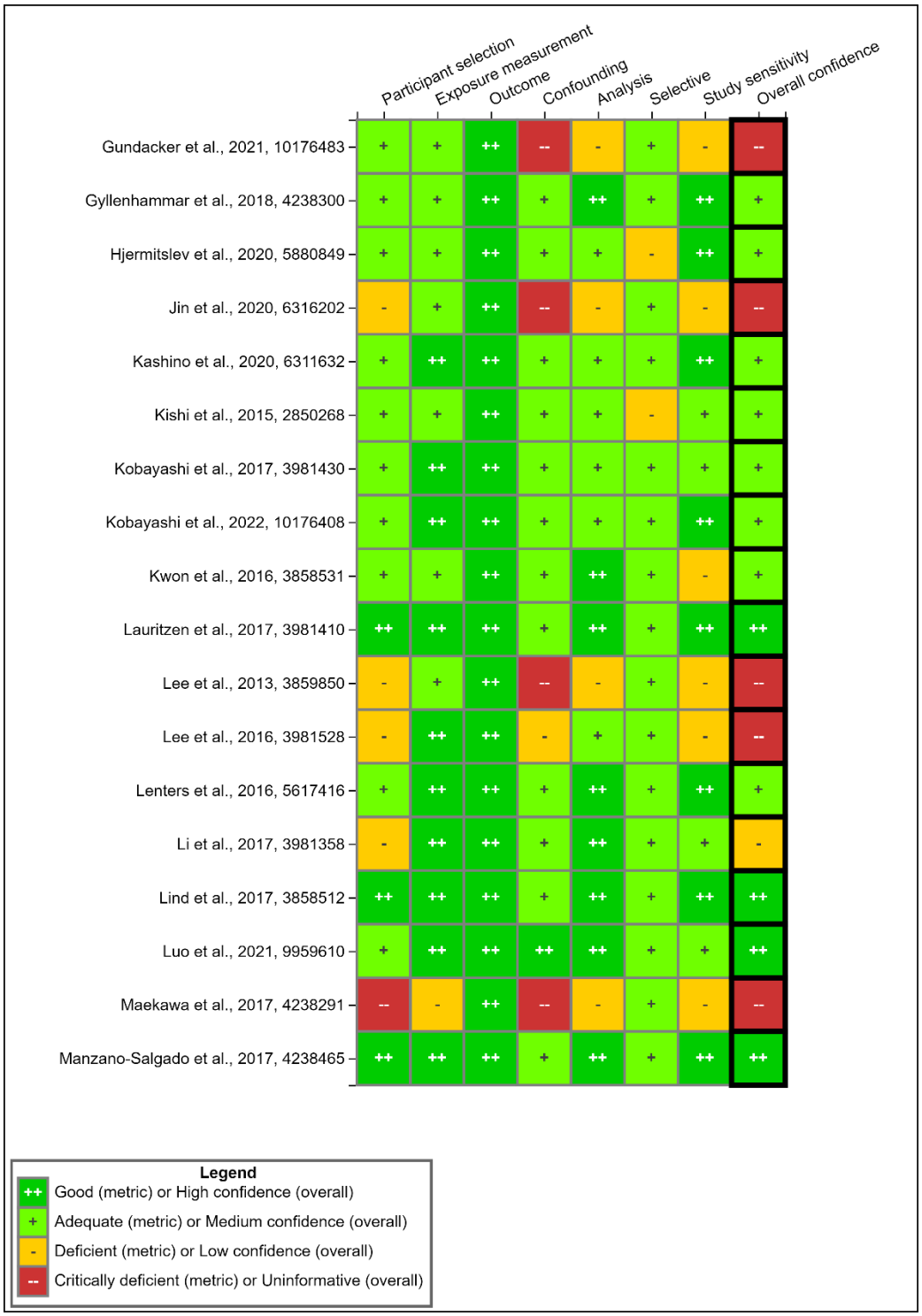


Figure 3-46. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

[a] Includes six overlapping studies (Bjerregaard-Olesen et al., 2019; Rokoff et al., 2018; Kobayashi et al., 2017; Li et al., 2017; Minatoya et al., 2017; Kishi et al., 2015).

Figure 3-47. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1.1 Mean Birth Weight Study Results: Overall Population Studies

Thirty of the 34 included studies that examined mean BWT data in the overall population (Chang et al., 2022; Chen et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Kashino et al., 2020; Wikström et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Xu et al., 2019a; Bell et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018b; Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Shi et al., 2017; Starling et al., 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Callan et al., 2016; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015; Wu et al., 2012), while four only reported sex-specific data only (Marks et al., 2019; Ashley-Martin et al., 2017; Lind et al., 2017a; Robledo et al., 2015). Nineteen of the 30 PFOS studies with analyses based on an overall population reported some mean BWT deficits, albeit some of these were not statistically significant (Figure 3-48, Figure 3-49, Figure 3-50, Figure 3-51, and Figure 3-52**)**.

Nine mean BWT studies in the overall population reported null associations (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Cao et al., 2018; Manzano-Salgado et al., 2017a; Woods et al., 2017; Govarts et al., 2016), while two reported increased mean BWT deficits (Shi et al., 2017; de Cock et al., 2016). Only two studies (Sagiv et al., 2018; Starling et al., 2017) out of 10 studies which examined categorical data (Chang et al., 2022; Eick et al., 2020; Wikström et al., 2020; Gao et al., 2019; Meng et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Bach et al., 2016 Cao, 2018, 5080197; Govarts et al., 2016) showed inverse monotonic exposure-response relationships. Although two studies (Meng et al., 2018; Bach et al., 2016) also showed large BWT deficits consistent in magnitude in the upper two quartiles (–50 to –62 g and –50 to –48 g relative to their quartile 1 referents, respectively).

Although there was a wide distribution of BWT deficits (range: –14 to –417 grams) in the overall population (i.e., both sexes combined) across both categorical and continuous exposure estimates, 18 of these ranged from –14 to –93 grams per each PFOS unit increase. This included all 10 *high* confidence studies with five of these reporting deficits ranging from 14 to 18 grams per each unit PFOS increase. The six *medium* confidence studies reporting deficits showed larger associations with an even narrower distribution ranging –35 to –69 grams per each unit PFOS increase. The three low confidence studies reporting deficits showed the largest associations ranging from –0 to –417 grams per each unit PFOS increase including three studies ranging from –50 to –69 grams. Thus, there was some suggestion of larger and more variable BWT deficits in *low* confidence studies which have a higher potential for bias. There was also a preponderance of inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) including 15 of the overall 19 studies and 7 of the 10 *high* confidence studies only; this may be related to pregnancy hemodynamic influences on the PFOS biomarkers during pregnancy. However, five (Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018; Bach et al., 2016) of eight *medium* and *high* confidence studies still reported evidence of mean BWT deficits based on early pregnancy biomarker samples.

3.4.4.1.5.1.2 Mean BWT-Overall Population Summary

Eighteen of the 19 studies that reported deficits based on either categorical or continuous expression ranged from –14 to –93 grams. A pattern of larger and more variable results was detected across study confidence with smaller and less variable BWT deficits among the higher

confidence studies. Overall, there was evidence of an adverse monotonic exposure-response in two of 10 studies, but an additional two studies showed large and consistent results in the upper two quartiles. Most of the evidence of mean birth weight difference was detected among the *medium* (6 of 12) or *high* (10 of 15) confidence studies. Study sensitivity was not an explanatory factor of the null BWT studies. There was some suggestion of a relationship between PFOS sample timing and magnitude of associations with the six of the largest deficits detected among studies that used maternal serum with some or all samples collected during trimester 3 or were based on umbilical cord samples. There was also a preponderance of inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) that may be related to pregnancy hemodynamic influences on the PFOS biomarkers during pregnancy.

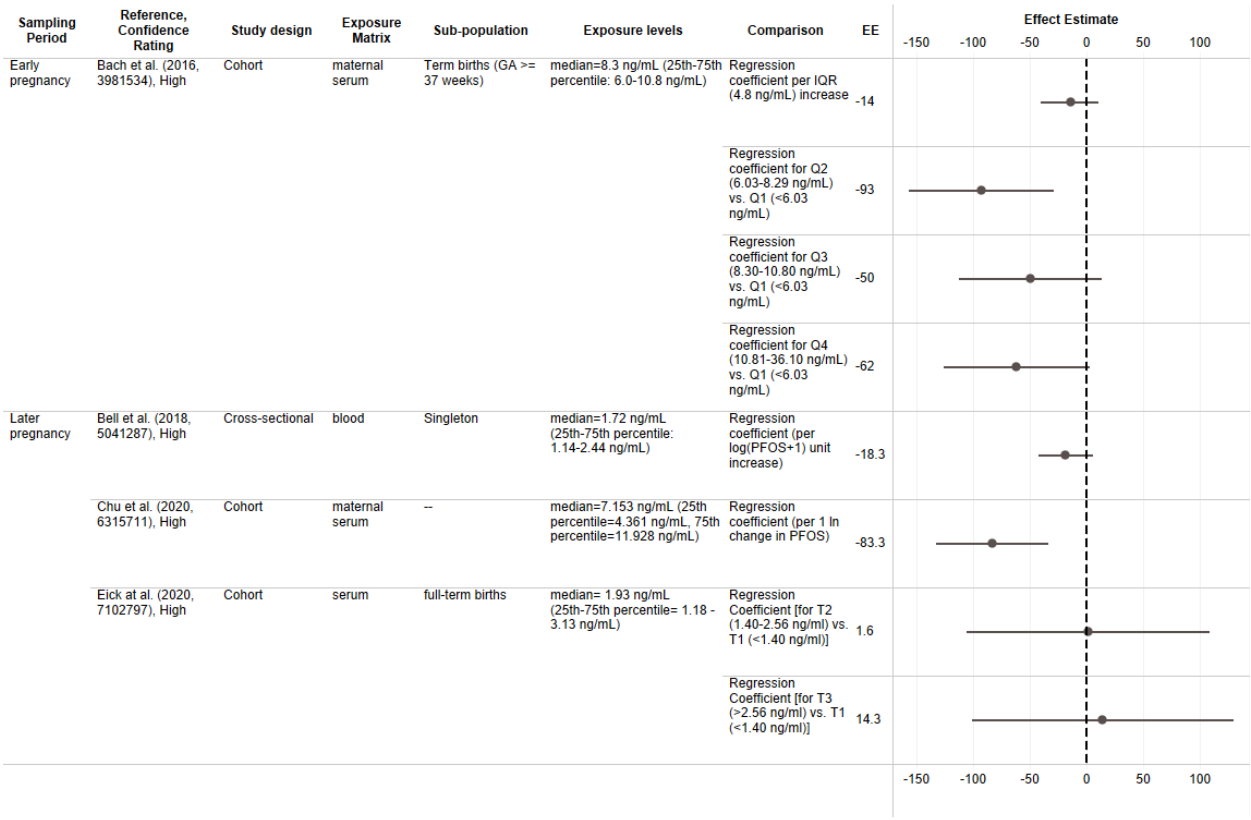


Figure 3-48. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

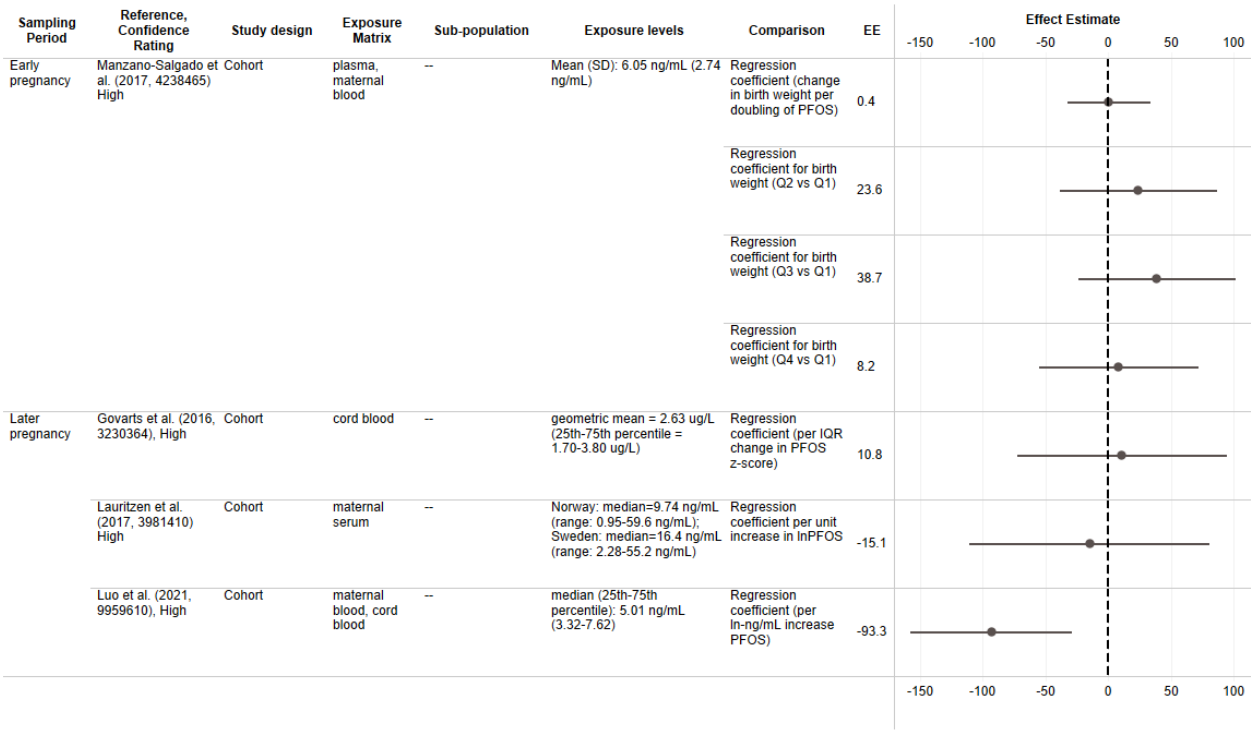
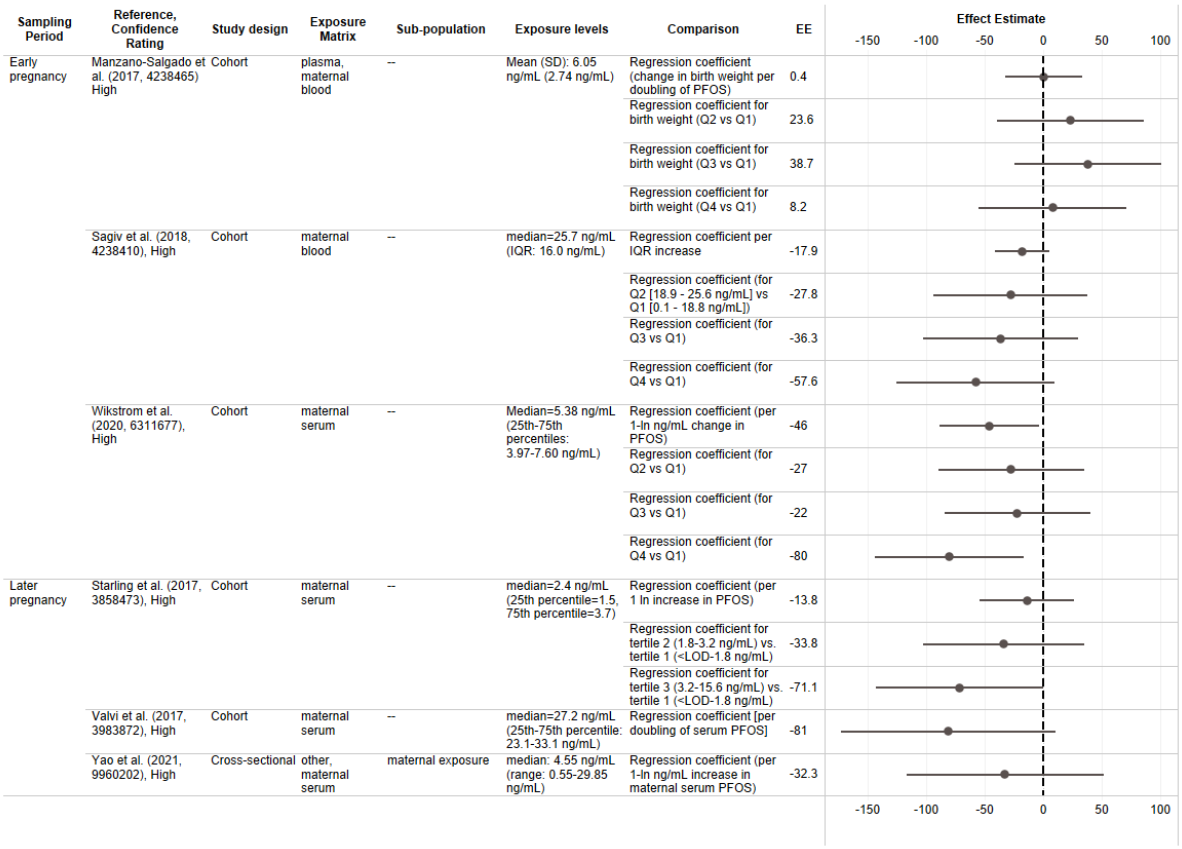


Figure 3-49. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.



Sampling Period	Reference, Confidence Rating	Study design	Exposure Matrix	Sub-population	Exposure levels	Comparison	EE
Early pregnancy	Manzano-Salgado et al. (2017, 4238465) High	Cohort	plasma, maternal blood	--	Mean (SD): 6.05 ng/mL (2.74 ng/mL)	Regression coefficient (change in birth weight per doubling of PFOS)	0.4
						Regression coefficient for birth weight (Q2 vs Q1)	23.6
						Regression coefficient for birth weight (Q3 vs Q1)	38.7
						Regression coefficient for birth weight (Q4 vs Q1)	8.2
	Sagiv et al. (2018, 4238410), High	Cohort	maternal blood	--	median=25.7 ng/mL (IQR: 16.0 ng/mL)	Regression coefficient per IQR increase	-17.9
						Regression coefficient (for Q2 [18.9 - 25.6 ng/mL] vs Q1 [0.1 - 18.8 ng/mL])	-27.8
						Regression coefficient (for Q3 vs Q1)	-36.3
						Regression coefficient (for Q4 vs Q1)	-57.6
	Wikstrom et al. (2020, 6311677), High	Cohort	maternal serum	--	Median=5.38 ng/mL (25th-75th percentiles: 3.97-7.60 ng/mL)	Regression coefficient (per 1-ln ng/mL change in PFOS)	-46
						Regression coefficient (for Q2 vs Q1)	-27
						Regression coefficient (for Q3 vs Q1)	-22
						Regression coefficient (for Q4 vs Q1)	-80
Later pregnancy	Starling et al. (2017, 3858473), High	Cohort	maternal serum	--	median=2.4 ng/mL (25th percentile=1.5, 75th percentile=3.7)	Regression coefficient (per 1 ln increase in PFOS)	-13.8
						Regression coefficient for tertile 2 (1.8-3.2 ng/mL) vs. tertile 1 (<LOD-1.8 ng/mL)	-33.8
						Regression coefficient for tertile 3 (3.2-15.6 ng/mL) vs. tertile 1 (<LOD-1.8 ng/mL)	-71.1
	Valvi et al. (2017, 3983872), High	Cohort	maternal serum	--	median=27.2 ng/mL (25th-75th percentile: 23.1-33.1 ng/mL)	Regression coefficient [per doubling of serum PFOS]	-81
	Yao et al. (2021, 9960202), High	Cross-sectional	other, maternal serum	maternal exposure	median: 4.55 ng/mL (range: 0.55-29.85 ng/mL)	Regression coefficient (per 1-ln ng/mL increase in maternal serum PFOS)	-32.3

Figure 3-50. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

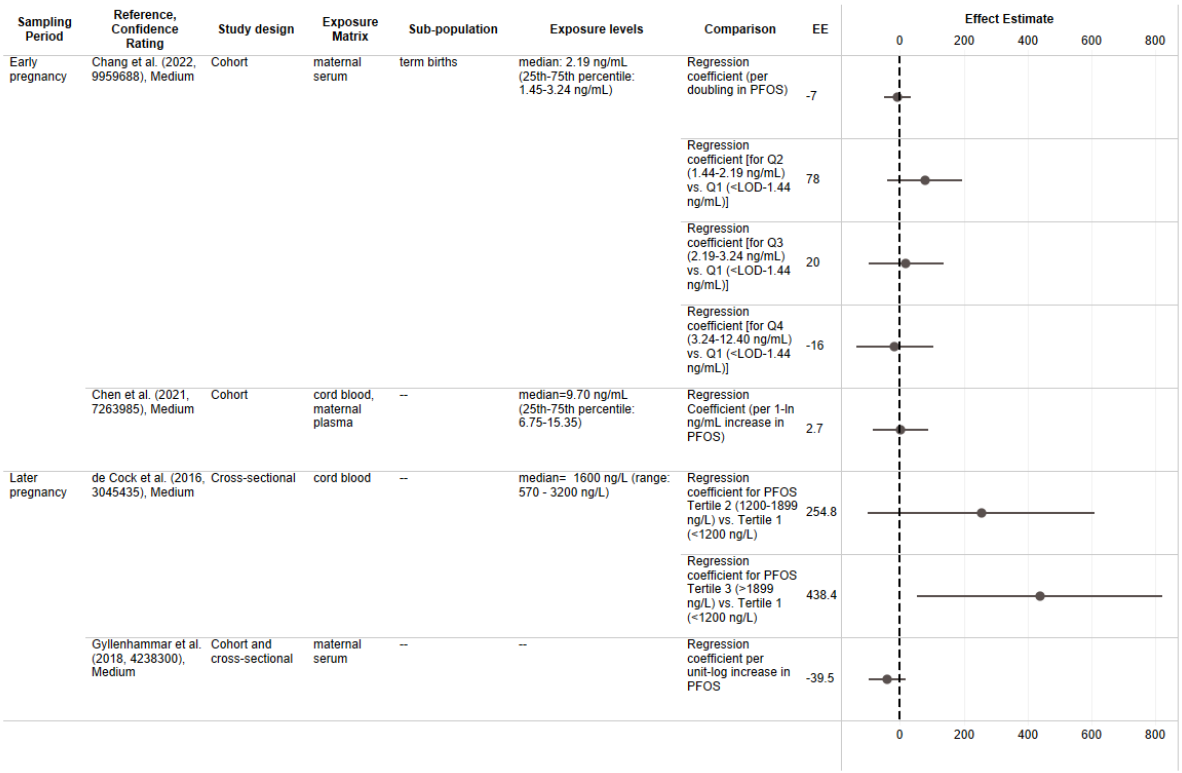


Figure 3-51. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

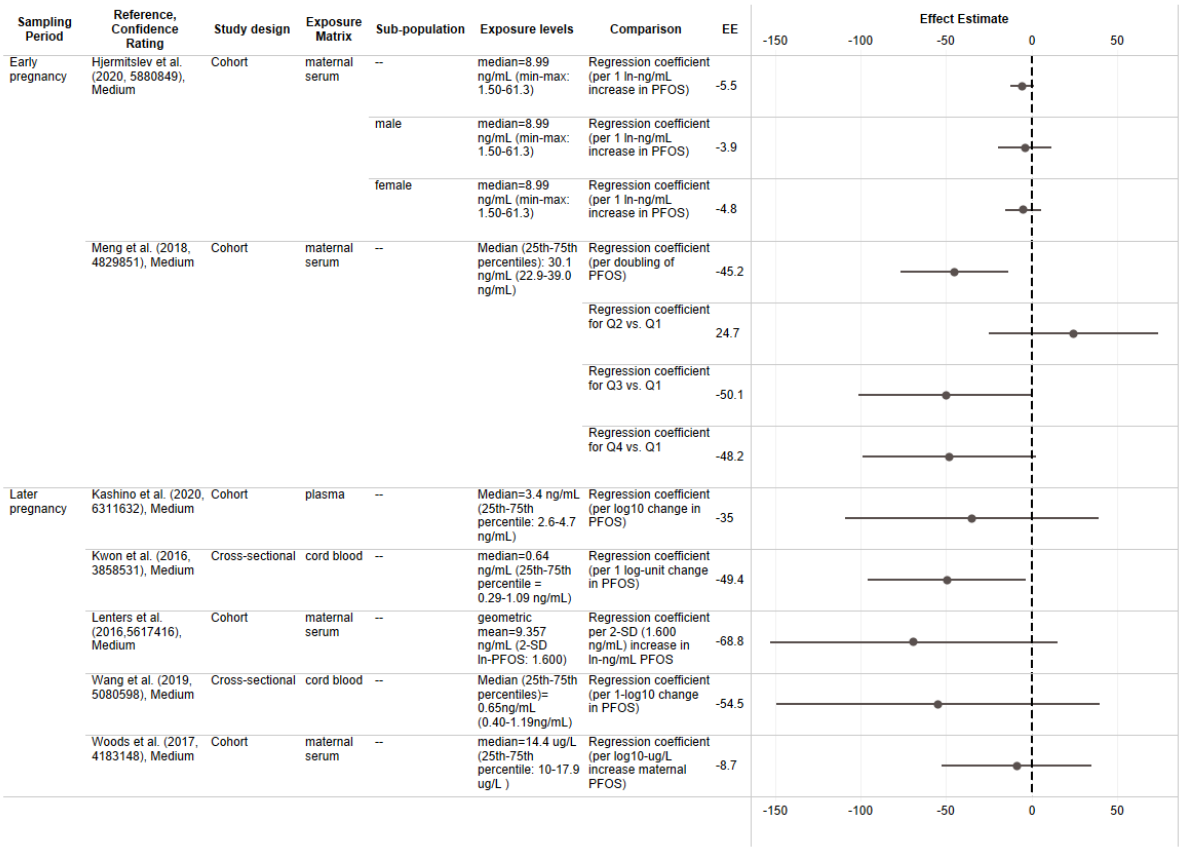


Figure 3-52. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1.3 Mean Birth Weight Study Results: Sex-Specific Studies

Ten of 16 epidemiological studies examining sex-specific results in male neonates showed some BWT deficits. The remaining six studies (Hjermitslev et al., 2019; Cao et al., 2018; Ashley-Martin et al., 2017; Shi et al., 2017; de Cock et al., 2016; Robledo et al., 2015) in male neonates were either null or showed larger birth weights with increasing PFOS exposures. Six of 15 epidemiological studies examining sex-specific results in female neonates showed some BWT deficits. The magnitude of associations was much more variable in boys (range: –9 to –150 grams) than in girls (range: –20 to –85 grams) per each unit PFOS increase. There was also little evidence of exposure-response relationships in either sex as only 1 out of 5 studies with categorical data showed monotonicity.

Six of the 15 studies examining mean BWT associations in both boys and girls detected some deficits in both sexes. Two of these six studies showed deficits comparable in magnitude among boys and girls (Chu et al., 2020; Wang et al., 2019). Three of these studies (Wikström et al., 2020; Meng et al., 2018; Bach et al., 2016) showed larger deficits among girls and one showed larger deficits among boys (Kashino et al., 2020). The *low* confidence study by Marks et al. (2019) of males only detected a small statistically significant association (β per each ln-unit PFOS increase: −8.5 g; 95% CI: −15.9, −1.1) and showed an exposure-response with reported

large deficits in PFOS tertile 2 (β: −26.6 g; 95% CI: −147.3, 94.2) and tertile 3 (β: −83.9 g; 95% CI: −201.4, 33.7) compared with the tertile 1 referent. Four other studies reported mean BWT deficits only in boys (Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017); no studies reported deficits in girls only.

Overall, there was more evidence of inverse associations detected in boys, but the magnitude of associations detected was more consistent in girls. There was an exposure-response relationship detected in only one of five studies with categorical data in both sexes. Study confidence and most other study characteristics did not seem to be explanatory patterns for the results, as, for example, nearly all (9 of 10 in boys) or all (6 of 6 girls) were either *high* or *medium* confidence. Definitive patterns by sample timing were also not evident in the male neonates across all study confidence levels but a larger proportion of the later sampled studies (60%) showed inverse associations in females compared with early sampled studies (38%). Study sensitivity was not an explanatory factor among the null studies in either sex.

3.4.4.1.5.1.4 Standardized Birth Weight Measures

Fifteen studies examined standardized BWT measures including 14 studies reporting a change in BWT z-scores on a continuous scale per each PFOS comparison. Eight of the 15 studies were *high* confidence studies (Gardener et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016), four were *medium* (Wang et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Chen et al., 2017b) and three were *low* confidence (Espindola-Santos et al., 2021; Gross et al., 2020; Workman et al., 2019) (Figure 3-45, Figure 3-46, Figure 3-47).

Nine of the 15 studies showed some evidence of inverse associations between PFOS exposures and BWT z-scores. Six of these were *high* confidence (Gardener et al., 2021; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016), two were *medium* confidence (Wang et al., 2019; Chen et al., 2017b) and one was *low* confidence (Gross et al., 2020). None of the four studies reporting categorical data showed evidence of monotonicity across tertiles or quartiles. The *high* confidence study by Gardener et al. (2021) reported that participants in the highest PFOS exposure quartile (relative to the lowest quartile) had a higher odds ratio (OR = 1.41; 95% CI: 0.66, 2.03) of being in the lowest standardized birthweight category (vs. the top 3 BWT z-score quartiles). Four studies reporting associations in the overall population also reported standardized birth weight deficits in either or both male and female neonates. Two studies (Gardener et al., 2021; Gyllenhammar et al., 2018b) also reported that there were no statistically significant interactions for their BWT-z measures by sex.

Among the 14 studies examining continuous BWT z-score measures in the overall population, eight reported associations for different PFOS exposures. The *high* confidence study by Bach et al. (2016) reported a statistically significant association between mean BWT z-score and PFOS quartile 2 (β: −0.15; 95% CI: −0.29, −0.02) and quartile 4 (β: −0.11; 95% CI: −0.25, 0.02) only, with no exposure-response relationship detected. Although not statistically significant, both Wang et al. (2019) (β: −0.15; 95% CI: −0.41, 0.11) and Shoaff et al. (2018) reported associations similar in magnitude for their overall population (β: −0.12; 95% CI: −0.36, 0.13). The *medium* confidence study by Chen et al. (2017b) reported inverse associations in the overall population (β: −0.14; 95% CI: −0.26, −0.01) with comparable results in both male and female neonates (BWT z-score range: −0.13 to −0.15). The *high* confidence study by Sagiv et al. (2018) reported

associations for PFOS quartile 4 in the overall population (β: –0.13; 95% CI: 0.26, 0.00); the largest association in this study was found for male neonates (β: −0.19; 95% CI: −0.33, −0.05) per each interquartile range (IQR) increase. The *high* confidence study by Wikström et al. (2020) reported inverse associations (β per each ln-unit increase: –0.10; 95% CI: –0.20; −0.004) as well as in quartile 4 in the overall population (β: −0.17; 95% CI: −0.37, −0.03); these results appeared to be driven by associations detected in female neonates (β per each ln-unit increase: –0.17; 95% CI: –0.30, –0.03; β for quartile 4: −0.30; 95% CI: −0.49, –0.10). The *high* confidence study by Xiao et al. (2019) reported z-scores fairly similar in magnitude for the overall population (β: −0.47; 95% CI: −0.85, −0.09), male neonates (β: −0.40; 95% CI: −0.89, 0.08), and female neonates (β: −0.56; 95% CI: −1.12, 0). Among the eight studies showing some deficits, the largest association was detected in the *low* confidence study by Gross et al. (2020) for the overall population (β: –0.62; 95% CI: −0.96 to −0.29). The authors also reported large deficits for both males (β: –0.81; SE = 0.24; p-value = 0.001) and females (β: –0.46; SE = 0.29; p-value = 0.11) for PFOS levels greater than the mean level.

3.4.4.1.5.1.5 BWT Z-Score Summary

Nine out of 15 studies showed some associations between standardized BWT scores and PFOS exposures including eight *medium* or *high* confidence studies. None of the five studies with categorical data reported strong evidence of exposure-response relationships. No patterns by sample timing were evident as three of these studies had trimester one maternal samples; however, the strongest associations were seen in studies with later biomarker sampling. Study sensitivity did not seem to be an explanatory factor in the six null studies of standardized BWT most of these studies had moderate or large exposure contrasts and sufficient sample sizes. Although some studies may have been underpowered to detect associations small in magnitude relative to PFOS exposure, there was consistent lower BWT z-scores reported in these studies. There was no apparent pattern related to magnitude of deficits across study confidence, but more associations were evident across *high* confidence studies in general. Twice as many studies showing inverse associations were based on later (6 of 9) versus early (i.e., at least some trimester one maternal samples) pregnancy sampling (3 of 9); this might be reflective of some impact of pregnancy hemodynamics on biomarker concentrations over time. Few differences were seen across sexes including magnitude of associations as the majority of studies in both male (3 of 5 studies; 2 were *medium* or *high* confidence) and female (4 of 5 studies; 3 of 4 were *medium* or *high* confidence) neonates showed some associations between decreased standardized birth weights and increasing PFOS exposures. Overall, 9 different studies out of 15 showed some suggestion of inverse associations in the overall population or either or both sexes.

3.4.4.1.5.2 Small for Gestational Age/Low Birth Weight

Ten informative and non-overlapping epidemiological studies examined associations between PFOS exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), LBW, or both (i.e., Manzano-Salgado et al. (2017a)). Overall, 11 studies examined either or both LBW or SGA in relation to PFOS exposure with 4 classified as *high* confidence (Chu et al., 2020; Wikström et al., 2020; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a), three as *medium* confidence (Hjermitslev et al., 2019; Govarts et al., 2018; Meng et al., 2018), three as *low* confidence, (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a) and one as *uninformative* (Arbuckle et al., 2013). Six of these studies had *good* sensitivity (Chu et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a), while five were considered

adequate (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a; Govarts et al., 2018; Arbuckle et al., 2013).

Four (Souza et al., 2020; Wikström et al., 2020; Xu et al., 2019a; Lauritzen et al., 2017) of the seven SGA studies reporting main effects showed some increased risk, while three studies were null (Chang et al., 2022; Govarts et al., 2018; Manzano-Salgado et al., 2017a). The magnitude of odds ratios (ORs) across the four studies showing increased risk in the overall population (OR range: 1.19 to 4.14) was variable whether the effect estimates were based on either categorical or continuous exposures (per each unit increase) (Figure 3-53 and Figure 3-54) with the two low confidence studies showing the largest risks. For example, Xu et al. (2019a) reported an OR of 4.14 (95% CI: 1.07, 16.0) for each log10 unit increase in PFOS. Souza et al. (2020) reported an OR of 3.67 (1.38–9.74) in quartile 4 relative to quartile 1. The *high* confidence Lauritzen et al. (2017) study did not show an increased risk in the overall population per each ln-unit PFOS increase, but they did show a larger association among participants from Sweden (OR = 2.51; 95% CI: 0.93, 6.77). The *high* confidence study by Wikström et al. (2020) reported an OR of 1.56 (95% CI: 1.09; 2.22 per each ln-unit increase) with a larger OR in girls (OR = 2.05; 95% CI: 1.00, 4.21) than boys (OR = 1.30; 95% CI: 0.70, 2.40). Similarly, a slight increased risk in their overall population (OR per each ln-unit change = 1.19; 95% CI: 0.87, 1.64) was largely driven by results in girls (OR = 1.40; 95% CI: 0.83, 2.35).

Overall, four (2 *high* and 2 *low* confidence studies) reported increased risks for SGA with increasing PFOS exposures (Figure 3-53 and Figure 3-54). SGA findings from *low* confidence studies are not included in figures. The magnitude in risk across many of these studies were relatively large, but neither of two studies examining categorical exposures showed any evidence of an exposure-response relationship. Few patterns were discernible across study characteristics or study confidence for these SGA findings, although the number of studies was small.

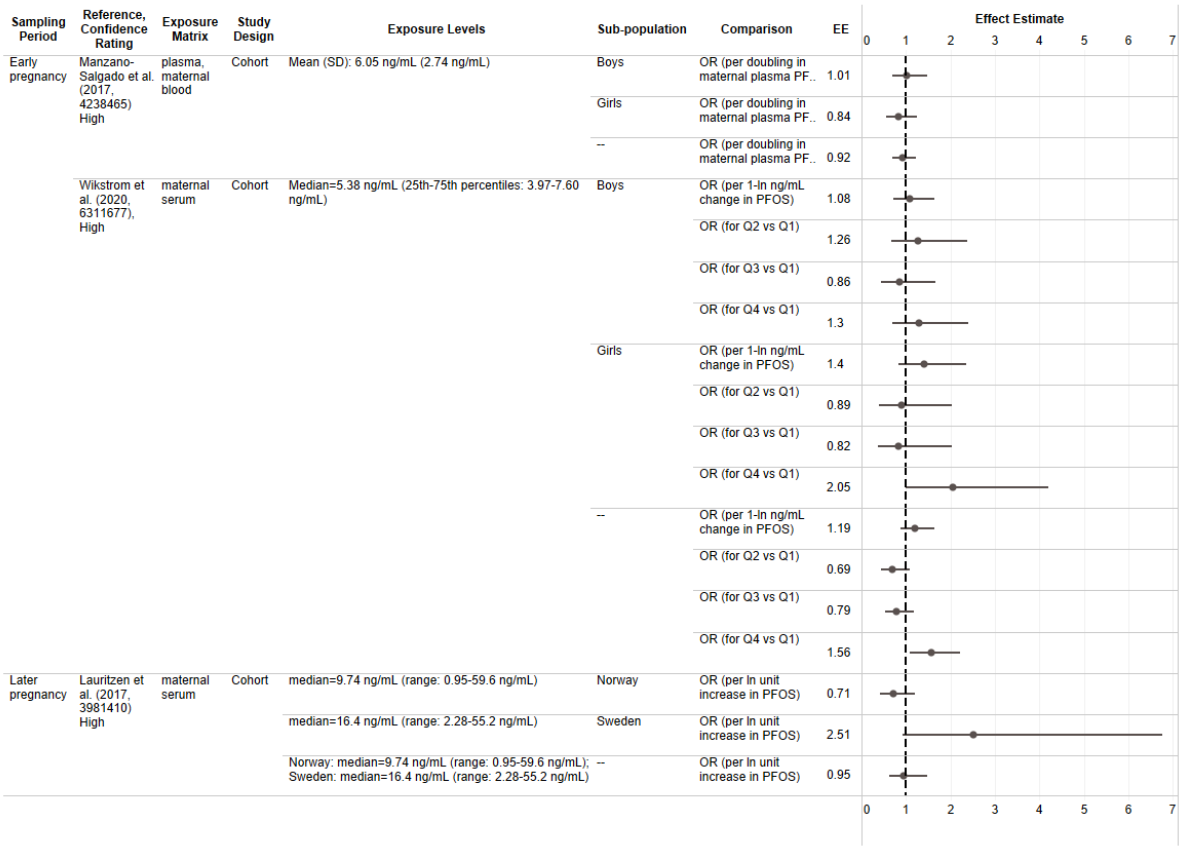


Figure 3-53. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Small for gestational age defined as birthweight below the 10th percentile for the reference population.

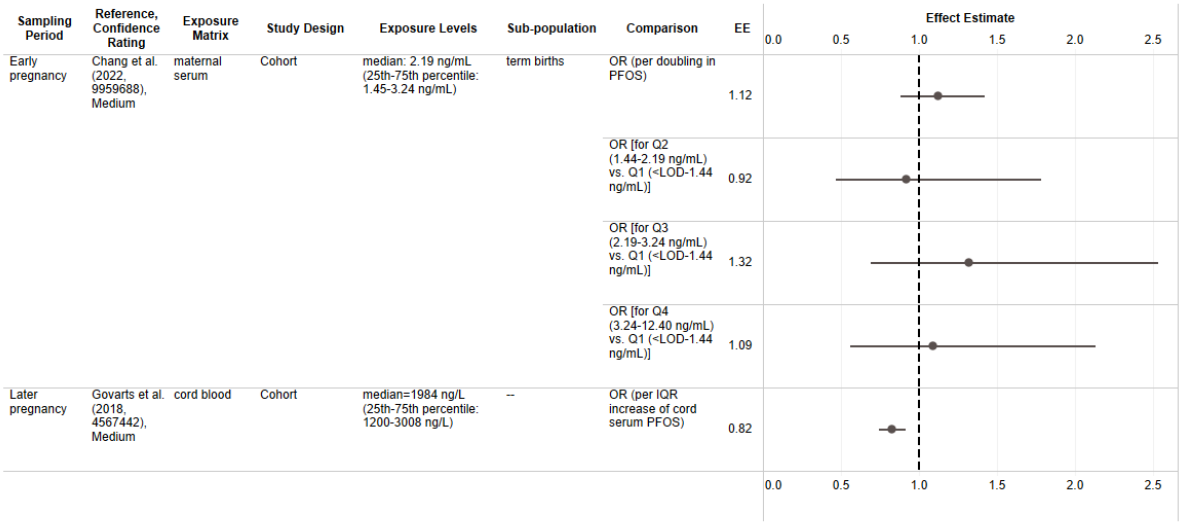


Figure 3-54. Odds of Small for Gestational Age in Children from Medium Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Small for gestational age defined as birthweight below the 10th percentile for the reference population.

Five studies examined LBW in relation to PFOS including one considered *uninformative* (Arbuckle et al., 2013) and two each that were either *high* (Chu et al., 2020; Manzano-Salgado et al., 2017a) or *medium* confidence (Hjermitslev et al., 2019; Meng et al., 2018). All but two (Hjermitslev et al., 2019; Arbuckle et al., 2013) of the five LBW studies reported some associations with either the overall population, or in either boys or girls (Figure 3-55) although no evidence of exposure-response relationships were reported in those studies analyzing categorical exposures.

Although the number of studies was small, few discernible patterns by study characteristics or confidence levels were evident across these LBW findings. The three LBW studies that showed increased risks were all either *medium* or *high* confidence with two of these showing fairly small ORs (Figure 3-55). The *high* confidence study by Manzano-Salgado et al. (2017a) did not detect associations in the overall population but showed an increased risk for term LBW among boys only (OR = 1.68; 95% CI: 0.62, 4.54). The *medium* confidence study by Meng et al. (2018) reported nonsignificant increased ORs (range 1.2–1.8) in the overall population across all quartiles but no evidence of an exposure-response relationship. The *high* confidence study by Chu et al. (2020) reported limited evidence of an exposure-response relationship in the overall population with imprecise increased risks shown for PFOS exposure quartile 3 (OR = 1.41; 95% CI: 0.23, 8.82) and quartile 4 (OR = 3.70; 95% CI: 0.61, 22.6) compared with the quartile one referent.

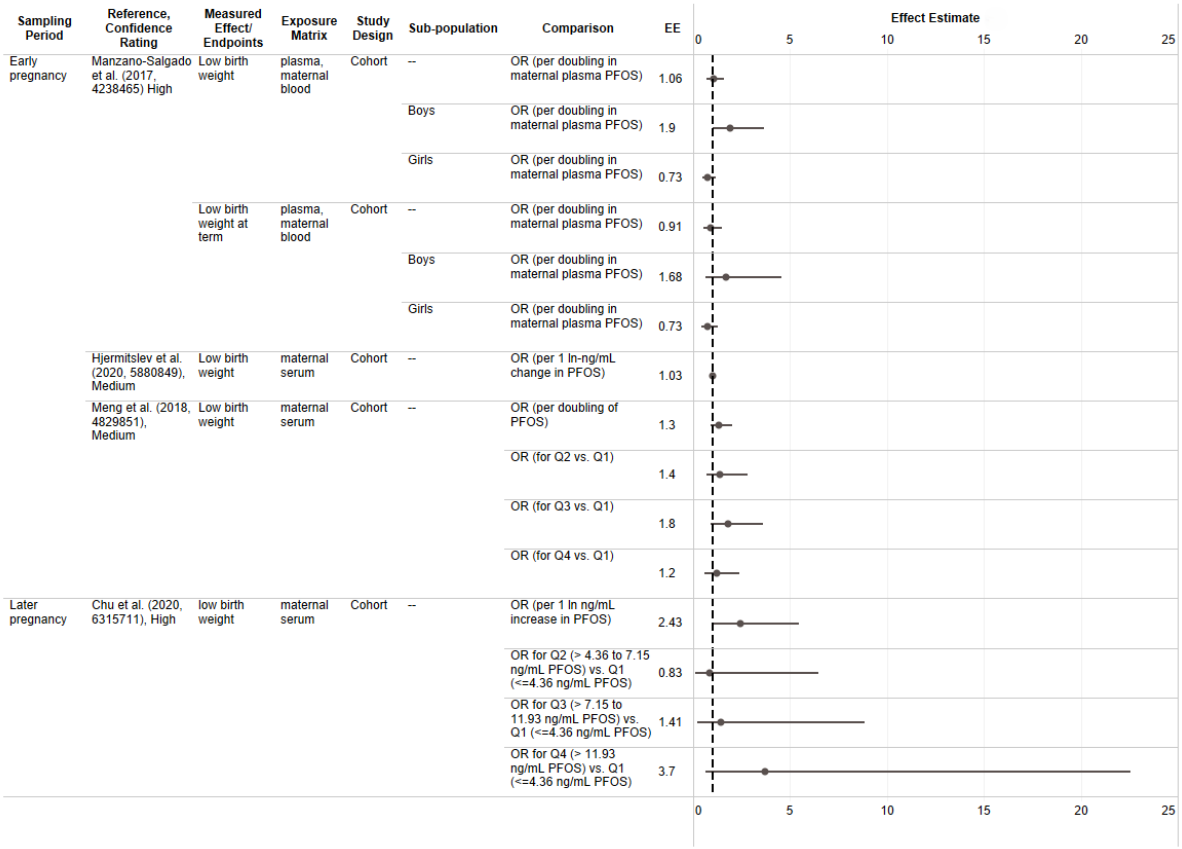


Figure 3-55. Odds of Low Birthweight in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Low birthweight defined as birthweight <2,500 g.

Collectively, the majority (7 of 10) of SGA and LBW studies were supportive of an increased risk with increasing PFOS exposures. The increased odds ranged from 1.19 to 4.14 although evidence of exposure-response relationships was lacking. There was no evidence of differences by study confidence as five of these seven were either *high* (n = 4) or *medium* (n = 1) confidence. There was also no evidence of sample timing differences as the majority of studies with associations were reported in studies based on early sampling periods.

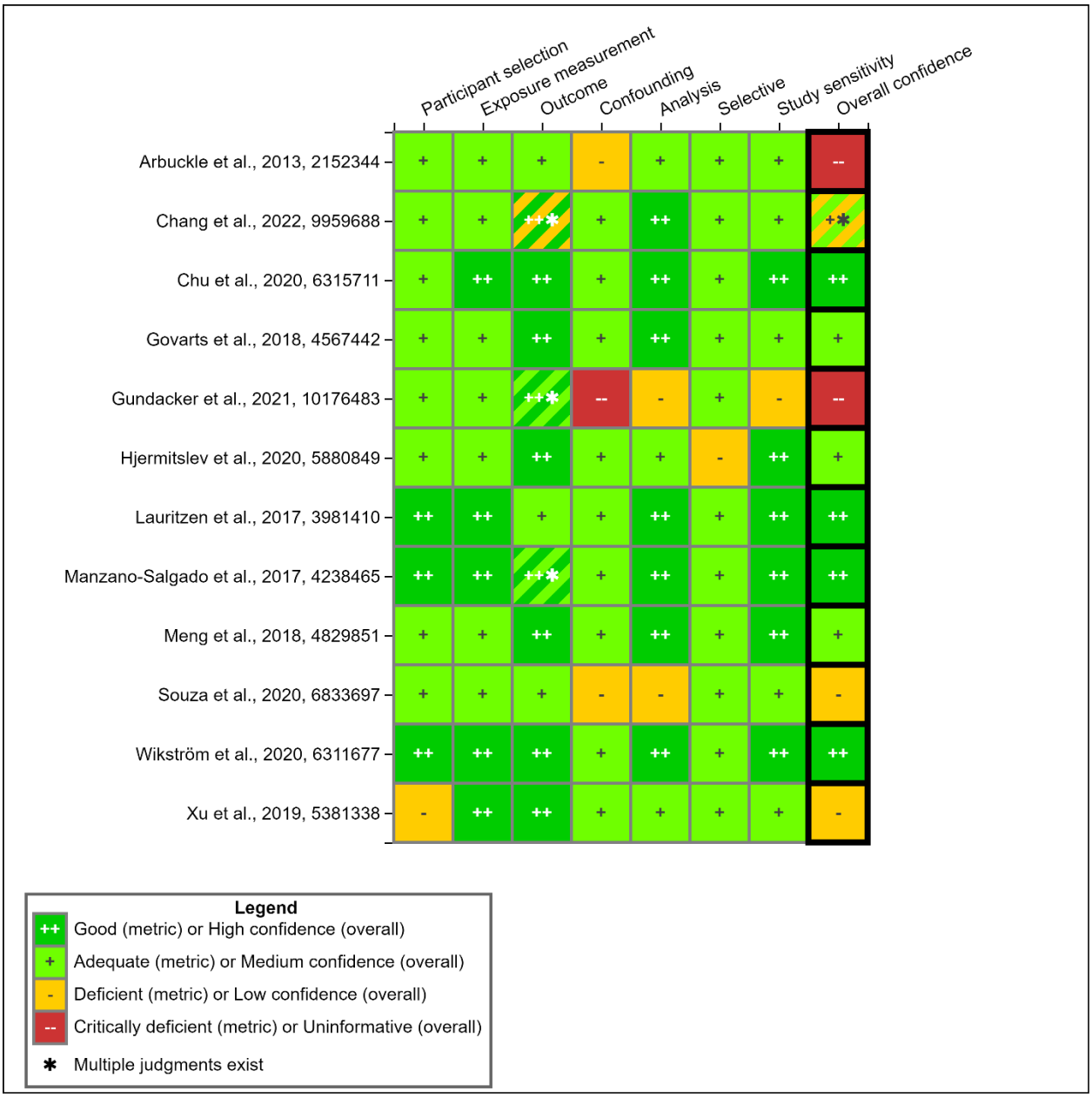


Figure 3-56. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Small for Gestational Age and Low Birth Weight Effects

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.3 Birth Length

Thirty-one birth length studies were considered as part of the study evaluation as shown in Figure 3-57. and Figure 3-58. Four studies were considered *uninformative* (Gundacker et al., 2021; Jin et al., 2020; Alkhalawi et al., 2016; Lee et al., 2013) and four more studies noted above (Kobayashi et al., 2022; Bach et al., 2016; Kishi et al., 2015 Kobayashi, 2017, 3981430) were not further considered for multiple publications from the same cohort studies. Twenty-three non-overlapping and informative studies examined birth length in relation to PFOS with five of these

examining standardized birth length measures only (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b), and one evaluating both measures (Workman et al., 2019). Twelve studies examined sex-specific data with two studies (Marks et al., 2019; Robledo et al., 2015) reporting only sex-specific results. Eighteen studies examined mean birth length differences in the overall study population.

Seven of these 23 included studies were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017), eight were *medium* confidence (Chen et al., 2021; Luo et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Chen et al., 2017b; Robledo et al., 2015) and eight were *low* confidence studies (Espindola-Santos et al., 2021; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016). Twelve PFOS studies had *good* study sensitivity (Chen et al., 2021; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Robledo et al., 2015), while eight had *adequate* sensitivity (Luo et al., 2021; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016) and three (Espindola-Santos et al., 2021; Wang et al., 2019; Bell et al., 2018) were considered *deficient*.

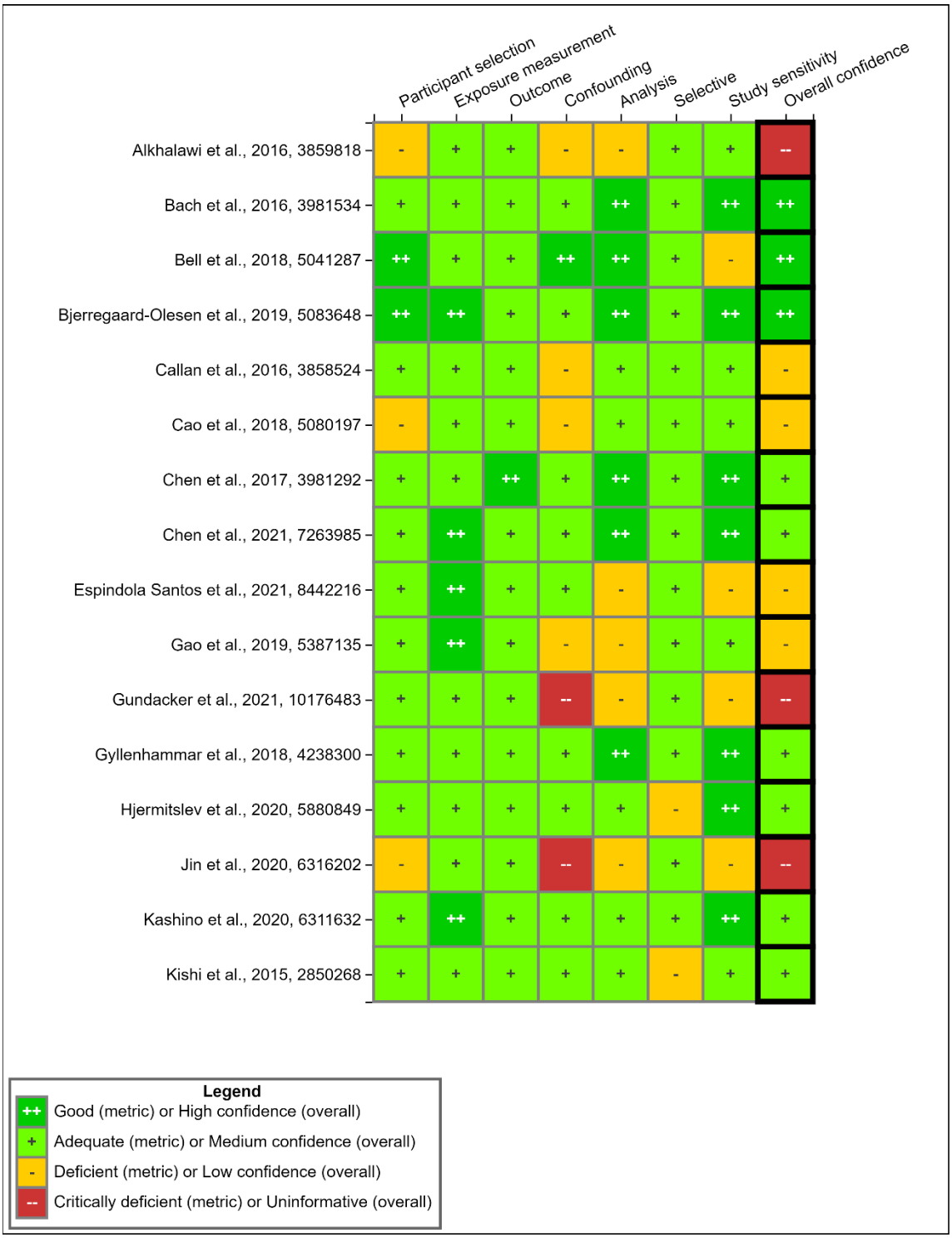


Figure 3-57. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Length Effects [a]

Interactive figure and additional study details available on HAWC.

[a] Includes three overlapping studies: Bjerregaard-Olsen et al. (2019); Kishi et al. (2015); Kobayashi et al. (2017).

Figure 3-58. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Length Effects (Continued)a

Interactive figure and additional study details available on HAWC.

[a] Includes three overlapping studies: Bjerregaard-Olsen et al. (2019); Kishi et al. (2015); Kobayashi et al. (2017).

Of the 23 studies examining either standardized birth length or mean birth length measures, seven studies showed some inverse associations based on the overall population. This included three of the six (Espindola-Santos et al., 2021; Workman et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b) studies that reported standardized birth length data. The *high* confidence study by Xiao et al. (2019) reported reduced birth length z-scores (β per each log2 increase in PFOS: −0.33; 95% CI: −0.69, 0.03) in the overall population, as well as for both male (β: −0.41; 95% CI: −0.87, 0.05) and female neonates (β: −0.23; 95% CI: −0.75, 0.30). Although smaller in magnitude, the *medium* confidence study by Chen et al. (2017b) also reported a birth length deficit of −0.16 per each ln-unit PFOS increase (95% CI: −0.31, −0.02) in the overall population as well as male (β: −0.15; 95% CI: −0.33, 0.03) and female neonates (β: −0.20; 95% CI: −0.44, 0.05). The other *high* confidence study by Shoaff et al. (2018) of standardized birth length measures showed a deficit only for tertile 3 (β: −0.24; 95% CI: −0.64, 0.15) compared with tertile 1.

Four (Chen et al., 2021; Workman et al., 2019; Lauritzen et al., 2017; Callan et al., 2016) of the 16 studies examining mean birth length in the overall population in relation to PFOS showed some evidence of reductions. The *high* confidence study by Lauritzen et al. (2017) showed a small deficit in the overall population (β: –0.3 cm; 95% CI: −0.7, 0.1), but detected the strongest association when restricted to the Swedish population (β: −1.2 cm; 95% CI: −2.1, −0.3). The *medium* confidence study by Chen et al. (2021) reported birth length deficits in the overall population (β per each PFOS ln-unit increase: –0.27 cm; 95% CI: –0.51, –0.02), males (β: –0.14 cm; 95% CI: –0.55, 0.26), and females (β: –0.40 cm; 95% CI: –0.74, –0.06). The *low* confidence study by Workman et al. (2019) reported a non-statistically significant birth length reduction (β per each ln-unit PFOS increase: –0.16 cm; 95% CI: –0.92, 0.60). The *low* confidence study by Callan et al. (2016) reported a slightly larger birth length reduction of –0.22 cm (95% CI: –1.0, 0.57) per each ln-unit PFOS increase.

Five different sex-specific studies reported some birth length deficits in either or both male (4 of 11) and female (2 of 10) neonates including the Chen et al. (2021) results noted above. Among the two sex-specific only studies (Marks et al., 2019; Robledo et al., 2015), the Marks et al. (Marks et al., 2019) *low* confidence study of boys only showed inverse associations (β for tertile 3 vs. tertile 1: −0.52 cm; 95% CI: −1.05, 0.01). The *high* confidence study by Valvi et al. (2017) reported no associations in the overall population but did detect a nonsignificant birth length deficit in male neonates (β per each PFOS log2 exposure increase: −0.18 cm; 95% CI: −0.60, 0.23). The *low* confidence study Wang et al. (2019) study also reported a nonsignificant birth length deficit in males that was similar in magnitude (β: −0.17 cm; 95% CI: –0.71, 0.37). Although it was not statistically significant, the *high* confidence study by Bjerregaard-Olesen et al. (2019) detected a difference in mean birth length among girls only (β per each IQR PFOS increase: −0.3 cm; 95% CI: −0.7, 0.0). One study not reporting sex-specific differences did report that there were no statistically significant interactions by sex for their birth length and PFOS measures (Gyllenhammar et al., 2018b).

In summary, of the 23 birth length studies, 11 different ones showed some inverse associations either in the overall population, or in either or both sexes. Two of 10 studies in females and four of 11 studies in males reported some birth length deficits. Although there were more studies in males that reported decreased birth length, there was little consistency across sex or even compared with the overall population. None of the five studies examining categorical data in

either sex or the overall population showed any evidence of an adverse exposure-response relationship. Few patterns were evident across study characteristics or confidence levels, although the database may be prone to bias due to pregnancy hemodynamics as eight of the studies that showed associations relied on later biomarker samples.

3.4.4.1.5.4 Head Circumference at Birth

Nineteen informative studies that examined head circumference were considered in the synthesis. Seven studies were rated as *medium* (Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Lind et al., 2017a; Robledo et al., 2015) confidence, while six were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017) and six were *low* confidence (Espindola-Santos et al., 2021; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Callan et al., 2016). Three studies were *deficient* in study sensitivity (Espindola-Santos et al., 2021; Wang et al., 2019; Bell et al., 2018), while 11 had *good* (Chen et al., 2021; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Robledo et al., 2015) and five had *adequate* study sensitivity (Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Callan et al., 2016).

Figure 3-59. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Head Circumference Effects

Interactive figure and additional study details available on HAWC.

Sixteen of the 19 included studies examined PFOS in relation to mean head circumference differences including 13 studies with results in the overall population and 11 different studies with sex-specific data. Three of the mean head circumference studies (Marks et al., 2019; Lind et al., 2017a; Robledo et al., 2015) only reported sex-specific data, including the *low* confidence study by Marks et al. (2019) which only examined male neonates. The three remaining studies (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018b) examined unitless standardized measures.

Five of the 16 studies with data based on the overall population reported some associations between PFOS and different head circumference measures. This included one study based on standardized head circumference and four studies examining mean head circumference. The *high* confidence study by Xiao et al. (2019) showed consistent head circumference z-score deficits across their overall population (β: −0.26; 95% CI: −0.68, 0.16), as well as male (β: −0.15; 95% CI: −0.68, 0.39) and female neonates (β: −0.42; 95% CI: −1.05, 0.21) per each log2 increase in PFOS. Although the *high* confidence study by Lauritzen et al. (2017) reported a null association in the combined Norwegian and Swedish population, they did detect a large head circumference reduction amongst their Swedish population only (β per each ln-unit PFOS change: −0.4 cm; 95% CI: −0.9, 0.04).

Only three of the 14 studies examining mean head circumference differences in the overall population reported any evidence of associations with none of these reaching statistical significance. The *high* confidence study by Bach et al. (2016) showed a small, nonsignificant head circumference differences (β per each PFOS IQR increase: –0.1 cm; 95% CI: −0.2, 0.1). In their *low* confidence study, Cao et al. (2018) reported a nonsignificant inverse association in the overall population (β per each ln-unit PFOS: −0.23 cm; 95% CI: −1.19, 0.73) as did the *low* confidence study by Callan et al. (2016) (β per each ln-unit PFOS: −0.39 cm; 95% CI: −0.98, 0.20).

Two of 10 studies examining female neonates and four of 11 examining male neonates reported some inverse associations between increasing PFOS and mean head circumference. One study not reporting sex-specific differences did report that there were no statistically significant interactions by sex for their head circumference and PFOS measures (Gyllenhammar et al., 2018b). The head circumference reductions were consistently around –0.3 cm in males in three (one each *low*, *medium,* and *high* confidence) of four studies. The *medium* confidence study by Lind et al. (2017a) reported deficits across all quartiles (range: –0.3 to –0.4 cm) but only in males. The *high* confidence study by Valvi et al. (2017) also reported deficits only in male neonates (β per each doubling of serum PFOS: −0.28 cm; 95% CI: −0.65, 0.09), while head circumference increases were found for female neonates (β: 0.48 cm; 95% CI: 0.05, 0.90). The *low* confidence study of boys only by Marks et al. (2019) reported monotonic deficits across PFOS tertiles 2 (β: −0.13 cm; 95% CI: −0.45, 0.19) and 3 (β: −0.31 cm; 95% CI: −0.62, 0.01) compared with tertile 1. The *medium* confidence study by Kashino et al. (2020) reported smaller deficits only in male neonates (β per each log10 PFOS: −0.14 cm; 95% CI: −0.61, 0.32). Although it was not statistically significant, the *high* confidence study by Bjerregaard-Olesen et al. (2019) detected a small difference in mean head circumference among girls only (β per each IQR PFOS increase: −0.1 cm; 95% CI: −0.3, 0.1). The low confidence study by Cao et al. (2018) found a large head circumference difference (β for tertile 3 vs. 1: –1.22 cm; 95% CI: −2.70, 0.25) among females with some evidence of an exposure-response relationship.

Although there were nine different studies that showed some evidence of associations between PFOS and head circumference in the overall population or different subsets by countries or sex, there was limited epidemiological evidence of associations among the overall population with only four of 13 studies showing any inverse associations. Mean sex-specific head circumference deficits were detected in six different studies including four in male neonates and two others in females only. An additional study with standardized head circumference measures showed deficits in both sexes, but larger deficits were noted among females. One of two studies in each sex showed some evidence of an exposure-response relationship. A very large association was seen in one *low* confidence study among females, but more consistent results were seen across four studies in males (two *high*, one *medium* and one *low* confidence). Although limited numbers across different study characteristic or overall confidence level subgroups precluded a detailed assessment, few patterns were evident across the 10 different studies that showed some inverse associations with head circumference. Only two (Bjerregaard-Olesen et al., 2019; Lind et al., 2017a) of these nine studies had any early pregnancy (i.e., trimester 1) samples, with seven studies (Kashino et al., 2020; Marks et al., 2019; Xiao et al., 2019; Cao et al., 2018; Lauritzen et al., 2017; Valvi et al., 2017; Callan et al., 2016) based on either second and/or third trimester maternal samples or later. Overall, nine of 19 studies showing some evidence of inverse associations with some uncertainty as to what degree these results may be influenced by pregnancy hemodynamics due to later sample timing. There was considerable heterogeneity of results within and across both sexes and different studies.

3.4.4.1.5.5 Fetal Growth Restriction Summary

The majority of studies examining fetal growth restriction showed some evidence of associations with PFOS exposures especially those that included BWT data (i.e., SGA, low BWT, as well as mean and standardized BWT measures). The evidence for two fetal growth measures such as head circumference and birth length were less consistent. For many of these endpoints, there was a preponderance of associations amongst studies with later biomarker samples that may be more prone to potential biases from pregnancy hemodynamic impacts. However, there were also inverse associations observed in multiple studies based on early pregnancy biomarker samples. There was limited evidence of exposure-response relationships in either analyses specific to the overall population or different sexes, although the categorical data generally supported the linearly expressed associations that were detected.

Among the most accurate fetal growth restriction endpoints examined here, there was generally consistent evidence for BWT deficits across different measures and types of PFOS exposure metrics considered. BWT deficits were detected in the roughly two-thirds of included studies whether measured as mean BWT or standardized z-scores. This included 19 out of 30 mean BWT studies in the overall population and 16 of 27 *medium* or *high* confidence studies. Most of the sex-specific mean BWT studies showed some inverse associations in either male or female neonates, and although it was not consistent across studies, more deficits were found in male neonates. As noted above, many of the individual study results lacked precision and were not statistically significant especially the sex-stratified results which may have been largely underpowered to detect sex-specific differences.

The magnitude of some fetal growth measures were at times considered large, especially when considering the per unit PFOS increases across the exposure distributions. Although some of the other endpoints were fairly small in magnitude, the birth weight deficits and odds ratios for

birthweight-related measures were more sizable especially when considering most were expressed on a per-unit increase basis. For example, for all but one of the 19 studies showing mean BWT deficits in the overall population, reported deficits ranging from –14 to –93 grams per each PFOS unit increase. Associations were also seen for the majority of studies examining small for gestational age and low birth weight measures.

The current database (studies published since the 2016 PFOS HESD) is fairly strong given the wealth of studies included here, with most studies considered *high* or *medium* confidence (e.g., 23 out of 30 mean BWT) and most having adequate or good study sensitivity. As noted earlier, one source of uncertainty is that the meta-analyses of PFOS by Dzierlenga et al. (2020a) and PFOA by Steenland et al. (2018a) have shown that some measures like mean BWT may be prone to bias from pregnancy hemodynamics especially in studies with sampling later in pregnancy. Although a limited number of studies across some strata does not fully lend itself to differentiating patterns across different study characteristics, like study confidence and sample timing, some patterns emerged across the study results. For many of these endpoints, there was a preponderance of associations, such as birth weight measures, amongst studies with later biomarker samples (i.e., either exclusive trimester 2 maternal sample or later, such as umbilical cord or postpartum maternal samples) that may be more prone to pregnancy hemodynamic impacts. This observation is in agreement with the results of Dzierlenga et al. (2020a), though there was also evidence of associations in studies less likely to be biased by pregnancy hemodynamics (i.e., preconception or trimester 1 sampling). Therefore, despite consistency in evidence across some of these fetal growth endpoints, some important uncertainties remain mainly around the degree that some of the results examined here may be influenced by sample timing.

3.4.4.1.6 Postnatal growth

Eleven studies examined PFOS exposure in relation to postnatal growth measures (Figure 3-60). The synthesis here is focused on postnatal growth measures including mean and standardized weight (Starling et al., 2019; Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Manzano-Salgado et al., 2017b; de Cock et al., 2014) and height (Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; de Cock et al., 2014), as well as body mass index (BMI)/adiposity measures (Gross et al., 2020; Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Chen et al., 2017b; de Cock et al., 2014) and estimates of rapid growth during infancy (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b).

Four postnatal growth studies were *high* confidence (Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018), four were *medium* confidence (Gyllenhammar et al., 2018b; Chen et al., 2017b; Manzano-Salgado et al., 2017b; de Cock et al., 2014), and three were *low* confidence (Gross et al., 2020; Cao et al., 2018; Lee et al., 2018b). As shown in Figure 3-60, seven postnatal growth studies had good study sensitivity (Jensen et al., 2020; Starling et al., 2019; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Manzano-Salgado et al., 2017b), two each were adequate (Yeung et al., 2019; Cao et al., 2018) or deficient (Gross et al., 2020; de Cock et al., 2014). The *medium* confidence study by de Cock et al. (2014) did not report effect estimates but indicated that there were no statistically significant associations between PFOS quartiles and infant BMI (p-value = 0.59), infant weight

(p-value = 0.80), and infant height (p-value = 0.98) measures up to 11 months of age. But their lack of reporting of effect estimates precluded consideration of magnitude and direction of any associations and are not further examined below in the summaries.

The medium confidence study by Manzano-Salgado et al. (2017b) reported null associations for their overall population, female, and male neonates for weight gain z-score measured at 6 months per each log2 PFOS increase. The *low* confidence study by Lee et al. (2018b) reported statistically significant inverse associations for height at age 2 years (β per each PFOS ln-unit increase: −0.77 cm; 95% CI: −1.27, −0.15) as well as height change from birth to 2 years (β: −0.71 cm; 95% CI: −1.27, −0.15). Small differences were seen for mean weight differences at age 2 years (β: −0.17 cm; 95% CI: −0.38, 0.04) but not for weight change from birth to 2 years. Although no exposure-response relationships were detected when examined across PFOS categories, those with the highest exposure saw smaller statistically significant height increases at age 2 compared with lower exposures. Although a statistically significant birth length association was detected, the *medium* confidence study by Chen et al. (2017b) reported no association with infant height z-score up to 24 months. They did report statistically significant lower infant weight z-scores among female neonates comparable in magnitude for 6 to 12 months (β per each ln-unit PFOS increase: −0.25; 95% CI: −0.47, −0.04) or 12 to 24 months (β: −0.25; 95% CI: −0.41, −0.06). Females seemed to drive the deficit detected in the overall population (β per each ln-unit PFOS increase: −0.13; 95% CI: −0.32, 0.07) for the 6-to-12-month window. The *medium* confidence study by Gyllenhammar et al. (2018b) did not detect standardized BWT deficits per each IQR PFOS change, but they showed slight weight deficits (~ –0.2) at 3 months that persisted throughout 60 months of age. In contrast, standardized birth length measures were null for increasing PFOS exposures regardless of the time windows examined. Compared with the tertile 1 referent, the low confidence study of infants followed up to a median age of 19.7 months by Cao et al. (2018) reported slight increases in postnatal length (i.e., height) (β: 1.37 cm; 95% CI: −0.5, 3.28), while large postnatal weight deficits were reported for PFOS tertiles 2 (β: −138 g; 95% CI: −574, 298) and 3 (β: −78 g; 95% CI: −532, 375).

Associations at five months of age in the overall population (β: –0.28; 95% CI: –0.51, –0.05) and females (β: –0.56; 95% CI: –0.87, –0.26) from the *high* confidence study by Starling et al. (2019) were detected for weight-for-age z-scores, as well as weight-for-length z-scores (β: overall: –0.26; 95% CI: –0.53, 0.00; females: –0.52; 95% CI: –0.88, –0.17). Exposure-response relationships were observed across tertiles for both of these measures. In their *high* confidence study of repeated measures at 4 weeks, 1 year and 2 years of age, Shoaff et al. (2018) detected statistically significant deficits and exposure-response relationships for infant weight-for-age z-score (β: −0.33; 95% CI: −0.65, −0.01) and weight-for-length z-score (β: −0.34; 95% CI: −0.59, −0.08) in PFOS tertile 3 compared with tertile 1. Small deficits that were not statistically significant were observed in tertile 3 for length for age z-score (β: −0.22; 95% CI: −0.49, 0.04). In their *high* confidence study, Yeung et al. (2019) reported statistically significant negative growth trajectories weight-for-length z-scores in relation to each log SD increase in PFOS exposures among singletons followed for 3 years. No associations were detected for infant length (i.e., height) measures. Some sex-specific results were detected with larger associations seen in singleton females for weight-for-length z-score (β: −0.10; 95% CI: −0.16, −0.05) and weight z-score (β: −0.07; 95% CI: −0.13, −0.01). An infant weight deficit of –22.0 g (95% CI: −59.5, 15.6

per each 1 log SD PFOS increase) was also observed that was driven by results in females (β: –51.6 g; 95% CI: –102.3, –0.8).

Overall, seven of 8 studies with quantitative estimates (including 5 *high* and *medium* confidence studies) showed some associations between PFOS exposures and different measures of infant weight. Two of four studies with categorical data showed some evidence of inverse monotonic exposure-response relationships. Two of six studies with quantitative estimates examining different infant height measures showed some evidence of inverse associations with PFOS. Study quality ratings, including study sensitivity and overall confidence, did not appear to be explanatory factors for heterogeneous results across studies.

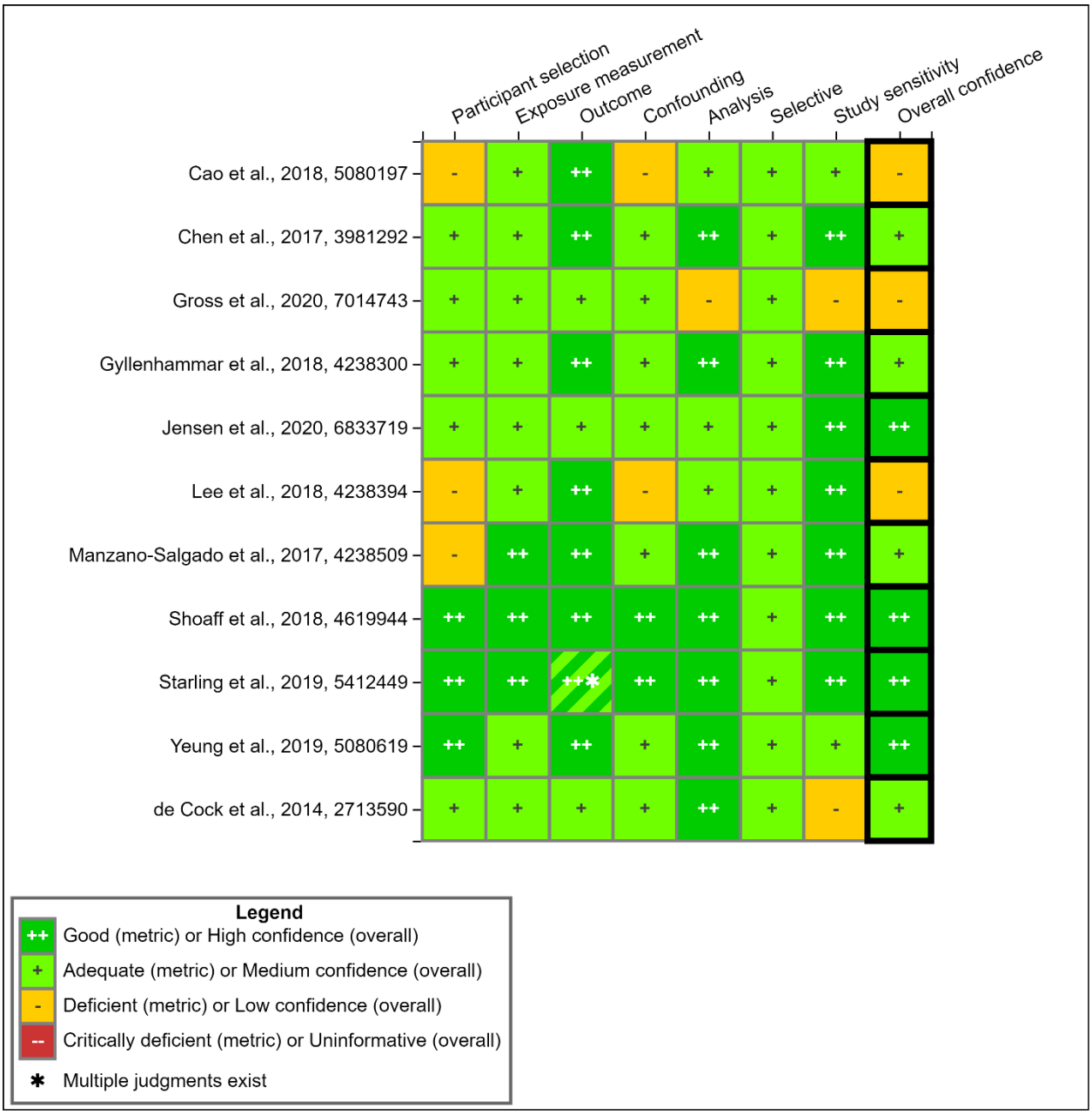


Figure 3-60. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Postnatal Growth

Interactive figure and additional study details available on HAWC.

3.4.4.1.6.1 Adiposity/BMI

In their *high* confidence study of repeated measures at 4 weeks, 1 year and 2 years of age, Shoaff et al. (2018) detected statistically significant decreases in infant BMI z-score (β: −0.36; 95% CI: −0.60, −0.12). Although they were not statistically significant, the *medium* confidence Chen et al. (2017b) reported consistently small BMI z-scores across infant developmental windows (range: −0.08 to −0.10) per each ln-unit PFOS. These results seem to be driven by results in females especially for the 6 to 12 months (β: −0.33; 95% CI: −0.59, −0.08) and 12 to 24 months

(β: −0.25; 95% CI: −0.45, −0.05) developmental periods. In their *high* confidence study, Yeung et al. (2019) reported statistically significant negative growth trajectories for BMI and BMI z-score in relation to each log SD increase in PFOS exposures among singletons followed for 3 years. No exposure-response relationship was detected for BMI z-scores. Some sex-specific results were detected with larger associations seen in singleton females BMI z-score (β: −0.11; 95% CI: –0.17, –0.05) and BMI (β: –0.16 kg/m2; 95% CI: –0.24, –0.08). In the *high* confidence study by Starling et al. (2019), decreased adiposity (β: –2.08; 95% CI: –3.81, –0.35) among girls was detected in PFOS tertile 3 compared with the tertile 1 referent. The *high* confidence study by Jensen et al. (2020) reported null associations between adiposity and per each 1-unit increase in PFOS measured at 3 and 18 months. The low confidence study by Gross et al. (2020) reported an inverse association (OR = 0.43; 95% CI: 0.17 to 1.09) of being overweight at 18 months for PFOS levels greater than the mean level. They also reported a lower odds ratio of being overweight at 18 months in males (OR = 0.19; p-value = 0.04) than females (OR = 0.85; p-value = 0.85). Mixed results were seen for measures of adiposity and increased BMI with increasing PFOS exposures.

3.4.4.1.6.2 Rapid Weight Gain

Four *high* confidence studies (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) examined rapid infant growth. Limited evidence of associations was reported, as only one (Starling et al., 2019) of four studies (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) showed increased odds or rapid weight gain with increasing PFOS. For example, Starling et al. (2019) reported a small OR of 1.36 for rapid growth in the overall population based on either weight-for-length-based z-scores. Study sensitivity was not an explanatory factor for the null studies.

3.4.4.1.6.3 Postnatal Growth Summary

Seven (3 *high*, 2 *medium*, and 2 *low* confidence) of the 8 studies with quantitative estimates examining different infant weight measures showed some evidence of adverse associations with PFOS exposures either in the overall population or either/or both male or female neonates. There was some evidence of exposure-response relationships as two of the four studies on infant weight showed adverse monotonic relationships across PFOS categories. No patterns by study characteristics or study confidence were evident. Only two (one *low* and one *high* confidence) of the seven studies with quantitative estimates examining different infant height measures showed some evidence of inverse associations with PFOS exposures. Two of the six postnatal growth studies with quantitative estimates showed increased infant BMI or adiposity with increasing PFOS exposures, while three showed decreased risk of higher BMI or adiposity. Only one out of four *high* confidence studies showed any evidence of rapid growth among infants following PFOS exposures. Although the data for some endpoints was less consistent, the majority of infant weight studies indicated that PFOS may be associated with postnatal growth measures up to 2 years of age.

3.4.4.1.7 Gestational Duration

Twenty-two different studies examined gestational duration measures (i.e., PTB or gestational age measures) in relation to PFOS exposures. Nine of these studies examined both PTB and gestational age measures, while two studies only examined PTB (Gardener et al., 2021; Liu et al., 2020d).

3.4.4.1.7.1 Gestational Age

Seventeen of the 20 studies reporting gestational age estimates in relation to PFOS exposures were considered (Figure 3-61). Two studies were deemed *uninformative* (Gundacker et al., 2021; Lee et al., 2013) and were excluded and one study was excluded based on an overlapping cohort (Li et al., 2017). Sixteen non-overlapping and informative studies examined mean gestational age (in weeks) in relation to PFOS exposures and one study reported sex-specific results only (Lind et al., 2017a).

Among the 17 different studies included here, nine were *high* confidence (Chu et al., 2020; Eick et al., 2020; Huo et al., 2020; Bell et al., 2018; Sagiv et al., 2018; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), four were *medium* (Yang et al., 2022In Press; Hjermitslev et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018) and four were *low* confidence (Bangma et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a). Ten of these studies had good study sensitivity, six were adequate (Yang et al., 2022In Press; Bangma et al., 2020; Eick et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a) and one was deficient (Bell et al., 2018).

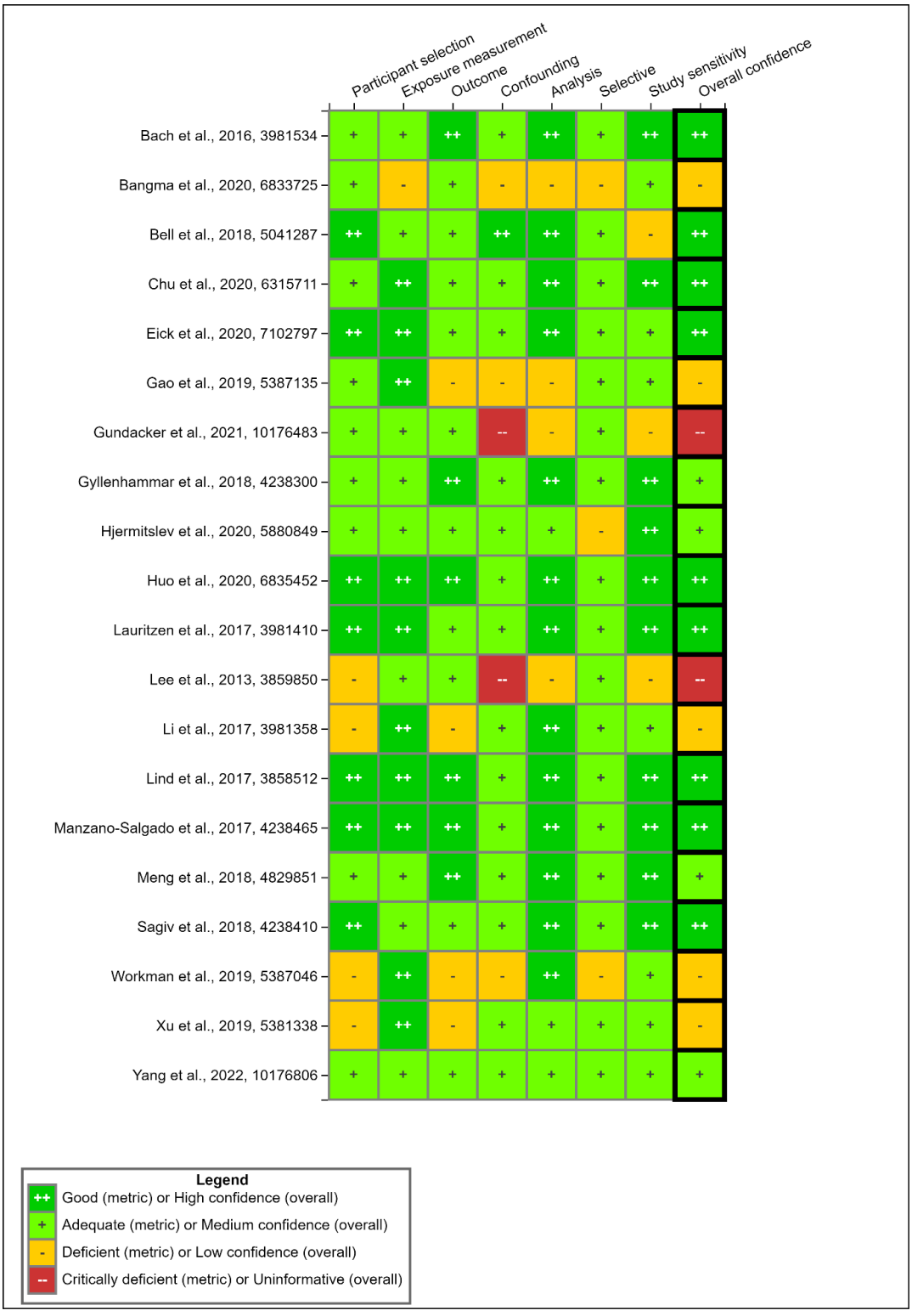


Figure 3-61. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Gestational Age

Interactive figure and additional study details available on HAWC.

Nine of the 16 studies examining mean gestational age change in the overall population reported some deficits. Among these nine studies, four were *high* confidence, and three were *medium* and two were *low* confidence. The *medium* confidence study by Gyllenhammar et al. (2018b) reported a deficit of –0.29 weeks (95% CI: –0.59, 0.01) per each IQR PFOS change; they also reported that there were no statistically significant interactions by sex for their PFOS measures. The *high* confidence study by Sagiv et al. (2018) reported a similar gestational age reduction in the overall population (β: –0.36 weeks; 95% CI: −0.64, −0.09) for PFOS quartile 4 versus quartile 1; this seemed to be driven by associations among boys only (β per each IQR increase: −0.19 weeks; 95% CI: −0.33, −0.05). The *high* confidence study by Chu et al. (2020) reported similar deficits in the overall population (β: –0.32 weeks; 95% CI: –0.53, –0.11) which was driven by female neonates (β: –0.61 weeks; 95% CI: –0.90, –0.32). The *high* confidence study by Lauritzen et al. (2017) only showed deficits among their Swedish population (β: –0.4 weeks; 95% CI: –0.9, 0.2). Compared with tertile 1, the *low* confidence study by Gao et al. (2019) reported deficits in tertile 2 (β: –0.40 weeks; 95% CI: –0.92, 0.12) and tertile 3 (β: –0.20; 95% CI: –0.61, 0.20). The *high* confidence study by Manzano-Salgado et al. (2017a) reported deficits in quartile 4 among the overall population (β: –0.31 weeks; 95% CI: –0.55, –0.06) compared with quartile 1. Despite relatively low overall PFOS concentrations, the *medium* confidence study by Yang et al. (2022In Press) showed reduced gestational age only among pre-term births for both total PFOS (β: −1.26 weeks; 95% CI: − 2.46, –0.05) and linear PFOS (β per each IQR increase: −1.80 weeks; 95% CI: −3.24, −0.37), with results larger results in female (β: −1.06 weeks; 95% CI: −2.87, 0.74) than male neonates (β: −0.41 weeks; 95% CI: −2.20, 1.37). The *medium* confidence study by Meng et al. (2018) reported statistically significant gestational age deficits (range: –0.16 to –0.29 weeks) across all quartiles but no evidence of an exposure-response relationship. The *low* confidence study by Workman et al. (2019) reported a nonsignificant decrease (β per each ln-unit PFOS change: –0.17 weeks; 95% CI: –0.52, 0.18).

Lind et al. (2017a) reported sex-specific changes in mean gestational age only. Inverse associations were observed for both boys (β per ln-unit increase: –0.5 days, 95% CI: –3.4, 2.3) and girls (β: –1.0, 95% CI: –4.2, 2.1), but neither was significant.

Overall, nine of the 16 studies based on the overall population showed some evidence of inverse associations between PFOS and gestational age. This included seven *medium* or *high* confidence studies. The four *high* confidence studies showed deficits in the overall population consistent in magnitude (range: –0.30 to –0.40 weeks). Apart from one study with very large deficits, the remaining two *medium* and *two* low confidence studies all ranged from –0.17 to –0.30 weeks for different PFOS contrasts). No exposure-response relationships were detected in any study, and no definitive patterns were seen based on other study characteristics or in the other few studies with sex-specific data. For example, 3 of 7 studies showed decreased gestational ages in relation to PFOS exposures among both male or female neonates. Study sensitivity did not seem to be an explanatory factor as five of six studies that did not show inverse associations had good or adequate study sensitivity. Lastly, sample timing did not seem to be an explanatory factor of the results as an equal proportion (60%) of studies showing inverse associations between PFOS and gestational age deficits were based on earlier and later biomarker sampling.

3.4.4.1.7.2 Preterm Birth

As shown in Figure 3-62, 11 studies examined the relationship between PFOS and preterm birth (PTB); all of the studies were either *medium* (Yang et al., 2022In Press; Liu et al., 2020d;

Hjermitslev et al., 2019; Meng et al., 2018) or *high* confidence (Gardener et al., 2021; Chu et al., 2020; Eick et al., 2020; Huo et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016). Nine of the 11 studies were prospective birth cohort studies, while the two studies by Liu et al. (2020d) and Yang et al. (2022In Press) were case-control studies nested with prospective birth cohorts. Four studies had maternal exposure measures that were sampled during trimester one (Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016), or trimester three (Gardener et al., 2021). The *high* confidence study by Chu et al. (2020) sampled during the late third trimester or within three days of delivery. Four studies collected samples across multiple trimesters (Eick et al., 2020; Huo et al., 2020; Liu et al., 2020d; Hjermitslev et al., 2019). One study used umbilical cord serum samples (Yang et al., 2022In Press). The *medium* confidence study by Meng et al. (2018) pooled umbilical cord blood and maternal serum (trimester 1 and 2) exposure data from two study populations. Seven studies had good study sensitivity, while four others were considered adequate (Yang et al., 2022In Press; Gardener et al., 2021; Eick et al., 2020; Liu et al., 2020d) with the median exposure values in the overall population ranging from 1.79 ng/mL (Liu et al., 2020d) to 30.1 ng/mL (Meng et al., 2018). Lower levels were also seen for a total PFOS measure in Yang et al. (2022In Press) for both cases (median (IQR) = 0.27 (0.30) ng/mL) and controls (0.21 (0.37) ng/mL).

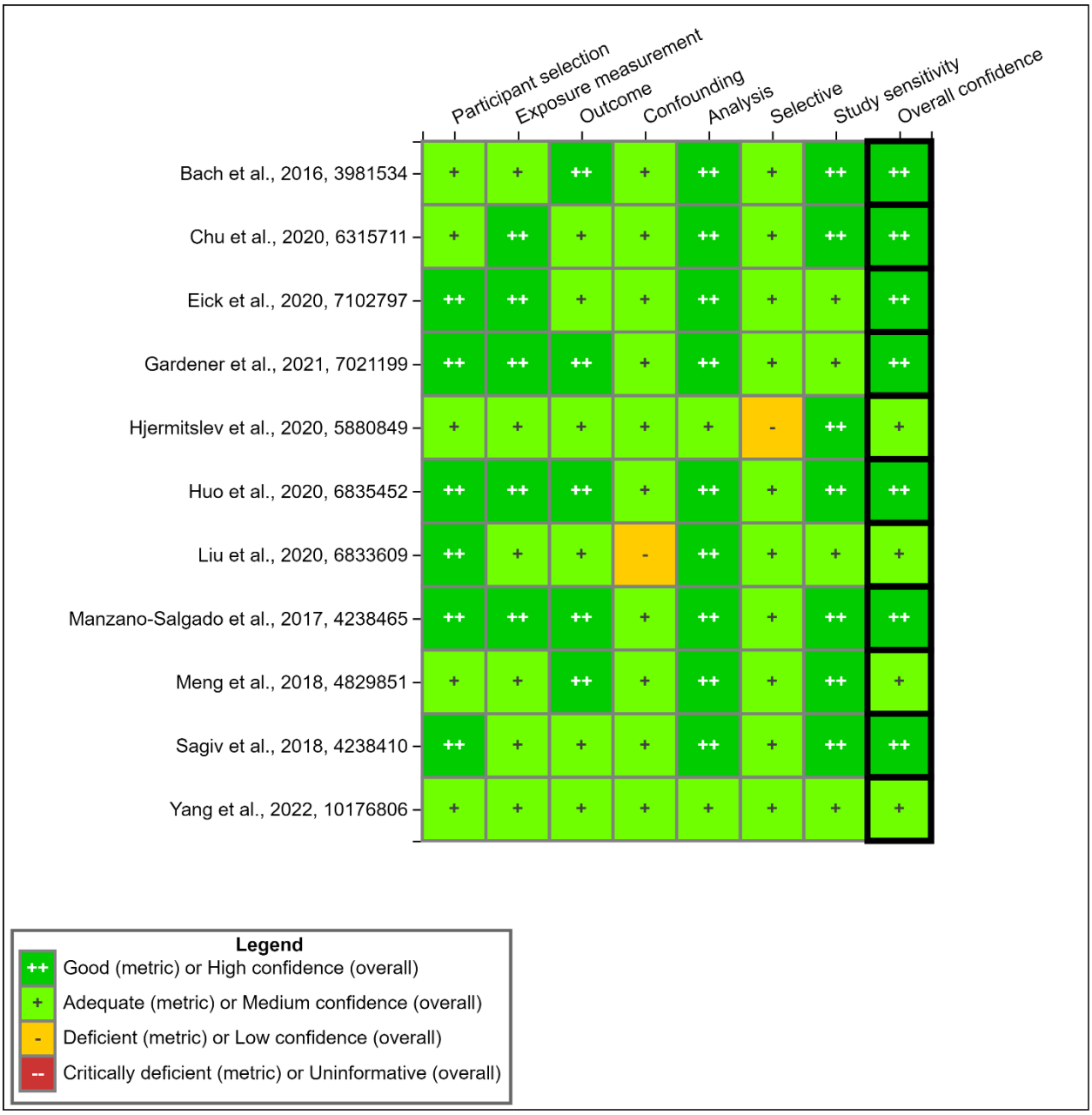


Figure 3-62. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Preterm Birth Effects

Interactive figure and additional study details available on HAWC.

An increased risk was reported in seven of the 11 PTB studies with ORs from 1.5- to 5-fold higher for elevated PFOS exposures. The *medium* confidence study by Meng et al. (2018) study reported statistically significant non-monotonic increased ORs for PTB in the upper three PFOS quartiles (OR range: 1.9–3.3), as well as per each doubling of PFOS exposures (OR = 1.5; 95% CI: 1.1, 2.2). The *high* confidence study by Chu et al. (2020) reported some statistically significant increased ORs per each ln unit increase (OR = 2.03; 95% CI: 1.24, 3.32) as well as an exposure-response relationship across upper three quartiles (OR range: 2.22–4.99) exposures

when compared with the referent. The *high* confidence study by Eick et al. (2020) reported an exposure-response relationship as well (tertile 2 OR = 1.21; 95% CI: 0.50, 2.91; tertile 3 OR = 1.87; 95% CI: 0.72, 4.88, compared with tertile 1). Although they were not statistically significant, the *medium* confidence study by Liu et al. (2020d) reported increased ORs of similar magnitude per each $\log_{10}$ unit increase (OR = 1.30; 95% CI: 0.76, 2.21) or when quartile 3 (OR = 1.51; 95% CI: 0.85, 2.69) and quartile 4 (OR = 1.35; 95% CI: 0.74, 2.45) exposures were compared with the referent. The *high* confidence study by Sagiv et al. (2018) study reported consistently elevated non-monotonic ORs for PTB in the upper three PFOS quartiles (OR range: 2.0–2.4), but smaller ORs when examined per each IQR PFOS increase (OR = 1.1; 95% CI: 1.0, 1.3). The *high* confidence study by Gardener et al. (2021) reported that participants in the PFOS exposure quartiles 2 (OR = 1.94; 95% CI: 0.66, 5.68) and 4 (OR = 1.41; 95% CI: 0.46, 4.33) had higher odds of preterm birth (relative to the lowest quartile). Despite low overall PFOS concentrations, the *medium* confidence study by Yang et al. (2022In Press) showed statistically significant increased odds of preterm birth per each IQR increase in total PFOS (OR = 1.44; 95% CI: 1.18, 1.79), linear PFOS (OR = 1.41; 95% CI: 1.19, 1.73), and branched PFOS (OR = 1.11; 95% CI: 1.01, 1.29). No differences were observed for male or female stratified results (OR range: 1.40–1.45). Null or inverse associations were reported by Bach et al. (2016), Huo et al. (2020), Manzano-Salgado et al. (2017a) and Hjermitslev et al. (2019). Overall, only two (Chu et al., 2020; Eick et al., 2020) out of eight studies showed evidence of exposure-response relationships.

Overall, 7 of 11 studies reported increased odds of preterm birth in relation to PFOS with some sizable relative risks reported. There was some limited evidence of exposure-response relationships as well. Although small numbers limited the confidence in many of the sub-strata comparisons, few patterns in the PTB results emerged based on study confidence (all 11 studies were *medium* or *high* confidence), sample timing or other study characteristics. For example, three of the four null studies were considered to have good sensitivity to detect associations that may be present. The results for preterm birth are strong with respect to an increased risk detected with increasing PFOS exposures.

Few patterns in the PTB results emerged based on study confidence or other study characteristics. Since nearly all studies had good study sensitivity, study sensitivity did not largely appear to be a concern in this database. In addition, only one out of the four studies that did not show increased risk had limited exposure contrasts.

3.4.4.1.7.3 Gestational Duration Summary

Overall, there is *robust* evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as most studies showed some increased risk of gestational duration deficits. This was strengthened by consistency in the reported magnitude of gestational age deficits despite different exposure levels and metrics examined. Although they were not as consistent in magnitude (60% of the PTB studies showed some increased risk), some of the effect estimates were large for preterm birth in relation to PFOS exposures with limited evidence of exposure-response relationships. Few patterns were evident as explanatory factors for heterogeneous results based on the qualitative analysis.

3.4.4.1.8 Fetal Loss

As shown in Figure 3-63, five (two *high*, two *medium,* and one *low* confidence) studies examined PFOS exposure and fetal loss. All of these studies had good study sensitivity owing largely to very large sample sizes (Wang et al., 2021; Wikström et al., 2021; Liew et al., 2020; Buck Louis et al., 2016; Jensen et al., 2015).

The *high* confidence study by Wikström et al. (2021) showed little evidence of association between PFOS and miscarriages (OR = 1.13; 95% CI: 0.82, 1.52 per doubling of PFOS exposures). The authors did not report an exposure-response relationship across PFOS quartiles but did show elevated nonsignificant ORs of approximately 1.2 and 1.3 for the upper two quartiles. Although the ORs were not statistically significant in the *medium* confidence study by Liew et al. (2020), there was some suggestion of an exposure-response relationship for miscarriages across PFOS quartiles (OR range: 1.1–1.4). Similarly, the *low* confidence study by Jensen et al. (2015) reported increased nonsignificant risks across tertiles 2 and 3 (OR range: 1.15–1.33). No association was detected in the *high* confidence study by Wang et al. (2021) (OR = 0.95; 95% CI: 0.87, 1.04) or the *medium* confidence study by Buck Louis et al. (2016) (hazard ratio (HR) = 0.81; 95% CI: 0.65, 1.00 per each SD PFOS increase).

Overall, there was positive evidence for fetal loss with increased relative risk estimates in three out of five studies. In those three studies, the magnitude of associations detected were low but consistently reported in the range of 1.1 of 1.4 with an exposure-response relationship detected in one study. No patterns in the results were detected by study confidence ratings including sensitivity.

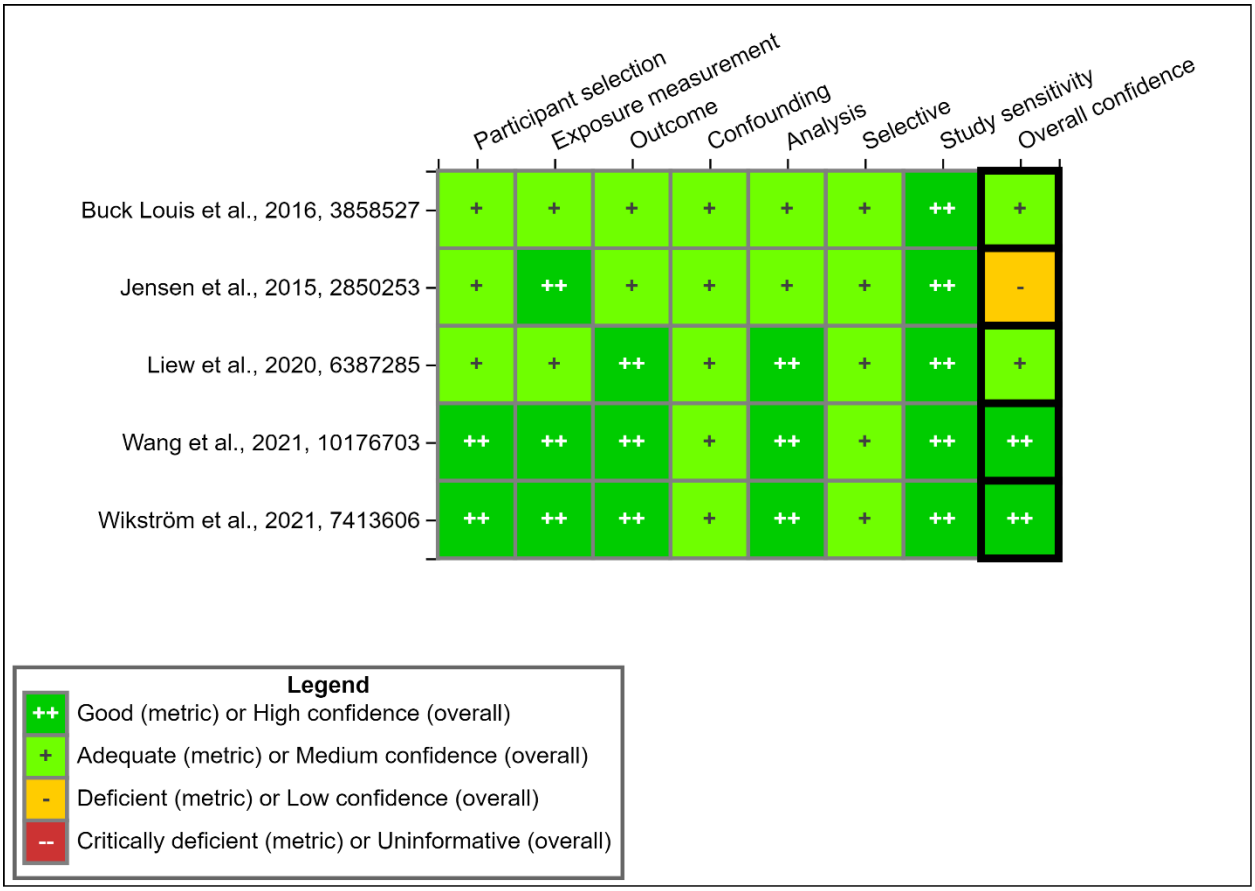


Figure 3-63. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Fetal Loss

Interactive figure and additional study details available on HAWC.

3.4.4.1.9 Birth Defects

As shown in Figure 3-64, five (three *medium* and two *low* confidence) studies examined PFOS exposure in relation to birth defects. Four of the five studies had adequate sensitivity. This included a *medium* confidence study by Ou et al. (2021) that reported increased risks for septal defects (OR = 1.92; 95% CI: 0.80, 4.60), conotruncal defects (OR = 1.65; 95% CI: 0.59, 4.63) and total congenital heart defects (OR = 1.61; 95% CI: 0.91, 2.84) among participants with maternal serum levels over the 75th PFOS percentile level (relative to those <75th percentile). A *low* confidence study of a non-specific grouping of all birth defects (Cao et al., 2018) reported a small but imprecise increased risk (OR = 1.27; 95% CI: 0.59, 2.73). Interpretation of all birth defect groupings is challenging given that etiological heterogeneity may occur across individual defects.

Three studies examined PFOS exposures in relation to cryptorchidism. The *medium* confidence study by Vesterholm Jensen et al. (2014) detected an inverse association for cryptorchidism (OR per each ln-unit increase in PFOS = 0.51; 95% CI: 0.21–1.20). This risk seemed to be largely driven by boys from Finland. The *medium* confidence study by Toft et al. (2016) reported null associations per each ln-unit increase in PFOS exposures and both cryptorchidism (OR = 0.99; 95% CI: 0.75, 1.30) and hypospadias (OR = 0.87; 95% CI: 0.57, 1.34). The *low* confidence study

by Anand-Ivell et al. (2018) did not find statistically significant PFOS exposure differences among cryptorchidism or hypospadia cases compared with controls, but they did not examine this in a multivariate fashion adjusting for confounders.

Overall, there was very limited evidence of associations between PFOS and birth defects based on the available epidemiological studies. This was based on cryptorchidism, hypospadias or all birth defect groupings. As noted previously, there is considerable uncertainty in interpreting results for broad any defect groupings which are anticipated to have decreased sensitivity to detect associations.

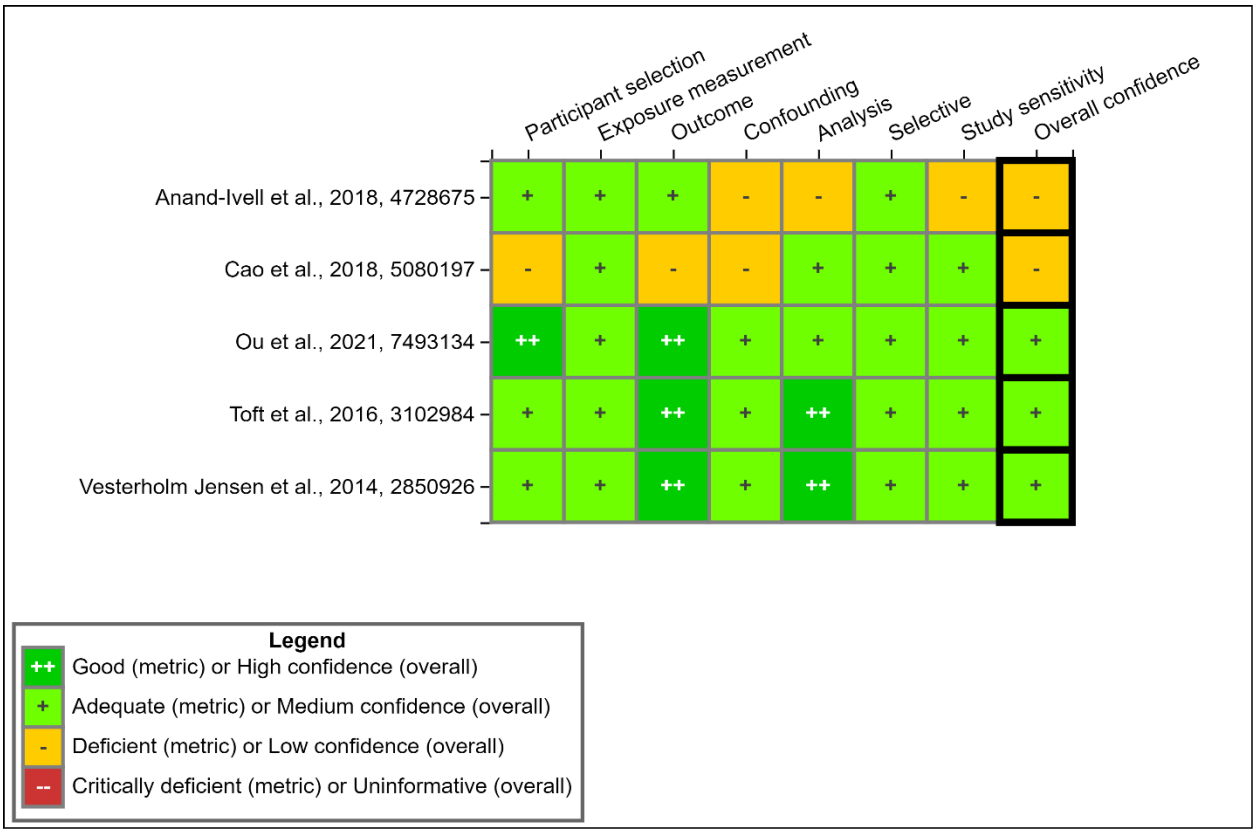


Figure 3-64. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Defects

Interactive figure and additional study details available on HAWC.

3.4.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 4 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and 16 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and developmental effects. Study quality evaluations for these 20 studies are shown in Figure 3-65.

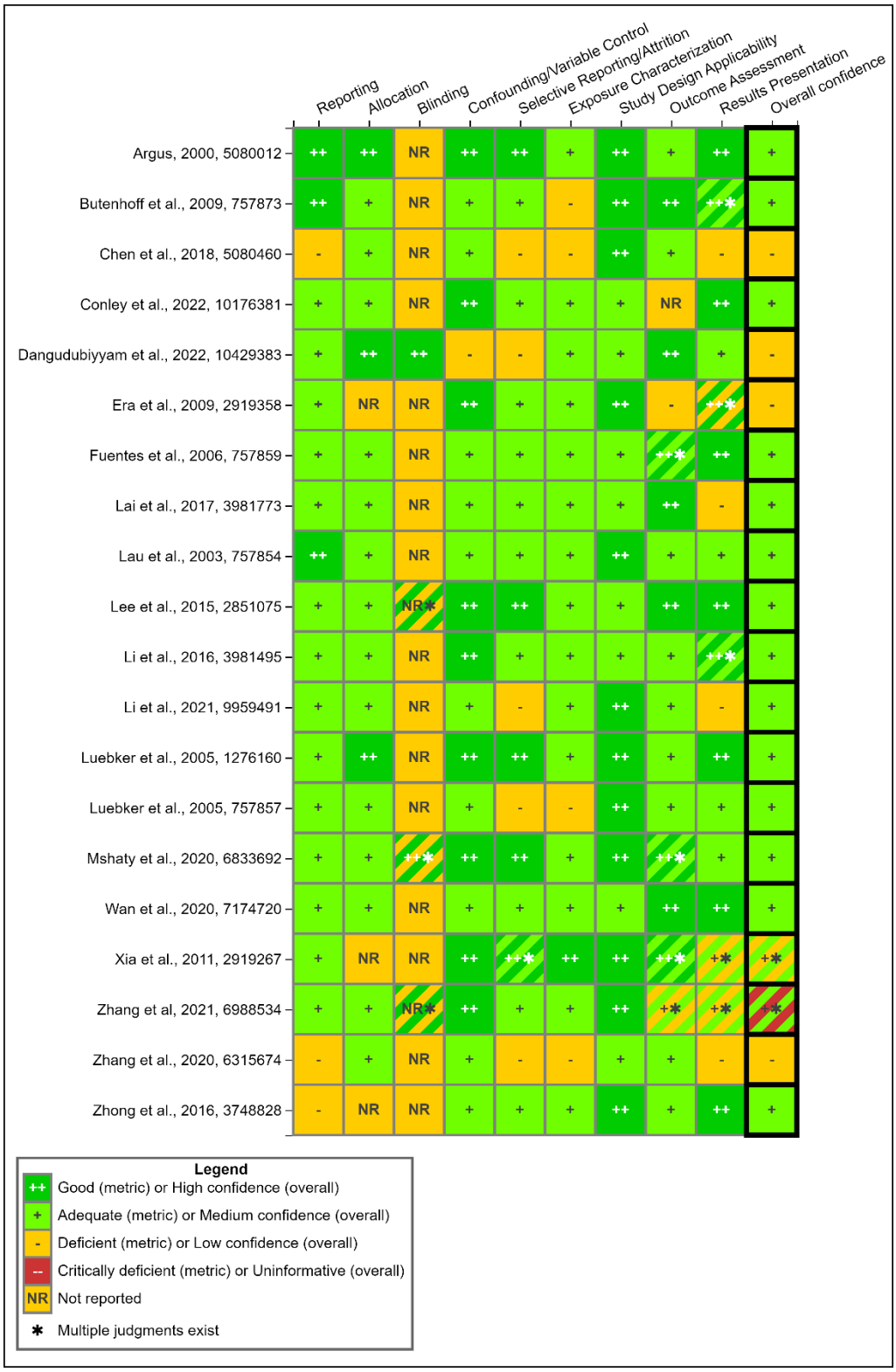


Figure 3-65. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Developmental Effects

Interactive figure and additional study details available on HAWC.

Evidence indicates that PFOS exposure can adversely affect development. Oral studies in mice, rats, and rabbits report effects in offspring including decreased survival, decreased body weights, structural abnormalities (e.g., reduced skeletal ossification), histopathological changes in the lung, and delayed eye opening, among others. Effects in offspring primarily occurred at similar doses as those seen in the maternal animals. Adverse effects observed in dams include alterations in gestational weight and gestational weight gain, as well as evidence of altered placental histology. In some cases, adverse developmental effects of PFOS exposure that relate to other health outcomes may be discussed in the corresponding health outcome section (e.g., fetal and neonatal pulmonary effects are discussed in the respiratory section found in Appendix C (U.S. EPA, 2024a)).

3.4.4.2.1 Maternal Effects

Multiple developmental studies evaluated maternal weight outcomes in rats, mice, and rabbits (Figure 3-66). Yahia et al. (2008) observed a decrease in body weight in ICR mouse dams administered 20 mg/kg/day PFOS from gestational day 1 to 17 (GD 1 to GD 17) or GD 18. The dams exhibited no clinical signs of toxicity. Thibodeaux et al. (2003) observed significantly decreased maternal body weight gain in CD-1 mice at exposed to 20 mg/kg/day PFOS (highest dose tested in the study); food and water consumption were not affected by treatment. Lee et al. (2015) also reported reduced maternal body weight gain in CD-1 mice treated with 2 or 8 mg/kg/day PFOS (not 0.5 mg/kg/day) compared with controls. Dams in the 2 and 8 mg/kg/day dose groups had significantly lower mean body weights on GD 14–17. In contrast, Lai et al. (2017a) did not observe a significant difference in maternal body weight in CD-1 mouse dams orally exposed to 0, 0.3, or 3 mg/kg/day throughout gestation (GD 1–20). The authors determined that there were no observable maternal effects related to PFOS exposure at the relatively low doses evaluated. Wan et al. (2020) found no effect of PFOS on maternal body weight in CD-1 mouse dams orally dosed with 0, 1, or 3 mg/kg/day from GD 4.5 to GD 17.5. Likewise, Fuentes et al. (2006) found no treatment-related effects on maternal body weight, maternal body weight gain, or maternal food consumption in CD-1 mouse dams orally exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. Mshaty et al. (2020) orally administered PFOS to C57BL/6J mice from postnatal day 1 (PND 1) to PND 14, resulting in lactational exposure to pups. Mean maternal body weights were evaluated at PND 21 and determined to be comparable between the control and the 1 mg/kg/day dose groups.

Thibodeaux et al. (2003) observed significant, dose-dependent decreases in maternal body weight, food consumption, and water consumption in Sprague-Dawley rats dosed with ≥2 mg/kg/day PFOS from GD 2 to GD 20. Xia et al. (2011) also observed reduced body weight on GD 21 in Sprague-Dawley rats dosed with 2 mg/kg/day from GD 2 to GD 21. In a 2-generation reproductive toxicity study in rats, Luebker et al. (2005a) similarly observed dose-dependent decreases in maternal body weight in the 3.2 mg/kg/day dose group of the parental generation (P_0) from day 15 of the premating exposure through lactation day 1 (LD 1), the last recorded weight; this dose group also had significantly decreased maternal weight gain from GD 0 to GD 20. The 1.6 mg/kg/day dams experienced transient decreases in maternal weight compared with controls in the window between GD 3 and GD 11. There were no reported differences in the maternal weight of adult first generation (F_1) females during pre-cohabitation until the end of lactation, though the highest dose tested in these females was only 0.4 mg/kg/day. Following the 2-generation study, Luebker et al. (2005b) conducted a follow-up 1-generation study that examined additional PFOS doses during development. Crl:Cd(Sd)Igs

Vaf/Plus rat dams were gavaged with 0, 0.4, 0.8, 1, 1.2, 1.6, or 2 mg/kg/day PFOS. Dosing started 6 weeks prior to mating and continued through mating and gestation with the final dose on LD 4. The authors observed no treatment-related effects on body weight change during gestation, but body weight gain was reduced in the 0.8, 1, 1.6, and 2 mg/kg/day groups relative to controls during lactation. They also reported a general trend for reduced food consumption with increasing dose during gestation and lactation (Luebker et al., 2005b). In another study with Sprague-Dawley rats dosed with 0, 5, or 20 mg/kg/day PFOS from GD 12 to GD 18, Li et al. (2016) also reported reduced mean maternal body weights in the 20 mg/kg/day dose group. In another study, Conley et al. (2022) reported a significant 43% weight gain reduction relative to controls in Sprague-Dawley (Crl:CD(SD)) rat dams dosed with 30 mg/kg/day PFOS from GD 14 to GD 18; no significant effects were observed for the 0.1, 0.3, 1, 3, or 10 mg/kg/day PFOS groups. Zhang et al. (2021) also reported no significant treatment-related effects on maternal body weight in Sprague-Dawley rat dams dosed with 0, 1, or 5 mg/kg/day PFOS from GD 12 to GD 18. Butenhoff et al. (2009) observed comparable maternal body weight and body weight gain during gestation in Sprague-Dawley rat dams dosed with 0, 0.1, 0.3, or 1 mg/kg/day PFOS from GD 0 to LD 20 but observed significantly lower absolute body weights during lactation (PND 4–20) in dams treated with 1 mg/kg/day PFOS. Transient decreases in food consumption were observed in the 0.3 and 1.0 mg/kg/day groups throughout the study, though these findings were not considered treatment-related or adverse.

In a single rabbit study, Argus Research Laboratories (2000) reported significantly decreased maternal body weight gain from GD 7 to GD 21 at PFOS doses ≥1 mg/kg/day (mean body weight change of 0.38, 0.3, 0.2, and −0.01 kg with 0, 1, 2.5, and 3.75 mg/kg/day PFOS, respectively); no significant effect was observed from GD 21 to GD 29. There were observations of scant or no feces for some does in the 1.0, 2.5, and 3.75 mg/kg/day groups. Observations of scant feces were significant relative to control at 3.75 mg/kg/day. Significant reductions in absolute (g/day) and relative (g/kg/day) feed consumption was also observed in the 2.5 and 3.75 mg/kg/day dose groups.

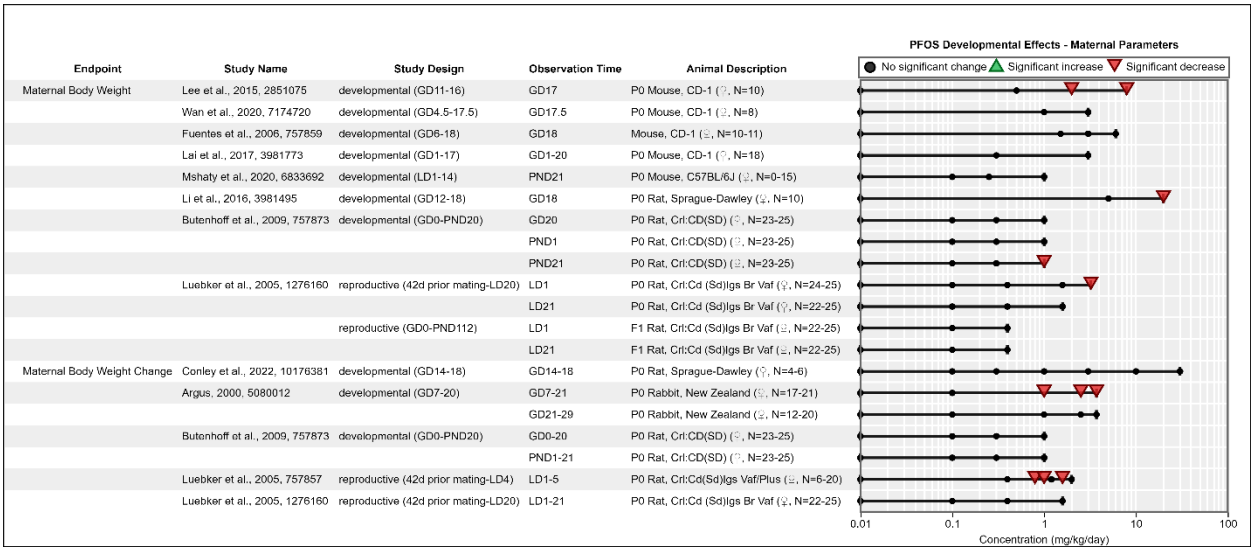


Figure 3-66. Maternal Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day.

3.4.4.2.2 Viability

Decreases in both fetal and pup survival and viability with perinatal PFOS exposure were observed in multiple studies (Figure 3-67). Lee et al. (2015) reported a significantly higher incidence of resorptions, post-implantation loss, and dead fetuses at GD 17 after dosing pregnant CD-1 mice by gavage with 0.5, 2, or 8 mg/kg/day from GD 11 to GD 16; however, there was no significant difference in the mean number of implantations. A significant decrease in mean number of live fetuses was also observed in the 2.0 and 8.0 mg/kg/day dose groups versus controls. A decrease in the mean number of live fetuses was reported in the 0.5 mg/kg/day dose group but this difference was not significant relative to control. Administration of 0, 1, 5, 10, 15, or 20 mg/kg/day PFOS to CD-1 mice from GD 1 to GD 17 did not affect the number of implantation sites but resulted in a significant increase in post-implantation loss, as measured by decrease in mean percentage of live fetuses, in dams administered 20 mg/kg/day (Thibodeaux et al., 2003). In another study, CD-1 mouse dams were dosed with 0, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. The authors found no treatment-related effects on the number of litters with dead fetuses, the total number of dead fetuses, dead fetuses per litter, or live fetuses per litter, and there were no effects of PFOS on the number of implantation sites, the percentage of post-implantation loss, the number of early or late resorptions, or fetal sex ratio (Fuentes et al., 2006).

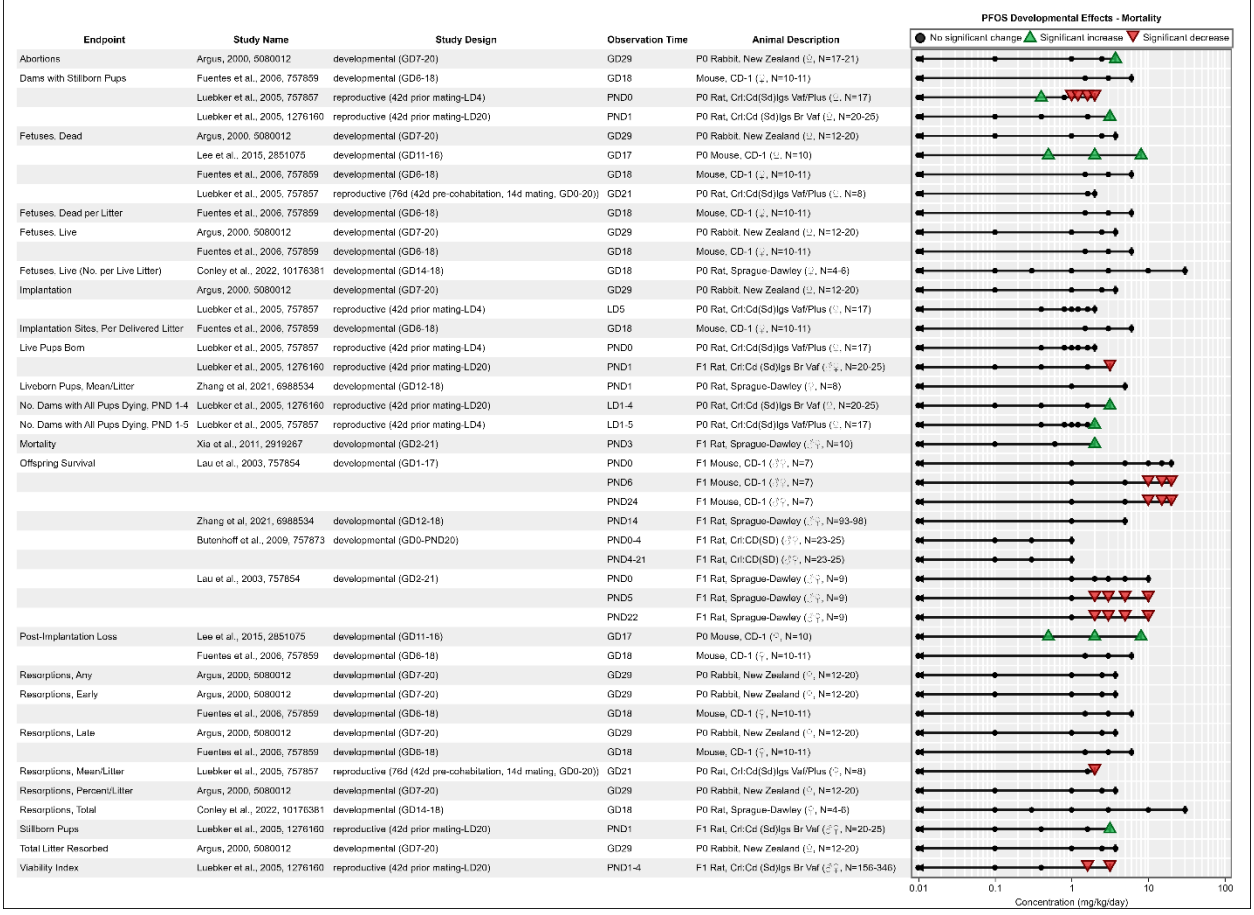
Mice appear to be more sensitive to alterations in fetal viability than rats. Thibodeaux et al. (2003) dosed pregnant Sprague-Dawley rats with 0, 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from GD 2 to GD 20. The number of implantations was not affected by treatment and there were no treatment-related effects observed on the live rat fetuses at term. Likewise, Zhang et al. (Zhang et al., 2021) dosed Sprague-Dawley rat dams with 0, 1, or 5 mg/kg/day PFOS from GD 12 to GD 18 and found no treatment-related effects on liveborn pups per litter, pup survival, or pup sex ratio. Butenhoff et al. (2009) also observed no treatment-related effects on the number of implantation sites or resorptions in pregnant Sprague-Dawley rats exposed to 0.1, 0.3, or 1.0 mg/kg/day by gavage from GD 0 to PND 20. Similarly, Conley et al. (2022) found no effects of PFOS on the number of live fetuses per litter or total resorptions in a study wherein Sprague-Dawley (Crl:CD(SD)) rat dams were dosed with 0, 0.1, 0.3, 1, 3, 10, or 30 mg/kg/day PFOS from GD 14 to GD 18.

In pregnant New Zealand white rabbits cesarean sectioned on GD 29 after gestational exposure to PFOS, Argus Research Laboratories (2000) reported no significant effects on implantations or resorptions. However, Argus Research Laboratories (2000) did report abortions among New Zealand white rabbits orally dosed with 2.5 mg/kg/day (1/17 does, 5.9%) or 3.75 mg/kg/day (9/21 does, 42.8%) from GD 7 to GD 20. The abortion rate was statistically greater relative to control for the 3.75 mg/kg/day dose group. Argus Research Laboratories (2000) reported no significant effects on the mean number of live fetuses per doe, number of dead fetuses per doe, mean litter size, and offspring viability.

Altered pup viability was observed in studies of both rats and mice. In one- and two-generation reproductive toxicity studies in Sprague-Dawley rats, Luebker et al. (2005b; 2005a) observed reduced pup viability index (ratio of the number of pups alive at PND 5 to the number of live pups born) with higher maternal PFOS doses. A significant decrease in pup viability for the one-

generation study was associated with a dose of 1.6 mg/kg/day (Luebker et al., 2005b); the number of dams with all pups dying between PND 1 and PND 5 was also significantly increased in the 2 mg/kg/day dose group. The dose associated with a decreased viability index in F_1 pups was also 1.6 mg/kg/day in the two-generation study (Luebker et al., 2005a); between PND 1 and PND 4, 100% of dams had all pups dying in the 3.2 mg/kg/day dose group. Following gestational exposure to PFOS on GD 19–20, Grasty et al. (2006) observed survival of 98%, 66%, and 3% of rat pups in the control, 25, and 50 mg/kg/day groups, respectively, on PND 5. Similarly, Xia et al. (2011) found decreased number of delivered pups per litter and increased pup mortality between birth and PND 3 for rats treated with 2 mg/kg/day on GD 2 to GD 21. Chen et al. (2012b) also observed decreased pup survival through PND 3 in rat pups exposed to 2 mg/kg/day PFOS from GD 1 to GD 21. Thibodeaux et al. (2003) and Lau et al. (2003) similarly observed decreased pup survival in rats exposed to ≥2.0 mg/kg/day PFOS from GD 2 to GD 21.

Lau et al. (2003) also reported PFOS-related effects on survival in mice following gestational exposure to PFOS. Briefly, most mouse pups from dams administered 15 or 20 mg/kg/day did not survive for 24 hours after birth. Fifty percent mortality was observed at 10 mg/kg/day. Survival of pups in the 1 and 5 mg/kg/day treated dams was similar to controls. Yahia et al. (2008) also observed significant effects on pup survival. In this study, pregnant ICR mice/group were administered 0, 1, 10, or 20 mg/kg of PFOS daily by gavage from GD 1 to GD 17 or GD 18. All neonates in the 20 mg/kg/day dose group were born pale, weak, and inactive, and all died within a few hours of birth. At 10 mg/kg/day, 45% of those born died within 24 hours. Survival of the 1 mg/kg/day group was similar to that of controls. Of the developmental studies identified in the most recent literature search, only Mshaty et al. (2020) evaluated the impact of lactational (PND 1–14) PFOS exposure on pup survival. Mshaty et al. (2020) observed no difference in C57BL/6J mouse pup survival through PND 21 between control group pups and pups exposed to 1 mg/kg/day PFOS (quantitative data not provided).



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Abortions	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=17-21)
Dams with Stillborn Pups	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	PND0	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=20-25)
Fetuses, Dead	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Lee et al., 2015, 2851075	developmental (GD11-16)	GD17	P0 Mouse, CD-1 (♀, N=10)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
	Luebker et al., 2005, 757857	reproductive (76d (42d pre-cohabitation, 14d mating, GD0-20))	GD21	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=8)
Fetuses, Dead per Litter	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Fetuses, Live	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Fetuses, Live (No. per Live Litter)	Conley et al., 2022, 10176381	developmental (GD14-18)	GD18	P0 Rat, Sprague-Dawley (♀, N=4-6)
Implantation	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	LD5	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
Implantation Sites, Per Delivered Litter	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Live Pups Born	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	PND0	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=20-25)
Liveborn Pups, Mean/Litter	Zhang et al, 2021, 6988534	developmental (GD12-18)	PND1	P0 Rat, Sprague-Dawley (♀, N=8)
No. Dams with All Pups Dying, PND 1-4	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	LD1-4	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=20-25)
No. Dams with All Pups Dying, PND 1-5	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	LD1-5	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
Mortality	Xia et al., 2011, 2919267	developmental (GD2-21)	PND3	F1 Rat, Sprague-Dawley (♂♀, N=10)
Offspring Survival	Lau et al., 2003, 757854	developmental (GD1-17)	PND0	F1 Mouse, CD-1 (♂♀, N=7)
			PND6	F1 Mouse, CD-1 (♂♀, N=7)
			PND24	F1 Mouse, CD-1 (♂♀, N=7)
	Zhang et al, 2021, 6988534	developmental (GD12-18)	PND14	F1 Rat, Sprague-Dawley (♂♀, N=93-98)
	Butenhoff et al., 2009, 757873	developmental (GD0-PND20)	PND0-4	F1 Rat, Crl:CD(SD) (♂♀, N=23-25)
			PND4-21	F1 Rat, Crl:CD(SD) (♂♀, N=23-25)
	Lau et al., 2003, 757854	developmental (GD2-21)	PND0	F1 Rat, Sprague-Dawley (♂♀, N=9)
			PND5	F1 Rat, Sprague-Dawley (♂♀, N=9)
			PND22	F1 Rat, Sprague-Dawley (♂♀, N=9)
Post-Implantation Loss	Lee et al., 2015, 2851075	developmental (GD11-16)	GD17	P0 Mouse, CD-1 (♀, N=10)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Any	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Resorptions, Early	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Late	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Mean/Litter	Luebker et al., 2005, 757857	reproductive (76d (42d pre-cohabitation, 14d mating, GD0-20))	GD21	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=8)
Resorptions, Percent/Litter	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Resorptions, Total	Conley et al., 2022, 10176381	developmental (GD14-18)	GD18	P0 Rat, Sprague-Dawley (♀, N=4-6)
Stillborn Pups	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=20-25)
Total Litter Resorbed	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Viability Index	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1-4	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=156-346)

Figure 3-67. Mortality and Viability in Mice, Rats, and Rabbits Following Exposure to PFOS (Logarithmic Scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day.

3.4.4.2.3 Skeletal, Soft Tissue, and Gross Effects

Skeletal defects in offspring, including bone ossification, have been observed in mice, rats, and rabbits gestationally exposed to PFOS. In one study, 0, 1, 10, or 20 mg/kg of PFOS was administered daily by gavage to pregnant ICR mice from GD 1 to GD 17 or GD 18 (Yahia et al., 2008). Five dams/group were sacrificed on GD 18 for fetal external and skeletal effects. In the fetuses from dams treated with 20 mg/kg/day, there were significant increases in the numbers of fetuses with cleft palates (98.56%), sternal defects (100%), delayed ossification of phalanges (57.23%), wavy ribs (84.09%), spina bifida occulta (100%), and curved fetus (68.47%). In mice, Thibodeaux et al. (2003) observed significantly increased incidences of cleft palate at 15 and 20 mg/kg/day PFOS, sternal defects at 5, 10, 15, and 20 mg/kg/day PFOS, and ventricular septal defects at 20 mg/kg/day PFOS. Thibodeaux et al. (2003) also observed significantly increased incidences of these deformities in rats. The authors reported incidences of cleft palate at 10 mg/kg/day PFOS and sternal defects at 2 and 10 mg/kg/day PFOS. In another study, CD-1 mouse dams were exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18 (Fuentes et al., 2006). The authors reported a lower incidence of incomplete calcaneus ossification in the

3 mg/kg/day group (6% fetal incidence, 20% litter incidence) relative to controls (46% fetal incidence, 80% litter incidence). The same study observed no treatment-related effects on fetal or litter incidence of the following skeletal development outcomes: supernumerary ribs, asymmetric sternebra, incomplete ossification of vertebra, or total skeletal malformations (Fuentes et al., 2006).

Skeletal malformations in fetal and neonatal rabbits were reported in Argus Research Laboratories (2000) at comparatively lower PFOS doses than those described in rat and mouse studies. A significant decrease in the mean number of isolated ossification sites of the metacarpal per fetus per litter was observed in the 3.75 mg/kg/day dose group versus control (4.82 vs. 4.98, respectively); no significant change in mean number of ossification sites per fetus per litter was reported in the 0.1 (4.97), 1 (4.99), or 2.5 mg/kg/day (4.97) dose groups. A significant decrease in the mean number of sternal center ossification sites per fetus per litter was observed in the 2.5 and 3.75 mg/kg/day dose groups relative to control (3.81 and 3.82, respectively, relative to 3.98 for the control group); no significant change in the mean number of sternal center ossification sites per fetus per litter was detected in the 0.1 (3.92) and 1 mg/kg/day (3.95) dose groups. A significant difference in fetal incidence of irregular ossification of the skull was reported in both the 2.5 and 3.75 mg/kg/day dose groups relative to control (0.8% and 9.2% incidence respectively, relative to 4% in the control); no significant difference was observed in the 0.1 (5.6%) and 1 mg/kg/day (2%) dose groups. There were no significant differences in litter incidence of irregular ossification of the skull in the 0.1, 1, 2.5, and 3.75 dose groups versus control (38.9%, 15.8%, 6.2%, and 25%, respectively, vs. 30%). A significant decrease in mean number of ossification sites in the hyoid body per fetus per litter was reported in the 3.75 mg/kg/day dose group (0.92) versus Control (1); no change in mean number of hyoid ossification sites was reported in other dose groups (mean of 1 for the 0.1, 1, and 2.5 mg/kg/day dose groups). A significant increase in fetal incidence of a hole in the parietal bone was observed in the 3.75 mg/kg/day dose group versus Control (6.5% vs. 0%); no holes were detected in the 0.1, 1, and 2.5 mg/kg/day dose groups. Litter incidence of a hole in the parietal was 1 (8.3%) in the 3.75 mg/kg/day dose group and 0 (0%) in the 0, 0.1, 1, and 2.5 mg/kg/day dose groups. Fetal incidence of unossified pubis was also significantly increased in the 3.75 mg/kg/day group versus Control (3.7% vs. 0%). No other dose groups exhibited unossified pubis. A significant increase in litter incidence of unossified pubis was observed in the 3.75 mg/kg/day group versus Control (16.7% vs. 0%). The rest of the dose groups exhibited 0% litter incidence of unossified pubis. However, fetal alterations were observed in a similar percentage of litters across all dose groups (70%, 61.1%, 47.4%, 25%, and 66.7% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively). No significant difference was seen in the mean percentage of fetuses per litter with any alteration (14.1%, 17%, 9.5%, 3.6%, and 17.4% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively).

3.4.4.2.4 Fetal or Pup Body Weight

Several studies in different species reported data on fetal body weight (Figure 3-68). In a study in CD-1 mice with gestational PFOS exposure from GD 11 to GD 16, Lee et al. (2015) reported mean fetal body weights on GD 17 of 1.72, 1.54, 1.3, and 1.12 g in the 0, 0.5, 2, and 8 mg/kg/day dose groups, respectively. The mean fetal weights reported for the 2 and 8 mg/kg/day groups were significantly lower than those reported for the control dose group. In another study with CD-1 mice that were exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5 to GD 17.5, Wan et al. (2020) reported a significant reduction in fetal body weight in the 3 mg/kg/day group compared

with controls. In contrast, Fuentes et al. (2006) found no treatment-related effects on mean fetal weight per litter on GD 18 in CD-1 mice exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. Li et al. (2021a) observed a dose-dependent decrease in fetal body weight in mice (strain not specified) exposed to 0, 0.5, 2.5, or 12.5 mg/kg/day PFOS from GD 1 to GD 17, whereby the mean fetal weights in the 2.5 and 12.5 mg/kg/day groups were decreased by approximately 17% and 24%, respectively, relative to controls. However, the reduction in weight did not reach statistical significance, though it should be noted that the sample size was small (n = 3 litters/group). Li et al. (2016) reported mean GD 18.5 fetal body weights of 2.73, 2.68, and 2.48 g in the 0, 5, and 20 mg/kg/day dose groups (sexes combined) following exposure of Sprague-Dawley rat to PFOS from GD 12 to GD 18. Mean fetal body weight for the 20 mg/kg/day dose group was significantly different from that of the control group. Mean fetal body weight in males alone was also significantly decreased at 20 mg/kg/day (2.79, 2.74, and 2.43 g for the 0, 5, and 20 mg/kg/day dose groups, respectively). Thibodeaux et al. (2003) similarly observed a decrease in rat fetal weight following gestational exposure to 10 mg/kg/day PFOS. In a one-generation reproductive study in Sprague-Dawley rats, Luebker et al. (2005b) reported no effect on pooled fetal body weights with PFOS doses up to 2 mg/kg/day. Similarly, Conley et al. (2022) found no effects of PFOS on fetal body weight on GD 18 in Sprague-Dawley rats (Crl:CD(SD)) exposed to 0, 0.1, 0.3, 1, 3, 10, or 30 mg/kg/day from GD 14 to GD 18. In a study in New Zealand white rabbits, Argus Research Laboratories (2000) reported mean live fetal body weights of 44.15, 41.67, 42.37, 39.89, and 33.41 g/litter in 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively. Fetal body weights for the 2.5 and 3.75 mg/kg/day dose groups were significantly lower than fetal body weight reported in the control group.

Several other studies measured body weights of pups after birth (Figure 3-68). The most sensitive endpoint in the one- and two-generation reproductive studies in Sprague-Dawley rats (dams treated with PFOS pre-conception through gestation for 63 or 84 days, respectively) was decreased pup body weight (Luebker et al., 2005b; Luebker et al., 2005a). The NOAEL and LOAEL for pup body weight effects was 0.1 and 0.4 mg/kg/day, respectively, in the two-generation study (Luebker et al., 2005a); the lowest dose of 0.1 mg/kg/day was not tested in the one-generation study (Luebker et al., 2005b) where the LOAEL was the lowest dose tested of 0.4 mg/kg/day for decreased pup body weight, decreased maternal body weight, and decreased gestation length. In both the one- and two-generation studies, the decreased pup body weight was observed across multiple time points (PND 0 and LD 5 and PND 1, 4, 7, 14, and 21, respectively) in the first generation. In the second generation, decreased pup weight was only observed in the highest dose group tested (0.4 mg/kg/day) on PND 7 and 14 (Luebker et al., 2005a). Lau et al. (2003) also reported significant weight deficits in Sprague-Dawley rat pups on PND 0 after gestational PFOS exposures of 2, 3, or 5 mg/kg/day, but not 1 mg/kg/day. Similarly, Xia et al. (2011) observed significantly reduced pup body weights in Sprague-Dawley rats on PND 0 and PND 21 following gestational exposure to 2 mg/kg/day PFOS. In contrast, Zhang et al. (2021) found no PFOS-related effects on pup body weight on PND 1, 3, 7, and 14 in Sprague-Dawley rat pups exposed to 0, 1, or 5 mg/kg/day from GD 12 to GD 18.

For this endpoint, rats appear to be more sensitive than mice. Yahia et al. (2008) reported significant decreases in ICR mouse neonatal weight at relatively high doses of 10 and 20 mg/kg/day. Lau et al. (2003) did not report statistically significant reductions in pup body weights of CD-1 mice gestationally exposed to PFOS doses up to 20 mg/kg/day. Zhong et al. (2016) measured body weights of C57BL/6 mouse pups that had been exposed to 0, 0.1, 1, or

5 mg/kg/day PFOS in utero from GD 1 to GD 17. They did not see significant differences in body weight measurements of male or female mice at 4 and 8 weeks of age. Mshaty et al. (2020) also reported no effects on C57BL/6J mouse pup body weight at PND 21 following lactational exposure to 1 mg/kg/day PFOS from PND 1 to PND 14.

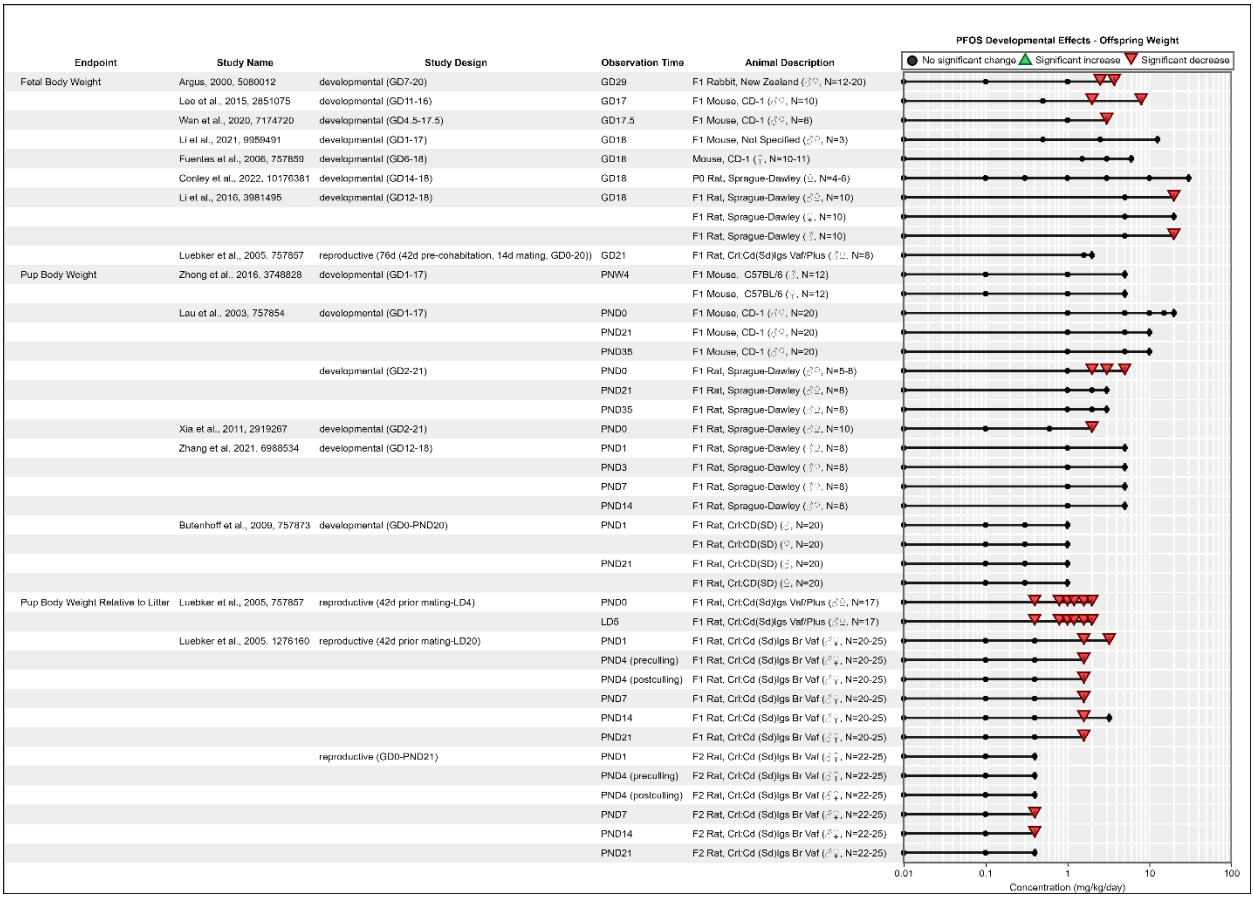


Figure 3-68. Offspring Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS (Logarithmic Scale, Sorted by Observation Time)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; F_1 = first generation; F_2 = second generation; d = day.

3.4.4.2.5 Placenta

Placental endpoints were reported in six studies with rats, mice, or rabbits. Li et al. (2016) reported a significant decrease in mean placental weight in Sprague-Dawley rat dams exposed to 20 mg/kg/day PFOS from GD 12 to GD 18 relative to control (442.8 mg vs. 480.4 mg in controls). No significant difference in placental weights was detected in dams exposed to 5 mg/kg/day PFOS relative to control. At ≥0.5 mg/kg/day, Lee et al. (2015) observed significant decreases in mean absolute placental weight (185.63, 177.32, 163.22, and 151.54 mg at 0, 0.5, 2, and 8 mg/kg/day, respectively) and placental capacity (ratio of fetal weight/placental weight; 9.3, 8.68, 7.96, and 7.39 at 0, 0.5, 2, and 8 mg/kg/day, respectively) in mice exposed to PFOS from GD 11 to GD 16 and sacrificed at GD 17. In the same study, microscopic evaluation revealed necrotic changes and dose-dependent decreases in the frequency of glycogen trophoblast cells

and sinusoidal trophoblast cells at dose levels ≥2.0 and ≥0.5 mg/kg/day, respectively (Lee et al., 2015). Li et al. (2021a) dosed mouse dams (strain not specified) with 0, 0.5, 2.5, or 12.5 mg/kg/day PFOS from GD 1 to GD 17 and observed smaller placental diameter in the 12.5 mg/kg/day group compared with controls, though the biological significance of that effect is unclear. Wan et al. (2020) found no effects on absolute or relative placenta weight, junctional zone area, labyrinth zone area, or the ratio of labyrinth to junctional zone area in CD-1 mice exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5 to GD 17.5. Argus Research Laboratories (2000) did not observe any placental effects in exposed rabbits and Luebker et al. (2005b) observed no changes in placental size, color, or shape in exposed rats.

3.4.4.2.6 Postnatal Development

Gestational PFOS exposure is associated with effects on postnatal development. Lau et al. (2003) observed delayed eye opening in rats and mice following developmental exposure to PFOS. A significant, treatment-related delay in eye opening was reported in mice following gestational exposure to PFOS (eye opening at PND 14.8 in control vs. eye opening at PND 15.1, PND 15.5, and PND 15.6 at 1, 5, and 10 mg/kg/day, respectively). The NOAEL for delays in eye opening in rats was 1 mg/kg/day PFOS. A two-generation reproduction study in rats (Luebker et al., 2005a) evaluated various developmental landmarks in the F_1 offspring and observed significant delays in pups attaining pinna unfolding, eye opening, surface righting, and air righting in the 1.6 mg/kg/day dose group. Eye opening was also slightly, but significantly, delayed in pups exposed to 0.4 mg/kg/day. Mshaty et al. (2020) evaluated age at eye opening in mice exposed to 1 mg/kg/day from PND 1 through PND 14 and found no significant effects.

Developmental PFOS exposure also had adverse effects on lung development, further described in the Respiratory Section of Appendix C (U.S. EPA, 2024a).

3.4.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse developmental outcomes is discussed in Section 3.3.4 of the 2016 PFOS HESD (U.S. EPA, 2016b). There are 34 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to developmental effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-69.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	5	6	4	14
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	15	20
Cell Signaling Or Signal Transduction	5	1	5	10
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	3	1	2	6
Hormone Function	2	0	1	2
Inflammation And Immune Response	0	1	1	2
Oxidative Stress	1	1	3	5
Xenobiotic Metabolism	1	0	2	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	14	7	16	34

Figure 3-69. Summary of Mechanistic Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on HAWC.

Mechanistic data available from in vitro, in vivo, and epidemiological studies were evaluated to inform the mode of action of developmental effects of PFOS. Outcomes included early survival, general development, and gross morphology; fetal growth and placental effects; metabolism; lung development; hepatic development; testes development; cardiac development; and neurological development.

3.4.4.3.1 Early Survival, General Development, Gross Morphology

Mechanisms through which PFOS exposure may alter survival and development were studied in several zebrafish embryo bioassay studies. Several of these studies identified in the current assessment were included in a recent review of developmental effects of PFOS in zebrafish models (Lee et al., 2020). In general, PFOS can lead to embryo and/or larva malformation, delays in hatching, and decreases in body length. Wang et al. (2017) exposed embryos to 0.2, 0.4, 0.8, or 1.6 mg/L PFOS and observed significant and dose-dependent reductions in hatching rate and heart rate as well as significant increases in mortality and malformations in the spine and swim bladder. The overt effects in general development and gross morphology coincided with dose-dependent increases in reactive oxygen species (ROS), lipid peroxidation, and antioxidant enzyme activity (including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px)). Interestingly, co-exposure of the embryos with PFOS and multi-walled carbon nanotubes (MWCNTs) reduced toxicity in several of these endpoints and attenuated the increase in oxidative stress biomarkers caused by PFOS, suggesting that oxidative stress is a key event that mediates alterations in development and gross morphology following exposure to PFOS. Another zebrafish embryo bioassay conducted by Dang et al. (2018) reported that

exposure to 0.1, 1, or 10 μM PFOS did not affect hatching and survival rates, but did increase malformation rates by 7%, possibly due to downregulation of the growth hormone/insulin-like growth factors (GH/IGFs) axis. Blanc et al. (2019) determined the lethal/effect concentrations (LC/ECs) for zebrafish embryos at 96-hours post-fertilization (hpf). The 50% lethal effect concentration (LC_{50}) was 88 μM, which is lower than the previously determined value of 109 μM by Hagenaars et al. (2011). The 10% lethal effect concentration (LC_{10}) was 35 μM and was used in subsequent experiments to explore mechanisms that may contribute to the developmental toxicity at the transcriptional and epigenetic level, which are described in the Section below (Blanc et al., 2019). Lastly, Chen et al. (2014) found that PFOS exposure of zebrafish embryos led to several malformations, including uninflated swim bladder, underdeveloped gut, and curved spine, which paralleled histological alterations in the swim bladder and gut. To complement the functional data, the authors examined differential gene expression by microarray analysis, which revealed upregulated genes involved in nucleic and macromolecule metabolism, cell differentiation and proliferation, neuron differentiation and development, and voltage-gated channels. Genes that were downregulated were associated with cellular protein metabolic processes, macromolecular complex assembly, protein-DNA complex assembly, and positive regulation of translation and multicellular organism growth. The authors also used the genomic data to identify the top predicted developmental toxicity pathways initiated by PFOS exposure, including Peroxisome Proliferator-Activated Receptor alpha (PPARα)-mediated pathways, decreases of transmembrane potential of mitochondria and mitochondrial membrane, and cardiac necrosis/cell death.

Two in vitro studies by Xu et al. (2015; 2013) examined the effects of PFOS on changes in mouse embryonic stem cell (mESC) pluripotency markers, which control normal cell differentiation and development. Xu et al. (2013) found that PFOS exposure did not affect cell viability. However, PFOS exposure decreased mRNA and protein levels of the pluripotency markers Sox2 and Nanog, but not Oct4. They also measured several miRNAs, including miR-145 and miR-490-3p, which can regulate Sox2 and Nanog, and found them to be increased, supporting the epigenetic mechanisms of control of these markers. In Xu et al. (2015), cell differentiation effects on mouse embryoid bodies (mEBs) were examined. eBs are formed when embryonic stem cells spontaneously differentiate into the three germ cell layers, mimicking early gastrulation. The authors found that mEB formation was unaffected by PFOS, but that PFOS exposure increased the mRNA and protein levels of the previously studied pluripotency markers (Oct4, Sox2, and Nanog); this is notably a reversal of the findings from their previous study in mESCs (Xu et al., 2013). Xu et al. (2015) found that PFOS exposure in mEBs decreased differentiation markers (Sox17, FOXA2, SMA, Brachyury, Nestin, Fgf5), as well as Polycomb group (PcG) proteins and several miRNAs also involved in differentiation. These alterations could disturb the dynamic equilibrium of embryonic differentiation and induce developmental toxicity. Altogether, the results suggest that PFOS exposure can disturb the expression of pluripotency factors that are essential during early embryonic development, potentially via miRNA dysregulation, which may reflect mechanisms of toxicity that are relevant during a critical window of embryonic development.

Global epigenetic changes in response to PFOS exposure were measured in several studies, including in one zebrafish study and two epidemiological studies. Blanc et al. (2019) found that PFOS induced global DNA hypermethylation, minor alterations in gene expression of several epigenetic factors (including DNA methylation, histone deacetylation, and histone demethylation

factors) following PFOS exposure. Moreover, the genes encoding the DNA methyltransferase *dnmt3ab* and the H3K4 histone demethylase *kdm5ba* were significantly downregulated. H3K4 methylation is associated with open, transcriptionally active regions and depleted of DNA methylation. The authors did not measure methylation patterns on H3K4 or other histones; to confirm alterations to H3K4 methylation status, additional studies are required.

In cord blood samples from a Japanese birth cohort study, Miura et al. (2018) measured PFOS levels in tandem with epigenetic modifications during fetal development. The authors found significant associations between global hypermethylation and PFOS exposure. The top differentially methylated regions (DMRs) of the genome that were associated with PFOS exposure included hypermethylation of CpG sites of *CYP2E1*, *SMAD*, and *SLC17A9*; however, the authors did not measure the expression level of these genes to confirm the effect of the epigenetic alterations. In contrast, another study of human cord blood samples conducted by Liu et al. (2018a) found that PFOS exposure was associated with low methylation of Alu retrotransposon family in cord blood DNA samples, indicating global hypomethylation. Demethylation of Alu elements has been proposed to induce insertion and/or homologous recombination and cause alterations to genomic stability and, subsequently, gene transcription. In another study of human cord blood samples, PFOS exposure was associated with DNA methylation changes at key CpG sites associated with genes in pathways important for several physiological functions and diseases, including nervous system development, tissue morphology, digestive system development, embryonic development, endocrine system development, cancer, eye disease, organ abnormalities, cardiovascular disease, and connective tissue disorders (Leung et al., 2018).

Lastly, in a study of human cord blood in a prospective cohort in China, PFOS exposure was associated with significantly shorter leukocyte telomere lengths and increased ROS in female newborns. Interestingly, the effects were not observed in male newborns, suggesting sex-specific effects in early-life sensitivity to PFOS exposure at the molecular level. The authors determined that the effect of PFOS on shortened leukocyte telomere length was partially mediated through ROS in females, indicating a programming role of PFOS on telomere length during gestation (Liu et al., 2018c).

3.4.4.3.2 Fetal Growth and Placental Development

Growth was measured in developing zebrafish larvae in three studies. Wang et al. (2017), reported a dose-dependent reduction in body length that coincided with dose-dependent increases in ROS generation, lipid peroxidation, and the activities of antioxidant enzymes in larvae exposed to 0.2, 0.4, 0.8, or 1.6 mg/L PFOS. Reduction in body length was likely due to PFOS-related increased oxidative stress and lipid peroxidation. In Jantzen et al. (2016a), the morphometric endpoints of interocular distance, total body length, and yolk sac area were measured in zebrafish embryos. PFOS exposure significantly decreased all three parameters relative to controls, indicating slowed embryonic development, at values 5- to 25-fold below previously calculated LC_{50} values. The authors found alterations in the expression of several genes involved in development, including calcium ion binding (*calm3a*), cell cycle regulation (*cdkn1a*), aromatic compound metabolism (*cyp1a*), and angiogenesis (*flk1*), as well as increased *tfc3a* (muscle development) expression and decreased *ap1s* (protein transport). Lastly, Dang et al. (2018) found that PFOS significantly inhibited body length and growth of larvae. This appeared to be mediated through the growth hormone/insulin-like growth factor (GH/IGF) axis,

as several GH/IGF axis genes had decreased expression, including the genes *gro*wth hormone releasing hormone (*ghrh*), growth hormone receptors a and b (*ghra* and *ghrb*), insulin-like growth factor 1 receptor a and b (*igf1ra* and *igf1rb*), insulin-like growth factor 2 receptor (*igf2r*), insulin-like growth factor 2a (*igf2a*), and insulin-like growth factor binding protein 2a and 2b (*igfbp2a* and i*gfbp2b*).

In three in vivo rodent studies, fetal growth and placental disruption in response to maternal PFOS exposure were measured. In a mouse study, Lee et al. (2015) reported a relationship between gene expression of prolactin-family hormones and placental and fetal outcomes following maternal exposure to 0, 0.5, 2.0, or 8.0 mg/kg/day PFOS from GD 11–16 via gavage. Dose-dependent increases in placental histopathological lesions and reductions in placental weights, fetal weights, and number of live fetuses were significantly correlated with reductions in gene expression of mouse placental lactogen (*mPL-II*), prolactin-like protein Cα (*mPLP-Cα*), and prolactin-like protein K (*mPLP-K*). Given the alterations in prolactin-family gene expression, the authors propose that this placental disruption is related to endocrine (i.e., prolactin) dysfunction. Li et al. (2016) also found that maternal PFOS exposure reduced fetal and placental weight, which coincided with increased corticosterone in fetal serum. In the placenta, activity of 11b-hydroxysteroid dehydrogenase 2, and expression of several genes involved in development (i.e., extracellular matrix, growth factors and hormones, ion transporters, signal transducers, and structural constituents) were downregulated, suggesting intrauterine growth restriction was related to altered placental development and functionality. Li et al. (2020b) also found that PFOS exposure was associated with reduced placental size in mice and proposed that the disruption was mediated by the dysregulation of a long non-coding RNA, H19 which plays a role in regulation of embryonic growth (Monnier et al., 2013), which was altered in placental tissues (i.e., hypomethylation of the H19 promoter and increased expression of the gene). In vitro experiments in human placental trophoblast cells (HTR-8/sVneo) provided further support for a mechanism involving H19; cell growth that was inhibited by PFOS was partially alleviated following suppression of H19 via transfection with si-H19 (Li et al., 2020b).

Sonkar et al. (2019) also used HTR-8/sVneo cells to evaluate the epigenetic mechanisms through which PFOS exposure adversely effects the placenta. The authors reported increased ROS production, possibly due to alterations of several DNA methyltransferases and sirtuins, which consequently led to a reduction in global DNA methylation and increased protein lysine acetylation. The authors propose that ROS production could lead to pregnancy complications, such as preeclampsia and intrauterine growth restrictions.

In a human placental choriocarcinoma cell line (JEG-3), PFOS exposure was found to induce placental cell cytotoxicity and inhibition of aromatase activity (Gorrochategui et al., 2014). In Yang et al. (2016), 0.1 μM PFOS inhibited decidualization of the first trimester human decidual stromal cells (collected from the uterine lining). PFOS also downregulated 11-hydroxysteroid dehydrogenase 1 (11β-HSD1), an enzyme that converts the inactive form of cortisol to the active form of cortisol, and inhibited the glucocorticoid-driven reduction of the proinflammatory cytokines IL-6 and IL1-β, which could result in a reduced immune-tolerance environment in early pregnancy. In human amnion and fetal lung cells exposed to PFOS in vitro, PFOS exposure upregulated the gene expression of Caspase3 and apoptotic peptidase activating factor 1 (*APAF1)*, genes that initiate apoptosis. This effect was concentration (between 10^{-4} and 10^{-6} M PFOS) and time-dependent (between 24 and 48 hours) (Karakas-Celik and Aras, 2014).

Lastly, in humans, Ouidir et al. (2020) recruited pregnant women and measured plasma PFOS levels during the first trimester of the pregnancy and examined global methylation in the placenta at birth. The authors found significant associations between PFOS exposure and DNA methylation changes in the placenta, and the associated downregulation of certain genes, particularly the reduced gene expression of several genes associated with anthropometry parameters such as shorter birth length, reduced birth weight, and reduced head circumference that were previously associated with PFAS exposure (Buck Louis et al., 2018). These data suggest that the prenatal toxicity of PFOS might be driven by epigenetic changes in the placenta (Ouidir et al., 2020).

3.4.4.3.3 Metabolism

Metabolomic profiles in relation to PFOS exposure were analyzed in humans in two studies. In a cross-sectional study in 8-year-old children in Cincinnati, OH, the authors conducted untargeted, high-resolution metabolomic profiling in relation to serum PFOS concentrations. They found that PFOS exposure was associated with several lipid and dietary factors, including arginine, proline, aspartate, asparagine, and butanoate metabolism (Kingsley et al., 2019). In a study of mothers that were part of the Child Health and Development Studies (CHDS) cohort, maternal serum was analyzed for PFOS as well as underwent metabolomics profiling to determine if metabolic alterations reflected in measurements from maternal serum could possibly contribute to later health outcomes in their children (Hu et al., 2019a). PFOS exposure was associated with a distinct metabolic profile, including a positive association with urea cycle metabolites and a positive association with carnitine shuttle metabolites. This profile indicates disruption of fatty acid metabolism, which could possibly cause developmental alterations in offspring (Hu et al., 2019a).

3.4.4.3.4 Lung Development

In a human fetal lung fibroblast cell line (Hel299), PFOS exposure upregulated the expression of *Caspase3* and *Apaf1*, genes that initiate apoptosis. This effect was dose and time-dependent (Karakas-Celik and Aras, 2014). These results indicate that PFOS can cause in vitro toxicity (via apoptotic mechanisms) in embryonic cells, possibly affecting the development.

3.4.4.3.5 Hepatic Development

Liang et al. (2019) studied the effects of developmental exposure to PFOS on metabolic liver function in Kunming mice, in postnatal day 1 offspring. They found that PFOS exposure during gestation increased liver triglycerides, total cholesterol, and low-density lipoprotein (LDL), and decreased high-density lipoprotein (HDL) in the offspring. The mRNA of several factors involved in fatty acid oxidation, update, and hepatic export of livers were altered, indicating developmental perturbation of lipid metabolic function. These in vivo results show that PFOS may disrupt hepatic lipid metabolism through negative effects on hepatocellular lipid trafficking in mice developmentally exposed to PFOS.

3.4.4.3.6 Cardiac Development

Several in vitro studies examined developmental toxicity of PFOS using embryonic stem cell-derived cardiomyocytes (ESC-CMs) as a model of the early stages of heart development (Liu et al., 2020a; Yang et al., 2020c; Tang et al., 2017; Zhou et al., 2017a; Zhang et al., 2016; Cheng et al., 2013). Most of the studies utilized mouse ESC-CMs but one study, Yang et al. (2020c), used

a human ESC-CM model of cardiac differentiation. Cardiac differentiation was inhibited in PFOS-treated mouse ESC-CMs, shown by a concentration-dependent decrease in the contract positive rate (i.e., percentage of beating embryoid bodies) on differentiation days 8–10 (Tang et al., 2017; Zhou et al., 2017a; Zhang et al., 2016; Cheng et al., 2013) and a decreased proportion of α-actinin-positive cells (a marker of cardiomyocytes) on differentiation day 10 (Tang et al., 2017; Zhang et al., 2016). The median inhibition of differentiation (ID_{50}), defined as the concentration at which PFOS inhibited the development of contracting cardiomyocytes by 50%, ranged from 40 μM (Zhang et al., 2016) to 73 μM (Zhou et al., 2017a). Collectively, these results provide in vitro evidence of potential developmental cardiotoxicity following PFOS exposure.

Several in vitro studies have demonstrated that PFOS can significantly alter gene and protein expression at multiple time points during differentiation of cardiomyocytes from mouse or human ESCs, specifically for genes in the myosin heavy chain, myosin light chain, and cardiac troponin T families. In human ESC-CMs, 0.1–60 μM PFOS significantly inhibited the expression of cardiac-specific homeobox gene Nk2 homeobox 5 (*NKX2.5*), myosin heavy chain 6 (*MYH6)*, and myosin light chain 7 (*MYL7),* and significantly reduced protein levels of NKX2.5 and cardiac troponin T2 (TNNT2) on day 8 and/or day 12 of differentiation (Yang et al., 2020c). In mouse ESC-CMs, on differentiation day 5, PFOS (20–40 μM) reduced gene and protein levels of Brachyury (mesodermal marker), cardiac transcription factors GATA binding protein 4 (GATA4), and myocyte enhancer factor 2C (MEF2C) (Zhang et al., 2016). On differentiation days 9–10, PFOS reduced the expression of *Myh6* and *Tnnt2* (i.e., *cTnT*) in a dose-dependent manner from 2.5 to 160 μg/mL PFOS (Zhou et al., 2017a; Cheng et al., 2013). Cheng et al. (2013) found that PFOS significantly altered the chronological order of gene expression during in vitro cardiogenesis. Expression of important cardiac genes were significantly lower in PFOS-treated cells compared with controls on day 9, but expression of *Nkx2.5* and *Mlc1a* were significantly higher in PFOS-treated cells by day 14 of differentiation (Cheng et al., 2013).

Proteomic analysis during cardiac differentiation of mouse ESCs revealed 176 differentially expressed proteins (67 upregulated and 109 downregulated) (Zhang et al., 2016). The differentially expressed proteins were mainly associated with catalytical activity, protein binding, nucleotide binding, and nucleic acid binding. PFOS significantly affected 32 signaling pathways, with metabolic pathways the most affected. The PPAR signaling pathway and mitogen-activated protein kinase (MAPK) signaling pathways were also significantly affected by PFOS.

Yang et al. (2020c) studied global gene expression during cardiac differentiation of human ESCs exposed to 60 μM PFOS. Their analysis revealed 584 differentially expressed genes (247 upregulated and 337 downregulated) on differentiation day 8, and 707 differentially expressed genes (389 upregulated and 318 downregulated) on differentiation day 12. In total, 199 genes were affected on both days 8 and 12. The majority of affected genes are related to extracellular matrix and cell membrane. Seven Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were affected by PFOS on both days (mostly neural-related pathways and a few general pathways), but cardiac pathways were not greatly affected. PFOS downregulated cardiac markers such as natriuretic peptide A (*NPPA)*, natriuretic peptide B (*NPPB)*, *NKX2.5*, *MYH6*, *MYL2*, and *MYH7*, but upregulated epicardial markers WT1 transcription factor (*WT1*) and T-box transcription factor 18 (*TBX18*). Wingless-related integration site (WNT) signaling pathway-related genes (secreted frizzled-related protein 2 (*SFRP2)* and frizzled-related protein (*FRZB*)) and IGF signaling pathway genes (*IGF2* and IGF binding protein 5 (*IGFBP5)*) were significantly

upregulated in PFOS-treated cells. The authors postulated that PFOS stimulated differentiation to epicardial cells more than to cardiomyocytes by stimulating the WNT signaling pathway.

Mouse ESC cardiac differentiation assays have demonstrated that exposure to PFOS can cause mitochondrial toxicity in these cells. In contrast, one study in human ESCs-derived cardiomyocytes (Yang et al., 2020c) found that PFOS did not affect mitochondrial integrity on day 12 of differentiation.

Cheng et al. (2013) found that PFOS reduced ATP production, increased accumulation of ROS, and stimulated apoptosis in mouse ESC-CMs. However, Tang et al. (2017) demonstrated that PFOS decreased intracellular ATP and lowered mitochondrial membrane potential in mouse ESC-CMs without inducing apoptosis. Exposure to PFOS during cardiac differentiation also caused structural damage to mitochondria (e.g., swelling, vacuolar structure, loss of cristae) and the mitochondria-associated endoplasmic reticulum membrane (MAM). Furthermore, PFOS increased intracellular lactate production, fatty acid content, and disrupted calcium fluxes. Analysis of protein expression demonstrated that destruction of the MAM structure occurred along with activation of Rictor/mTORC2 signaling pathway via phosphorylation of epidermal growth factor receptor, which led to accumulation of intracellular fatty acid and resulted in blocking of the $[Ca^{2+}]_{mito}$ transient.

The mechanisms behind PFOS mitochondrial toxicity were further explored by Liu et al. (2020a) who found that PFOS-treated ESC-CMs displayed autophagosome accumulation accompanied by increased levels of p62 and ubiquitinated proteins, increased lysosomal pH, and decreased the levels of lysosome-associated membrane protein (Lamp2a) and the mature form of Cathepsin D (lysosomal protease), suggesting an impairment of autophagy-lysosome degradation. PFOS also blocked mitophagy, the removal of damaged mitochondria through autophagy, thereby disrupting the balance between mitophagy and biogenesis (Liu et al., 2020a). The authors postulated that the mechanism of PFOS-induced toxicity to ESC-CMs involves reduced lysosomal acidification, inhibited maturation of cathepsin D, blocked fusion between lysosomes and autophagosomes, accumulation of autophagosomes, and dysfunctional mitochondria.

One study included in the prior 2016 PFOS HESD (U.S. EPA, 2016b) investigated cardiac mediated apoptosis in weaned rats exposed to PFOS (0, 0.1, 0.6, or 2 mg/kg/day) on GD 2–21 (Zeng et al., 2014). The pups were sacrificed at the end of the lactation period, and trunk blood and the heart were recovered. Apoptotic cells in the heart tissue from six animals per dose group were measured using a Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assay. PFOS exposure was associated with a dose-dependent increase in the percentage of TUNEL positive nuclei. The 0.6 mg/kg/day dose was the LOAEL and the 0.1 mg/kg/day dose the NOAEL. The researchers found that biomarkers for apoptosis were supportive of the TUNEL results. The expression of BCL2-associated X protein and cytochrome c were upregulated and bcl-2 downregulated. The concentration of caspase 9 was significantly increased above the control levels at all doses and caspase 3 levels were significantly increased for all but the lowest dose level.

3.4.4.3.7 Testicular Development

Two rat studies examined PFOS effects on testicular development. Zhang et al. (2013a) isolated primary Sertoli cells and gonocytes from 5-day-old rat pups and created a Sertoli cell/gonocyte coculture system to mimic in vivo interactions. PFOS exposure reduced cell viability and

induced ROS production in a concentration-dependent manner, although PFOS did not appear to increase apoptosis. PFOS exposure altered and inhibited the cytoskeletal proteins vimentin and F-actin in Sertoli cells, indicating PFOS could adversely affect developing testes via ROS and cytoskeleton disruption. Li et al. (2018a) examined the effects of PFOS on pubertal Leydig cell development, both in vitro and in vivo. In vitro, PFOS inhibited androgen secretion via the downregulation of 17b-hydroxysteroid dehydrogenase 3 (HSD17B3, gene *Hsd18b3*), as measured by *Hsd18b3* mRNA expression. PFOS also promoted apoptosis of immature Leydig cells in vitro but did not affect cell proliferation. In vivo, PFOS exposure reduced serum testosterone levels, and reduced sperm production. LHCGR, CYP11A1, and CYP17A1 levels in Leydig cells were reduced, suggesting that PFOS exposure downregulates critical Leydig cell gene expression, indicating delayed maturation of these cells.

3.4.4.3.8 Neurological Development

PFOS effects on neurodevelopment and behavior in zebrafish were examined in two studies. In the zebrafish embryo assay by Jantzen et al. (2016a), embryonic exposure to PFOS resulted in hyperactive locomotor activity in larvae, possibly mediated through altered expression of development-associated genes (*calm3a, cdkn1a, cyp1a, flk1, tfc3a*, and *ap1s*). Stengel et al. (2018) developed a neurodevelopmental toxicity test battery in zebrafish embryos and evaluated the effect of PFOS exposure. Although PFOS exposure had significant adverse effects on neuromast cells, including degeneration, no changes were observed in the olfactory or retinal toxicity assays.

Rat embryonic neural stem cells (NSCs) were used to examine the effects of PFOS on neuronal and oligodendrocytic differentiation. PFOS exposure at 25 or 50 nM reduced cell proliferation but showed increased protein levels in markers associated with differentiation (TuJ1, CNPase). Exposure also reduced the number of cells with spontaneous calcium activity. These effects appeared to be mediated through PPAR pathways, as indicated by increases in *PPARγ* and the downstream target *UCP2*. Results were confirmed using a PPARγ agonist that showed similar effects in the cells. This study also evaluated effects of PFOS exposure on the PPAR system in vivo. In PFOS-treated neonatal mice, *PPARγ* and *UCP3* were upregulated in brain cortical tissue (Wan Ibrahim et al., 2013).

Lastly, Leung et al. (2018) conducted a genome-wide methylation study on mothers and infants from the Faroese birth cohort study, which has been extensively studied for associations between neurodevelopmental deficits in children exposed to various chemicals, including PFAS. In cord blood samples from males, PFOS exposure was significantly associated with 10,598 methylation changes in CpG sites, 15% of which were enriched in cytobands of the X chromosome associated with neurological disorders. Other CpG sites were associated with genes in pathways of key physiological functions and diseases, including nervous system development, tissue morphology, digestive system development, embryonic development, endocrine system development, cancer, eye disease, organ abnormalities, cardiovascular disease, and connective tissue disorders. The same effects were not observed in cord blood from females.

3.4.4.3.9 Conclusion

The available mechanistic studies suggest that the developing liver, developing heart, and placenta may be affected by PFOS at the molecular level (i.e., differential methylation of genes, gene expression changes, mitochondrial dysregulation), which may be related to developmental

health effects described in Sections 3.4.4.1 and 3.4.4.2. Some effects tend to vary by sex or by developmental timepoint of outcome evaluation (e.g., early gastrulation, late gestation, lactation). Oxidative stress in parallel with epigenetic alterations in the placenta were consistently reported.

3.4.4.4 Evidence Integration

The evidence of an association between PFOS and developmental effects in humans is *moderate* based on the epidemiological literature reviewed in the 2016 PFOS HESD and the updated literature searches. As noted in the epidemiological fetal growth restriction summary, there is *robust* evidence that PFOS may impact fetal growth restriction in humans. Several meta-analyses also support evidence of associations between maternal or cord blood serum PFOS and BWT or BWT-related measures (Yang et al., 2022; Cao et al., 2021; Dzierlenga et al., 2020a; Negri et al., 2017; Verner et al., 2015) (see Appendix A, (U.S. EPA, 2024a)). Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catchup periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT.

The consistent and strong evidence for decreases in birth weight in PFOS-exposed population is further supported by coherent evidence for other developmental effects. There is evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) with most of the studies showing increased risk of gestational duration deficits. This was strengthened by consistency in the reported magnitude of gestational age deficits despite different exposure levels and metrics examined. Although they were not as consistent in magnitude (60% of the PTB studies showed some increased risk), some of the effect estimates were large for preterm birth in relation to PFOS exposures with limited evidence of exposure-response relationships. Few patterns were evident as explanatory factors for heterogeneous results based on our qualitative analysis.

Overall, there was inconsistent evidence of PFOS impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available for studies of associations between PFOS exposure and other endpoints such as fetal loss and birth defects. The evidence for an association between PFOS exposure and cryptorchidism or hypospadias were primarily negative but overall inconsistent. Several meta-analyses also show associations between PFOS and preterm birth (Yang et al., 2022; Deji et al., 2021; Gao et al., 2021) (see Appendix A, (U.S. EPA, 2024a)).

As noted previously, there is some uncertainty as to what degree the available evidence may be impacted by pregnancy hemodynamic and sample timing differences across studies, as this may result in either confounding or reverse causality (Steenland et al., 2018a). Additional uncertainty exists due to the potential for confounding by other PFAS, and Section 5.1.1 provides a further discussion on considerations for potential confounding by co-occurring PFAS. Very few of the existing studies performed multipollutant modeling in comparison with single pollutant estimates of PFOS associations. For studies that provided this comparison, the results were often mixed, with some estimates increasing and some decreasing although PFOS was rarely chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants. There is some concern that controlling for other highly correlated co-exposures in the same model may amplify the potential confounding bias of another co-exposure rather than removing it (Weisskopf et al., 2018). Given these interpretation difficulties

and potential for this co-exposure amplification bias, it remains unclear whether certain mutually adjusted models give a more accurate representation of the independent effect of specific pollutants for complex PFAS mixture scenarios.

The animal evidence for an association between PFOS exposure and developmental toxicity is *moderate* based on 16 *medium* confidence animal toxicological studies. Dose-dependent maternal and offspring effects were reported in mice, rats, and rabbits; however, a few studies in rodents did not observe effects. The studies evaluated demonstrate that PFOS exposure is associated with various developmental toxicity endpoints including increased mortality (pup mortality, fetal death, stillbirth, abortion), decreased body weight or body weight change (fetal, pup, and maternal), skeletal and soft tissue effects (e.g., ossification), and developmental delays (e.g., delayed eye opening). The most consistent effects observed across studies were decreased maternal body weight (encompassing decreases in maternal body weight and maternal body weight change), decreased offspring weight during the perinatal developmental period (encompassing fetal weight and pup weight prior to weaning), and increased mortality (encompassing all metrics of fetal or pup viability).

Reductions in litter size or fetal weight may be the driver of reductions seen in maternal weight. For all but one study, decreased maternal weight was observed at the same doses as the potential confounding effects of reduced fetal weight, increased incidence of abortion, increased stillbirth, and others. However, Argus Research Laboratories (2000) reported reduced maternal body weight change in the absence of statistically significant effects on pups that could influence maternal weight. In this case, maternal body weight may be an influential precursor to or sensitive indicator of potential offspring mortality.

Similarly, Luebker et al. (2005b; 2005a) observed decreased pup weights as an average per litter at lower dose levels than effects on viability endpoints including decreases in implantations, increased number of dams with all pups dying, and decreased number of live pups per litter. These results are supported by Lau et al. (2003) who found significant decreases in rat pup body weight at birth and increases in pup mortality in the first 24–48 hours after birth. Significant reductions in both endpoints occurred at the same dose of 2 mg/kg/day. A final study (Lee et al., 2015) also observed increased fetal death and decreased fetal weight. However, in this study, increased incidence of fetal death was statistically significant at all dose levels whereas fetal weight was not affected at the lowest dose of 0.5 mg/kg/day. It is unclear at this time whether one effect should be considered a precursor for the other.

The mechanistic data are primarily focused on gene expression changes and epigenetic alterations related to exposure to PFOS during developmental stages. The PFOS-induced alterations to the expression of genes related to growth and development support the observations of developmental effects in animals and humans (e.g., fetal growth restriction). Molecular alterations (primarily epigenetic alterations) measured in human cord blood were related to PFOS levels in the same biological samples. Specifically, global DNA hypomethylation, a marker of genomic instability, was associated with PFOS exposure, as was hypermethylation of genes related to xenobiotic metabolism. Another study in human cord blood reported changes in DNA methylation at genomic sites associated with genes related to normal development of several tissue and organ systems (e.g., nervous system development and endocrine system development, among others). The authors of these studies of epigenetic

alterations did not measure gene expression changes to confirm that the epigenetic alterations indeed affected gene expression, nor were adverse postnatal outcomes measured in the same study. In addition to human data, mechanistic data related to developmental effects and PFOS have been collected in vivo in zebrafish and rodent studies, as well as in human and rodent in vitro models. In zebrafish embryos exposed to PFOS, changes in genes that are related to growth and development (e.g., growth factors, among others) were observed along with growth inhibition, decreased hatch rate, embryonic malformations, and other metrics of development, indicating that PFOS-induced effects on growth and development are related to alterations to the transcriptome of developing zebrafish. Alterations to individual genes or pathways that are also seen in tissues from adult animals in laboratory studies (e.g., PPAR and markers of apoptosis in the liver, or cardiac-specific pathways) were observed in developing animals and/or embryonic cell lines. Alterations to the epigenome were observed in several animal toxicological studies, including in the placenta of pregnant rodents exposed to PFOS. Such alterations occurred at the global and gene-specific levels, indicating that epigenetic regulation of normal development can be altered by PFOS exposure.

3.4.4.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the available human and animal ***evidence indicates*** that PFOS exposure is likely to cause developmental toxicity in humans under relevant exposure circumstances (Table 3-17). This conclusion is based primarily on evidence of decreased birth weight from epidemiologic studies in which PFOS was measured during pregnancy, primarily with median PFOS ranging from 5.0 to 30.1 ng/mL. The conclusion is supported by coherent epidemiological evidence for measures of decreased gestational duration and other biologically related effects (e.g., decreased postnatal growth and birth length) and consistent findings of dose-dependent decreases in fetal and maternal weight, with the effects observed in animal models gestationally exposed to PFOS at doses as low as 0.4 mg/kg/day. The available mechanistic information provides support for the biological plausibility of the phenotypic effects observed in exposed animals and humans.

Table 3-17. Evidence Profile Table for PFOS Exposure and Developmental Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.4.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Fetal growth restriction 21 *High* confidence studies 26 *Medium* confidence studies 11 *Low* confidence studies 2 *Mixed* confidence studies	Deficits in mean birth weight were observed in most studies (27/39) in the overall population. Studies on changes in standardized birth weight measures reported some inverse associations (12/18) in the overall population or among boys or girls. Ten of 17 studies observed increased risk of low birth weight or SGA. Deficits in birth weight-related measures were supported by decreases in related FGR outcomes such as birth length (15/28) and head circumference (12/23).	• *High* and *medium* confidence studies • *Coherence* across different measures of FGR • *Good* or *adequate sensitivity* in most studies	• *Limited* evidence of exposure-response relationships based on categorical data • *Potential bias* due to hemodynamic differences noted in studies using samples from later pregnancy	⊕⊕⊙ *Moderate* Evidence for developmental effects is based on consistent adverse effects for FGR including birthweight measures which are the most accurate endpoint. Inverse associations were consistently reported for birth weight and standardized birth weight in many *high* and *medium* confidence cohort studies. Effects on birth weight were supported by findings for other measures of FGR, including birth length and head circumference, and impacts on gestational duration. Some uncertainty arises due to the potential impact of hemodynamics in later pregnancy due to use of biomonitoring samples from the second and third trimester or postpartum. However,	*Primary basis and cross-stream coherence:* Evidence consisted of decreased birth weight from epidemiologic studies in which PFOS was measured during pregnancy. This is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length). Further support is provided by consistent inverse associations with gestational age measures in *high* or *medium* confidence epidemiological studies in the overall population and consistent findings of dose-dependent decreases in fetal weight in animal models gestationally exposed to PFOS. *Human relevance and other inferences:* The available mechanistic information provides support for the biological plausibility of the
Gestational duration 10 *High* confidence studies 11 *Medium* confidence studies 7 *Low* confidence studies	Some inverse associations with gestational age measures were observed in *high* or *medium* confidence studies in the overall population (10/18). Increased risk of preterm birth was also observed in *high* or *medium* confidence studies	• *High* and *medium* confidence studies • *Consistency* in the magnitude of gestational age deficits	• *Limited* evidence of exposure-response relationships in studies examining preterm birth		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	(12/17).			several studies present associations for samples collected pre-pregnancy or in the first trimester.	phenotypic effects observed in exposed animals in support of the human relevance of the animal findings.
Fetal loss 3 *High* confidence studies 3 *Medium* confidence studies 1 *Low* confidence study	Increased risk of fetal loss was observed (4/7) although results were mostly nonsignificant. One *high* confidence study observed a significant increase in risk for miscarriage for some quintiles of exposure in subgroup analyses. One *medium* confidence study reported an inverse association.	• *High* and *medium* confidence studies • *Good sensitivity* across all studies • *Consistent* magnitude of effect • *Exposure-response* relationship	• No factors noted		
Postnatal growth 4 *High* confidence studies 7 *Medium* confidence studies 3 *Low* confidence studies	Most studies (8/10) reported an inverse association for infant weight or BMI changes. There was some evidence of an exposure-response relationship in two studies (2/4) reporting categorical exposures. Decreases in infant height were mixed (2/4). Inverse associations were observed for infant weight in most *medium* and *high* confidence studies (6/10), while two studies observed positive associations (2/10). In	• *High* and *medium* confidence studies • *Exposure-response* relationship • *Good* or *adequate sensitivity* for most studies	• *Inconsistent* timing of follow-up evaluation		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	medium and *high* confidence studies, inverse associations with infant BMI or adiposity were observed in some studies (4/9), but other studies reported positive associations (1/8) or mixed associations by sex and timepoint (2/8).				
Birth defects 4 *Medium* confidence studies 3 *Low* confidence studies	One *low* confidence study (1/2) observed a small increased risk for total or combined birth defects. One *medium* confidence study reported increased risk for septal defects, conotruncal defects, and total congenital heart defects, but results were imprecise. Cryptorchidism was examined in three studies. Of the two *medium* confidence studies, one reported a nonsignificant inverse association and the other reported a null association.	• *Medium* confidence studies	• *Low* confidence studies • *Imprecision* of some positive associations may suggest statistical power was limited • *Limited number* of studies examining individual defects		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from In Vivo Animal Studies (Section 3.4.4.2)					
Maternal body weight 12 *Medium* confidence studies	Maternal body weight and/or body weight gain during gestation and lactation were dose-dependently reduced in several studies in rats, mice, and rabbits (8/12). Remaining studies (4/12) in mice found no effects on maternal body weight	• *Medium* confidence studies • *Exposure-response* relationship	• *Inconsistent direction* of effects across species	⊕⊕⊙ *Moderate* Evidence based on 16 *high* or *medium* confidence animal studies indicates that the developing fetus is a target of PFOS toxicity. Dose-dependent maternal and offspring effects were reported in mice, rats, and rabbits; however, a few studies did not observe effects. The studies evaluated demonstrate that PFOS exposure is associated with various developmental toxicity endpoints including increased mortality (pup mortality, fetal death, stillbirth, abortion), decreased body weight or body weight change (fetal, pup, and maternal), skeletal and soft tissue effects, and delayed eye opening.	
Offspring body weight 15 *Medium* confidence studies	Fetal body weights were dose-dependently reduced (4/8) in studies in rats, mice, and rabbits. Pup birth weights and/or body weights during lactation were dose-dependently reduced (4/9), with significant effects observed in rats but not mice.	• *Medium* confidence studies • *Dose-dependent* response	• *Inconsistent direction* of effects across species for postnatal body weight		
Offspring mortality 11 *Medium* confidence studies	Increased fetal mortality (2/7) was reported in rats, mice, and rabbits that evaluated endpoints such as abortion, implantation, resorption, and dead/live fetus counts prior to parturition. Two studies exposed female rats prior to mating through lactation, and the study with higher doses	• *Medium* confidence studies • *Consistent direction* of effects • *Dose-dependent* response	• No factors noted		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	observed decreased number of implantation sites per delivered litter and liveborn litter size, and increased number of stillborn pups per litter (1/2). Four studies began exposure during gestation and allowed natural delivery of litters, and only one (1/4) observed decreased liveborn litter size. No studies reported an effect on sex ratio (percentage of male pups delivered per litter) (0/6). Postnatal survival was dose-dependently decreased in several studies in mice and rats (5/8). For the two studies with exposure prior to mating through lactation, both reported decreased pup viability index and increased numbers of dams with all pups dying in the first 4–5 days postpartum.				
Placental effects 6 *Medium* confidence studies	Decreased placental weight (2/3), decreased placental diameter (1/1), and decreased placental capacity (1/1) were	• *Medium* confidence studies • *Dose-response* relationship	• *Inconsistent direction* of effects • *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	observed in rat and mouse studies, but two other studies in rats and rabbits reported normal placental size and appearance. Histopathology was evaluated in two mouse studies; one study observed no changes in the placenta while the other study observed necrotic changes and dose-dependent decreases in trophoblasts.	• *Coherence* of findings			
Structural abnormalities 2 *Medium* confidence studies	No external or visceral abnormalities were detected in mouse or rabbit fetuses (2/2). Lower incidence of diminished calcaneus ossification was observed in mice (1/1) and delayed skeletal ossification was observed in rabbits (1/1).	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		
Developmental timing and organ maturation 4 *Medium* confidence studies	Delayed eye opening (2/3) was reported in rats and mice following gestational PFOS exposure. In a two-generation study in rats, delayed pinna unfolding, air righting, and surface	• *Medium* confidence studies • *Coherence* of effects with other developmental delays	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	righting was also observed (1/1). In contrast, eye opening in mice exposed from PND 1–14 was unaffected (pup body weight was also unaffected in that study). In general, the studies that observed developmental delays also reported growth deficits and decreased viability during the lactation period. PFOS exposure from GD 12–18 affected lung development and maturation in rats when observed on PND 1–14 (1/1).				

Mechanistic Evidence and Supplemental Information (Section 3.4.4.3)		
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**	
Key findings and interpretation: • Evidence from zebrafish embryo assays demonstrate that PFOS exposure can lead to embryonic and/or larval malformation and delays/reduction in hatching. • Alterations to the expression of genes related to growth and development in vivo in zebrafish and rodents, and in human embryonic cell lines. • Alterations to DNA methylation (global hypomethylation and gene-specific hypermethylation) in human cord blood and in placenta from rodent studies. **Limitations:** • The role of epigenetic mechanisms in changes at the mRNA level is not clear, nor is the relationship between molecular changes and apical developmental outcomes.	The evidence demonstrates that PFOS exposure during development can alter the epigenome and the expression of genes that control regular growth and development; it is possible that such changes are related,	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
				although the relationship has not been directly measured.	

Notes: SGA = small-for-gestational age; FGR = fetal growth restriction; PND = postnatal day; GD = gestational day; BMI = body mass index; DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid.

3.4.5 Evidence Synthesis and Integration for Other Noncancer Health Outcomes

Consistent with the SAB's recommendation (U.S. EPA, 2022e), EPA concluded that the noncancer health outcomes with the strongest evidence are hepatic, immune, cardiovascular, and developmental. For all other health outcomes (e.g., reproductive and endocrine), EPA concluded that the epidemiological and animal toxicological evidence available from the preliminary scoping considered in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonate (PFOS) (CASRN 1763-23-1) in Drinking Water* is either *suggestive* of associations or *inadequate* to determine associations between PFOS and the health effects described (U.S. EPA, 2021b). Based on this analysis, these outcomes were not prioritized for the subsequent literature search update efforts; the evidence synthesis and integration for these outcomes are presented in Appendix C (U.S. EPA, 2024a). In addition, Section 5.5 further describes rationale for evidence integration judgments for health outcomes which EPA determined had *evidence suggestive* of associations between PFOS and related adverse health effects, though the databases for those health outcomes shared some characteristics with the *evidence indicates* judgment.

3.5 Cancer Evidence Study Quality Evaluation, Synthesis, Mode of Action Analysis and Weight of Evidence

EPA identified 17 epidemiological and 1 animal toxicological study (2 overlapping publications) that investigated the association between PFOS and cancer. Of the epidemiological studies, eight were classified as *medium* confidence, seven as *low* confidence, and two were considered *uninformative* (Section 3.5.1). The single animal toxicological study was considered a *high* confidence study (Section 3.5.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.5.1 Human Evidence Study Quality Evaluation and Synthesis

3.5.1.1 Introduction and Summary of Evidence from the 2016 PFOS HESD

There are eight epidemiological studies (nine publications[15]) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cancer effects. Study quality evaluations for these seven studies are shown in Figure 3-70.

The 2016 PFOS HESD (U.S. EPA, 2016b) concluded that there was no evidence of carcinogenic effects for PFOS from human studies, but that the small number, breadth, and scope of the studies were not adequate to make definitive conclusions. Although an elevated risk of bladder cancer mortality was observed in an occupational study of workers at the 3M Decatur, Alabama plant (Alexander et al., 2003), a subsequent study to ascertain cancer incidence in the cohort observed elevated but nonsignificant incidence ratios that were 1.7- to twofold higher among

[15] Ghisari, 2014, 2920449 analyzes interactions between gene polymorphisms and PFOS exposure on breast cancer risk in the same population analyzed in Bonefeld-Jørgensen, 2011, 2150988.

exposed workers (Alexander and Olsen, 2007). Mean PFOS serum levels were 94.1 ng/mL. In the same 3M cohort, Grice et al. (2007) observed that prostate, melanoma, and colon cancer were the most frequently reported malignancies. When cumulative exposure measures were analyzed, elevated odds ratios were reported for melanoma, colon, and prostate cancer, however, they did not reach statistical significance. Length of follow-up may not have been adequate to detect cancer incidence in this cohort as approximately one-third of the participants had worked <5 years in their jobs, and only 41.7% were employed ≥20 years.

No elevated risk was observed for bladder, liver, or pancreatic cancer in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment ranging 1–130.5 ng/mL (Eriksen et al., 2009). No elevated risk of colorectal cancer was observed in community participants of the C8 Health Project (Innes et al., 2014). Elevated nonsignificant ORs for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009), and no association was reported by another case-control study in Denmark (Hardell et al., 2014). A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (1.5–172 ng/mL) reported an association of low magnitude that could not be separated from other perfluorosulfonated acids, and the association was not confirmed in a Danish population (Bonefeld-Jørgensen et al., 2014; Bonefeld-Jorgensen et al., 2011). Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis and the impact of this potential exposure misclassification on the estimated risks is unknown (Hardell et al., 2014; Bonefeld-Jorgensen et al., 2011). No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

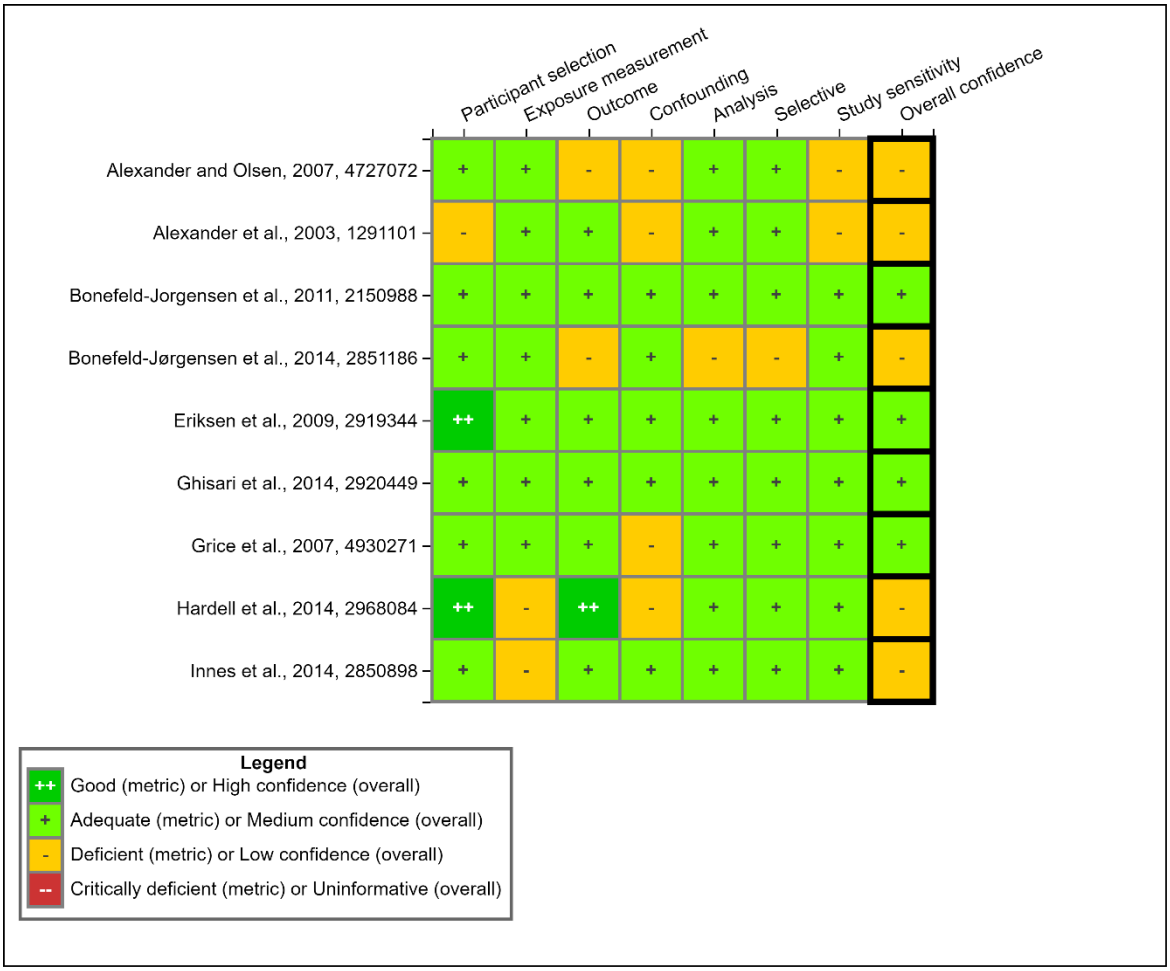


Figure 3-70. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cancer Effects Published Before 2016 (References from 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 17 studies have been published that investigated the association between PFOS and cancer (see Appendix D, (U.S. EPA, 2024a)). All studies were conducted on the general population with one in a high-exposure community (i.e., C8 population). Different study designs were also used including two cohort studies (Li et al., 2022; Fry and Power, 2017), six case-control studies (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Wielsøe et al., 2017), six nested case-control studies (Goodrich et al., 2022; Shearer et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Hurley et al., 2018; Ghisari et al., 2017), and three cross-sectional studies (Omoike et al., 2021; Christensen et al., 2016; Ducatman et al., 2015). The studies were conducted in different study populations including populations from China (Cao et al., 2022; Liu et al., 2021; Lin et al., 2020b), Denmark (Ghisari et al., 2017), France (Mancini et al., 2020), Greenland (Wielsøe et al., 2017), Japan (Itoh et al., 2021), Sweden (Li et al., 2022), Taiwan (Tsai et al., 2020), and the United States (Goodrich et al., 2022; Omoike et al., 2021; Shearer et al., 2021; Cohn et al., 2020; Hurley et al., 2018; Fry and Power, 2017; Christensen et al., 2016; Ducatman et al., 2015). All

the studies measured PFOS in study subject's blood components (i.e., serum or plasma) with one study measuring the levels in the maternal serum (Cohn et al., 2020). Cancers evaluated included breast (Li et al., 2022; Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017; Wielsøe et al., 2017), germ cell tumors (Lin et al., 2020b), kidney (Shearer et al., 2021), liver (Cao et al., 2022; Goodrich et al., 2022), melanoma (Li et al., 2022), ovarian (Omoike et al., 2021), prostate (Omoike et al., 2021; Ducatman et al., 2015), thyroid (Liu et al., 2021) uterine (Omoike et al., 2021), and any cancer (Li et al., 2022; Fry and Power, 2017; Christensen et al., 2016).

3.5.1.2 Study Quality

Study quality evaluations for the 17 studies identified since the 2016 PFOS HESD are shown in Figure 3-71. Of these 17 studies, eight were considered *medium* confidence and seven were *low* confidence (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Christensen et al., 2016). One study conducted in the high exposure to PFAS Ronneby Register Cohort in Sweden was *uninformative* (Li et al., 2022) because of concerns about exposure assessment and lack of data on important covariates. One study conducted in Greenland was considered *uninformative* (Wielsøe et al., 2017) because of concerns about exposure assessment and participant selection. As a result, these two studies are not further considered in this review. Concerns in the *low* confidence studies included the possibility of outcome misclassification, confounding or potential selection bias. Residual confounding was also a concern, including lack of considering co-exposures by other PFAS, and lack of appropriately addressing SES and other lifestyle factors, which could be associated with both exposure and cancer outcome. Although PFOS has a relatively long half-life in the blood, concurrent measurements may not be appropriate for cancers with long latencies. Temporality of exposure measure in terms of cancer development was noted to be a concern in several *low* confidence studies (Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Tsai et al., 2020). Many of the *low* confidence studies also had sensitivity issues due to small sample sizes. Lack of details or reporting issues were also a concern for some *low* confidence studies which resulted in difficulty in quantitatively interpreting analysis results (Cao et al., 2022). Cao et al. (2022) was determined to have *mixed* confidence (*low* and *uninformative*). The *uninformative* metric was the liver cancer biomarker analysis included in this study which did not provide sufficient information on biomarker measurement methods (Cao et al., 2022). The biomarker analysis portion of this study is not further considered in this review.

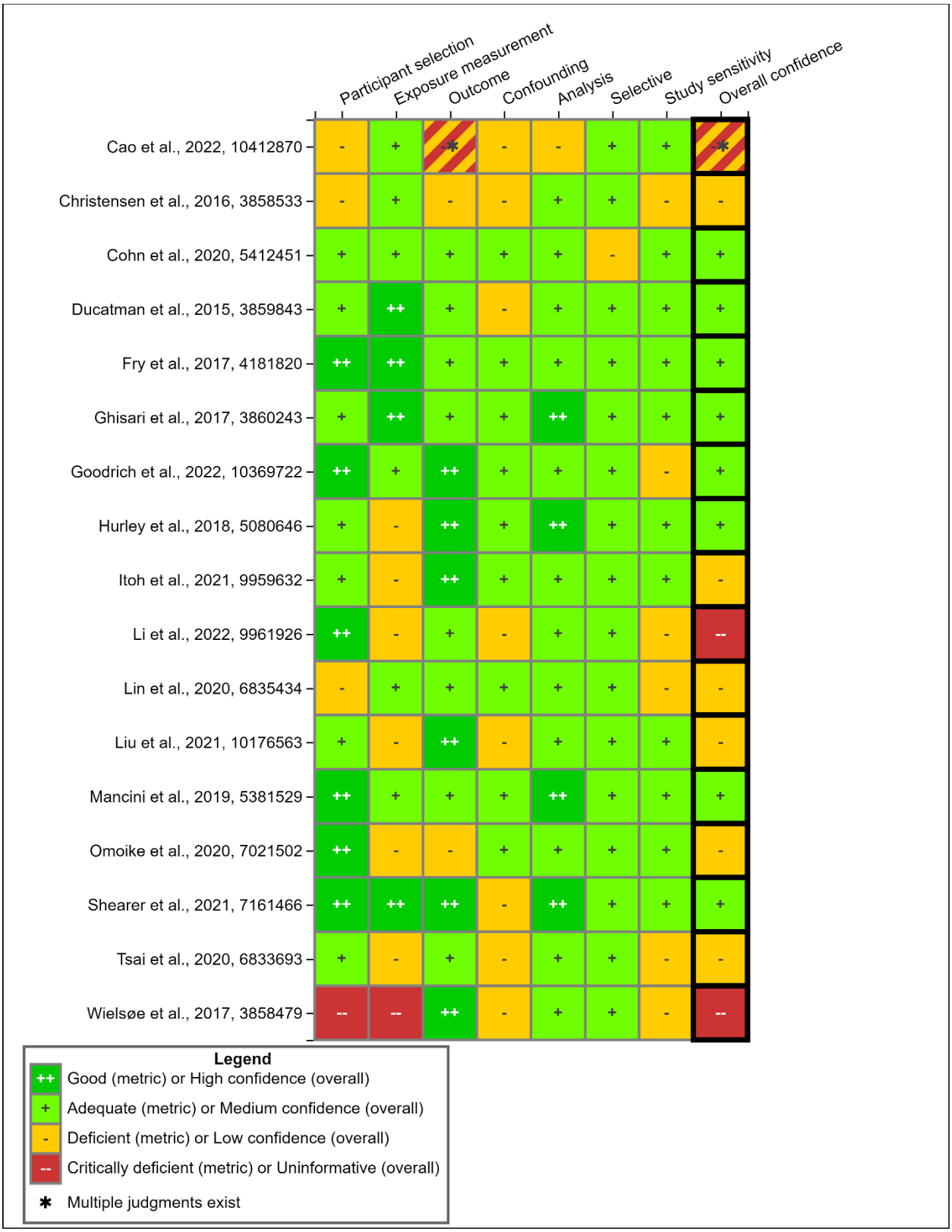


Figure 3-71. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

3.5.1.3 Findings From Children

One *low* confidence study examined cancers in children (Lin et al., 2020b) and reported a statistically significant higher median PFOS concentration in 42 pediatric germ cell tumor cases compared with 42 controls in blood samples collected from the children 1 week after diagnosis. However, the study did not observe an increased risk of germ cell tumors association with a per ng/mL increase in blood PFOS. One *low* confidence study examined liver cancers in children and adults (Cao et al., 2022), but since results are not presented separately by age group, this study will be reviewed in the following section.

3.5.1.4 Findings From the General Adult Population

PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a *medium* confidence study (Shearer et al., 2021). A case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial, reported a statistically significant positive trend in risk of renal cell carcinoma with pre-diagnostic serum levels of PFOS (OR = 2.51; 95% CI: 1.28, 4.92 for the highest *vs.* lowest quartiles; p-trend = 0.009, or per doubling of PFOS: OR: 1.39; 95% CI: 1.04, 1.86) (Shearer et al., 2021). Although the trend was significant across quartiles, the effect in the third quartile was null (OR = 0.92; 95% CI: 0.45, 1.88). Additionally, the association with PFOS was attenuated after adjusting for other PFAS (OR = 1.14; 95% CI: 0.45, 2.88 for the highest *vs.* lowest quartiles; p-trend = 0.64), and it was lower in the third quartile than in the second quartile, indicating potential confounding by correlated PFAS exposures. There was no association with a per doubling change in PFOS after adjusting for other PFAS.

Seven general population studies published since the 2016 PFOS HESD, evaluated PFOS and risk for breast cancer (Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017) with mixed results. All studies were case-control studies (with some nested case-controls), except for one cross-sectional NHANES-based study (Omoike et al., 2021). Three studies were considered *low* confidence (Itoh et al., 2021; Omoike et al., 2021; Tsai et al., 2020) because of concerns about temporality of exposure measurements and breast cancer development, the control status was not confirmed via examination or medical records (Tsai et al., 2020), and potential for residual confounding due to SES, lifestyle factors and exposure to other PFAS. The remaining studies were all *medium* confidence. A nested case-control study did not observe an association between breast cancer identified through California cancer registry and PFOS concentrations in serum after case diagnosis (max PFOS concentration of 99.8 ng/mL) (Hurley et al., 2018). A nested case-control study in a prospective (pregnancy) cohort study, the CHDS, suggested that maternal PFOS was associated with a decrease in the daughters' breast cancer risk in the first or fourth quartile of TC (Cohn et al., 2020), but the study did not examine breast cancer subtypes or genetic variants. Two nested case-control studies and one *low* confidence case-control study found associations between PFOS and breast cancer, but only in specific groups of participants (Mancini et al., 2020; Tsai et al., 2020; Ghisari et al., 2017). Ghisari et al. (2017) reported an increased risk for breast cancer identified from the cancer registry with increasing PFOS concentrations only in participants with a CC genotype (n = 36 cases and 47 controls) in the CYP19 gene (cytochrome P450 aromatase). A nested case-control study (194 pairs of breast cancer cases and controls)

within the French E3N cohort found an 86% higher risk of breast cancer in the 2nd and 3rd quartiles of PFOS (13.6–17.3 ng/mL, and 17.3–22.5 ng/mL) compared with the 1st quartile (5.8–13.6 ng/mL) (OR = 1.94; 95% CI: 1.00, 3.78, and OR = 2.03; 95% CI: 1.02, 4.04) in the full adjusted model (Mancini et al., 2020). Mancini et al. (2020) reported that the risk for breast cancer (93% verified pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increasing trend in estrogen receptor positive (ER+) and progesterone receptor positive (PR+) breast cancers. The study also observed a significant increase in estrogen receptor- (ER-) and progesterone receptor- (PR-) breast cancers in the second quartile with elevated risks also observed in the other quartiles, but with no trend. The sample size was small with 26 participants having ER- breast cancers and 57 having PR- breast cancers.

One *low* confidence study (Tsai et al., 2020) conducted in Taiwan observed a statistically significant increase in risk of breast cancer with increasing log transformed PFOS, but only in participants aged 50 years or younger and in ER+ breast cancer in participants aged 50 years or younger. Statistically significant increased odds of breast cancer were also observed in a *low* confidence NHANES study (2005–2012) (Omoike et al., 2021) both per ng/mL increase in PFOS (OR = 1.011; 95% CI: 1.011, 1.011) and in the two highest quartiles of exposure. The association was significantly inverse in the second quartile compared with the lowest (OR = 0.87; 95% CI: 0.86, 0.89). One *low* confidence case-control study conducted in Japanese women (Itoh et al., 2021) observed a significant inverse association across serum PFOS quartiles with a significant dose-response trend (p-value < 0.0001) (see Appendix D, (U.S. EPA, 2024a)). Median PFOS levels ranged from 7.6 ng/mL in the lowest quartile to 24.67 ng/mL in the highest quartile. The association remained significantly inverse in both pre- and postmenopausal women in the highest tertile of exposure, with a significant dose-response trend (p-values for trend = 0.007 and 0.001, respectively).

Two general population studies published since the 2016 PFOS HESD examined liver cancer (Cao et al., 2022; Goodrich et al., 2022). One study was considered *medium* confidence (Goodrich et al., 2022) and one study was considered *low* confidence (Cao et al., 2022). The *medium* confidence nested case-control study of U.S. adults observed a significant increase in risk of liver cancer when comparing participants with PFOS exposures above the 85th percentile (54.9 ng/mL) compared with those at or below (OR = 4.50, 95% CI: 1.20, 16.00) (Goodrich et al., 2022). The association remained elevated but not statistically significant in analyses of continuous PFOS exposure. The study was nested in the large Multiethnic Cohort study of California and Hawaii; however, the sample size was small (n = 50 cases and controls each) which likely limited study sensitivity. A significantly elevated risk of liver cancer was also observed in a *low* confidence case-control study of Chinese children and adults (OR per log-ng/mL increase in PFOS exposure = 2.609; 95% CI: 1.179, 4.029) (Cao et al., 2022). However, confidence in the study results was considered *low* due to limited or lacking information regarding selection of controls, diagnosis method for liver cancer, adjustment for potential confounding, and details on the statistical analysis.

One *medium* confidence study based on the C8 Health Project (Ducatman et al., 2015) examined prostate-specific antigen (PSA) as a biomarker for prostate cancer in adult males over age 20 years who lived, worked, or went to school in one of the six water districts contaminated by the DuPont Washington Works facility. No association was observed between PSA levels in

either younger (i.e., aged 20–49 years) or older (i.e., aged 50–69 years) men and concurrent mean serum PFOS concentrations up to 25 ng/mL. In an NHANES population, Omoike et al. (2021) observed a significantly inverse association with prostate cancer (OR = 0.994; 95% CI: 0.994, 0.994).

Omoike et al. (2021) also observed statistically significant increased odds of ovarian cancer both per ng/mL increase in PFOS (OR = 1.012; 95% CI: 1.012, 1.013) and in the two highest quartiles of exposure, although the association was significantly inverse for the second quartile of PFOS exposure (see Appendix D, (U.S. EPA, 2024a)). A significant inverse association also was observed for uterine cancer (OR = 0.945; 95% CI: 0.944, 0.945 per ng/mL increase in PFOS) (Omoike et al., 2021).

One *low* confidence study conducted in Shandong Province, in eastern China (Liu et al., 2021) observed a statistically significant inverse association with thyroid cancer across quartiles of serum PFOS (p-value for trend = 0.001). The median serum PFOS levels were higher in controls than in cases (7.5 *vs.* 5.5 ng/mL, p-value < 0.001). However, there is some concern about possible reverse causality. The ability to excrete PFAS could change when the thyroid becomes cancerous by causing abnormal thyroid hormone levels which can affect the glomerular filtration rate (Dzierlenga et al., 2020b), thereby changing the PFAS concentrations.

Two studies examined all cancers together, but collected different information on cancer (i.e., incidence verses mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was not associated with PFOS exposure in a *medium* confidence study of participants over 60 years of age from NHANES, with median PFOS concentration 4.3 ng/g lipid (Fry and Power, 2017); PFOS also was not found to be associated with self-reported cancer incidence in a *low* confidence study among male anglers over 50 years, median PFOS concentration 19 µg/L (Christensen et al., 2016). Christensen et al. (2016) was considered *low* confidence due to the potential of self-selection because participants were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There is one study (2 overlapping publications) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cancer effects. Study quality evaluation for this one study is shown in Figure 3-72.

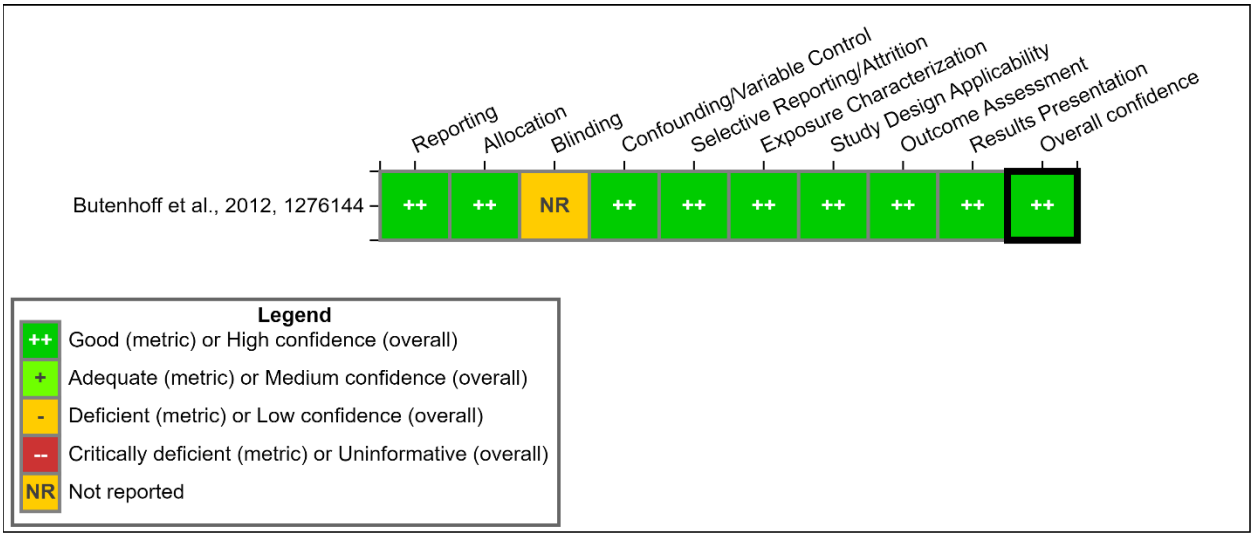


Figure 3-72. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

A single chronic cancer bioassay in animals was previously identified for PFOS (Butenhoff et al., 2012; Thomford, 2002a). In this study, conducted by Thomford (2002a) and published in part by Butenhoff et al. (2012), male and female Crl:CD®(SD)IGS BR rats were administered diets containing 0, 0.5, 2, 5, or 20 ppm PFOS for 103–104 weeks. Increased incidence of hepatocellular adenomas in the high-dose groups for male (7/43; 16%) and female rats (5/31; 16%) and combined adenomas/carcinomas in high-dose group females (6/32; 19%) were observed (Table 3-18). There was also a statistically significant positive trend of each of these responses in both male and female rats (all $p \leq 0.01$). At 105 weeks there was an accompanying increase in eosinophilic clear cell foci, and cystic hepatocellular degeneration in males given 2, 5, and 20 ppm PFOS. Low levels of single cell necrosis in all dose groups for both males and females were identified, though the increase compared with controls was significant only at the highest dose in each sex.

Table 3-18. Incidences[a] of Hepatocellular and Pancreatic Tumors in Male and Female Sprague-Dawley Rats as Reported by Thomford (2002b)

Sex	Tumor Type	Treatment group				
		0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
Male	Hepatocellular Adenomas	0/41 (0%)**	3/42 (7%)	3/47 (6%)	1/44 (2%)	7/43 (16%)**
Female	Hepatocellular Adenomas	0/28 (0%)**	1/26 (4%)	1/15 (7%)	1/28 (4%)	5/31 (16%)*
Female	Hepatocellular Carcinomas	0/28 (0%)	0/29 (0%)	0/16 (0%)	0/31 (0%)	1/32 (3%)
Female	Combined Hepatocellular Adenomas and Carcinomas	0/28 (0%)**	1/29 (3%)	1/16 (6%)	1/31 (3%)	6/32 (19%)*

Sex	Tumor Type	Treatment group				
		0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
Male	Pancreatic Islet Cell Adenomas	4/44 (9%)	3/45 (7%)	4/48 (8%)	4/46 (9%)	4/44 (9%)
Male	Pancreatic Islet Cell Carcinomas[b]	1/38 (3%)*	2/41 (5%)	2/44 (5%)	5/44 (11%)	5/40 (13%)
Male	Combined Pancreatic Islet Cell Adenomas and Carcinomas	5/44 (11%)	5/45 (11%)	6/48 (13%)	8/46 (17%)	9/44 (20%)

Notes: *Statistically significant compared with the control group at $p \leq 0.05$. **Statistically significant compared with the control group at $p \leq 0.01$. Denoted significance for the control groups indicate statistically significant trends.
[a] Tumor incidence is expressed as the number of animals with tumors over the number of animals alive at the time of first occurrence of the tumor.
[b] Statistical significance determined by EPA using the Cochran-Armitage test.

In addition to hepatocellular tumors, Thomford (2002b) reported increased incidences of pancreatic islet cell carcinomas in male rats (Table 3-18). Though the increases in the number of animals with carcinomas in the 5 and 20 ppm dose groups were not statistically different from the control group, there was a statistically significant trend of increased incidence with increased dose ($p \leq 0.05$; Cochran-Armitage test).

Thyroid and mammary gland tumors were also observed but did not exhibit linear dose-response relationships (Butenhoff et al., 2012; Thomford, 2002b). The most frequent thyroid tumor type in females was C-cell adenomas, but the highest incidence was that for the controls and there was a lack of dose response among the exposed groups. There was also a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose response for all tumor classifications.

3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cancer outcomes is discussed in Section 3.4.3 of the 2016 PFOS HESD (U.S. EPA, 2016b). There are 27 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to cancer effects. A summary of these studies by data source is shown in Figure 3-73.

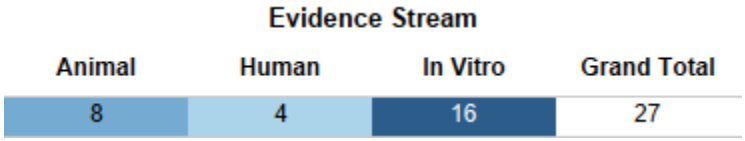


Figure 3-73. Summary of Mechanistic Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on HAWC..

In 2016, 10 key characteristics of carcinogens were selected by a multi-disciplinary working group of the International Agency for Research on Cancer (IARC), based upon common empirical observations of chemical and biological properties associated with human carcinogens (i.e., Group 1 carcinogens as determined by IARC) (Smith et al., 2016b). In contrast to the

"Hallmarks of cancer" as presented by Hanahan and Weinberg (Hanahan, 2022; Hanahan and Weinberg, 2011, 2000), the key characteristics focus on the properties of human carcinogens that induce cancer, not the phenotypic or genotypic traits of cancers. The 10 key characteristics provide a framework to systematically identify, organize, and summarize mechanistic information for cancer hazard evaluations (Smith et al., 2016b).

To aid in the evaluation of the carcinogenic potential of PFOS, the studies containing mechanistic data were organized by the proposed key characteristics of carcinogens for the following section. Evidence related to 7 of the 10 key characteristics of carcinogens was identified in the literature included in this assessment: 'Is Genotoxic,' 'Induces Epigenetic Effects,' 'Induces Oxidative Stress,' 'Modulates Receptor-Mediated Effects,' 'Alters Cell Proliferation, Cell Death, and Nutrient Supply,' 'Is Immunosuppressive,' and 'Induced Chronic Inflammation.' No studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and recent systematic literature search and review efforts were identified for the following key characteristics: 'Is Electrophilic or Can Be Metabolically Activated to Electrophiles,' 'Alters DNA Repair and Causes Genomic Instability,' and 'Causes Immortalization.'

3.5.3.1 Key Characteristic #2: Is Genotoxic

Genotoxicity is a well-characterized mode of action for carcinogens, defined as alterations to DNA through single or double strand breaks, alterations to DNA synthesis, and DNA adducts, all of which can result in chromosomal aberrations, formation of micronuclei, and mutagenesis if not effectively repaired.

3.5.3.1.1 Gene Mutation

3.5.3.1.1.1 In Vivo Evidence

Male *gpt* delta transgenic mice, a strain that was designed to facilitate the quantification of point mutations and deletions, were exposed to PFOS (4 and 10 mg/kg/day) for 28 days (Wang et al., 2015b). The mutation frequencies at the targeted *redBA* and *gam* loci in the liver of exposed male mice were increased at concentrations of 4 and 10 mg/kg/day relative to controls, but the increase was not significant, and the variance of the high-dose group was relatively large. The evidence for mutagenicity of PFOS in vivo is negative based on this single study (Table 3-19).

3.5.3.1.1.2 In Vitro Evidence

Several studies have demonstrated that PFOS is not mutagenic in vitro (Table 3-20). Of the four publications that tested PFOS for mutagenicity in *Salmonella typhimurium, Saccharomyces cerevisiae,* and *Escherichia coli* (NTP, 2019; Mecchi, 1999; Litton Bionetics, 1979 10228135; Simmon, 1978), no evidence of DNA mutagenesis has been described in the presence or absence of metabolic activation. In contrast, Wang et al. (2015b) exposed *gpt* delta transgenic mouse embryonic fibroblast cells to PFOS and found concentration-dependent increases in mutation frequencies at the *redBA/gam* loci, a region often used to determine point mutations and deletions.

3.5.3.1.2 DNA Damage

3.5.3.1.2.1 In Vivo Evidence

3.5.3.1.2.1.1 Human Studies

One study reported on the genotoxic potential of PFOS exposure in humans (Table 3-21). Governini et al. (2015) collected semen samples from healthy nonsmoking men and evaluated aneuploidy, diploidy, and DNA fragmentation. The occurrence of aneuploidy and diploidy in sperm cells, which are normally haploid, was significantly higher in the PFAS-positive samples (PFOS was detected in 25% of the samples) when compared with PFAS-negative samples. This suggests that PFAS exposure is related to errors in cell division leading to aneugenicity. Additionally, fragmented chromatin levels were also significantly increased for the PFAS-positive group compared with the PFAS-negative group.

3.5.3.1.2.1.2 Animal Toxicological Studies

Evaluations of PFOS exposure in rat, mouse, and zebrafish models were identified, which predominantly demonstrated evidence of genotoxicity (Table 3-21). The majority of studies presented data on potential micronuclei formation in bone marrow, peripheral blood, and/or the liver, though some also reported different metrics of DNA damage. Quantifying micronuclei formation in rats via optimal and reliable methods has been previously described (WHO & FAO, 2020; WHO and FAO, 2009; Witt et al., 2000).

NTP (2019) reported using flow cytometry to analyze micronuclei formation in immature polychromatic erythrocytes from the peripheral blood of male and female Sprague-Dawley rats treated with 0.312–5 mg/kg/day PFOS by gavage for 28 days. No effects on the number of micronucleated polychromatic erythrocytes (PCEs) were observed in males, though there was a significant increase in the number of PCEs in the 5 mg/kg/day females. Importantly, NTP (2019) noted that while there was a statistically significant trend for increasing micronucleated PCEs, and that the response in the 5 mg/kg/day group was statistically significant compared with controls indicating a positive test, the response was nonetheless within the range of historical control levels. NTP (2019) also reported that there were significant dose-dependent decreases in the percentage of PCEs in the peripheral blood of both males and females, suggesting that PFOS exposure may induce bone marrow toxicity.

Three other studies published by the same primary authors also reported the induction of micronuclei formation in male or female Swiss Albino rats (Eke et al., 2017; Eke and Çelik, 2016; Çelik et al., 2013). Çelik et al. (2013) found that oral treatment with PFOS (≤2.5 mg/kg/day) administered every other day for 30 days induced genetic damage as measured with the comet assay, as well as the formation of micronuclei in female rat bone marrow samples. However, similar to the results from NTP (2019), the study also demonstrated that PFOS exposure decreased the ratio of PCEs to normochromic erythrocytes (NCEs), indicating that the genetic damage may be a result of bone marrow toxicity rather than direct genotoxicity of PFOS. Two subsequent studies in male rats using the same exposure paradigm (30-day exposure administered every other day) found similar results. Eke and Çelik (2016) reported increased micronuclei formation and genetic damage indices (calculated using results of a comet assay) in peripheral blood, while Eke et al. (2017) reported increased micronuclei formation and genetic damage indices in liver tissue. Notably, these two studies did not report the ratio of PCEs

to NCEs which limits the ability to interpret these data further. Given the results from Çelik et al. (2013) and considering the similarities in study design, it is reasonable to assume that the genetic damage observed may be due to bone marrow or hepatic toxicity.

Micronucleus frequency was slightly elevated in the bone marrow male *gpt* delta transgenic mice exposed to PFOS (4 and 10 mg/kg/day) for 28 days than in controls; however, these results were not statistically significant (Wang et al., 2015b). Similarly, EPA's 2016 PFOS HESD (U.S. EPA, 2016b) reported mouse bone marrow micronucleus assays to be negative after high-dose acute exposures (237.5, 475, and 950 mg/kg; measured after approximately 24, 48, and 72 hours) to PFOS (Murli, 1996). Subchronic 28-day exposure of Sprague-Dawley rats to PFOS did not alter micronuclei formation in reticulocytes in exposed males, while data derived from exposed female rats was equivocal (NTP, 2019).

In another study, male and female zebrafish embryos were exposed to PFOS concentrations of 0.4, 0.8, or 1.6 mg/L for 30 days (Du et al., 2014). Following exposure, Du et al. (2014) found significant dose-dependent increases in micronucleus formation. Du et al. (2014) also reported increases in the number of DNA single-strand breaks, though none of the PFOS doses tested resulted in significant effects. Notably, the high-dose exposure resulted in increased rates of developmental malformations, which could potentially confound these results.

3.5.3.1.2.2 In Vitro Evidence

3.5.3.1.2.2.1 Chromosomal aberrations

EPA's 2016 PFOS HESD (U.S. EPA, 2016b) reports that PFOS exposure did not induce chromosomal aberrations in human lymphocytes (Table 3-22) (Murli, 1999). No new studies were identified that measure chromosomal aberrations after PFOS exposure in the updated literature search.

3.5.3.1.2.2.2 DNA Synthesis

A study by Cifone (1999) evaluated the effects of 15 different PFOS concentrations ranging from 0.25 µg/mL to 4,000 µg/mL in Fisher 344 male rat hepatocytes. No evidence of increased DNA synthesis was observed, denoted by the lack of elevated mean net nuclear grains. Cytotoxicity significantly increased at approximately 50 µg/mL.

An additional study, detailed elsewhere, noted increased DNA synthesis (increased cells in S phase) following exposure in rodent hepatocytes. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.1.2.2.3 DNA Damage

Several assays of DNA damage have been performed on a variety of in vitro models (Table 3-22). Wang et al. (2015b) exposed gpt delta transgenic mouse embryonic fibroblasts to PFOS and found evidence of concentration-dependent increase in phosphorylated histone H2AX (γ-H2AX), a biomarker of DNA double strand breaks (DSBs), after exposure to 1 or 20 µM PFOS (no statistical analysis was reported). Direct exposure of suspended calf thymus DNA to 10 µM PFOS for 30 minutes modified DNA structure, attenuated DNA charge transport, and led to PFOS-DNA adduct formation (Lu et al., 2012).

In contrast, several studies found no evidence of DNA damage after exposure. Jacquet et al. (2012) exposed Syrian hamster embryos to PFOS (≤50 μg/mL) and found no evidence of DNA damage by a comet assay. Similarly, there was no evidence of DNA damage via a comet assay in the protist species *Paramecium caudatum* exposed to 10–100 μM for 24 hours (Kawamoto et al., 2010).

Florentin et al. (2011) exposed HepG2 cells to PFOS (5–300 μM) for 1 or 24 hours. There was no evidence of DNA damage in a comet assay nor change in micronucleus frequency at any concentration or time point. However, within the 24-hour exposure assay, significant cytotoxic effects were noted at 300 μM. In contrast, a study conducted by Wielsoe et al. (2015) exposed HepG2 cells to PFOS (2×10^{-7} to 2×10^{-5} M) for 24 hours and used a comet assay to measure DNA damage. Following exposure, the cells demonstrated a dose-dependent increase in DNA damage at all tested concentrations.

Table 3-19. Mutagenicity Data From In Vivo Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
Wang et al. (2015b)	Mouse, *Gpt* delta transgenic (Male)	Liver	Negative	1–10 mg/kg/day (daily via gavage for 28 days)

Table 3-20. Mutagenicity Data From In Vitro Studies

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of Exposure)
		S9-Activated	**Non-Activated**	
Litton Bionetics, Inc. (1979)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	0.1–1,000 µg/plate
Litton Bionetics, Inc. (1979)	*Saccharomyces cerevisiae* (D4)	Not Reported	Negative	0.1–1,000 µg/plate
Mecchi (1999)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Negative	Negative	0.333–5,000 µg/plate
Mecchi (1999)	*Escherichia coli* (WP2*uvr*A)	Negative	Negative	33.3–5,000 µg/plate
NTP (2019)	*Salmonella typhimurium* (TA98, TA100)	Negative	Negative	100–5,000 µg/plate
NTP (2019)	*Escherichia coli* (WP2*uvr*A/pkM101)	Negative	Negative	100–10,000 µg/plate
Simmon (1978)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	10–5,000 µg/plate
Simmon (1978)	*Salmonella cerevisiae* (D3)	Negative	Negative	0.1–5 µg/plate
Wang et al. (2015b)	*gpt* Delta transgenic mouse embryonic fibroblasts	Not reported	Positive[a]	1–20 µM (24 hours)

Notes:
[a] Mutagens were present in cells exposed ≥10 µM.

Table 3-21. DNA Damage Data From In Vivo Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
DNA Strand Breakage				
Governini et al. (2015)	Human (Male)	Semen	Positive	Average Seminal Plasma Concentration of 5.37 ng/g f.w.
DNA Damage via Comet Assay				
Çelik et al. (2013)	Rat, Swiss Albino (Female)	Bone marrow	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Du et al. (2014)	Zebrafish, AB (Male and female)	Peripheral blood cells	Negative	0.4–1.6 mg/L (single dose to rearing water)
Eke and Çelik (2016)	Rat, Swiss Albino (Male)	Peripheral blood cells	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Eke et al. (2017)	Rat, Swiss Albino (Male)	Liver	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Micronuclei Formation				
Çelik et al. (2013)	Rat, Swiss Albino (Female)	Bone marrow	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Du et al. (2014)	Zebrafish, AB (Male and female)	Peripheral blood cells	Positive	0.4–1.6 mg/L (single dose to rearing water for 30 days)
Eke and Çelik (2016)	Rat, Swiss Albino (Male)	Peripheral blood cells	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Eke et al. (2017)	Rat, Swiss Albino (Male)	Liver	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Murli (1996)	Mouse, Crl:CD-1 (Male and female)	Bone marrow	Negative	—[a]
NTP (2019)	Rat, Sprague-Dawley (Male)	Peripheral blood cells	Negative	0.312–5 mg/kg/day (daily via gavage for 28 days)
NTP (2019)	Rat, Sprague-Dawley (Female)	Peripheral blood cells	Equivocal	0.312–5 mg/kg/day (daily via gavage for 28 days)
Wang et al. (2015b)	Mouse, *Gpt* delta transgenic (Male)	Bone marrow	Negative	1–10 mg/kg/day (daily via gavage for 28 days)

Notes: f.w. = formula weight.

[a] Findings based on the 2016 EPA's Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) (U.S. EPA, 2016b), concentration(s) unknown.

Table 3-22. DNA Damage Data From In Vitro Studies

Reference	In Vitro Model (Assay)	Results	Concentration (Duration of Exposure)
	Chromosomal Aberrations		
Murli (1999)	Human lymphocytes	Negative	10–470 µg/mL (3 hours)
	Unscheduled DNA Synthesis		
Cifone (1999)	Fisher 344 male rat hepatocytes	Negative	0.25–4,000 µg/mL
	DNA Damage		
Wang et al. (2015b)	*gpt* Delta transgenic mouse embryonic fibroblasts (γ-H2AX foci)	Positive	0–30 µM (24 hours)
Jacquet et al. (2012)	Syrian hamster embryo cells (comet assay)	Negative	2×10^{-4}–50 µg/mL (7 days)
Kawamoto et al. (2010)	*Paramecium caudatum* (comet assay)	Negative	10–100 µM (1–24 hours)
Lu et al. (2012)	Calf thymus DNA (X-ray photoelectron spectroscopic and electrochemical impedance spectroscopy)	Positive	10 µmol/L (30 minutes)
Wielsoe et al. (2015)	HepG2 (comet assay)	Positive	2×10^{-7}–2×10^{-5} M (24 hours)
Florentin et al. (2011)	HepG2 (comet assay)	Negative	5–300 µM (1 or 24 hours)

3.5.3.2 Key Characteristic #4: Induces Epigenetic Alterations

Epigenetic alterations are modifications to the genome that do not change genetic sequence. Epigenetic alterations include DNA methylation, histone modifications, changes in chromatin structure, and dysregulated microRNA expression, all of which can affect the transcription of individual genes and/or genomic stability (Smith et al., 2016b).

3.5.3.2.1 In Vivo Evidence

3.5.3.2.1.1 Humans

A cohort of singleton term births were recruited from Faroese hospitals over an eighteen-month period from 1986 to 1987 (Leung et al., 2018). At delivery, samples of umbilical cord whole blood and scalp hair from the mothers were collected and used to measure toxicant levels as well as evaluation of DNA methylation. PFOS levels were significantly correlated with the number of methylated CpG sites (10,598 sites) in male newborn umbilical cord whole blood samples. Data from the male samples were then used to evaluated potential gene networks or pathways enriched based on the genes related to the methylated CpG sites; specifically, to evaluate potential relationships between physiological functions/diseases and the PFOS-induced aberrant methylation patterns. The top physiological function related to the methylation changes was "nervous system development and function." Additionally, CpG sites for which PFOS exposure altered the methylation status were associated with individual genes related to cancer.

A subset of adults enrolled in the C8 Health Project between August 1, 2005 and August 31, 2006 were evaluated for exposure to perfluoroalkyl acids (PFAAs) via drinking water (Watkins et al., 2014). The cross-sectional survey consisted only of residents within the mid-Ohio River Valley. A second, short-term follow-up study including another sample collection was conducted in 2010 to evaluate epigenetic alterations in relation to serum PFOS concentrations. Serum concentrations of PFOS decreased slightly between enrollment (2005–2006) and follow-up (2010). Methylation of long interspersed nuclear elements (LINE-1) transposable DNA elements in peripheral blood leukocytes at the follow-up timepoint in 2010 was significantly associated with PFOS exposure, with an unadjusted 0.265% increase in LINE-1 methylation (per 12 ng/mL increase in mean serum PFOS). This association between LINE-1 methylation and PFOS exposure remained significant after adjusting for covariates; a 0.20% increase was observed when the data were adjusted for age, gender, BMI, smoking status, and drinking status.

Additional epidemiological studies of prenatal or birth cohorts have identified epigenetic alterations associated with PFOS, indicating exposure can induce global DNA methylation changes and alterations to methylation of CpG sites that are associated with genes involved in several physiological functions and diseases related to development. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.2.1.2 Animals

Dysregulation of long non-coding RNAs in rodent in vivo studies following PFOS exposure has been demonstrated, leading to reduced placental size. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details). It should be noted that such effects were not seen in other tissues or in relation to other effects that may be more relevant to cancer outcomes.

Additional rodent evidence examined liver microRNA (miRNA) expression and found an increase in the expression of *miR-34a-5p*, which is involved in p53-mediated apoptosis, following exposure to PFOS. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.2.2 In Vitro Evidence

Pierozan et al. (2020) evaluated PFOS (10 μM) in the MCF-10A breast cell line. After 72 hours of exposure, PFOS-treated cells exhibited decreased acetylation of histone H3K9 (H3K9ac). In contrast, no alterations were found in the levels of H3K9 methylation and H3K26 acetylation.

Several additional studies have evaluated the potential of PFOS to alter the epigenome within various in vitro systems designed to test developmental effects. The available mechanistic studies suggest that the developing liver, developing heart, and placenta may be affected by PFOS at the molecular level (i.e., differential methylation of genes, gene expression changes, mitochondrial dysregulation). For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.3 Key Characteristic #5: Induce Oxidative Stress

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are byproducts of energy production that occur under normal physiological conditions. An imbalance in the detoxification of reactive such species can result in oxidative (or nitrosative) stress, which can play a role in a variety of diseases and pathological conditions, including cancer. The primary mechanism by which oxidative stress leads to the carcinogenic transformation of normal cells is by inducing oxidative DNA damage that leads to genomic instability and/or mutations (Smith et al., 2016b).

3.5.3.3.1 In Vivo Evidence

3.5.3.3.1.1 Humans

Several human epidemiological studies have reported that PFOS exposure induces oxidative stress, leading to cardiological dysregulation (e.g., endothelial dysfunction, impaired vasodilation, increased 8-OHdG and 8-NO2Gua). For additional information, please see the cardiovascular mechanistic section (Section 3.4.3.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.3.1.2 Animals

Male Sprague-Dawley rats were administered 1 or 10 mg/kg/day PFOS orally for 28 days (Han et al., 2018a). Following exposure, significant increases in ROS production and nitric oxide synthase mRNA expression were noted in the liver. Elevation of oxidative stress was associated with decreased intracellular antioxidant defense by aberrant catalase and superoxide dismutase activities.

Liu et al. (2009) studied markers of oxidative stress in the liver and brain in KM mice exposed to PFOS and found that there was no treatment effect. The authors found that levels of malondialdehyde (MDA) did not differ between controls and exposed animals, and that

superoxide dismutase activity was lower in treated versus control mice. indicating that oxidative stress was not induced.

Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in antioxidant enzymes, was observed in rodents in vivo following oral exposure to PFOS. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.3.2 In Vitro Evidence

Several studies have evaluated ROS production in HepG2 cells exposed to PFOS, reporting varied results. A study by Hu and Hu (2009) demonstrated PFOS exposure (50–200 µmol/L; 24–72 hours) induced a significant increase in ROS. This effect correlated with decreased mitochondrial membrane potential and apoptosis. Furthermore, PFOS exposure caused increased superoxide dismutase, catalase, and glutathione reductase levels but decreased glutathione-*S*-transferase and glutathione peroxidase levels in cells. In contrast, Florentin et al. (2011) exposed HepG2 cells to PFOS (5–300 µM) for 24 hours and found a decrease in ROS generation by approximately 23%.

A study by Wang et al. (2015b) used mouse embryonic fibroblast (MEF) cells to identify intercellular ROS induced by PFOS exposure (1 or 20 µM). Using a fluorescent free radical probe CM-H_2DCFDA kit to evaluate ROS levels, cells exposed to 20 µM PFOS had a significantly higher level of florescence than controls, indicating PFOS induced intercellular oxidative stress. To better understand the role of H_2O_2 in this PFOS-induced cytotoxicity (Section 3.5.3.7) and genotoxicity (Section 3.5.3.1), Wang et al. treated cells concurrently with a cell membrane-permeating catalase to initiate the breakdown of H_2O_2 and protect cells from oxidative damage. In the presence of catalase, cytotoxicity and DNA double strand break frequency were decreased in PFOS-exposed cells. Mutation frequencies were also significantly suppressed in cells exposed to both PFOS and catalase when compared with cells exposed to PFOS alone. These results in Wang et al. (2015b) suggest that PFOS-induced genotoxicity is mediated by the induction of ROS.

Wielsoe et al. (2015) exposed HepG2 cells to PFOS (2×10^{-7} to 2×10^{-5} M) for 24 hours. Following exposure, the cells demonstrated significant increase in intercellular ROS at all tested PFOS concentrations.

Several studies have identified the potential of PFOS to induce oxidative stress within various in vitro testing systems that are designed to understand effects during developmental stages. The available mechanistic studies demonstrated that oxidative stress mediates alterations in development and gross morphology following PFOS exposure. PFOS. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details).

Further evidence of the ability of PFOS to induce oxidative stress is described elsewhere. PFOS exposure has been shown to be associated with increased markers of oxidative damage and decreased activity of protective antioxidants that play a role in the reduction of oxidative damage. PFOS. For additional information, please see the hepatic mechanistic section (Section

3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.4 Key Characteristic #6: Induces Chronic Inflammation

The induction of chronic inflammation includes increased white blood cells, altered chemokine and/or cytokine production, and myeloperoxidase activity (Smith et al., 2016b). Chronic inflammation has been associated with several forms of cancer, and a role of chronic inflammation in the development of cancer has been hypothesized. However, there are biological links between inflammation and oxidative stress and genomic instability, such that the contribution of each in carcinogenic progression is not always clear.

Several studies have identified the potential of PFOS to increase inflammation within various in vivo and in vitro models. It is important to note that in vitro models may be used for the evaluation of changes in inflammatory markers and response, they are generally not effective in modeling the events that are associated with chronic inflammation. For additional information, please see the immune (Section 3.4.2.3), hepatic (Section 3.4.1.3), developmental (Section 3.4.4.3), and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.5 Key Characteristic #7: Is Immunosuppressive

Immunosuppression refers to the reduction in the response of the immune system to antigen, which is important in cases of tumor antigens (Smith et al., 2016b). It is important to note that immunosuppressive agents do not directly transform cells, but rather can facilitate immune surveillance escape of cells transformed through other mechanisms (e.g., genotoxicity).

Studies have identified the immunosuppressive potential of PFOS in in vivo and in vitro testing systems. Specifically, PFOS has been associated with depression of natural killer cell activity, reduced macrophage function, and changes in the cellularity and immunophenotypes of lymphocytes. For additional information, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.6 Key Characteristic #8: Modulates Receptor-Mediated Effects

Modulation of receptor-mediated effects involves the activation or inactivation of receptors (e.g., PPAR, AhR) or the modification of endogenous ligands (including hormones) (Smith et al., 2016b).

3.5.3.6.1 In Vivo Evidence

Several studies have reported the potential of PFOS to modulate nuclear receptor- and hormone-mediated effects within various in vivo and in vitro testing systems, specifically models relevant to the hepatic system.

PFOS has been shown to activate several nuclear receptors, including PPARα, PPARγ, PPARβ/δ, CAR/PXR, and LXR/RXR. Many of these nuclear receptors, including PPARα and CAR, are known to play an important role in liver homeostasis and have been implicated in liver dysfunction. PFOS exposure may lead to liver toxicity through the activation of multiple nuclear

receptors in both rodents and humans. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.6.2 In Vitro Evidence

3.5.3.6.2.1 PPAR Mediated Effects

Liver-expressed peroxisome PPARα regulates transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism. Data for PFOS illustrates the ability of PFOS to activate PPARα (Wolf et al., 2014; Wolf et al., 2008; Martin et al., 2007; Shipley et al., 2004).

Jacquet et al. (2012) exposed Syrian hamster embryo (SHE) cells to PFOS (≤50 µg/mL) for 5 and 24 hours. Evaluation of PPAR gene expression by qPCR indicated a threefold increase of *ppar-b/d* mRNA level at a PFOS concentration of 0.2 µg/mL after 24 hours. Subsequent exposure of SHE cells to PFOS (0.02–20 µg/mL) for 1 week found overexpression of PPAR-target genes and a significant increase of *ppar-b/d* mRNA at 0.2 µg/mL (twofold increase) and 2 µg/mL (2.5-fold increase). mRNA levels of *ppar-y* were significant increased after 7 days at all PFOS exposure concentrations. Interestingly, upregulation of the *ppar-a* gene was found at the lowest concentration tested (0.2 µg/mL). A study using MCF-7 human breast cancer cells demonstrated that PFOS increased proliferation in a dose-dependent manner at concentrations of 0.01 and 30 µg/mL, a response that was observed in tandem with the maximal estrogen (E_2) response, suggesting that PFOS may be an estrogen receptor agonist at these concentrations (Henry and Fair, 2013).

3.5.3.7 Key Characteristic #10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

Aberrant cellular proliferation, cell death, and/or nutrient supply is a common mechanism among carcinogens. This mechanism includes aberrant proliferation, decreased apoptosis or other evasion of terminal programming, changes in growth factors, angiogenesis, and modulation of energetics and signaling pathways related to cellular replication or cell cycle control (Smith et al., 2016b).

3.5.3.7.1 In Vivo Evidence

3.5.3.7.1.1 Humans

Epidemiological studies found an association between PFOS exposure and increased markers of endothelial and platelet apoptosis. For additional information, please see the cardiovascular mechanistic section (Section 3.4.3.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.7.1.2 Animals

Proliferation of peroxisomes has been suggested as a mechanism of action for several non-genotoxic carcinogens that induce liver tumors upon chronic administration to rats and mice (Rao and Reddy, 1996; Ashby et al., 1994), and PFOS has been shown to activate PPARs. In a study of male and female Sprague-Dawley rats administered PFOS in the diet at 0, 0.5, 2, 5, or 20 ppm for 4 or 14 weeks, there was no evidence of increased hepatic cell proliferation (Seacat et

al., 2003). However, the same authors continued this same dietary PFOS exposure in Sprague-Dawley rats for up to 2 years and found liver effects consistent with PPAR activation (Butenhoff et al., 2012; Thomford, 2002b). This 2-year cancer bioassay found that the only neoplastic response that was attributable to PFOS exposure was an increased incidence of hepatocellular adenoma in both male and female rats in the 20 ppm PFOS group.

3.5.3.7.2 In Vitro Evidence

Two human giant cell tumor (GCT)-derived cell lines (COV434 and KGN) were exposed to PFOS (0.08–8,000 ng/mL) for 72 hours (Gogola et al., 2019). PFOS significantly increased proliferation in both cell lines in a dose-dependent manner. Specifically, PFOS treatment at 0.08 ng/mL increased COV434 and KGN proliferation by 1.4-fold and 1.9-fold, respectively. Follow-up studies by the same authors did not observe any change in caspase 3 or 7 activities in cells exposed to concentrations of PFOS (0.8, 8, or 80 ng/ml; 72 hours), both of which play a role in apoptosis (Gogola et al., 2020a; Gogola et al., 2020b).

The potential of PFOS to induce tumorigenic activity (proliferation, cell-cycle progression, and malignant phenotype) was evaluated in MCF-10A breast epithelial cells (Pierozan and Karlsson, 2018). Exposure to 10 μM promoted proliferation by accelerating G0/G1-to-S phase transition of the cell cycle after 24, 48, and 72 hours of exposure. PFOS exposure increased CDK4 while simultaneously decreased p27, p21, and p53 levels in MCF-10A cells. Furthermore, 10 μM PFOS exposure for 72 hours stimulated MCF-10A cell migration and invasion. A follow-up study evaluating PFOS (10 μM; 72 hours) in MCF-10A cells induced proliferation and alteration of regulatory cell-cycle proteins (cyclin D1, CDK6, p21, p53, p27, ERK1, ERK2, and p38) (Pierozan et al., 2020). Additionally, PFOS exposure increased cell migration and invasion in unexposed daughter cells of exposed cells, as evidenced by a reduction in the levels of E-cadherin, occludin, and β-integrin. A study in MCF-7 human breast cancer cells demonstrated that PFOS increased proliferation in a dose-dependent manner at concentrations of 0.01 and 30 μg/mL, a response that may be the result of estrogen receptor activation (Henry and Fair, 2013). These results elucidate PFOS's potential carcinogenic effects through alteration of cell proliferation.

In contrast to these results, no changes in cellular proliferation were observed in MCF-7 breast adenocarcinoma cells exposed to PFOS (0.1–100 μM) for 24 hours (Maras et al., 2006). However, a small but significant downregulation of estrogen-responsive genes (*TFFI* and *ESR1*) was noted following PFOS exposure.

In a study designed to determine the effect of PFOS effect on the tumor suppressor protein SHP-2, HepG2 cells were exposed to sub-cytotoxic concentrations of PFOS for 24 hours before SHP-2 was immunoprecipitated from the cell lysates (Yang et al., 2017). While PFOS exposure increased SHP-2 gene expression in a concentration-dependent manner, it was also found to have an inverse proportional decrease in SHP-2 enzyme activity. Interestingly, a 1.4-fold increase in SHP-2 protein levels was observed in exposed cells, indicating that PFOS inhibits SHP-2 by blocking enzymatic activity post-translationally.

For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.4 Weight Of Evidence for Carcinogenicity

3.5.4.1 Summary of Evidence

The carcinogenicity of PFOS has been documented in both epidemiological and animal toxicological studies. The available epidemiology studies report elevated risk of liver, bladder, kidney, prostate, and breast cancers after chronic PFOS exposure in some studies, though limited evidence for some tumor types (i.e., liver and renal) and mixed results for other tumor types (i.e., bladder, prostate, breast) provide plausible but not definitively causal evidence of a relationship between PFOS exposure and cancer outcomes from the epidemiological evidence alone. The animal chronic cancer bioassay provides additional support for carcinogenicity with the identification of multi-site tumorigenesis (liver and pancreas) in both male and female rats. The available mechanistic data suggest that multiple MOAs could play role in the hepatic and pancreatic tumorigenesis associated with PFOS exposure based on animal model study findings.

3.5.4.1.1 Evidence From Epidemiological Studies

Results for liver cancer from one *low* confidence occupational (Alexander et al., 2003) and one *medium* confidence general population-based (Eriksen et al., 2009) study of PFOS exposure published approximately 15–20 years ago were generally imprecise (i.e., null results with wide confidence intervals), but more recent studies have reported statistically significant increased risk of liver cancer associated with increased PFOS exposure (Cao et al., 2022; Goodrich et al., 2022). A *medium* confidence nested case-control study of adults from the Multiethnic Cohort (MEC) study reported a significant increased risk of liver cancer when comparing those in the 85th percentile of PFOS exposure to those at or below the 85th percentile (Goodrich et al., 2022). Positive, but not statistically significant, associations were observed in analyses of continuous PFOS exposure which supported the study's overall conclusion of an increased risk of liver cancer with increasing PFOS exposure. The study's sensitivity was limited by the small number of cases and controls (n = 50 each). Consistent with this finding, a Chinese general population case-control study of children and adults reported a significant increase in risk of liver cancer in analyses of continuous PFOS exposure; however, the study was considered *low* confidence due to lack of information on control selection, outcome ascertainment, and statistical analysis (Cao et al., 2022).

Studies of the association between PFOS serum concentrations and bladder cancer have mixed (positive and null) findings. An elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study (Alexander et al., 2003) but a subsequent study to ascertain cancer incidence in this cohort with four additional years of observation observed elevated but not statistically significant incidence ratios that were 1.7- to twofold higher among workers with higher cumulative exposure to PFOS (Alexander and Olsen, 2007). Some of the limitations of these studies include the lack of precision of the risk estimates due to the small number of cases, and the lack of control for the potential confounding of smoking. A nested case-control study in a general population Danish cohort did not observe elevated bladder cancer risk with increasing PFOS serum levels (Eriksen et al., 2009). Overall, there is plausible evidence of a relationship between PFOS exposure and bladder cancer, particularly for high-exposure communities.

One study in the general population reported a statistically significant increase in risk of RCC in the highest PFOS exposure quartile and in continuous analyses of PFOS exposure (i.e., per

doubling of PFOS concentration) (Shearer et al., 2021). Although the trend was significant across quartiles, the effect in the third quartile was null. Additionally, the association with PFOS was attenuated after adjusting for other PFAS, and it was lower in the third quartile than in the second quartile, indicating potential confounding by correlated PFAS exposures. There was no reported association when evaluated on a per doubling of PFOS after adjusting for other PFAS.

Elevated nonsignificant ORs for prostate cancer were reported for the occupationally exposed cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009). In the same occupational cohort studied by Alexander and Olsen (2007), Grice et al. (2007) observed that prostate cancers were among the most frequently reported cancers. When cumulative PFOS exposure measures were analyzed, elevated ORs were reported for prostate cancer, however, they did not reach statistical significance. Length of follow-up may not have been adequate to detect cancer incidence in this cohort as approximately one-third of the participants had worked <5 years in their jobs, and only 41.7% were employed ≥20 years (Grice et al., 2007). No association between PFOS exposure and prostate cancer was reported in either a second case-control study in Denmark (Hardell et al., 2014) or in a study of the association between PFOS serum concentrations and prostate-specific antigen (a biomarker of prostate cancer) from the C8 Health Project (Ducatman et al., 2015). In an NHANES population, Omoike et al. (2021) observed a significantly inverse association between PFOS exposure and prostate cancer.

The majority of studies examining associations between PFOS exposure and cancer outcomes were on breast cancer. One study of Inuit females in Greenland observed positive associations between PFOS levels and risk for breast cancer (Bonefeld-Jorgensen et al., 2011), although the association was of a low magnitude and could not be separated from the effects of other perfluorosulfonated compound exposures (i.e., PFHxS and PFOSA). Three studies indicated potential associations between PFOS exposure and increased breast cancer risk in specific subgroups or increased risk for specific breast cancer subtypes. Ghisari et al. (2017) reported that increased breast cancer risk was associated with increased PFOS serum concentrations in Danish individuals with a specific polymorphism in the CYP19 gene (for aromatase, associated with estrogen biosynthesis and metabolism). Mancini et al. (2020) reported that increased PFOS serum concentrations were associated specifically with increased risk of ER+ and PR+ tumors, whereas risk of ER– and PR– tumors did not follow a dose-dependent response. In a Taiwanese population, Tsai et al. (2020) observed a statistically significant increased risk of breast cancer in all women 50 years old or younger (including ER+ and ER– participants), and in ER+ participants aged 50 years or younger. Statistically significant increases in breast cancer risk were also observed in an NHANES population in the two highest quartiles of exposure, but the association was inverse in the second quartile (Omoike et al., 2021). No association was identified between PFOS and breast cancer in either case-control or nested case-control studies of Danish and California cancer registry populations, respectively (Hurley et al., 2018; Bonefeld-Jørgensen et al., 2014). Another general population study in the United States suggested that maternal PFOS exposure combined with high maternal cholesterol may decrease the daughters' risk of breast cancer but did not examine breast cancer subtypes or individuals with genetic variants that may have increased susceptibility (Cohn et al., 2020). A recent study in a Japanese population observed an inverse association across serum PFOS quartiles with a significant dose-response trend (Itoh et al., 2021). The association remained significantly inverse in both pre- and postmenopausal women in the highest tertile of exposure, with a significant dose-response trend.

However, in some of the studies PFOS levels were measured after or near the time of cancer diagnosis (Omoike et al., 2021; Tsai et al., 2020). Given the long half-life of PFOS in human blood, the exposure levels measured in these studies could represent exposures that occurred prior to cancer development. However, this is currently difficult to evaluate since data on the latency of PFOS exposure and subsequent cancer assessment is not available. Overall, study design limitations with specific studies, lack of replication of the results, and a lack of mechanistic understanding of specific breast cancer subtypes or susceptibilities of specific populations limit firm conclusions regarding PFOS and breast cancer. However, there is suggestive evidence that PFOS exposure may be associated with an increased breast cancer risk based on studies in susceptible populations, such as those with specific polymorphisms and for specific types of breast tumors.

3.5.4.1.2 Evidence From Animal Bioassays

One available chronic toxicity/carcinogenicity bioassay for PFOS, a 104-week dietary study in rats, provides evidence of multi-sex and multi-site tumorigenesis resulting from PFOS exposure (Butenhoff et al., 2012; Thomford, 2002b). This study was originally published as a 3M-sponsored report by Thomford (2002b) and some of the data were later published in a peer-reviewed study by Butenhoff et al. (2012). Statistically significant increases in the incidence of hepatocellular adenomas in the high-dose (20 ppm) male (7/43; 16%) and female (5/31; 16%) rat groups and combined adenomas/carcinomas in the females (6/32; 19%; five adenomas, one carcinoma) were observed. The observation of one carcinoma in the female rats is a relatively rare occurrence according to NTP's historical controls for female Sprague-Dawley rats (1/639 historical control incidence) (NTP, 2020a). Historical control incidence rates for these tumor types were not provided by Thomford (2002b). Additionally, there were statistically significant dose-related trends in the hepatic tumor responses of both males and females. A statistically significant trend of increased incidence of pancreatic islet cell carcinomas with increased PFOS dose was also observed in the male rats, though the individual dose groups were not statistically different from the control group. The percentages of animals with islet cell carcinomas in the highest dose group (12.5%) exceeds NTP's historical controls for male Sprague-Dawley rats by over an order of magnitude (12/638; 1.9%) (NTP, 2020a).

Thyroid tumors (follicular cell adenomas and carcinomas) were observed in males and females, though these responses were not statistically significant in any dose group, nor was there a linear dose-response trend (Butenhoff et al., 2012; Thomford, 2002b). In males, the incidence of thyroid tumors was significantly elevated only in the high-dose, recovery group males exposed for 52 weeks (10/39) but not in the animals receiving the same dose for 105 weeks. However, Thomford (2002b) indicated that the number of thyroid tumors observed in the recovery group males were outside the range of historical control values at that time, similar to what NTP (2020a) has reported for its laboratories (3/637 combined follicular cell adenoma or carcinoma). There were few follicular cell adenomas/carcinomas in the females (4 total, excluding the recovery group) with a nonlinear dose response. Mammary gland tumors, primarily combined fibroma adenoma and adenoma, were also observed in females, though there was a high background incidence of mammary gland tumors in the control animals, and the incidence lacked dose response for all tumor classifications.

3.5.4.2 Mode of Action Analysis

As PFOS has been associated with multi-site tumorigenesis in both epidemiological studies and animal toxicological studies, not always with site concordance, it is reasonable to assume that it may act through multiple carcinogenic MOAs. In the 2016 PFOS HESD (U.S. EPA, 2016b), EPA suggested that the induction of tumors may be related to nuclear receptor activation, mitochondrial effects, and gap junction intercellular communication. As described in the following subsections, the available mechanistic data continue to suggest that multiple MOAs could play a role in the tumorigenesis associated with PFOS exposure in animal models and human populations.

3.5.4.2.1 Mode of Action for Hepatic Tumors

The strongest evidence of the carcinogenicity of PFOS comes from a *high* confidence chronic rodent study identifying hepatocellular tumors in both male and female rats (Butenhoff et al., 2012; Thomford, 2002b). These findings in rats are supported by recent epidemiological studies that have reported associations between PFOS and hepatocellular carcinoma in humans (Cao et al., 2022; Goodrich et al., 2022).

The EPA previously concluded that, "the data are inadequate to support a PPARα-linked MOA for the liver and thyroid adenomas observed by Thomford (2002)/Butenhoff et al. (2012)" (U.S. EPA, 2016b). As described in the subsections below, the available mechanistic data continue to suggest that multiple MOAs may underlie the hepatocellular tumors observed after PFOS exposure. Specifically, the available studies provide varying levels of support for the role of several plausible MOAs: PPARα activation, CAR activation, HNF4α suppression, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression.

3.5.4.2.1.1 PPARα Activation

There is considerable debate over the relevance of PFAS-induced hepatic tumors to human health. Exposure to some PFAS have been shown to activate PPARα, which is characterized by downstream cellular or tissue alterations in peroxisome proliferation, cell cycle control (e.g., apoptosis and cell proliferation), and lipid metabolism (U.S. EPA, 2016b). Notably, human expression of PPARα mRNA and protein is only a fraction of what is expressed in rodent models, though there are functional variant forms of PPARα that are expressed in human liver to a greater extent than rodent models (Corton et al., 2018; Klaunig et al., 2003). Therefore, for PPARα activators that act solely or primarily through PPARα-dependent mechanisms (e.g., Wyeth-14,643, di-2-ethyl hexyl phthalate), the hepatic tumorigenesis observed in rodents may be expected to be reduced in frequency or severity or not observed in humans (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

The adverse outcome pathway (AOP) for the PPARα MOA for hepatic tumors has been characterized to include the following set of key events: 1) PPARα activation in hepatic cells; 2) alterations in cell growth signaling pathways (e.g., increases in Kupffer cell activation leading to increases in TNFα); 3) perturbations of hepatocyte growth and survival (i.e., increased cell proliferation and inhibition of apoptosis); and 4) selective clonal expansion of preneoplastic foci cells leading to 5) increases in hepatocellular adenomas and carcinomas (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003) (Table 3-23, Table 3-24). This AOP is associated with but not necessarily causally related to nonneoplastic effects including peroxisome proliferation,

hepatocellular hypertrophy, Kupffer cell-mediated events, and increased liver weight. There is also some overlap between signaling pathways and adverse outcomes, including tumorigenesis, associated with PPARα activation and the activation or degradation of other nuclear receptors, such as CAR, PXR, HNF4α, and PPARγ (Corton et al., 2018; Huck et al., 2018; Rosen et al., 2017; Beggs et al., 2016).

Table 3-23. Evidence of Key Events Associated With the PPARα Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**PPARα Activation[c]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.024	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.098	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.242	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.312	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.625	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.984	**↑ (4w) – (14w)**	**↑ (4w)**	**↑ (4w) – (14, 53w)**	**↓ (103w) – (14, 53w)**	NR	**↑ (103w)**
1	**↑ (F_1 PND 21)**	NR	NR	NR	NR	NR
1.25	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1.33/1.51	**– (4, 14w)**	NR	– (4w)	NR	NR	NR
1.66	**↑ (28d) – (1, 7d)**	NR	**↑ (7d) – (1, 28d)**	↑ (7d) – (1, 28d)	NR	NR
1.93	– (7d)	NR	**↑ (7d)**	**↓ (7d)**	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; d = day(s); w = week(s); F_1 = first generation of offspring; PND = postnatal day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); Elcombe et al. (2012b); Elcombe et al. (2012a); Seacat et al. (2003); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012). Dose for 1.33/1.51 mg/kg corresponds to 20 ppm in feed for animals exposed for 14 and 4 weeks, respectively, in Seacat et al., (2003). Dose for 1.66 mg/kg corresponds to 20 ppm in feed in Elcombe et al. (2012a). Dose for 1.93 mg/kg corresponds to 20 ppm in feed in Elcombe et al. (2012b).

[c] Indirect measurement of PPARα induction provided as *Cyp4a1*, *Cyp2b2*, or *ACoA* mRNA expression in Chang et al. (2009); as hepatic palmitoyl-CoA oxidase activity in Butenhoff et al. (2012)/Thomford (2002b), Seacat et al. (2003), Elcombe et al. (2012b), and Elcombe et al. (2012a); and as *Cyp4a1*, *Cyp2b1*, *Cyp2b2*, and *Acox1* gene expression or hepatic acyl-CoA oxidase activity in NTP (2019).

Table 3-24. Evidence of Key Events Associated With the PPARα Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**PPARα Activation[c]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.029	– (4, 14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.120	↓ (4w) – (14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.299	– (4, 14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.312	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.47	↓ (4w)	NR	– (4w)	NR	NR	NR
0.625	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1	**↑ (P_0 GD 1–20)**	NR	NR	NR	NR	NR
1.25	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1.251	– (4, 14w)	NR	– (4, 14, 53w)	**↓ (103w) – (14, 53w)**	NR	**↑ (103w)**
1.56/1.77	– (4, 14w)	NR	– (4w)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; w = week(s); P_0 = parental generation; GD = gestational day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); Seacat et al., (2003); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012). Dose for 0.47 corresponds to 5 ppm in feed in Seacat et al. (2003). Dose for 1.56/1.77 mg/kg corresponds to 20 ppm in feed for animals exposed for 14 and 4 weeks, respectively, in Seacat et al. (2003).

[c] Indirect measurement of PPARα induction provided as *Cyp4a1*, *Cyp2b2*, or *ACoA* mRNA expression in Chang et al. (2009), as hepatic palmitoyl-CoA oxidase activity at 4 and 14 weeks in Butenhoff et al. (2012)/Thomford (2002b), and as *Cyp4a1*, *Cyp2b1*, *Cyp2b2*, and *Acox1* gene expression in NTP (2019).

The published in vivo and in vitro literature suggests that PFOS is a relatively weak PPARα agonist compared with other known PPARα agonists such as PFOA (Behr et al., 2020b; Rosen et al., 2013; Wolf et al., 2012; Martin et al., 2007). While in vitro PPARα activation assay results indicate overall effective activation of PPARα by PFOS, the magnitude of that activation has been found to be relatively lower than chemicals that induce toxicity primarily through PPARα activation (e.g., di-2-ethyl hexyl phthalate). There is in vivo rodent assay evidence of PFOS-induced PPARα-associated transcriptional and enzymatic responses (e.g., upregulation of *Acox1* and acyl-CoA activity) as well. However, consistent with the in vitro activation assays, these in vivo responses were relatively weaker than PFOA and/or other PPARα activators and were often reported to be accompanied by transcriptional responses associated with other nuclear receptor signaling pathways (e.g., CAR and PPARγ), consistent with multiple modes of action (NTP, 2019; Dong et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a; Chang et al., 2009; Martin et al., 2007). For further details, see Section 3.4.1.3. Consistent with these findings,

studies of WT and PPARα-null mice reported that 808 differentially expressed genes responsive to a 7-day 10 mg/kg/day PFOS exposure were expressed in PPARα-null mouse livers while 906 genes were differentially expressed in WT mice, corroborating the likelihood of an active PPARα-independent MOA(s) (Rosen et al., 2010). Robust PPARα-independent effects in null mice were observed even at the lowest dose of PFOS (3 mg/kg/day; 630 differentially expressed genes in PPARα-null mice vs. 81 differentially expressed genes in WT mice) compared with responses in mice treated with 3 mg/kg/day Wyeth-14,643 (902 genes WT, 10 genes PPARα-null) or PFOA (879 genes WT, 176 genes PPARα-null) (Rosen et al., 2010), consistent with multiple MOAs for PFOS hepatic effects.

There is evidence from in vivo animal bioassays and in vitro studies of Kupffer cell activation, an indicator of alterations in cell growth, in response to PFOS treatment. Though this mechanism is itself PPARα-independent, factors secreted upon Kupffer cell activation may be required for increased cell proliferation by PPARα activators (Corton et al., 2018). Two short-term exposure in vivo rodent studies reported increased serum TNFα levels after 3–4 weeks of PFOS administration (Su et al., 2019; Han et al., 2018b); TNFα is a pro-inflammatory cytokine that can be released upon activation of Kupffer cells (Corton et al., 2018). In addition to serum TNFα levels, Han et al. (2018b) reported increased TNFα mRNA in hepatic tissues of PFOS-exposed rats. The authors also extracted primary Kupffer cells from untreated rats and cultured them with PFOS in vitro for 48 hours and reported increased supernatant TNFα levels and cellular TNFα mRNA levels. These results indicate that rodent hepatic tissues may be primed for perturbations of PPARα-dependent cell growth upon PFOS exposure. However, further study is needed to understand the potential role of other mediators of Kupffer cell activation since unlike PPARα, PPARγ is expressed in Kupffer cells and can also be activated by PFOS.

While there is some evidence of alterations in cell growth signaling pathways due to PFOS exposure, there is conflicting evidence related to the ability of PFOS to induce hepatic cell proliferation and inhibit apoptosis. The available rodent in vivo study results indicate that increases in proliferation may be dose- and exposure duration-dependent whereas changes in apoptosis may be species- or dose-dependent. In the only available chronic rodent bioassay for PFOS (Butenhoff et al., 2012; Thomford, 2002b), significant increases in the number of hepatic tumors were observed at the highest dose levels in each sex (20 ppm in diet or approximately 1 mg/kg/day) without corresponding increases in the incidence or severity of cell proliferation at 52 weeks in the livers of male or female rats. Additionally, there were transient effects on hepatic peroxisomal proliferation in males or females at weeks 4 and 14 as indicated by the palmitoyl-CoA assay (Seacat et al., 2003; Thomford, 2002b). In contrast, there is evidence of hepatic cell and/or peroxisome proliferation from short-term studies that administered higher PFOS dose levels than the Thomford report (2002b) (i.e., 2–10 mg/kg/day) (NTP, 2019; Han et al., 2018b; Elcombe et al., 2012b; Elcombe et al., 2012a). Results were not always consistent across time points or sexes and were accompanied by evidence of increased activation of other nuclear receptors (i.e., CAR and PXR), which could also influence cell proliferation. The characteristics of typical PPARα-induced cell proliferation includes an early burst that recovers to a level that is slightly higher than background, the latter of which is difficult to detect for compounds that are weak PPARα activators (Corton et al., 2018). This likely explains, at least in part, the inconsistencies in cell proliferation patterns across timepoints and lends support to the evidence of relatively weak PPARα activation by PFOS. Additionally, Elcombe et al. (2012a) reported substantially greater palmitoyl-CoA oxidation after 50 ppm Wyeth-14,643 administration in

male Sprague-Dawley rats compared with 20 or 100 ppm (approximately 1.7 and 7.9 mg/kg/day, respectively) PFOS administration for up to 28 days, lending further support for PFOS as a relatively weak PPARα activator.

In addition to the observation of increased hepatic cell proliferation on day 1 of recovery in male rats administered 20 or 100 ppm PFOS (approximately 1.93 and 9.65 mg/kg/day, respectively) for 7 days, Elcombe et al. (2012b) also reported decreased hepatic apoptotic indices (i.e., the percent of apoptotic nuclei out of the total number cell nuclei in a unit of area) in both dose groups, which is an indication of PPARα-dependent hepatotoxicity. However, these results were inconsistent with the results of the second Elcombe et al. (2012a) study, which reported an increased apoptotic index after 7 days of 20 ppm dietary PFOS administration. The authors observed no other statistically significant changes in the apoptotic indices of rats from the 20 ppm group in the two additional timepoints tested (1 day and 28 days), though they did report decreases in the apoptotic indices of rats in the 100 ppm group at all three time points, similar to the results of Elcombe et al. (2012b; 2012a). The underlying reason for the inconsistent apoptosis findings in the 20 ppm dose groups between the two studies is unclear. Increased hepatic apoptosis was observed in mice administered 2.5–10 mg/kg/day PFOS for 30 days (Xing et al., 2016), and short-term PFOS studies in both rats and mice reported increases in apoptosis-related hepatic gene expression and/or protein activity/expression (Han et al., 2018a; Lv et al., 2018; Eke et al., 2017; Wan et al., 2016). Further descriptions of these in vivo studies, as well as in vitro studies examining hepatic cell proliferation and apoptosis can be found in Section 3.4.1.3.

There are several studies of the hepatic effects resulting from PFOS exposure observed in PPARα-null mice with either short-term or gestational exposure durations but therefore, lack an ability to assess tumor incidence or chronic histopathological effects. The studies of Qazi et al. (2009b), Abbott et al. (2009), and Rosen et al. (2010) all observed increased absolute and/or relative liver weight in PPARα-null adults orally administered PFOS or pups exposed to PFOS in utero. Along with the PPARα-independent cell signaling effects in PPARα-null mice reported by Rosen et al. (2017; 2010), these studies corroborate that the hepatomegaly observed in WT rodents administered PFOS is not entirely PPARα-dependent. Several other signaling pathways may contribute to the observed hepatomegaly due to PFOS exposure, though the relationship of these liver effects with tumor formation is unclear. Further descriptions of studies utilizing PPARα-null mice can be found in Section 3.4.1.3.

In general, PPARα activators are not necessarily expected to induce cell proliferation or suppress apoptosis of hepatocytes in humans (Corton et al., 2018). Specifically, some have argued that the MOA for liver tumor induction by PPARα activators in rodents has limited-to-no relevance to humans, due to differences in cellular expression patterns of PPARα and related proteins (e.g., cofactors and chromatin remodelers), as well as differences in binding site affinity and availability (Corton et al., 2018; Klaunig et al., 2003). Nonetheless, several studies have reported increased cell proliferation or markers of cell proliferation in vitro in human liver cell lines exposed to PFOS (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a) (see Section 3.4.1.3). For example, Cui et al. (2015a) found increased proliferation using the MTT assay in the non-tumor fetal human liver cell line HL-7702. These increases in cell proliferation were accompanied by corresponding proteomic changes indicative of increased proliferation. Using flow cytometry, Cui et al. (2015a) also found that increased percentages of cells were in cell phases associated with DNA synthesis and/or interphase growth and mitosis (S and G2/M

phases), depending on the length of exposure and dose of PFOS. Corroborative transcriptional results were observed in two additional human cell lines (HepG2 and HepaRG) (Louisse et al., 2020; Song et al., 2016). There was no mention of changes in apoptosis accompanying increased cell proliferation in two of the studies of human hepatocytes (Louisse et al., 2020; Cui et al., 2015a), while Song et al. (2016) reported that genes related to "regulation of apoptosis" were significantly altered, although the direction of the change is not specified. Beggs et al. (2016) reported that a human primary cell line exposed to PFOS predominantly showed changes in the expression of genes involved in carcinogenesis and cell death signaling, among other biological pathways/functions related to hepatotoxicity and hepatic diseases. The authors linked these transcriptional changes to the loss of HNF4α functionality which is known to promote the development of hepatocellular carcinoma, providing evidence of a PPARα-independent mechanism of hepatotoxicity and carcinogenicity. In addition to HNF4α-mediated hepatocarcinogenicity, Benninghoff et al. (2012) proposed that promotion of hepatocarcinogenesis by PFOS in an initiation-promotion model in rainbow trout, which are similarly insensitive to PPARα as humans, is potentially the result of activation of the trout liver estrogen receptor. Specifically, dietary PFOS treatment promoted hepatocarcinogenesis (i.e., increased the incidence of hepatocellular carcinomas and adenomas) and increased tumor promotion and cell proliferation in rainbow trout exposed to aflatoxin B_1 as a cancer initiator (Benninghoff et al., 2012).

3.5.4.2.1.2 Other Nuclear Receptors

In addition to PPARα, there is some evidence that other nuclear receptors may play a role in the MOA for hepatic tumors resulting from PFOS exposure. For example, CAR, which has an established adverse outcome pathway of key events similar to PPARα, has been implicated in hepatic tumorigenesis in rodents. The key events of CAR-mediated hepatic tumors are: 1) activation of CAR; 2) altered gene expression specific to CAR activation; 3) increased cell proliferation; 4) clonal expansion leading to altered hepatic foci; and 5) liver tumors (Felter et al., 2018) (Table 3-25, Table 3-26). Associative events include hypertrophy, induction of CAR-specific CYP enzymes (e.g., CYP2B) and inhibition of apoptosis. As described in Section 3.4.1.3, there is both in vivo and in vitro evidence that PFOS can activate CAR and initiate altered gene expression and associative events (NTP, 2019; Rosen et al., 2017; Dong et al., 2016; Rosen et al., 2013; Elcombe et al., 2012b; Elcombe et al., 2012a; Rosen et al., 2010; Chang et al., 2009; Martin et al., 2007). Some studies, such as NTP (2019), report greater activation of CAR with PFOS treatment compared with PPARα, depending on the sex and/or model of interest. As with PPARα-mediated tumorigenesis, there are claims that CAR-mediated tumorigenesis is not relevant to humans because CAR activators such as phenobarbital have been shown to induce cell proliferation and subsequent tumorigenesis in rodents but do not induce cell proliferation in human cell lines (Elcombe et al., 2014). However, as outlined above, several studies have reported increased cell proliferation or markers of cell proliferation due to PFOS treatment in human cell lines (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a). Further study is needed to understand the mechanistic underpinnings of PFOS-induced hepatic cell proliferation and whether it is related to CAR activation.

Table 3-25. Evidence of Key Events Associated With the CAR Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.024	NR	NR	– (4, 14w)	NR	– (103w)
0.098	NR	NR	– (4, 14w)	NR	– (103w)
0.242	NR	NR	– (4, 14w)	NR	– (103w)
0.312	NR	**↑ (4 w)**	NR	NR	NR
0.625	NR	**↑ (4 w)**	NR	NR	NR
0.984	NR	NR	**↑ (4w) – (14, 53w)**	NR	**↑ (103w)**
1	NR	**↑ (F_1 PND 21)**	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; w = week(s); GD = gestational day; F_1 = first generation of offspring; PND = postnatal day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Felter et al. (2018).

[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

Table 3-26. Evidence of Key Events Associated With the CAR Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.029	NR	NR	– (4, 14w)	NR	– (103w)
0.120	NR	NR	– (4, 14w)	NR	– (103w)
0.299	NR	NR	– (4, 14w)	NR	– (103w)
0.312	NR	**↑ (4w)**	NR	NR	NR
0.625	NR	**↑ (4w)**	NR	NR	NR
1.251	NR	**↑ (P_0 GD 1–20)**	– (4, 14, 53w)	NR	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; w = week(s); P_0 = parental generation; GD = gestational day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Felter et al. (2018)

[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

HNF4α is known as a master regulator of hepatic differentiation and plays a role in tumor suppression as well as general liver maintenance and function (Beggs et al., 2016). Interestingly, PFOS exposure appears to downregulate HNF4α and its target genes. Studies utilizing primary human hepatocytes, HepG2 cells, and in vivo mouse models have reported decreased HNF4α protein expression as well as corresponding changes in downstream HNF4α target genes with PFOS treatment (Behr et al., 2020a; Beggs et al., 2016). Beggs et al. (2016) reported that PFOS induced changes in genes involved in carcinogenesis and cell death signaling and linked the loss of HNF4α functionality to potential hepatocellular tumor promotion. The authors also suggested that loss of HNF4α functionality may play a role in noncancer hepatic effects including hepatomegaly, steatosis, altered lipid metabolism, and fatty liver disease. Beggs et al. (2016) exposed human primary hepatocytes to 0.01–10 μM PFOS and determined after 48 and 96 hours of 10 μM PFOS, HNF4α protein expression was significantly decreased. Beggs et al. (2016) also observed a decrease in HNF4α protein in the livers of 10-week-old CD-1 mice exposed to 10 mg/kg/day PFOS once daily by oral gavage for 7 days. A study in HepaRG cells exposed to 1–100 μM PFOS for 24 or 48 hours corroborated these findings, as downregulations in both HNF4α and its target gene CYP7A1 were observed (Behr et al., 2020a).

There is additional evidence from in vivo and in vitro studies that PFOS has the ability to activate and modulate the targets of other nuclear receptors. As described in Section 3.4.1.3, PFOS has been reported to modulate the activity of PPARs other than PPARα (i.e., PPARβ/δ and PPARγ), as well as PXR, LXR, RXR, RAR, and Erβ, though the evidence of activation is sometimes conflicting across different cell lines, assays, and species. Several of these nuclear receptors, such as PPARγ, are known to play a role in liver homeostasis and disease and may be driving factors in the hepatotoxicity observed after PFOS exposure, though their role in tumorigenesis is less clear. As described in Section 3.5.3, there is also evidence that PFOS modulates endogenous ligands for nuclear receptors, most notably thyroid and reproductive hormones. However, it is also unclear what role, if any, these receptors and ligands may be playing in PFOS-induced hepatic tumorigenesis.

3.5.4.2.1.3 Cytotoxicity

There is suggestive evidence that PFOS may act through a cytotoxic MOA. Felter et al. (2018) identified the following key events for establishing a cytotoxicity MOA: 1) the chemical is not DNA reactive; 2) clear evidence of cytotoxicity by histopathology such as the presence of necrosis and/or increased apoptosis; 3) evidence of toxicity by increased serum enzymes indicative of cellular damage that are relevant to humans; 4) presence of increased cell proliferation as evidenced by increased labeling index and/or increased number of hepatocytes; 5) demonstration of a corresponding dose response for cytotoxicity and formation of tumors; and 6) reversibility upon cessation of exposure (Table 3-27,Table 3-28). As discussed above in the genotoxicity section (Section 3.5.4.2.1.4), there is no experimental support that PFOS can induce DNA damage and/or micronuclei formation in liver tissue, which supports the first key event in the cytotoxicity MOA. Quantitative liver histopathology is limited to three studies, however the one available chronic study (Butenhoff et al., 2012) reported significant trends in increased individual hepatocyte necrosis in male and female Sprague-Dawley rats which was also statistically significant in the highest dose groups. Liver histopathology in humans is also

limited, however, Jin et al. (2020) reported higher odds (not necessarily statistically significant) of non-alcoholic steatohepatitis ($p < 0.05$), ballooning, fibrosis, and portal inflammation.

Table 3-27. Evidence of Key Events Associated With the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**Cytotoxicity**	**Serum Enzymes**	**Regenerative Proliferation**	**Hyperplasia and/or Preneoplastic Lesions**	**Hepatic Tumors**
0.024	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.098	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.242	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.312	– (4w)	– (4w)	NR	– (4w)	NR
0.625	– (4w)	**↑ (4w)**	NR	– (4w)	NR
0.984	**↑ (103w) – (4, 14, 53w)**	**↑ (4, 14, 53w) – (27w)**	**↑ (4w) – (14, 53w)**	**↑ (103w) – (14, 53w)**	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; w = week(s); NR = not reported.
Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2019) and Butenhoff et al. (2012)/Thomford (2002b).
[a] Reviewed in Felter et al. (2018).
[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

Table 3-28. Evidence of Key Events Associated With the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**Cytotoxicity**	**Serum Enzymes**	**Regenerative Proliferation**	**Hyperplasia and/or Preneoplastic Lesions**	**Hepatic Tumors**
0.029	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.120	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.299	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.312	– (4w)	– (4w)	NR	– (4w)	NR
0.625	– (4w)	– (4w)	NR	– (4w)	NR
1.251	**↑ (103w) – (4, 14, 53w)**	– (4, 14, 27, 53w)	– (4, 14, 53w)	**↑ (103w) – (14, 53w)**	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; w = week(s); NR = not reported.
Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2019) and Butenhoff et al. (2012)/Thomford (2002b).
[a] Reviewed in Felter et al. (2018).
[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

There is evidence in both humans and animals that exposure to PFOS increases serum liver enzymes. Specifically, statistically significant positive associations between ALT and PFOS (i.e., increased ALT as a continuous measure with higher PFOS exposure levels) were observed in several studies (Jain, 2019; Nian et al., 2019; Salihovic et al., 2018; Gallo et al., 2012; Costa et al., 2009; Olsen et al., 2003). These individual findings are supported by a meta-analysis of epidemiological studies reporting biomarkers of liver injury reporting a statistically significant ($p < 0.001$) weighted z-score suggesting a positive association between PFOS and increased ALT in adults and children (Costello et al., 2022). Statistically significant increases in serum enzymes (i.e., ALT, AST, ALP, and GGT) were also observed in several animal toxicological studies, though these increases were generally less than twofold (100% change relative to control) compared with control (NTP, 2019; Han et al., 2018b; Xing et al., 2016; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). However, these changes in serum enzyme levels were accompanied by histopathological evidence of damage, as outlined above, and coherence is observed in humans.

As highlighted in the PPARα activation section, several studies have reported increased cell proliferation or markers of cell proliferation in human cell lines (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a), though there is limited quantitative histopathological data to determine the ability of PFOS to induce hepatic hyperplasia. Finally, the available data indicate a corresponding dose response for cytotoxicity and the formation of liver tumors as evidence in Table 3-29 and Table 3-30, though dose spacing (i.e., the gap in dosing between the mid-high and high doses administered) may limit the precision of a dose-response curve.

Table 3-29. Incidences of Liver Tumor and Nonneoplastic Lesions in Male Sprague-Dawley Rats at 103 Weeks, as Reported by Thomford (2002b)

	0 mg/kg/day	0.024 mg/kg/day	0.098 mg/kg/day	0.242 mg/kg/day	0.984 mg/kg/day
Hepatocellular Adenomas	0/41**	3/42	3/47	1/44	7/43**
Necrosis, Individual Hepatocyte	3/50	2/50	6/50	4/50	10/50
Altered Hepatocellular, Clear/Eosinophilic Cell	13/50	21/50	23/50	24/50	24/50
Cystic Degeneration	5/50	15/50	19/50	17/50	22/50
Hyperplasia, Bile Duct	19/50	20/50	25/50	24/50	25/50

Notes: Statistical significance for an exposed group indicates a significant pairwise test compared with the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

Table 3-30. Incidences of Liver Tumor and Nonneoplastic Lesions in Female Sprague-Dawley Rats at 103 Weeks, as Reported by Thomford (2002b)

	0 mg/kg/day	0.029 mg/kg/day	0.120 mg/kg/day	0.299 mg/kg/day	1.251 mg/kg/day
Combined Hepatocellular Adenomas & Carcinomas	0/28**	1/29	1/16	1/31	6/32*
Necrosis, Individual Hepatocyte	3/50	4/50	4/50	5/50	9/50
Infiltrate, Macrophage, Pigmented	2/50	3/50	5/50	6/50	20/50
Infiltrate, Lymphohistiocytic	33/50	37/50	33/50	36/50	42/50
Hyperplasia, Bile Duct	21/50	25/50	19/50	17/50	27/50

Notes: Statistical significance for an exposed group indicates a significant pairwise test compared with the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

3.5.4.2.1.4 Genotoxicity

Several relatively recent studies, primarily published by the same laboratory, have shown the potential for PFOS to act as a genotoxicant (see Section 3.5.3); previously, EPA had not identified evidence supporting genotoxicity as a potential MOA for PFOS (U.S. EPA, 2016b). Two in vivo studies, the first a 30-day study in male Swiss Albino rats and the second a 28-day study in male *gpt* delta transgenic mice, provided evidence of DNA damage and/or micronuclei formation in liver tissue of animals administered up to 2.5 or 10 mg/kg/day PFOS, respectively (Eke et al., 2017; Wang et al., 2015b). However, there are concerns about the interpretation of these studies regarding the genotoxicity and mutagenicity of PFOS because results reported as not statistically significant, concerns about the study design, or unclear relationship of the observed effects to genotoxicity of PFOS versus secondary effects from hepatoxicity (e.g., oxidative stress).

Several other 28–30-day studies in male and female rats and mice also observed DNA damage and/or micronuclei formation in bone marrow or peripheral blood cells (NTP, 2019; Eke and Çelik, 2016; Çelik et al., 2013), though there are similar concerns about whether these responses are attributable to direct genotoxicity of PFOS. For example, NTP (2019) reported increased numbers of micronucleated polychromatic erythrocytes in the blood of female rats administered 5 mg/kg/day PFOS (highest dose group) for 28 days, but also reported concomitant decreases in the percentage of polychromatic erythrocytes in the peripheral blood, indicative of bone marrow toxicity. This potential bone marrow toxicity may be driving micronuclei formation rather than the direct mutagenicity of PFOS. NTP (2019) also noted that the observed responses of the high-dose females were within historical control ranges and considered these results to be equivocal. From this very limited database, it does not appear that genotoxicity in male and female Sprague-Dawley rats occurs at doses at or below those that result in tumorigenesis.

In addition to rodent studies, Du et al. (2014) reported increased DNA strand breaks and micronuclei formation in peripheral blood cells of male and female zebrafish exposed to PFOS

for 30 days and several other studies reported increased DNA damage in vitro (Wang et al., 2015b; Wielsøe et al., 2015; Lu et al., 2012). However, the majority of in vitro studies (described in Section 3.5.3) report negative results for genotoxic endpoints including chromosomal aberrations, unscheduled DNA synthesis, mutagenicity, and various types of DNA damage.

The available in vivo evidence suggests that exposure to PFOS at levels resulting in cytotoxicity (e.g., hepatotoxicity, bone marrow toxicity) can lead to secondary genotoxicity in target tissues. At this time, there are no generally accepted mechanistic explanations for PFOS directly interacting with genetic material. Additionally, while there is some in vivo evidence of PFOS-induced mutagenicity as primarily evidenced by micronuclei formation in rats, mice, and zebrafish, there are several uncertainties that limit the interpretation of these results. There is currently no robust evidence to support a mutagenic MOA for PFOS, though overall, genotoxicity cannot be ruled out as a potential MOA or key event in PFOS tumor formation.

3.5.4.2.1.5 Consideration of Other Plausible MOAs

In addition to the evidence supporting modulation of receptor-mediated effects, and potential genotoxicity, PFOS also exhibits several other key characteristics (KCs) of carcinogens (see Section 3.5.3), some of which are directly evident in hepatic tissues.

For example, PFOS appears to induce oxidative stress, another KC of carcinogens, particularly in hepatic tissues (see Section 3.4.1.3). Several studies in rats and mice showed evidence of increased oxidative stress and reduced capacity for defense against oxidants and oxidative damage in hepatic tissues. Two studies, one 28-day study in rats and one 30-day study in mice, reported reduced Nrf2 protein levels or expression in hepatic tissues after PFOS exposure (Lv et al., 2018; Wan et al., 2016). Nrf2 is an important regulator of antioxidant response elements and is generally activated in response to pro-oxidant exposure and oxidative stress. Accordingly, these studies and others noted a reduction in the hepatic expression of genes that are implicated in antioxidant, anti-inflammatory, and/or stress response functions (e.g., *hmox1, nqo1*) as well as reduced antioxidant enzyme levels and activities (e.g., CAT, SOD) (Han et al., 2018a; Lv et al., 2018; Wan et al., 2016; Xing et al., 2016; Liu et al., 2009). Several in vivo exposure studies also noted increases in hepatic ROS and markers of oxidative damage (e.g., MDA) (Han et al., 2018a; Lv et al., 2018; Wan et al., 2016; Xing et al., 2016; Liu et al., 2009). Notably, Han et al. (2018a) reported several indicators of oxidative stress in male Sprague-Dawley rats gavaged for 28 days with 1 mg/kg/day PFOS (lowest dose tested in the study), a comparable dose to that which caused tumorigenesis in the chronic study in male rats. Taken together, these results provide some support for disruption of the oxidative stress response in hepatic tissues leading to accumulation of ROS and subsequent oxidative damage.

Immunosuppression is the reduction of an individual's immune system to respond to foreign cells or antigens, including tumor cells (Smith et al., 2020). The immune system plays an important role in the identification and eventual destruction of cancer cells; immunosuppression may allow for the evasion of this process by cancer cells and subsequently lead to tumorigenesis. As discussed in Section 3.4.2.1.1, PFOS serum levels are associated with markers of immunosuppression, particularly in children. Several studies reported inverse associations between PFOS serum concentrations and antibody production following vaccinations in children (Zhang et al., 2023; Timmermann et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Stein et al., 2016b; Mogensen et al., 2015a; Granum et al.,

2013; Grandjean et al., 2012). Additionally, one *medium* confidence study reported higher odds of total infectious diseases with increasing PFOS serum concentrations (Goudarzi et al., 2017), though it should be noted that studies reporting odds ratios for specific infectious diseases had mixed results. Animal toxicological studies also report markers of immunosuppression, including reductions in natural killer cell activity. As described in Section 3.4.2.2, there are several reports of decreased natural killer cell activity in male and female, adult and F_1 generation mice from short-term, subchronic, and gestational studies (Zhong et al., 2016; Dong et al., 2009; Zheng et al., 2009; Keil et al., 2008; Peden-Adams et al., 2008). While one short-term study in male mice reported increases in splenic T-helper (CD3 + $CD4^+$) and T-cytotoxic (CD3 + $CD8^+$) lymphocytes (Lv et al., 2015), two gestational studies reported reductions in thymic $CD4^+$ cells in male offspring (Zhong et al., 2016; Keil et al., 2008). There is also limited evidence of immunosuppression in the form of reduced white blood cell counts (primarily lymphocytes) from two short-term rodent studies in male mice and rats, respectively (NTP, 2019; Qazi et al., 2009a). This short-term report is the only available study in Sprague-Dawley rats and does not indicate that immunosuppressive effects are occurring at or below doses that result in tumorigenesis (NTP, 2019). However, it is difficult to discount immunosuppression as a potential MOA for PFOS, given the limited database for rats and stronger databases indicating immunosuppression in mice and humans.

3.5.4.2.2 Mode of Action for Pancreatic Tumors

Additional evidence of the carcinogenicity of PFOS comes from a *high* confidence chronic rodent study identifying pancreatic islet cell carcinomas in male rats (Thomford, 2002b). From a review of the literature, no established MOA was identified for pancreatic islet cell carcinogenicity in animals. Considerable uncertainty remains in the underlying mechanisms of PFOS-induced pancreatic islet tumors.

A recent review of the molecular mechanisms of pancreatic islet cell (i.e., neuroendocrine) tumors indicates pancreatic neuroendocrine tumors primarily originate from aberrant cell proliferation in the endocrine pancreas (Maharjan et al., 2021). However, these tumors can also develop from pluripotent cells of the exocrine pancreas (Maharjan et al., 2021). The human islet is similar to the rodent islet, with similarities in β-cell numbers, islet cell patterns, and blood vessel-islet structure and interactions (Bonner-Weir et al., 2015). Some evidence suggests a role for PPARα and PPARγ in rat and human pancreatic islet cell function (Eibl et al., 2001; Sugden et al., 2001; Dubois et al., 2000; Roduit et al., 2000), though PPARα activation has been argued to be related to pancreatic acinar cell tumors rather than to islet cell tumors (Klaunig et al., 2003). Other studies have shown that PFOS exposure can reduce pancreatic islet cell size and viability and can induce ROS (Qin et al., 2022).

Although an established MOA is currently unknown for this tumor type, the observation of pancreatic islet cell tumors in rodents provides additional evidence for the carcinogenic potential of PFOS.

3.5.4.2.3 Conclusions

Based on the weight of evidence evaluation of the available literature, PFOS has the potential to induce hepatic tumors in humans and rodents via multiple MOAs, most notably via the modulation of nuclear receptors (i.e., PPARα and CAR) and cytotoxicity. There is also limited evidence supporting additional potential MOAs of genotoxicity, immunosuppression, and

oxidative stress. The conclusions from the weight of evidence analysis of the available data for PFOS are consistent with literature reviews recently published by two state health agencies which concluded that the hepatotoxic effects of PFOS are not entirely dependent on PPARα activation (CalEPA, 2021; NJDWQI, 2018). No established MOA was identified for pancreatic islet cell carcinogenicity in rats.

As described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "[i]n the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data; animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity." For the available data regarding the MOA of PFOS-induced hepatic and pancreatic carcinogenesis, there is an absence of definitive information supporting a single, scientifically justified MOA; in fact, there is evidence supporting the potential for multiple plausible MOAs. Therefore, EPA concludes that the hepatic and pancreatic tumors observed by Thomford (2002, 5029075) and Butenhoff et al. (2012, 1276144) can be relevant to human health and support the positive, albeit, limited, tumor findings, particularly findings of increased risk of hepatocellular carcinoma, from epidemiological studies.

Several health agencies have reviewed the available mechanistic literature and have come to similar conclusions regarding the multiple potential MOAs for PFOS-induced tumorigenesis. For example, CalEPA's Office of Environmental Health Hazard Assessment recently concluded that PFOS "possess[es] several of the key characteristics of carcinogens, including the ability to induce oxidative stress, inflammation, and modulate receptor-mediated effects. Additionally, there is suggestive evidence that… PFOS [is] genotoxic, thus a genotoxic MOA for cancer remains plausible" (CalEPA, 2021). Zahm et al. (2023, 3982387) also concluded that there is moderate evidence for many potential mechanisms for PFOS-induced toxicity and specifically noted that PFOS can induce epigenetic alterations, immunosuppression, and oxidative stress and cause endocrine- and receptor-mediated effects.

3.5.5 Cancer Classification

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EPA reviewed the weight of the evidence and determined that PFOS is *Likely to Be Carcinogenic to Humans*, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor *Carcinogenic to Humans*." The *Guidelines* provide descriptions of data that may support the *Likely to Be Carcinogenic to Humans* descriptor; the available PFOS data are consistent with the following factors:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be

associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case" (U.S. EPA, 2005a).

The available evidence indicates that PFOS has carcinogenic potential in one animal model for multiple sites and both sexes, as well as supporting evidence from human studies, consistent with the examples described in the *Guidelines for Carcinogen Risk Assessment* for the *Likely* descriptor. The epidemiological evidence of associations between PFOS and cancer found mixed results across tumor types. However, the available study findings support a plausible correlation between PFOS exposure and carcinogenicity in humans. The single chronic cancer bioassay performed in rats is positive for multi-site and -sex tumorigenesis (Butenhoff et al., 2012; Thomford, 2002b). In this study, statistically significant increases in the incidences of hepatocellular adenomas or combined adenomas and carcinomas were observed in male and female rats, respectively. There was also a statistically significant trend of this response in both sexes indicating a relationship between the magnitude/direction of response and PFOS dose. As described in Section 3.5.4.2, the available mechanistic evidence is consistent with multiple potential MOAs for this tumor type; therefore, the hepatocellular tumors observed by Thomford (2002b)/Butenhoff et al. (2012) may be relevant to humans. These findings in rats and their potential human relevance are supported by recent epidemiological studies that have reported associations between PFOS and hepatocellular carcinoma in humans (Cao et al., 2022; Goodrich et al., 2022).

In addition to hepatocellular tumors, Thomford (2002b) reported increased incidences of pancreatic islet cell carcinomas with a statistically significant dose-dependent positive trend, as well as modest increases in the incidence of thyroid follicular cell tumors. The findings of multiple tumor types provide additional support for potential multi-site tumorigenesis resulting from PFOS exposure. Importantly, site concordance is not always assumed between humans and animal models; agents observed to produce tumors may do so at the same or different sites in humans and animals (U.S. EPA, 2005a). While site concordance was present between human studies of liver cancer and animal studies reporting increased incidence of hepatocellular tumors, evidence of carcinogenicity of PFOS from other cancer sites where concordance between humans and animals is not present is still relevant to the carcinogenicity determination for PFOS. See Table 3-31 below for specific details on how PFOS aligns with the examples supporting the *Likely to Be Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 3-31. Comparison of the PFOS Carcinogenicity Database With the *Likely* Cancer Descriptor as Outlined in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

***Likely to Be Carcinogenic* to Humans**	
"An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments." (U.S. EPA, 2005a)	**PFOS data are consistent with this description**. Epidemiological evidence supports a plausible association between PFOS exposure and liver cancer which is consistent with evidence of liver cancer in animals. Epidemiological studies evaluating the association between human exposure to PFOS and other cancers are mixed. Supporting carcinogenicity data are available from animal experiments.
"An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure	**PFOS data are consistent with this description**. PFOS has tested positive in animal experiments in more than

Likely to Be Carcinogenic to Humans	
route, with or without evidence of carcinogenicity in humans." (U.S. EPA, 2005a)	one sex and site. Hepatic tumors were observed in male and female rats (statistically significant at high dose and statistically significant trend tests for each) and islet cell carcinomas show a statistically significant positive trend in male rats.
"A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset." (U.S. EPA, 2005a)	**This description is not applicable to PFOS.**
"A rare animal tumor response in a single experiment that is assumed to be relevant to humans." (U.S. EPA, 2005a)	**PFOS data are consistent with this description**. The hepatocellular carcinoma observed in the high-dose female rats is a rare tumor type in this strain (NTP, 2020b).
"A positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case." (U.S. EPA, 2005a)	**PFOS data are consistent with this description.** The positive multi-site, multi-sex chronic cancer bioassay is supported by mechanistic data indicating that PFOS is associated with events generally known to be associated with tumor formation such as inducing nuclear receptor activation, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression.

Notes: MOA = mode of action.

EPA recognizes that other state and international health agencies have recently classified PFOS as either "possibly carcinogenic to humans" (IARC as reported in Zahm et al. (2023)) or carcinogenic to humans (CalEPA, 2021). As the SAB PFAS Review Panel (U.S. EPA, 2022e) noted, "the criteria used by California EPA, for determination that a chemical is a carcinogen, are not identical to the criteria in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*" and, similarly, IARC's classification criteria are not identical to EPA's guidelines (IARC, 2019). Rationale for why PFOS exceeds the *Suggestive Evidence of Carcinogenic Potential* descriptor and does not meet the *Carcinogenic to Humans* descriptor according to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) is detailed in Section 5.4.

4 Dose-Response Assessment

Considerations in Selecting Studies and Endpoints for Dose-Response Analysis

There is evidence from both human epidemiological and animal toxicological studies that oral perfluorooctane sulfonic acid (PFOS) exposure can result in adverse health effects across a range of health outcomes. In response to recommendations made by the EPA's Science Advisory Board (SAB) and the conclusions presented in the U.S. Environmental Protection Agency's (EPA's) preliminary analysis, the 2021 SAB review draft *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b), EPA focused its final toxicity value derivation efforts herein "on those health outcomes that have been concluded to have the strongest evidence" (U.S. EPA, 2022e). Therefore, EPA prioritized health outcomes and endpoints with the strongest overall weight of evidence which were the health outcomes with evidence ***demonstrates*** or evidence ***indicates*** integration judgments based on human, animal, and mechanistic evidence (Sections 3.4 and 3.5) for points of departure (POD) derivation using the systematic review methods described in Section 2 and Appendix A (U.S. EPA, 2024a). For PFOS, the health outcomes with the strongest weight of evidence are cancer (described in Section 4.2) and the noncancer health outcomes of immunological, developmental, cardiovascular (serum lipids), and hepatic effects (described in Section 4.1). For all other health outcomes (e.g., reproductive, endocrine, nervous, hematological, musculoskeletal), the evidence integration summary judgment for the human epidemiological and animal toxicological evidence was ***suggestive*** or ***inadequate*** and these outcomes were not assessed quantitatively. For transparency, health outcomes for which the results were *suggestive* are discussed in the evidence profile tables provided in Appendix C (U.S. EPA, 2024a).

In the previous sections describing the hazard judgment decisions (Sections 3.4 and 3.5), EPA qualitatively considered high, medium, and sometimes *low* confidence studies of PFOS exposure to characterize the weight of evidence for each health outcome. For the quantitative analyses described in the following subsections, EPA focused exclusively on *high* or *medium* confidence human epidemiological and animal toxicological studies for POD derivation, as recommended in Chapter 7.2 of the IRIS Handbook (U.S. EPA, 2022d). While the IRIS Handbook also includes consideration of *low* confidence studies for dose-response analysis under certain circumstances, this EPA assessment did not consider *low* confidence studies because of the relatively large and robust database for PFOS. At this stage, EPA considered additional study attributes to enable extrapolation to relevant exposure levels in humans. These attributes are described in Table 7-2 of the IRIS Handbook and include relevance of the test species, relevance of the studied exposure to human environmental exposures, quality of measurements of exposure and outcomes, and other aspects of study design including specific reconsideration of the potential for bias in the reported association between exposure and outcomes (U.S. EPA, 2022d).

Consideration of these attributes facilitates the transparent selection of studies and data for dose-response modeling and potential RfD or CSF derivation. Studies exhibiting these attributes are expected to provide more accurate human equivalent toxicity values and are therefore preferred in the selection process. Consideration of these attributes in the study selection process are described below for noncancer and cancer endpoints.

4.1 Noncancer

4.1.1 Study and Endpoint Selection

For study and endpoint selection for noncancer health outcomes, the human studies that provided all necessary analytical information (e.g., exposure distribution or variance, dose-response data) for POD derivation, analyzed the outcome of interest in the general population or susceptible population, and demonstrated the dose-response attributes outlined above were preferred. If available, *high* and *medium* confidence studies with exposures levels near the range of typical environmental human exposures, especially exposure levels comparable to human exposure in the United States, were preferred over studies reporting considerably higher exposure levels (e.g., occupational exposure levels). Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to the lower dose range of exposure levels that are relevant to the overall population. When available for a given health outcome, studies with analyses that addressed potential confounding factors affecting exposure concentrations (e.g., addressing temporal variations of PFOS concentrations during pregnancy due to hemodynamics) were also preferred. Additionally, when studies presented overlapping data on the same cohort or study population, various factors were considered to facilitate selection of one study for POD derivation. These factors included the duration of exposure, the length of observation of the study cohort, and the comprehensiveness of the analysis of the cohort in order to capture the most relevant results for dose-response analysis.

The preferred animal toxicological studies consisted of *medium* and *high* confidence studies with exposure durations appropriate for the endpoint of interest (e.g., chronic or subchronic studies vs. short-term studies for chronic effects) or with exposure during sensitive windows of development and with exposure levels near the lower dose range of doses tested across the evidence base. These types of animal toxicological studies increase the confidence in the RfD relative to other animal toxicological studies because they are based on data with relatively low risk of bias and are associated with less uncertainty related to low-dose and exposure duration extrapolations. See Section 5.3 for a discussion of animal toxicological studies and endpoints selected for POD derivation for this updated assessment compared with those selected for the 2016 PFOS HESD (U.S. EPA, 2016b).

4.1.1.1 Hepatic Effects

As reviewed in Section 3.4.1.4, ***evidence indicates*** that elevated exposures to PFOS are associated with hepatic effects in humans. As described in Table 3-6, the majority of epidemiological studies assessed endpoints related to serum biomarkers of hepatic injury (12 *medium* confidence studies), while fewer studies reported on liver disease or injury (3 *medium* confidence studies) and other serum markers of liver function (2 *medium* confidence studies). EPA prioritized studies that evaluated endpoints related to serum biomarkers of injury for quantitative analyses because the reported effects on these endpoints were well-represented within the database and were generally consistent across the available *medium* confidence studies. Additionally, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered reliable markers of hepatocellular function/injury, with ALT considered more specific and sensitive (Boone et al., 2005). Specifically, all five *medium* confidence studies in general population adults from the updated literature searches reported positive associations between PFOS serum concentrations and ALT, three of which reported

statistically significant responses (Jain, 2019; Jain and Ducatman, 2019c; Nian et al., 2019; Salihovic et al., 2018; Gleason et al., 2015). These more recently published studies provided additional evidence for increased ALT in adults beyond the three *medium* confidence reporting positive associations for ALT from the 2016 PFOS HESD (Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010). Findings from studies of other liver enzymes, AST and GGT, in adults generally reported a positive association, though less consistently than studies of ALT; therefore, studies of AST and GGT are supportive of the selection of ALT as an endpoint for POD derivation because these results demonstrate coherence across the different liver serum enzyme endpoints.

As mentioned above, serum ALT measures are considered a reliable indicator of impaired liver function because increased serum ALT is indicative of leakage of ALT from damaged hepatocytes (Liu et al., 2014; Boone et al., 2005; U.S. EPA, 2002a). Additionally, evidence from both human epidemiological and animal toxicological studies indicates that increased serum ALT is associated with liver disease (Roth et al., 2021; Kwo et al., 2017; Ioannou et al., 2006b; Ioannou et al., 2006a). Human epidemiological studies have demonstrated that even low magnitude increases in serum ALT can be clinically significant when extrapolated to the overall population (Gilbert and Weiss, 2006). For example, a Scandinavian study in people without any symptoms of liver disease but with relatively small increased serum ALT levels were later diagnosed with liver diseases such as steatosis and chronic hepatitis C (Mathiesen et al., 1999). Additionally, a study in Korea found that the use of lowered thresholds for "normal" serum ALT values showed good prediction power for liver-related adverse outcomes such as mortality and hepatocellular carcinoma (Park et al., 2019a).

Numerous studies have also demonstrated an association between elevated ALT and liver-related mortality (reviewed by Kwo et al. (2017)). Furthermore, the American Association for the Study of Liver Diseases (AASLD) recognizes serum ALT as an indicator of overall human health and mortality (Kim et al., 2008). For example, as reported by Kwo et al. (2017), Kim et al. (2004) observed that higher serum ALT concentrations corresponded to an increased risk of liver-related death in Korean men and women; similarly, Ruhl and Everhart (2013, 2009) analyzed NHANES data and observed an association between elevated serum ALT and increased mortality, liver-related mortality, coronary heart disease in Americans, and Lee et al. (2008) found that higher serum ALT was associated with higher mortality in men and women in Olmstead County, Minnesota. Furthermore, the American College of Gastroenterology (ACG) recommends that people with ALT levels greater than 33 (men) or 25 IU/L (women) undergo screenings and assessments for liver diseases, alcohol use, and hepatotoxic medication use (Kwo et al., 2017). Taken together, results of human epidemiological and animal toxicological studies as well as the positions of the AASLD and the ACG demonstrate the clinical significance of increased serum ALT. It is also important to note that while evaluation of direct liver damage is possible in animal studies, it is difficult to obtain biopsy-confirmed histological data in humans. Therefore, liver injury in humans is typically assessed using serum biomarkers of hepatotoxicity (Costello et al., 2022).

Among the available *medium* confidence epidemiological studies reporting alterations in serum ALT in humans, studies of adults in the general population were prioritized over studies in other populations (e.g., occupational) or life stages (e.g. children), as the adult studies provided the most consistent evidence of increases in ALT (see Section 3.4.1.1). Several of these *medium*

confidence studies reporting increases in ALT in adults were excluded from POD derivation for reasons such as combined adolescent and adult populations (Gleason et al., 2015), populations consisting of only elderly adults (Salihovic et al., 2018), use of correlation analyses only (Yamaguchi et al., 2013), and reporting analyses stratified by glomerular filtration without stratifying by exposure level, which were not amenable to modeling (Jain, 2019).

Exclusions of these studies resulted in the consideration of three *medium* confidence studies for POD derivation (Nian et al., 2019; Gallo et al., 2012; Lin et al., 2010) (Table 4-1). These studies exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). For example, Gallo et al. (2012), is the largest study assessing PFOS and ALT in adults which was conducted in over 30,000 individuals from the general population, aged 18-years and older, as part of the C8 Health Project in the United States. Further, Gallo et al. (2012) demonstrated a statistically significant trend in increased ALT across deciles. Two additional studies (Nian et al., 2019; Lin et al., 2010) were considered for POD derivation because they reported associations in general populations in the United States and a Chinese population located near a PFAS manufacturing facility, respectively. Nian et al. (2019) examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project and reported significantly increased level of ALT associated with PFOS. Lin et al. (2010) was also considered for POD derivation since it is a large (2,216 men and 1,063 women) nationally representative study in an NHANES adult population and observed increased ALT levels per log-unit increase in PFOS in the models adjusted for age, gender, and race/ethnicity. However, the association no longer remained in the fully adjusted models, or in the models additionally adjusted for PFOA, PFHxS, and PFNA. Additionally, several methodological limitations precluded its use for POD derivation. Limitations include lack of clarity about the base of logarithmic transformation applied to PFOS concentrations in regression models, and the choice to model ALT as an untransformed variable, which is a departure from the lognormality assumed in most of the ALT literature. Therefore, two *medium* confidence epidemiological studies were prioritized for POD derivation (Nian et al., 2019; Gallo et al., 2012) (Table 4-1).

Liver toxicity results reported in animal toxicological studies after PFOS exposure are concordant with the observed increased ALT indicative of hepatic damage observed in epidemiological studies. Specifically, studies in rodents found that oral PFOS treatment resulted in increased liver weight (11/14 *high* and *medium* confidence studies), increased levels of serum biomarkers of liver injury, particularly in male rodents (i.e., ALT (7/7 studies), AST (4/7 studies), ALP (3/4 studies), and GGT (1/1 study)), and evidence of histopathological alterations including hepatocellular damage (5/7 *high* and *medium* confidence studies). These hepatic effects, particularly the increases in serum enzymes and histopathological evidence of liver damage are supportive of increased ALT observed in human populations. Mechanistic studies in mammals and evidence from in vitro studies and nonmammalian animal models provide additional support for the biological plausibility and human relevance of the PFOA-induced hepatic effects observed in animals. These studies suggest multiple potential MOAs for the observed liver toxicity, including PPARα-dependent and -independent mechanisms of action (MOAs). The observed increases in liver enzymes (e.g., ALT) in rodents are supportive of the hepatic damage confirmed during histopathological examinations in several studies. Taken together, the study results suggest that at least some mechanisms for PFOS-induced hepatic effects are relevant to humans.

For animal toxicological hepatic endpoints, EPA preferred studies reporting quantitative biologically or statistically significant specific measures of severe toxicity (i.e., histopathological lesions related to cell or tissue death or necrosis) with study designs best suited for quantitative analysis (e.g., large sample size, reported effects in the lower dose range). Of the three studies that quantitatively reported incidences of hepatic histopathological alterations, two were excluded because they had relatively small sample sizes (i.e., $n \leq 10$) and used short-term exposure durations (i.e., 28 days) (NTP, 2019; Curran et al., 2008) as compared to Butenhoff et al., (2012). Butenhoff et al. (2012) was a chronic dietary study which conducted histopathological examinations of liver tissue in male and female rats and reported dose-dependent increases in the incidence of individual hepatocellular necrosis. As this is the only available chronic PFOS toxicity study with a large sample size (i.e., $n = 50$) , numerous and relatively low-dose levels, and data examining a suite of endpoints, individual cell necrosis in the liver in females was considered for derivation of PODs (Table 4-1). This endpoint was supported by the observation of non-monotonic increases in single cell necrosis in males from the same study.

4.1.1.2 Immunological Effects

As reviewed in Section 3.4.2.4, ***evidence indicates*** that elevated exposures to PFOS are associated with immunological effects in humans. As described in Table 3-12, the majority of epidemiological studies assessed endpoints related to immunosuppression (1 *high* and 21 *medium* confidence studies) and immune hypersensitivity (1 *high* and 20 *medium* confidence studies), while one study (*medium* confidence) also reported on endpoints related to autoimmune disease. Studies that reported on specific autoimmune diseases were excluded from POD derivation because there were a limited number of studies that assessed the same diseases (e.g., rheumatoid arthritis, celiac disease). Studies that evaluated endpoints related to immune hypersensitivity (e.g., asthma) were also not considered for POD derivation because there were inconsistencies in the direction and precision of effects across gender or age subgroups in the available studies. These inconsistencies limited the confidence needed to select particular studies and populations for dose-response modeling. Other immune hypersensitivity endpoints, such as odds of allergies and rhinoconjunctivitis, reported differing results across *medium* and *high* confidence studies and were therefore excluded from further consideration, though they provide qualitative support of an association between PFOS exposure and altered immune function.

Evidence of immunosuppression in children associated with exposure to PFOS reported by epidemiological studies were consistent across studies and endpoints. Specifically, epidemiological studies reported associations between PFOS exposure and reduced humoral immune response to routine childhood immunizations, including lower levels of tetanus and diphtheria (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012) and rubella (Zhang et al., 2023; Stein et al., 2016b; Granum et al., 2013) antibody titers. Reductions in antibody response were observed at multiple timepoints during childhood (specifically ages between 3-19 years in these studies), for either prenatal or postnatal childhood PFOS exposure levels, and were consistent across studies in children populations from *medium* confidence studies. Therefore, reduced antibody response in children was selected as an endpoint for POD derivation.

Measurement of antigen-specific antibodies following vaccination(s) is a measure of the overall ability of the immune system to respond to a challenge. The antigen-specific antibody response

is extremely useful for evaluating the entire cycle of adaptive immunity, which is a type of immunity that develops when a person's immune system responds to a foreign substance or microorganism, and it has been used as a comprehensive approach to detect immunosuppression across a range of cells and signals (Myers, 2018). The SAB's PFAS review panel noted that reduction in the level of antibodies produced in response to a vaccine represents a "failure of the immune system to respond to a specific challenge and is considered an adverse immunological health outcome" (U.S. EPA, 2022e). This is consistent with a review article by Selgrade (2007) who suggested that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards—which has the potential to be a more adverse effect that just a single immunotoxic effect. Thus, decrements in the ability to maintain effective levels of antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFOS.

As noted by Dewitt et al. (2019; 2017; 2016) and in comments from other subject matter experts on the SAB's PFAS review panel (U.S. EPA, 2022e), the clinical manifestation of a disease after chemical exposure is not required for a chemical to be classified as an immunotoxic agent and the ability to measure clinical outcomes as a result of mild to moderate immunosuppression in response to chemical exposure in traditional epidemiological studies can be challenging. Specifically, the SAB noted that "[d]ecreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease" (U.S. EPA, 2022e). The WHO *Guidance for immunotoxicity risk assessment for chemicals* similarly recommends measures of vaccine response as a measure of immune effects as "childhood vaccine failures represent a significant public health concern" (WHO, 2012). Decreases in antibody response, even at smaller magnitudes in individuals, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This response also translates across multiple species, including rodents, and extensive historical data indicate that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity.

Studies of developmental exposure to environmental toxicants demonstrate an association with immune suppression (Selgrade, 2007). When immunosuppression occurs during immune system development, the risks of developing infectious diseases and other immunosuppression-linked diseases may increase (Dietert et al., 2010). The immune system continues developing postnatally; because of this, exposures to PFAS and other immunotoxic agents during development may have serious, long-lasting, and irreversible health consequences (Dewitt et al., 2019; Macgillivray and Kollmann, 2014; Selgrade, 2007). Indeed, Hessel et al. (2015) reviewed the effect of exposure to nine toxicants on the developing immune system and found that the developing immune system was at least as sensitive or more sensitive than the general (developmental) toxicity parameters that were assessed. Developmental immunotoxicity as a result of chemical exposure is generally observed at doses lower than required to elicit immunotoxicity in adults (vonderEmbse and DeWitt, 2018). Therefore, developmental immunotoxicity is generally a highly sensitive health outcome, both when considering other types of developmental toxicity and when comparing it to immunotoxicity observed in exposed adults. Luster et al. (2005) similarly noted that the specific immunotoxic endpoint of responses to childhood vaccines may be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent.

One *high* and 10 *medium* confidence studies (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Pilkerton et al., 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Stein et al., 2016b; Mogensen et al., 2015a; Granum et al., 2013; Grandjean et al., 2012) reported findings on antibody response to tetanus, diphtheria, or rubella in children or adolescents. At least two *medium* confidence studies representing two different populations of children or adolescents reported inverse associations or increased risks of falling below seroprotective levels between each vaccine type and PFOS concentrations. For diphtheria and tetanus, this included five *medium* and one *high* confidence studies on the Faroe Island cohort (Shih et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a; Grandjean et al., 2012) and one *medium* confidence study in Greenlandic children (Timmermann et al., 2021). For rubella, this included one *medium* confidence study in Norwegian children (Granum et al., 2013) and two *medium* confidence studies on partially overlapping sets of children from the United States (Zhang et al., 2023; Stein et al., 2016b). Given the consistency of this response across multiple vaccine types and populations, including children from the United States, EPA considered studies reporting on all three vaccines for POD derivation. Specifically, EPA selected one *medium* confidence study representing each population (i.e., children or adolescents from the United States, Faroe Islands, Norway, and Greenland) for POD derivation.

Five separate studies (Shih et al., 2021; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a; Grandjean et al., 2012) reported on diphtheria and tetanus antibody responses in the same population (i.e., the same individuals) of Faroese children. One study reported on the same Faroese children cohort in a more recent *medium* confidence publication (Budtz-Jørgensen and Grandjean, 2018). Because this most recent *medium* confidence study is the only one of the five studies that provided dose-response data with untransformed PFOA concentrations which are more amenable to BMD modeling, only results from Budtz-Jørgensen and Grandjean (2018) were prioritized for POD derivation and the four other studies conducted in the Faroe Island population were excluded. For rubella, the NHANES populations examined in Zhang et al. (2023), Stein et al. (2016b), and Pilkerton et al. (2018) partially overlapped, and Zhang et al. (2023) was selected for POD derivation as it reported more recent data and a significant inverse response.

In total, four *medium* confidence epidemiologic studies (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018) exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a) and were considered for POD derivation (Table 4-1). Budtz-Jørgensen and Grandjean (2018) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and Timmerman et al. (2021) investigated anti-tetanus and anti-diphtheria responses in Greenlandic children aged 7–12. Granum et al. (2013) investigated rubella responses in Norwegian children aged 3 and Zhang et al. (2023) investigated rubella responses in U.S. adolescents.

Immunotoxicity results reported in animal toxicological studies are concordant with the observed immunosuppression in epidemiological studies. Specifically, studies in rodents found that oral PFOS treatment resulted in reduced immune responses (e.g., reduced plaque forming cell (PFC) responses, reduced natural killer (NK) cell activity) (4 *medium* confidence studies) and altered immune cell populations (e.g., bone marrow hypocellularity, altered splenic and thymic cellularity, white blood cell counts) (two *high* and three *medium* confidence studies). EPA

prioritized endpoints from both categories for quantitative analyses for several reasons. First, immunosuppression evidenced by functional assessments of the immune responses, such as analyses of PFC and NK responses, are concordant with decreased antibody responses seen in human populations. EPA prioritized PFC responses over NK cell activity for POD derivation because several studies (Zhong et al., 2016; Dong et al., 2009; Peden-Adams et al., 2008) reported non-monotonic dose-response curves for NK cell activity, increasing the uncertainty in the dose-response relationship for that endpoint. Of the six studies reporting reductions in PFC response in rodents (Zhong et al., 2016; Dong et al., 2011; Dong et al., 2009; Zheng et al., 2009; Keil et al., 2008; Peden-Adams et al., 2008), one *medium* confidence study (Zhong et al., 2016)was selected for POD derivation because the study tested a relatively low-dose range compared with the other five studies, the response was observed in both males and females, and the effect was measured in pups treated with PFOS in utero, consistent with the sensitive lifestage (i.e., children) identified from human studies (Table 4-1). Second, altered immune cell populations were reported in two *high* confidence studies and supported by several *medium* confidence studies, strengthening the weight of evidence for these immunological endpoints. EPA prioritized results from NTP (2019) for POD derivation over the other *high* confidence study (Lv et al., 2015) because it reported consistent effects of PFOS treatment on a suite of endpoints related to immune cellularity which were confirmed by histopathological evidence (if applicable), including increased bone marrow hypocellularity, increased splenic extramedullary hematopoiesis, and reduced leukocytes, neutrophils, and white blood cell counts in male and female rats. The endpoint of splenic extramedullary hematopoiesis was observed in both sexes and was consistent with other *high* and *medium* confidence studies that reported alterations in circulating immune cells, splenic cellularity, and thymic cellularity, both of which increase the confidence in this endpoint (Table 4-1).

4.1.1.3 Cardiovascular Effects

As reviewed in Section 3.4.3.4, ***evidence indicates*** that elevated exposures to PFOS are associated with cardiovascular effects in humans. As described in Table 3-15, the majority of epidemiological studies assessed endpoints related to serum lipids (2 *high*, 28 *medium*, and 12 *mixed*[16] confidence studies) and blood pressure and hypertension (2 *high* and 17 *medium* confidence studies), while some studies also reported on cardiovascular disease (1 *high* and 4 *medium* confidence studies) and atherosclerosis (1 *high* and 4 *medium* confidence studies). Endpoints related to cardiovascular disease and atherosclerosis were excluded from consideration for POD derivation because there were limited high and medium confidence studies and they reported mixed (i.e., positive and inverse associations) or mostly null results. Endpoints related to blood pressure and hypertension were also excluded from quantitative analyses because there was higher confidence in analytically determined serum lipid levels compared with blood pressure measurements and there was a larger evidence base for serum lipids as compared to blood pressure. However, there was evidence of associations between PFOS exposure and at least one measure of continuous blood pressure in adults and increased risk of hypertension. These results are qualitatively supportive of an association between PFOS and cardiovascular effects in humans.

[16] *Mixed* confidence studies on serum lipids were primarily of medium confidence for total cholesterol and HDL cholesterol, and *low* confidence for LDL cholesterol and triglycerides.

The majority of studies in adults from the general population, including high-exposure communities, reported positive associations between PFOS serum concentrations and serum lipids. Studies in adults were prioritized due to the current understanding that serum lipid changes in children are age-dependent and fluctuate during puberty (Daniels et al., 2008), which may impact the consistency of results from studies in children. Specifically, *medium* confidence epidemiological studies in the general population reported positive associations between PFOS exposure and total cholesterol (TC) (18/23 studies) and low-density lipoprotein (LDL) (13/18 studies). Associations between PFOS and high-density lipoprotein (HDL) or triglycerides in the general population were inconsistent and were therefore excluded from POD derivation. EPA selected TC for quantitative assessments because the association was the most consistently observed in adults, and studies for TC were of higher confidence for outcome measurements compared with LDL. Additionally, the positive associations with TC in these studies were further supported by a recent meta-analysis that included 14 general population studies in adults (U.S. EPA, 2024b) and reported an association between increased cholesterol and increased PFOS exposure.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), ischemic stroke (IS), and cardiovascular mortality occurring in populations without prior CVD events (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Additionally, disturbances in cholesterol homeostasis contribute to the pathology of nonalcoholic fatty liver disease (NAFLD) and to accumulation of lipids in hepatocytes (Malhotra et al., 2020). Cholesterol is made and metabolized in the liver, and thus the evidence indicating that PFOS exposure disrupts lipid metabolism, suggests that toxic disruptions of lipid metabolism by PFOS are indications of hepatoxicity. Increases in serum cholesterol, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude increases in serum cholesterol could shift the distribution of serum cholesterol in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for cardiovascular disease. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to…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" (U.S. EPA, 2022e).

A total of 13 *medium* confidence studies (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020d; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Eriksen et al., 2013; Fitz-Simon et al., 2013; Château-Degat et al., 2010; Nelson et al., 2010; Steenland et al., 2009; Olsen et al., 2003) reported on positive associations between exposure to PFOS and total cholesterol in adults from the general population. One study evaluated occupational adult populations only (Olsen et al., 2003) was not considered as exposure pathways and concentrations in this population did not represent typical exposure scenarios for human environmental exposure. Three studies (Canova et al., 2020; Lin et al., 2020d; Eriksen et al., 2013) were excluded from POD derivation due to narrow age ranges (i.e., 50–65 years of age,

55–75 years of age, and 20–39 years of age, respectively) of the study populations that were less comprehensive than the age groups included by other studies and therefore, may not apply across the general adult population. One study (Jain and Ducatman, 2019b) was excluded form POD derivation because the study reported findings stratified by BMI status but was not stratified by exposure.

Although the positive associations between PFOS and TC were supported by a recent meta-analysis that included 14 general population studies of adults (U.S. EPA, 2024b), EPA did not use the pooled effect from this meta-analysis for POD derivation. This meta-analysis was not comprehensive of the entire database of studies on PFOS and TC because it was performed specifically with the purpose of informing aspects of the Pooled Cohort Atherosclerotic Cardiovascular Disease (ASCVD) model which relies on CVD risk reduction analysis for those ages 40–89 (U.S. EPA, 2024b). The results of another recent meta-analysis on PFOS and serum lipids (Abdullah Soheimi et al., 2021) was excluded from POD derivation because the pooled effects reported combined 11 studies with TC, triglycerides and LDL in multiple populations (i.e., children, adolescents, pregnant women, and adults). As previously noted, serum lipids rise in early childhood and fluctuate in puberty (Daniels et al., 2008), and combining study populations at different lifestages would likely result in unaddressed confounding by age.

Four studies presented overlapping data from NHANES (Fan et al., 2020; Dong et al., 2019; Liu et al., 2018d; Nelson et al., 2010). Of these four, Dong et al. (2019) was selected for POD derivation because this larger study included data from all NHANES cycles between 2003 and 2014, while the other three studies reported results for only one or two cycles within the 2003-2014 range and were therefore not further considered. Similarly, two studies (Fitz-Simon et al., 2013; Steenland et al., 2009) presented data on the C8 Health Project population. Fitz-Simon et al. (2013) was not selected for POD derivation because it was a part of a short-term follow-up and was not as comprehensive as the population examined by Steenland et al. (2009). Likewise, another higher exposure community study (Château-Degat et al., 2010) reported TC changes in approximately 700 Nunavik Inuit adults which was not as comprehensive as Steenland et al. (2009) which investigated over 46,000 adults. Therefore, Steenland et al. (2009) was also selected for POD derivation. Finally, Lin et al. (2019) was also selected for POD derivation because it provided data for a large number of adults (n = 940) in the general U.S. population from the Diabetes Prevention Program (DPP) population, with PFOS levels at baseline comparable to those from NHANES 1999–2000.

In summary, three *medium* confidence epidemiologic studies were considered for POD derivation (Table 4-1) (Dong et al., 2019; Lin et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOS exposure measures across various populations and exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Lin et al. (2019) collected data from prediabetic adults from the DPP and DPPOS at baseline (1996–1999).

Though results reported in animal toxicological studies support the alterations in lipid metabolism observed in epidemiological studies, there are species differences direction of effect with dose. As a result of these differences, there is some uncertainty about the human relevance of these observed responses in rodents. Additionally, the available mechanistic data do not

increase the understanding about the non-monotonicity of serum lipid levels and decreased serum lipid levels at higher dose levels in rodents (Section 3.4.3.3). Due to the uncertainties regarding human relevance of the animal toxicology studies, EPA did not derive PODs for animal toxicological studies reporting cardiovascular effects, such as altered serum lipid levels.

4.1.1.4 Developmental Effects

As reviewed in Section 3.4.4.4, ***evidence indicates*** that elevated exposures to PFOS are associated with developmental effects in humans. As described in Table 3-17, the majority of epidemiological studies assessed endpoints related to fetal growth restriction (21 *high* and 26 *medium* confidence studies) and gestational duration (10 *high* and 11 *medium* confidence studies), while fewer studies reported on endpoints related to fetal loss (3 *high* and 3 *medium* confidence studies) and birth defects (4 *medium* confidence studies). Evidence for birth defects was limited in that there are only 4 *medium* confidence studies and those studies provided mixed findings. Therefore, birth defects not prioritized for POD derivation. Although half of the available *high* and *medium* confidence studies reported increased incidence of fetal loss (3/6), EPA did not prioritize this endpoint for dose-response analyses as there were a relatively limited number of studies compared with endpoints related to gestational duration and fetal growth restriction and the evidence from *high* confidence studies was mixed. The impacts observed on fetal loss are supportive of an association between PFOS exposure and adverse developmental effects.

The majority of the available studies reporting metrics of gestational duration observed increased risk associated with PFOS exposure, including among *high* confidence studies. Seven of the 13 *medium* or *high* confidence studies reported inverse associations for gestational age at birth and 7 of the 11 *medium* or *high* confidence studies reported an association with preterm birth. These findings are supportive of an association between PFOS exposure and adverse developmental effects. There were generally consistent associations with increased risk of preterm birth, particularly from the *high* confidence studies, with several studies reporting statistically significant results. While overall there appears to be consistent associations between PFOS exposure and gestational duration, the database for fetal growth restriction demonstrated consistent associations between PFOS and fetal growth restriction and was also both larger and consisted of more *medium* and *high* confidence studies than gestational duration. Therefore, studies demonstrating fetal growth restriction were prioritized for POD derivation.

The majority of *high* and *medium* confidence epidemiological studies (16/27) reported associations between PFOS and decreased mean birth weight in infants. Studies on changes in standardized birth weight measures (i.e., z-scores) also reported inverse associations (8/12 studies; 6 *high* and 2 *medium* confidence). Endpoints characterizing fetal growth restriction were included for POD derivation multiple studies reported effects on these endpoints, particularly decreased birth weight, and reported generally consistent findings across *high* and *medium* confidence studies. As noted in the Developmental Human Evidence Study Evaluation Considerations (Section 3.4.4.1.2), measures of birth weight were considered higher confidence outcomes compared with other measures of fetal growth restriction such as birth length, head circumference, or ponderal index because birth weight measures are less prone to measurement error (Shinwell and Shlomo, 2003). Studies reporting changes in mean birth weight were more amenable to modeling compared with those reporting changes in standardized (e.g., z-score) birth weight measurements. Standardized measurements depend on sources of standardization

and are harder to interpret and compare across studies. As a result, measures of mean changes in birth weight were considered for quantitative analysis.

Low birth weight (LBW) is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999). LBW is widely considered a useful population level public health measure (Vilanova et al., 2019; Cutland et al., 2017; WHO and UNICEF, 2004; Lira et al., 1996) and is on the World Health Organization's (WHO's) global reference list of core health indicators (WHO, 2018a, 2014). Decreases in birthweight, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude decreases in birthweight could shift the distribution of birthweight in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for decreased birthweight and subsequent effects related to decreased birthweight. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern" (U.S. EPA, 2022e).

Substantial evidence links LBW to a variety of adverse health outcomes at various stages of life. It has been shown to predict prenatal mortality and morbidity (Cutland et al., 2017; WHO, 2014; U.S. EPA, 2013) and is a leading cause of infant mortality in the United States (CDC, 2021). Low-birth-weight infants are also more likely to have underdeveloped and/or improperly-functioning organ systems (e.g., respiratory, hepatic, cardiovascular), clinical manifestations of which can include breathing problems, red blood cell disorders (e.g., anemia), and heart failure (U.S. EPA, 2013; Zeleke et al., 2012; Guyatt and Snow, 2004; WHO and UNICEF, 2004; JAMA, 2002). Additionally, low-birth-weight infants evaluated at 18 to 22 months of age demonstrated impaired mental development (Laptook et al., 2005).

LBW is also associated with increased risk for diseases in adulthood, including obesity, diabetes, and cardiovascular disease ((Smith et al., 2016a; Risnes et al., 2011; Gluckman et al., 2008; Ong and Dunger, 2002; Osmond and Barker, 2000), as reported in Yang et al. (2022)). Poor academic performance, cognitive difficulties (Hack et al., 2002; Larroque et al., 2001), and depression (Loret de Mola et al., 2014) in adulthood have also been linked to LBW. These associations between LBW and infant mortality, childhood disease, and adult disease establish LBW as an adverse effect. Considering the established consequences of LBW, as well as the consistency of the database and large number of *medium* and *high* confidence studies reporting mean birth weight and other binary birth weight-related measures, the endpoint of decreased birth weight in infants was selected for POD derivation.

Given the abundance of *high* confidence epidemiological studies evaluating decreases in birth weight, *low* and *medium* confidence studies were excluded from POD derivation. Thus, 15 *high* confidence studies reporting inverse associations between exposure to PFOS and mean birth weight (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Bell et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Darrow et al.,

2013; Whitworth et al., 2012) were considered for POD derivation. Four studies (Gardener et al., 2021; Xiao et al., 2019; Ashley-Martin et al., 2017; Whitworth et al., 2012) were excluded because they reported sex-stratified results rather than results in both sexes or results for the overall population in terms of standardized measurements (e.g., z-score) only. Analyses utilizing standardized measurements as the dependent variable are internally valid, but this type of analysis estimates a change in birthweight relative to the study population, which would not be generalizable to other populations. Two studies (Luo et al., 2021; Bell et al., 2018) were not considered due to the use of non-preferred exposure characterizations such as infant whole blood samples from a heel stick and postpartum maternal exposure samples, which are prone to exposure misclassification. Three studies (Lauritzen et al., 2017; Lind et al., 2017a; Valvi et al., 2017) were not considered further due to inconsistencies by sex or location with no clear biological explanation for the inconsistency.

As a result of these exclusions, six remaining *high* confidence epidemiologic studies (Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Darrow et al., 2013) met the preferred criteria outlined in Section 4.1.1 and were considered for POD derivation (Table 4-1). The candidate epidemiological studies offer a variety of PFOS exposure measures across different developmental windows (i.e., preconception, fetal, neonatal). All six studies reported their exposure metric in units of ng/mL and reported the β coefficients per ng/mL or ln(ng/mL), along with 95% confidence intervals, estimated from linear regression models. Two of the six studies examined PFOS primarily during trimester one (Sagiv et al., 2018 Wikström, 2020, 6311677), one during trimesters two and three (Starling et al., 2017) and one examined PFOS during trimester three (Yao et al., 2021). One study examined PFOS collected within days of birth (Chu et al., 2020) and another study (Darrow et al., 2013) examined PFOS collected at the time of enrollment in the C8 Health Project. In the latter cohort, two sets of analyses were conducted: one analysis including all births identified from women enrolling in the study and one analysis of only the mother's first prospective birth following enrollment (i.e., only births following blood collected during enrollment). EPA identified the first prospective birth analysis as the analysis to consider for POD derivation due to increased confidence in the temporal relationship between exposure measurement and outcome assessment (i.e., not including mothers with samples collected after pregnancy). The Wikström et al. (2020) study reported on the large Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study cohort with samples collected between 2007 and 2010. Sagiv et al. (2018) reported associations between first trimester PFOS samples collected between 1999–2002 in a Project Viva birth cohort in the United States. Darrow et al. (2013) reported large inverse associations between PFOS collected during C8 Health Project enrollment (2005–2006) in the Mid-Ohio Valley. Chu et al. (2020) reported on associations between maternal PFOS collected within three days of delivery and birth weight in the Chinese Guangzhou Birth Cohort Study (2013). Starling et al. (2017) reported on associations between PFOS collected in later pregnancy (range: 20 to 34 weeks gestational age) in the Healthy Start prospective cohort in Colorado (2009–2014). Yao et al. (2021) reported associations between PFOS measured in maternal blood collected three days prior to delivery and decreased birth weight in the Chinese Laizhou Wan Birth Cohort (2010–2013).

Developmental toxicity results reported in animal toxicological studies are concordant with the observed developmental effects in epidemiological studies. Specifically, studies in rodents found that gestational PFOS exposure resulted in reduced offspring weight (8/14 *medium* confidence

studies) and decreased offspring survival (5/9 *medium* confidence studies). Though limited in number, several other studies also reported consistent effects on placental endpoints, reduced ossification, and developmental delays. Some of these developmental effects seen in the offspring of rodents treated with PFOA (e.g., reduced offspring weight) are consistent with the decreases in birth weight and adverse effects associated with LBW observed in human populations.

Given the large number of adverse effects identified in the animal toxicological database for the developmental health outcome, EPA prioritized only the most sensitive effects (i.e., those observed at lower dose levels and/or higher magnitude) in offspring that were supported by multiple studies for derivation of PODs. EPA focused on the animal studies with effects in the offspring, as opposed to maternal effects, because these effects provide concordance with the approximate timing of decreased birth weight observed in human infants. The one study reporting altered maternal weight without confounding effects on the offspring (Argus Research Laboratories, 2000) could not be considered for POD derivation because the study was in rabbits and the pharmacokinetic model EPA used to predict internal dose in the animal models is parameterized for mice, rats, and monkeys but not rabbits (see Section 4.1.3). EPA also focused on endpoints for which results from multiple animal toxicological studies corroborated the observed effect, thereby increasing the confidence in that effect. EPA additionally focused on studies with exposure durations lasting through the majority of gestation and/or lactation (i.e., from GD 1 through early postnatal development) rather than those that targeted a specific period of gestation or postnatal development as they were more likely to be sensitive for detection of developmental effects. Multiple animal toxicological studies observed effects at low dose levels and demonstrated a dose-related response in decreased fetal weight, offspring body weight and decreased offspring survival. Therefore, these endpoints were prioritized for dose-response analysis.

Five studies in rats and mice reported decreased pup body weight with PFOS exposure (Xia et al., 2011; Yahia et al., 2008; Luebker et al., 2005b; Luebker et al., 2005a; Lau et al., 2003). For this endpoint, EPA selected studies in rats as the effect was observed more consistently in this species and rats appeared to be more sensitive to pup weight changes than mice. Of the four studies reporting this effect in rats, EPA selected the data presented in the 1- and 2-generation studies by Luebker et al. (2005b) and Luebker et al. (2005a) (F_1 generation only) because the exposure duration spanned prior to mating through lactation, there were more dose groups tested than any of the other available studies, the dosing paradigm encompassed relatively low-dose levels, the authors reported pup weight relative to litter weight, and the effect was observed at multiple time points. Specifically, EPA selected the time points of LD 0 and LD 5 from Luebker et al. (2005b) and PND 1 (F1 only) from Luebker et al. (2005a).

Six studies in mice, rats, and rabbits reported decreased fetal body weight with gestational PFOS exposure (Li et al., 2021a; Wan et al., 2020; Li et al., 2016; Lee et al., 2015; Thibodeaux et al., 2003; Argus Research Laboratories, 2000). While the majority of studies reporting this endpoint did not use an exposure paradigm that encompassed the earliest period of gestation (i.e., GD 1–4), thus increasing the uncertainty about the sensitivity of the data selected for dose-response modeling, EPA modeled this endpoint due to the consistency of the response across species and for comparison to PODs derived for pup weight. EPA selected studies in mice as this species appeared to be more sensitive to fetal weight changes than rats at lower dose levels and as

described above, the PK model used in this assessment is not parameterized for rabbits. Ultimately, Lee et al. (2015) was selected for POD derivation as it reported fetal weight for a relatively greater number of dose groups, incorporated a lower dose level than other studies reporting this effect, and reported more than one dose group with a statistically significant response.

Reduced offspring survival or viability was also observed with developmental PFOS exposure in both rats and mice. Various metrics were used to assess prenatal mortality, including measures of post-implantation loss, stillbirths, abortions, resorptions, and fetal death. Though the response was not entirely consistent between studies, potentially due to study design and differences in the endpoint measurement, reduced prenatal viability was observed in mice, rats, and rabbits and qualitatively supports the observation of reduced pup survival in rats and mice. Given these considerations, reduced fetal survival was not selected for dose-response modeling. Eight studies reporting reduced pup survival; seven in rats and two in mice (Lau et al. (2003) reported on both species). Therefore, EPA considered studies in rats for POD derivation. EPA then selected the metric of pup survival (Chen et al., 2012b; Xia et al., 2011; Grasty et al., 2006; Lau et al., 2003; Thibodeaux et al., 2003) over pup viability (Luebker et al., 2005b; Luebker et al., 2005a) since more studies reported on the former (5 vs. 2). Ultimately, EPA selected pup survival at PND 5 and PND 22 as reported by Lau et al. (2003) for POD derivation because this was a *medium* confidence study that presented data for a greater number of dose groups as compared to the other studies, provided data at multiple time points, incorporated relatively low-dose levels as compared to the other studies, used an exposure duration that encompassed the majority of gestation (GD 2–21), and reported more than one dose group with a statistically significant.

Table 4-1 summarizes the studies and endpoints considered for POD derivation.

Table 4-1. Summary of Endpoints and Studies Considered for Dose-Response Modeling and Derivation of Points of Departure for All Effects in Humans and Rodents

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Justification
		Immune Effects		
Reduced Antibody Concentrations for Diphtheria, Tetanus, and Rubella	Budtz-Jørgensen and Grandjean (2018)[a] *Medium* Timmerman et al. (2021) *Medium* Granum et al. (2013) *Medium* Zhang et al. (2023) *Medium*	Human, male and female children or adolescents	Yes	Decreases in antibody responses to pathogens such as diphtheria, tetanus, and rubella were observed at multiple timepoints in childhood and during adolescence, using both prenatal and childhood PFOS exposure levels. Effect was large in magnitude and generally coherent with epidemiological and animal toxicological evidence for other immunosuppressive effects. Effects were observed in multiple populations, including adolescents from the United States.
Decreased PFC Response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, F_1 males	Yes	Functional assessment indicative of immunosuppression indicative of immunosuppression. Effect was consistently observed across multiple studies: Peden-Adams et al. (2008), Dong et al. (2009), Zheng et al. (2009), and Keil et al. (2008). Zhong et al. (2016) was selected because the study tested a relatively low-dose range and the effect was measured in a sensitive lifestage and time point (pups at PNW 4).
Extramedullary Hematopoiesis in the Spleen	NTP (2019) *High*	Sprague-Dawley Rats, adult male and female	Yes	Blood cell production outside of the bone marrow which occurs when normal cell production is impaired. Selected for POD derivation because the results were from a *high* confidence study, histopathologically confirmed, consistent across both sexes, accompanied by evidence of bone marrow hypocellularity, and consistent with other studies that reported alterations in circulating immune cells, splenic cellularity, and thymic cellularity.
		Developmental Effects		
Decreased Birth Weight	Chu et al. (2020) *High* Darrow et al. (2013) *High*	Human, male and female infants	Yes	Evidence for developmental effects is based on consistent inverse effects for FGR including birthweight measures which are the most accurate endpoint examined. Some deficits were consistently reported for birth weight and standardized birth weight in many *high* and *medium* confidence cohort studies. Effect was generally large in magnitude and coherent with epidemiological evidence for other biologically related effects.

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Justification
	Sagiv et al. (2018) *High* Starling et al. (2017) *High* Wikström et al. (2020) *High* Yao et al. (2021) *High*			
Decreased Fetal Body Weight	Lee et al. (2015) *Medium*	CD-1 Mice, F_1 males and females	Yes	Effect was consistently observed across six studies and three species (Li et al., 2021a; Wan et al., 2020; Li et al., 2016; Lee et al., 2015; Thibodeaux et al., 2003; Argus Research Laboratories, 2000) and is coherent with epidemiological evidence of decreased birth weight and evidence of reduced pup weight in rodents. Lee et al. (2015) was selected because there is a pharmacokinetic model available to extrapolate from exposures in mice to exposures in humans, the study tested a relatively low-dose range, incorporates a relatively greater number of dose groups, and reported more than one dose group with a statistically significant response compared with other studies reporting this effect, and because mice appear to be a more sensitive model for this endpoint than rats.
Decreased Pup Body Weight (relative to litter)	Luebker et al. (2005b) *Medium* Luebker et al. (2005a)	Sprague-Dawley Rats, F_1 male and female (LD 0 and LD 5 (Luebker et al., 2005b); PND 1 (Luebker et al., 2005a))	Yes	Effect was consistently observed across five studies and two species and is coherent with epidemiological evidence of decreased birth weight and evidence of decreased fetal weight in rodents. Luebker et al. (2005b) and Luebker et al. (2005a) were selected because rats appear to be more sensitive than mice to this endpoint, the studies are designed to be sensitive to this effect (i.e., multigeneration studies testing relatively large numbers of dose groups and low-dose ranges), the studies reported effects as relative to litter and the studies observed effects in multiple generations or multiple time points and in multiple dose groups.
Decreased Pup Survival	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and	Yes	Decreased offspring survival was consistently observed across eight studies and two species and is also supported by reduced fetal survival observed in rodents. Lau et al. (2003) was selected because rats appeared to be more sensitive to this effect than mice and because the study presented data for a greater number of dose groups and at multiple time points compared with

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Justification
		female (PND 5 and PND 22)		the other four studies in rats, incorporated relatively low-dose levels, used an exposure duration that encompassed the majority of gestation (GD 2–21), and reported more than one dose group with a statistically significant response.
Serum Lipid Effects				
Increased Total Cholesterol	Dong et al. (2019) *Medium* Lin et al. (2019) *Medium* Steenland et al. (2009)[b] *Medium*	Human, male and female adults	Yes	Effect was consistent and observed across multiple adult populations including general population adults in NHANES (Dong et al., 2019); from prediabetic adults from the DPP and DPPOS cohort (Lin et al., 2019) and the C8 Health project high-exposure community (Steenland et al., 2009), as well as when study designs excluded individuals prescribed cholesterol medication, minimizing concerns of bias due to medical intervention (Dong et al., 2019; Steenland et al., 2009). Endpoint is supported by associations between PFOS and blood pressure.
Hepatic Effects				
Increased ALT	Gallo et al. (2012) *Medium* Nian et al. (2019) *Medium*	Human (male and female adults)	Yes	Effect was consistent and observed across multiple populations including general population adults (Lin et al., 2010) (NHANES) and high-exposure communities (Gallo et al., 2012) (C8 Health Project); (Nian et al., 2019) (Isomers of C8 Health Project in China).
Increased ALT	Lin et al. (2010) *Medium*	Human (male and female adults)	No	While this is a large nationally representative population, several methodological limitations preclude its use for POD derivation. Limitations include lack of clarity about base of logarithmic transformation applied to PFOS concentrations in regression models, and the choice to model ALT as an untransformed variable, a departure from the typically lognormality assumed in most of the ALT literature.
Individual Cell Necrosis in the Liver	Butenhoff et al. (2012) *High*	Sprague-Dawley rats, females	Yes	Effect was supported by a non-monotonic response in males from the same study (Butenhoff et al., 2012). Effect was qualitatively observed in Xing et al. (2016) and Cui et al. (2009), and further supported by increases in serum enzyme levels associated with hepatic damage in both animals and humans.

Notes: PNW = postnatal week; ALT = alanine transaminase; F_1 =first generation.

[a] Supported by Grandjean et al. (2012); Grandjean et al. (2017a); Grandjean et al. (2017b).

[b] See Section 5.6.3 for discussion on the approach to estimating BMDs from regression coefficients.

4.1.2 Estimation or Selection of Points of Departure for RfD Derivation

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR intended to represent a minimal, biologically significant level of change. The *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) describes a hierarchy by which BMRs are selected, with the first and preferred approach being the use of a biological or toxicological basis to define what minimal level of response or change is biologically significant. If biological or toxicological information is lacking, the guidance document recommends BMRs that could be used in the absence of information about a minimal clinical or biological level of change considered to be adverse—specifically, a BMR of 1 standard deviation (SD) change from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data. When severe or frank effects are modeled, a lower BMR can be adopted. For example, developmental effects are serious effects that can result in irreversible structural or functional changes to the organism, and the *Benchmark Dose Technical Guidance* suggests that studies of developmental effects can support lower BMRs. BMDs for these effects may employ a BMR of 0.5 SD change from the control mean for continuous data or a BMR of 5% for dichotomous data (U.S. EPA, 2012a). A lower BMR can also be used if it can be justified on a biological and/or statistical basis. The *Benchmark Dose Technical Guidance* (page 23; (U.S. EPA, 2012a)) shows that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of 1 SD results in a ~10% extra risk of being at risk of having an adverse effect. A BMR smaller than 0.5 SD change from the control mean is generally used for severe effects (e.g., 1% extra risk of cancer mortality).

Based on rationales described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the IRIS Handbook (U.S. EPA, 2022d) and past IRIS assessment precedent, BMRs were selected for dose-response modeling of PFOS-induced health effects for individual study endpoints as described below and summarized in Table 4-2 along with the rationales for their selection. For this assessment, EPA took statistical and biological considerations into account to select the BMR. For dichotomous responses, the general approach was to use 10% extra risk as the BMR for borderline or minimally adverse effects and either 5% or 1% extra risk for adverse effects, with 1% reserved for the most severe effects. For continuous responses, the preferred approach for defining the BMR was to use a preestablished cutoff for the minimal level of change in the endpoint at which the effect is generally considered to become biologically significant (e.g., greater than or equal to 42 IU/L serum ALT in human males (Valenti et al., 2021)). In the absence of an established cutoff, a BMR of 1 SD change from the control mean, or 0.5 SD for effects considered to be severe, was generally selected. Specific considerations for BMR selection for endpoints under each of the priority noncancer health outcomes are described in the subsections below and alongside the modeling methods and results provided in Appendix E (U.S. EPA, 2024a). Considerations for BMR selection for cancer endpoints are described in Section 4.24.2 and Appendix E (U.S. EPA, 2024a).

4.1.2.1 Hepatic Effects

For the hepatic endpoint of increased serum ALT in adults associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically

significant adverse serum ALT level (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for the endpoint of elevated ALT. Specifically EPA modeled elevated human ALT using cutoff levels of 42 IU/L for males and 30 IU/L for females (Valenti et al., 2021). These are the most updated clinical consensus cutoffs which post-date the American Association for the Study of Liver Diseases (AASLD) journal of Clinical Liver Disease recommended values of 30 IU/L for males, and 19 IU/L for females (Ducatman et al., 2023; Kasarala and Tillmann, 2016). Valenti et al. (2021, 1036989) determined the values using the same approach at the same center, but using an updated standardized method, a large cohort of apparently healthy blood donors (ages 18-65 years) and showed that the updated cutoffs were able to better predict liver disease.

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of USEPA (2012a)) to estimate the probability of an individual with an adverse serum ALT level as a function of PFOS exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the endpoint of elevated serum ALT.

EPA selected a BMR of 5% extra risk because studies have demonstrated that ALT levels at or slightly above the selected cutoff levels can be associated with more severe liver diseases (Wedemeyer et al., 2010; Mathiesen et al., 1999), increased risk of liver-related mortality (Park et al., 2019a; Ruhl and Everhart, 2009; Kim et al., 2004), and mortality (Lee et al., 2008). Based on the severity of the health effects associated with increased ALT, EPA determined that a BMR of 5% extra risk is warranted (U.S. EPA, 2012a); a 10% extra risk would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing more severe liver diseases such as advanced fibrosis, chronic liver disease, and even liver-related death. Since there is currently a relatively high prevalence of elevated ALT in the general population (14% and 13% of U.S. male and female adults, respectively, aged 20 and older (Valenti et al., 2021)), a small increase in the prevalence of elevated ALT associated with PFOA exposure would likely increase the number of individuals with severe liver-related health effects. This also supports using a more health protective BMR of 5% extra risk (rather than 10%) for POD derivation. EPA presents PODs with a 10% BMR for comparison purposes in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effect of individual cell necrosis observed in livers of adult rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of an effect-specific minimal biologically significant response level. Therefore, in accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a BMR of 10% extra risk was used because it is considered the standard reporting level for quantal (dichotomous) data and a minimally biologically significant response level (see Table 4-2).

4.1.2.2 Immune Effects

For the developmental immune endpoint of decreased diphtheria, rubella, and tetanus antibody response in children associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 0.5 SD change from the control mean (see Table 4-2). Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018), Timmerman et al. (2021), Granum et al. (2013), and Zhang et al. (2023) measured antibody concentrations in childhood and PFOS exposure during gestation or childhood, these are considered developmental studies based on EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), which includes the following definition**:**

> "Developmental toxicology - The study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the lifespan of the organism."

EPA guidance recommends the use of a 1 or 0.5 SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect. A blood concentration for tetanus and diphtheria antibodies of 0.1 IU/mL has been cited in the literature as a "protective level" (Grandjean et al., 2017b; Galazka and Kardymowicz, 1989). However, in the *Immunological Basis for Immunization Series* of modules (WHO, 2018b), the WHO argued that assay-specific "protective levels" of tetanus antitoxin may not actually guarantee immunity. Galazka et al. (1993) similarly argued that several factors give rise to variability in the vulnerability of individuals to diphtheria and there is no consensus on what level offers full protection. For rubella, 10 IU/mL has been cited in the literature as a protective level (Skendzel, 1996), however, the geographical variability, lack of consensus, and relatively dated assessment of this cutoff precludes its use as the basis of the BMR (Charlton et al., 2016). As such, EPA determined that there is no clear definition of an adverse effect threshold for the endpoints of reduced tetanus, rubella, or diphtheria antibody concentrations in children or adolescents.

With these two factors in mind, a 0.5 SD was selected as the BMR because: 1) the health outcome is developmental, and 2) there is no accepted definition of an adverse level of change or clinical cutoff for reduced antibody concentrations in response to vaccination. Therefore, EPA performed the BMDL modeling using a BMR equivalent to a 0.5 SD change in log2-transformed antibody concentrations, as opposed to a fixed change in the antibody concentration distributions. EPA also presented BMDL modeling using a BMR equivalent to a 1 SD change, as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effects of decreased PFC response to SRBC observed in PNW 4 mice and splenic extramedullary hematopoiesis in adult rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of minimal biologically significant response levels. In accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a BMR of 1 SD change from the control mean was employed for

the effect on PFC response (continuous data) and a BMR of 10% extra risk was used for the increased incidence of extramedullary hematopoiesis (dichotomous data) (see Table 4-2).

4.1.2.3 Cardiovascular Effects

For the cardiovascular endpoint of increased serum TC in adults associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant adverse serum TC concentration (Dong et al., 2019; Steenland et al., 2009) or a BMR of 0.5 SD (Lin et al., 2019), depending on the data provided by the study (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect and identified the definition of hypercholesterolemia from the American Heart Association (NCHS, 2019) as providing the most recent upper reference limit for clinically adverse serum TC. Specifically, when possible, EPA modeled human cholesterol using a cutoff level of 240 mg/dL for elevated serum total cholesterol (NCHS, 2019).

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of USEPA (2012a)) to estimate the probability of an individual with an adverse TC level as a function of PFOS exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the effect of elevated serum TC. For Lin (2019), EPA relied on the mean serum TC concentrations reported across PFOS quartiles (i.e., continuous data) provided by the study, and therefore considered a BMR of a change in the mean equal to 0.5 SD or 1 SD from the control mean.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), IS, and cardiovascular mortality occurring in populations without prior CVD events (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Based on the severity of the cardiovascular-related health effects associated with increased cholesterol, EPA determined that selection of a BMR of 5% extra risk or 0.5 SD is warranted (U.S. EPA, 2012a); a 10% extra risk or 1SD would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing increased incidence of cardiovascular disease events. Since there is currently a relatively high prevalence of elevated TC in the general population (11.5% of U.S. adults aged 20 and older (NCHS, 2019)), a small increase in the prevalence of elevated TC associated with PFOA exposure would likely increase risk of severe health outcomes, such as cardiovascular-related events. Thus, this supports using a more conservative BMR of 5% extra risk or 0.5 SD for POD derivation. EPA presents PODs with a BMR of 10% extra risk (Dong et al., 2019; Steenland et al., 2009) or 1 SD (Lin et al., 2019) for comparison purposes in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

4.1.2.4 Developmental Effects

For the developmental endpoint of decreased birth weight associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant birth weight deficit (see Table 4-2). As described in Appendix E (U.S. EPA, 2024a), LBW is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include but is not exclusive to babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999).

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Low birthweight is associated with increased risk for adverse health effects throughout life (Tian et al., 2019; Reyes and Mañalich, 2005; Hack et al., 1995) and therefore, supports a more stringent BMR below 10% (for dichotomous data) or 1 SD (for continuous data). Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of U.S. EPA (2012a)) to estimate the probability of an individual with a birth weight deficit as a function of PFOS exposure. This approach effectively dichotomized the data, resulting in a BMR defined as a 5% increase in the number of infants with birth weights below 2,500 g.

For decreased fetal and pup weights and decreased pup survival observed in animal studies, BMRs of 5% relative deviation and 0.5 SD from the control were employed, respectively (see Table 4-2). As with human data, these BMRs are consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) and the IRIS Handbook (U.S. EPA, 2022d), which note that studies of adverse developmental effects represent a susceptible lifestage and can support BMRs that are lower than 10% extra risk (dichotomous data) and 1 SD change from the control mean (continuous data). A 5% relative deviation in markers of growth in gestational exposure studies (e.g., fetal weight) has generally been considered an appropriate biologically significant response level and has been used as the BMR in final IRIS assessments (e.g., U.S. EPA (2003), U.S. EPA (2004), and U.S. EPA (2012b)). Additionally, the 5% BMR selection is statistically supported by data which compared a BMR of 5% relative deviation for decreased fetal weight to NOAELs and other BMR measurements, including 0.5 SD, and found they were statistically similar (Kavlock et al., 1995).. EPA presented modeling results using a BMR of 0.5 SD for decreased fetal and pup body weight and a BMR of 0.1 SD for the frank effect of decreased pup survival for comparison purposes, as recommended by EPA guidance (U.S. EPA, 2012a) (see Appendix, (U.S. EPA, 2024a)).

Table 4-2. Benchmark Response Levels Selected for BMD Modeling of Health Outcomes

Endpoint	BMR	Rationale
		Immune Effects
Reduced antibody concentrations for diphtheria, rubella, and tetanus in children or adolescents	0.5 SD	Consistent with EPA guidance**.** EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect and selects a 1 or 0.5 SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a)

Endpoint	BMR	Rationale
Decreased PFC Response to SRBC (PNW 4)	1 SD	Insufficient information available to determine minimal biologically significant response level. The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 1 SD was used as per EPA guidance (U.S. EPA, 2012a)
Extramedullary Hematopoiesis in the Spleen	10%	Insufficient information available to determine minimal biologically significant response level. The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 10% was used as per EPA guidance (U.S. EPA, 2012a)
Developmental Effects		
Decreased Birth Weight in Infants	5% extra risk of exceeding adversity cutoff (hybrid approach)	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a). The use of the hybrid approach results in dichotomization of the data and therefore a 5% BMR was selected (U.S. EPA, 2012a)
Decreased Fetal or Pup Weight	5%	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Decreased Pup Survival	0.5 SD	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Cardiovascular Effects		
Increased Cholesterol	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect.
	0.5 SD	Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects, a 0.5 SD was used as the BMR. A change from the mean of 0.5 SD limits further increases in the prevalence of this effect
Hepatic Effects		
Increased ALT	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data,

Endpoint	BMR	Rationale
		response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased ALT is not a frank effect but is associated with increased incidence of severe liver-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect
Individual Cell Necrosis	10%	Insufficient information available to determine minimal biologically significant response level. The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 10% was used as per EPA guidance (U.S. EPA, 2012a)

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMR = benchmark response; CDC = Centers for Disease Control; SD = standard deviation.

4.1.3 Pharmacokinetic Modeling Approaches to Convert Administered Dose to Internal Dose in Animals and Humans

4.1.3.1 Pharmacokinetic Model for Animal Internal Dosimetry

Following review of the available models in the literature (see Section 3.3.2), EPA chose the Wambaugh et al. (2013) model to describe PFOS dosimetry in experimental animals based on the following criteria:

- availability of model parameters across the species of interest,
- agreement with out-of-sample datasets (see Appendix, (U.S. EPA, 2024a)), and
- flexibility to implement life course modeling.

These criteria originated from the goal of accurately predicting internal dose metrics for toxicology studies that were selected for dose-response analysis. The species used in the toxicological studies (i.e., species of interest) were rats, mice, and nonhuman primates; model parameters for these species of interest were available. Good agreement with training and test (out-of-sample) datasets shows that the model performance is good compared with both the data used to identify model parameters and to external data. This was assessed using the mean square log error (MSLE) to compare model predicted concentration values to observed PFOS serum concentrations following single dose exposure to animals. Training set data demonstrated an MSLE of 0.17 for PFOS, respectively. For test set data, the MSLE was 0.38 for PFOS. The general agreement between test and training datasets increases confidence that the model can be used to make accurate predictions of internal dose metrics for the dose magnitudes used in the available toxicology studies. The ability to implement life-course modeling was necessary to properly predict internal dose metrics for developmental studies and endpoints as the animal transitioned through numerous lifestages.

In this case, an oral dosing version of the original model structure introduced by Andersen et al. (2006) and summarized in Section 3.3.2 was selected for having the fewest number of parameters that would need estimation. In addition, the Wambaugh et al. (2013) approach allowed for a single model structure to be used for all species in the toxicological studies

allowing for model consistency for the predicted dose metrics associated with LOAELs and NOAELs from 13 animal toxicological studies of PFOS.

The Wambaugh et al. (2013) model was selected for pharmacokinetic modeling for animal internal dosimetry for several important reasons: 1) it allowed for sex-dependent concentration-time predictions for PFOS across all three species of interest, 2) it adequately predicted dosimetry of newer datasets published after model development, and 3) it was amendable to addition of a lifestage component for predicting developmental study designs. These analyses are further described below. Uncertainties and limitations of the selected modeling approach are described in Section 5.6.1.

4.1.3.1.1 Animal Model Parameters

Pharmacokinetic parameters for different species and strains represented in the Wambaugh et al. (2013) model are presented in Table 4-3.

Table 4-3. PK Parameters from Wambaugh et al. (2013) Meta-Analysis of Literature Data for PFOS

Parameter	Units	CD1 Mouse (F)[a]	CD1 Mouse (M)[a]	Sprague-Dawley Rat (F)[a]	Sprague-Dawley Rat (M)[a]	CynomolgusMonkey (M/F)[a]
Body weight[b] (BW)	kg	0.02	0.02	0.203	0.222	3.42
Cardiac Output[c] (Q_{cc})	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
Absorption rate (k_a)	1/h	1.16 (0.617–42,400)	433.4 (0.51–803.8)	4.65 (3.02–1,980)	0.836 (0.522–1.51)	132 (0.225–72,100)
Central Compartment Volume (V_{cc})	L/kg	0.264 (0.24–0.286)	0.292 (0.268–0.317)	0.535 (0.49–0.581)	0.637 (0.593–0.68)	0.303 (0.289–0.314)
Intercompartment transfer rate (k_{12})	1/h	0.0093 ($2.63 \times e^{-10}$–38,900)	2,976 ($2.8 \times e^{-10}$–$4.2 \times e^{4}$)	0.0124 ($3.1 \times e^{-10}$–46,800)	0.00524 ($2.86 \times e^{-10}$–43,200)	0.00292 ($2.59 \times e^{-10}$–34,500)
Intercompartment ratio ($R_{V2:V21}$)	Unitless	1.01 (0.251–4.06)	1.29 (0.24–4.09)	0.957 (0.238–3.62)	1.04 (0.256–4.01)	1.03 (0.256–4.05)
Maximum resorption rate (T_{maxc})	µmol/h	57.9 (0.671–32,000)	$1.1 \times e^{4}$ ($2.1–7.9 \times e^{4}$)	1,930 (4.11–83,400)	$1.34 \times e^{-6}$ ($1.65 \times e^{-10}$–44)	15.5 (0.764–4,680)
Renal resorption affinity (K_T)	µmol	0.0109 ($1.44 \times e^{-5}$–1.45)	381 ($2.6 \times e^{-5}$–$2.9 \times e^{3}$)	9.49 (0.00626–11,100)	2.45 ($4.88 \times e^{-10}$–60,300)	0.00594 ($2.34 \times e^{-5}$–0.0941)
Free fraction	Unitless	0.00963 (0.00238–0.0372)	0.012 (0.0024–0.038)	0.00807 (0.00203–0.0291)	0.00193 (0.000954–0.00249)	0.0101 (0.00265–0.04)
Filtrate flow rate (Q_{filc})	Unitless	0.439 (0.0125–307)	27.59 (0.012–283)	0.0666 (0.0107–8.95)	0.0122 (0.0101–0.025)	0.198 (0.012–50.5)
Filtrate volume (V_{filc})	L/kg	0.00142 ($4.4 \times e^{-10}$–6.2)	0.51 ($3.5 \times e^{-10}$–6.09)	0.0185 ($8.2 \times e^{-7}$–7.34)	0.000194 ($1.48 \times e^{-9}$–5.51)	0.0534 ($1.1 \times e^{-7}$–8.52)

Notes: F = female; M = male.
Means and 95% credible intervals (in parentheses) from Bayesian analysis are reported. For some parameters the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).
[a] Datasets modeled for the mouse and rat were from Chang et al. (2012) and for the monkey from Seacat et al. (2002) and Chang et al. (2012).
[b] Average body weight for species:individual-specific bodyweights.
[c] Cardiac outputs obtained from Davies and Morris (1993).

4.1.3.1.2 Out-of-Sample Comparisons

To evaluate the model's ability to predict PFOS concentration-time data in the species of interest, EPA compared model fits to in vivo datasets published following the 2016 PFOS HESD (Table 4-4). For rats, the data of Kim et al. (2016) and Huang et al. (2021) were used. Model simulations demonstrated good agreement with available data for adult time-course PFOS PK predictions in the rat. However, there was no comparable PK dataset for PFOS in mice. Therefore, only the original study used for parameter determination (Chang et al., 2012) was compared with model simulations. This comparison approach demonstrated agreement with the in vivo data.

Using the Wambaugh et al. (2013) model, EPA predicted the half-life, V_d, and clearance and compared these species-specific predictions to values obtained from in vivo studies when data were available.

Following out-of-sample dataset evaluation of the female rat PK parameters (Table 4-4) and visual inspection of the resulting concentration-time fits, EPA determined that only male PK model parameters would be used for all rat-specific modeling. This assumption agrees with Kim et al. (2016) where they report no PK differences between the sexes for PFOS ADME.

Table 4-4. Model-Predicted and Literature PK Parameter Comparisons for PFOS

	Male			Female		
	t1/2,β (days)	**Vd,β (L/kg)**	**CL (L/d/kg)**	**t1/2,β (days)**	**Vd,β (L/kg)**	**CL (L/d/kg)**
			Rat			
Model	44.13	0.638	0.01	282.05	0.538	0.0013
Literature	28.7[a], 39.7[b]	0.382[a], 0.681[b]	0.0092[a], 0.013[b]	24.8[a], 32.8[b]	0.288[a], 0.421[b]	0.008[a], 0.009[b]
			Mouse			
Model	134.83	0.472	0.0024	38.4	1.41	0.0255
Literature	–	–	–	–	–	–

Notes: CL = clearance; PK = pharmacokinetic; $t_{1/2,\beta}$ = terminal-phase elimination half-life; V_d, β = volume of distribution during the terminal phase.
[a] Information obtained from Kim et al. (2016).
[b] Information obtained from Huang et al. (2019).

4.1.3.1.3 Life Course Modeling

The Wambaugh et al. (2013) model was modified to allow for a gestation, lactation, and post-weaning phase (Figure 4-1). Using the original model structure and published parameters, simulations assumed that dams were dosed prior to conceptions and up to the date of parturition. Following parturition, a lactational phase involved PFOS transfer from the breastmilk to the suckling pup where the pup was modeled with a simple one-compartment PK model. Finally, a post-weaning phase utilized the body-weight scaled Wambaugh model to simulate dosing to the growing pup and accounted for filtrate rate as a constant fraction of cardiac output.

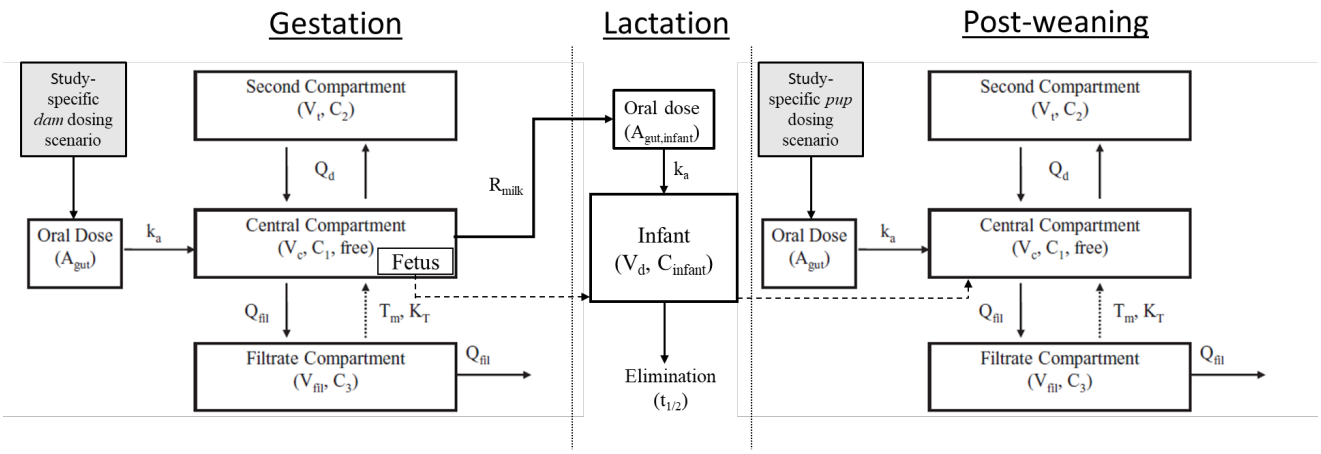


Figure 4-1. Model Structure for Lifestage Modeling

Model parameters for three-compartment model are the same as those described earlier. Pup-specific parameters include milk consumption in kg_{milk}/day (R_{milk}), infant-specific volume of distribution (V_d), and infant-specific half-life ($t_{1/2}$).

This methodology was adapted from Kapraun et al. (2022) and relies on the following assumptions for gestation/lactation modeling:

- During gestation and up through the instant birth occurs, the ratio of the fetal concentration (mg of substance per mL of tissue) to the maternal concentration is constant.
- Infant animal growth during the lactational period is governed by the infant growth curves outlined in Kapraun et al. (2022).
- Rapid equilibrium between maternal serum PFOS and milk PFOS is assumed and modeled using a serum:milk partition coefficient.
- All (100%) of the substance in the breast milk ingested by the offspring is absorbed by the offspring.
- The elimination rate of the substance in offspring is proportional to the amount of substance in the body and is characterized by an infant-specific half-life that is a fixed constant for any given animal species as described in Table 4-5 below.
- Following the lactation period, infant time course concentrations are tracked using the more physiologically based Wambaugh model to model post-weaning exposure and infant growth.

A simple one-compartment model for infant lactational exposure was chosen because of differences between PFOS V_d reported in the literature and Wambaugh et al. (2013) model-predicted V_d following extrapolation to a relatively low infant body weights. Because V_d is assumed to be extracellular water in humans, Goeden et al. (2019) adjusts for lifestage-specific changes in extracellular water using an adjustment factor where infants have 2.1 times more extracellular water than adults resulting in a larger V_d. However, this large difference in extracellular water is not observed in rats (Johanson, 1979). Johanson (1979) demonstrated a 5% decrease in blood water content from early postnatal life (~0.5 weeks) to adulthood (>7 weeks) in the rat. Therefore, EPA used the literature reported V_d (Kim et al., 2016; Chang et al., 2012) for the one-compartment model to describe infant toxicokinetics (Table 4-5). Finally, the

Wambaugh et al. (2013) model was not parameterized for a postpartum infant, and it was not possible to evaluate the mechanistic assumptions for renal elimination with postnatal toxicokinetic data. Therefore, the parameters listed in Table 4-5 in a one-compartment gestation/lactation model were used in conjunction with the parameters published in Wambaugh et al. (2013) to predict developmental dose metrics for PFOS.

Table 4-5. Additional PK Parameters for Gestation/Lactation for PFOS

Parameter	Units	Rat	Mouse
Maternal Milk:Blood Partition Coefficient (P_{milk})	Unitless	0.13[a]	0.32[e]
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.83[b]	0.41[f]
Elimination Half-Life ($t_{1/2}$)	Days	40[c]	36.87 g
Volume of Distribution (V_d)	L/kg	0.28[d]	0.26 g
Starting Milk Consumption Rate (r^0_{milk})	kg_{milk}/day	0.001[h]	0.0001[i]
Week 1 Milk Consumption Rate (r^1_{milk})	kg_{milk}/day	0.003[h]	0.0003[i]
Week 2 Milk Consumption Rate (r^2_{milk})	kg_{milk}/day	0.0054[h]	0.00054[i]
Week 3 Milk Consumption Rate (r^3_{milk})	kg_{milk}/day	0.0059[h]	0.00059[i]

Notes: PK = pharmacokinetic.
[a] Information obtained from Loccisano et al. (2013) (derived from Kuklenyik et al. (2004)).
[b] Information obtained from Lau et al. (2003).
[c] Average of male/female half-lives reported in Huang et al. (2019), Kim et al. (2016), and Chang et al. (2012).
[d] Information obtained from Kim et al. (2016).
[e] Assume same P_{milk} as PFOA (lack of mouse data).
[f] Information obtained from Wan et al. (2020).
[g] Information obtained from Chang et al. (2012).
[h] Information obtained from Kapraun et al. (2022) (adapted from Lehmann et al. (2014)).
[i] Information obtained from Kapraun et al. (2022) (mouse value is 10% of rat based on assumption that milk ingestion rate is proportional to body mass).

These developmental-specific parameters include the maternal milk:blood PFOS partition coefficient (P_{milk}), the ratio of the concentrations in the fetus(es) and the mother during pregnancy (R_{fm}), the species-specific in vivo determined half-life ($t_{1/2}$) and V_d for PFOS, and the species-specific milk consumption rate during lactation (r^i_{milk}) for the i^{th} week of lactation. Milk rate consumptions are defined as:

- r^0_{milk}, the starting milk consumption rate in kg milk per day (kg/d);
- r^1_{milk}, the (average) milk consumption rate (kg/d) during the first week of lactation (and nursing);
- r^2_{milk}, the (average) milk consumption rate (kg/d) during the second week of lactation; and
- r^3_{milk}, the (average) milk consumption rate (kg/d) during the third week of lactation.

where R_{milk} used in the model is a piecewise linear function comprising each r^i_{milk} depending on the week of lactation.

Using this gestation/lactation model, EPA fit one study for PFOS exposure in rats to ensure the model predicted the time-course concentration curves for both the dam and the pup. For all gestation/lactation studies, time zero represents conception followed by a gestational window (21 days for the rat). Dosing prior to day zero represents pre-mating exposure to PFOS.

Figure 4-2 demonstrates the model's ability to predict gestation and lactation study designs in rat dams exposed to 1.6 mg/kg/day PFOS that gave birth to pups who are exposed through gestation and lactation until weaning (Luebker et al., 2005a). For developmental PK simulations, the original Wambaugh et al. (2013) model with increasing maternal weight predicts dam concentrations in female rats while the one-compartmental lactational transfer model predicts infant concentrations for pups exposed both in utero and through lactation only.

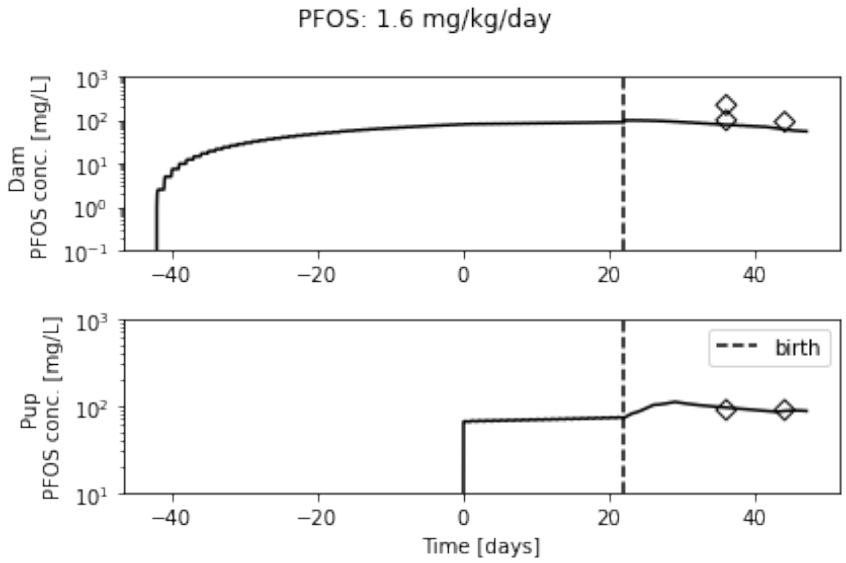


Figure 4-2. Gestation/Lactation Predictions of PFOS in the Rat

Top panel represents predicted dam concentrations with open diamonds (◊) representing the dam concentrations reported in Luebker et al. (2005a). Bottom panel represents predicted pup concentrations with open diamonds (◊) representing the reported pup concentrations in Luebker et al. (2005a) where the source of PFOS exposure is from the breast milk. Vertical dashed line represents birth.

The purpose of the animal PBPK model is to make predictions of internal dose in laboratory animal species used in toxicity studies and extrapolate these internal dose POD to humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose metrics across lifestages were selected for simulation in a mouse, rat, monkey, or human. Concentrations of PFOS in blood were considered for all the dose metrics. For studies in adult animals the dose-metric options were generally a maximum blood concentration (C_{max}, mg/L) and a time averaged blood concentration (i.e., the area under the curve over the duration of the study (AUC, mg * day/L)) or the blood concentration over the last 7 days of the study (C_{last7}, mg/L). In developmental studies, dose metrics were developed for the dam, the fetus (during gestation), and the pup (during lactation) for both time maximum blood concentrations (C_{max}) and average blood concentrations (C_{avg}). In the dam, the C_{max} and C_{avg} were calculated over a range of lifestages: during gestation ($C_{avg\_dam\_gest}$), during lactation ($C_{avg\_dam\_lact}$), or combined gestation and lactation ($C_{avg\_dam\_gest\_lact}$). In pups for C_{max}, two different lifestages were calculated either during gestation or lactation ($C_{max\_pup\_gest}$, $C_{max\_pup\_lact}$). In pups for time averaged metrics, a C_{avg} was calculated for during gestation, lactation or combined gestation and lactation ($C_{avg\_pup\_gest}$, $C_{avg\_pup\_lact}$ and $C_{avg\_pup\_gest\_lact}$).

EPA selected the metric of C_{last7} for studies examining noncancer effects using non-developmental exposure paradigms. This metric provides a consistent internal dose for use across disparate chronic and subchronic study designs where steady state may or may not have been reached in the animal following continuous dosing. When the animal has reached steady state, C_{last7} is equal to the steady-state concentration and for non-steady-state study designs, this metric averages the concentration variability over a week's worth of dosing rather than using a single, maximal concentration. This allows for extrapolation to the human model where a steady-state assumption is implemented for adult dose-metric calculations.

For developmental endpoints, the metric of C_{max} is typically used when there is a known MOA related to a threshold effect during a specific window of susceptibility. From the *Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation* (U.S. EPA, 2014), the choice of this metric "depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue." Furthermore, the guidance clarifies that "for chronic effects, in the absence of MOA information to the contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to the active toxicant is the most appropriate dose metric (e.g., AUC)" (U.S. EPA, 2014). Repeat dosing coupled with a lack of a defined MOA for the apical endpoints used for dose-response modeling resulted in EPA excluding C_{max} as an internal dose metric for animal toxicological endpoints. However, EPA provides modeling results using C_{max} for comparison purposes in Appendix E (U.S. EPA, 2024a).

EPA selected the metric of C_{avg} for studies with reproductive or developmental exposure designs encompassing gestation and/or lactation. One factor considered for this selection pertains to the long half-life of PFOA and the degree of accumulation throughout pregnancy and lactation. Because PFOA is not cleared within 24 hours, daily dosing throughout pregnancy/lactation will result in a C_{max} that falls on the final day of pregnancy or lactation or a C_{last7} only representative of the final days of gestation or lactation, even if dosing ceases after birth, due to ongoing lactational exposure. The endpoints in this assessment (decreased fetal or pup weight, decreased pup survival, delayed time to eye opening) do not have established MOAs or known windows of susceptibility and instead are expected to result from sustained internal dose from repeated exposures. If, as anticipated, this window of susceptibly for a given endpoint is not on the final day or the last week of exposure, the C_{max} or C_{last7} will not capture the exposure at the time associated with the adverse effect. A C_{avg} metric is more representative of the exposure throughout the potential window of susceptibility. This selection is also supported by the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), which state that when pharmacokinetic data are available, as is the case for PFOA, "adjustments may be made to provide an estimate of equal average concentration at the site of action for the human exposure scenario of concern." The selection of C_{avg} for developmental animal studies is therefore consistent with the guidance for humans.

4.1.3.2 Pharmacokinetic Model for Human Dosimetry

The key factors considered in model determination were to implement a human model from the literature that was able to model gestational and lactational exposure to infants, that was able to describe time course changes in serum concentration due to changes in body weight during growth, and that required minimal new development. Previous modeling efforts suggested that

limiting model complexity helps to prevent errors and facilitates rapid implementation (Bernstein et al., 2021). For the human epidemiological and animal toxicological endpoints of interests, serum concentration was identified as a suitable internal dosimetry target which provides support for using a simpler model that did not have individual tissue dosimetry. For these reasons, EPA selected the one-compartment human developmental model published by Verner et al. (2016). Several alternative models to EPA's updated version of the Verner et al. (2016) model for the calculation of POD_{HED} from an internal POD were considered. This included consideration of full PBPK models (i.e., the Loccisano family of models (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011) and a developmental PBPK model in rats (Chou and Lin, 2021)), as well as other one-compartment PK models (e.g., Goeden et al. (2019)). Discussion on the justification for selection of the Verner et al. (2016) model as the basis for the pharmacokinetic modeling approach used for PFOS is available in Sections 5.6.2 and 5.7.

Several adjustments were undertaken to facilitate the application of the model to our use. First, the model was converted from acslX language to an R/MCSim framework. This allows for the code to be more accessible to others by updating it to a contemporary modeling language, as acslX software is no longer available or supported. The starting point for the conversion to R/MCSim was another model with a similar structure that was in development by EPA at that time (Kapraun et al., 2022). Second, body weight curves for non-pregnant adults were revised based on U.S. Centers for Disease Control and Prevention (CDC) growth data for juveniles and values from EPA's *Exposure Factors Handbook* in adults (U.S. EPA, 2011b; Kuczmarski et al., 2002). Linear interpolation was used to connect individual timepoints from these two sources to produce a continuous function over time. Body weight during pregnancy was defined based on selected studies of maternal body weight changes during pregnancy (U.S. EPA, 2011b; Portier et al., 2007; Thorsdottir and Birgisdottir, 1998; Carmichael et al., 1997; Dewey et al., 1993). Age-dependent breastmilk intake rates were based on the 95th percentile estimates from EPA's *Exposure Factors Handbook* and was defined relative to the infant's body weight (U.S. EPA, 2011b).

A third modification was the update of parameters: the half-life, V_d, the ratio of PFOS concentration in cord blood to maternal serum, and the ratio of PFOS concentration in breastmilk and maternal serum. Details for how these parameters were updated are given in the following paragraphs. In the model, half-life and V_d are used to calculate the clearance, which is used in the model directly and is also used for calculation of steady-state concentrations in adults. Other than half-life and, because of that, clearance, the updated parameters were similar to the original parameters (Table 4-6). The results of the new R model and updated acslX model with the original parameters were essentially identical (see Appendix, (U.S. EPA, 2024a)). With the updated parameters, the predicted PFOS serum concentrations are approximately 60% of the original values during pregnancy, and the child's serum concentration is approximately 80% of the original values during the first year of life.

The use of the Verner model in humans presents a substantial advancement in approach for endpoints in children compared with the previous EPA assessment of PFOS (U.S. EPA, 2016b). The previous 2016 HESD did not explicitly model children, but instead applied an uncertainty factor to an RfD based on long-term adult exposure to account for the potential for increased susceptibility in children. The current approach explicitly models PFOS exposure to infants during nursing who are undergoing rapid development, including growth, through childhood, and

who do not reach steady state until near adulthood. This allows for a more accurate estimation of exposures associated with either serum levels in children or dose metric from developmental animal toxicological studies. The Verner model also explicitly models the mother from her birth through the end of breastfeeding which allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy. Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Application of the updated Verner model to three cohorts with paired maternal measurements and subsequent samples in children between ages of 6 months and 6 years showed good agreement between reported and predicted serum levels in the children (see Appendix, (U.S. EPA, 2024a)). This suggests that the assumptions made governing lactational transfer and the selected half-life value are reasonable. A local sensitivity analysis was also performed to better understand the influence of each parameter on model output (see Appendix, (U.S. EPA, 2024a)).

Table 4-6. Updated and Original Chemical-Specific Parameters for PFOS in Humans

Parameter	Updated Value	Original Value[a]
Volume of Distribution (mL/kg)	230[b]	230
Half-life (yr)	3.4[c]	5.5
Clearance (mL/kg/d)	0.128[d]	0.079
Cord Serum:Maternal Serum Ratio	0.40[e]	0.42
Milk:Serum Partition Coefficient	0.016[f]	0.014

Notes:
[a] Verner et al. (2016).
[b] Thompson et al. (2010a).
[c] Li et al. (2018b).
[d] Calculated from half-life ($t_{1/2}$) and volume of distribution (V_d). Clearance (Cl) = V_d * ln(2)/$t_{1/2}$.
[e] Average values for total PFOA Cord Serum:Maternal Serum ratios (see Appendix, (U.S. EPA, 2024a)). This is a similar approach to that used by Verner et al. (2016), but also includes studies made available after the publication of that model.
[f] Average value of studies as reported in Table 4-7. This is a similar approach to that used by Verner et al. (2016), but also includes studies made available after the publication of that model.

EPA selected a reported half-life value from an exposure to a study population that is demographically representative of the general population, with a clear decrease in exposure at a known time, with a high number of participants and a long follow-up time. Based on these criteria, a half-life of 3.4 years for PFOS was selected (Li et al., 2018b). This value for PFOS comes from a community with contaminated drinking water with serial blood samples of 106 individuals for a relatively short follow-up time of 2 years. A summary of PFOS half-life values is presented in the Appendix (U.S. EPA, 2024a). Uncertainties related to EPA's selected half-life are discussed in Section 5.6.2.

The updated value for human V_d, 230 mL/kg, was sourced from Thompson et al. (2010a). To estimate the V_d for PFOS, Thompson et al. (2010a) scaled the value they obtained for PFOA by the ratio of V_ds obtained by Andersen et al. (2006) in the parameterization of that PK model using PK data in monkey. That is, V_d PFOA, human) = V_d (PFOA, human*V_d (PFOS, monkey)/V_d (PFOA, monkey). V_d is a parameter that is relatively easily obtained from an analysis of PK data from a controlled experimental study, as it is related to the peak concentration observed after dosing and is expected to be similar between human and nonhuman

primates (Mordenti et al., 1991). For comparison, the optimized V_d value from oral dosing in monkeys was 220 mL/kg for PFOS (Andersen et al., 2006).

A summary of PFOS V_d values is presented in the Appendix (U.S. EPA, 2024a). Uncertainties related to EPA's selected V_d are discussed in Section 5.6.2.

In the original model, the ratio of PFOS concentration in cord blood to maternal serum, and the ratio of PFOS concentration in breastmilk and maternal serum were based on an average of values available in the literature; here, EPA identified literature made available since the original model was published and updated those parameters with the averages of all identified values (Table 4-7). The values for cord blood to maternal serum ratio are presented in the Appendix (U.S. EPA, 2024a). One restriction implemented on the measurements of the cord blood to maternal serum ratio was to only include reports where the ratio was reported, and not to calculate the ratio from reported mean cord and maternal serum values. This was due to potential bias that could be introduced if a greater proportion of cord blood measurements are below the limit of detection compared with maternal serum.

Table 4-7. Summary of Studies Reporting the Ratio of PFOS Levels in Breastmilk and Maternal Serum or Plasma

Source	HERO ID	Milk:Maternal Plasma Ratio	Included in Verner et al. (2016) Analysis
Haug et al. (2011)	2577501	0.014	No
Seung-Kyu Kim et al. (2011b)	2919258	0.011	No
Liu et al. (2011)	2919240	0.020	No
Kärrman et al. (2007)	1290903	0.010	No
Cariou et al. (2015)[a]	3859840	0.011	Yes
Sunmi Kim et al. (2011a)[b]	1424975	0.030	Yes
Verner et al. (2016)	3299692	0.014[c]	–
Additional Studies	–	0.016[d]	–

Notes:
Whether studies were included in the analysis of Verner et al. (2016) is noted. The reported values were based on the mean of ratios in the study populations except when noted otherwise.
[a] Median result based on the report of Pizzurro et al. (2019).
[b] Median result as reported by the authors.
[c] Average value of milk:maternal plasma ratio used by Verner et al. (2016).
[d] Average value of milk:maternal plasma ratio with the inclusion of additional studies not in the original analysis. This value was used in the human PK model.

This updated model was used to simulate the human equivalent doses (HED) from the animal PODs that were obtained from BMD modeling of the animal toxicological studies (see Appendix, (U.S. EPA, 2024a)). It was also used to simulate selected epidemiological studies (Section 4.1.4) to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling (see Appendix, (U.S. EPA, 2024a)). For PODs resulting from chronic exposure, such as a long-term animal toxicological study or an epidemiological study on an adult cohort, the steady-state approximation was used to calculate a POD_{HED} that would result in the same dose metric after chronic exposure. For PODs from exposure to animals in developmental scenarios, the updated Verner model was used to calculate a POD_{HED} that results in the same dose metric during the developmental window selected. The updated Verner model was also

used to calculate a POD_{HED} for PODs based on epidemiological observations of maternal serum concentration during pregnancy, cord blood concentration, and serum concentrations in children.

The pharmacokinetic modeling code for both the updated Wambaugh et al. (2013) and Verner et al. (2016) models that was used to calculate human equivalence doses is available in an online repository (https://github.com/USEPA/OW-PFOS-PFOA-MCLG-support-PK-models). The model code was thoroughly QA'd through the established EPA Quality Assurance Project Plan (QAPP) for PBPK models (U.S. EPA, 2018).

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOS Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Different approaches were taken to estimate POD_{HED}s depending on the species (i.e., human vs. animal model) and lifestage (e.g., developmental, adult). The PODs from epidemiological studies (immune, developmental, hepatic, and serum lipid endpoints) were derived using hybrid or benchmark dose modeling (see Appendix E.1, (U.S. EPA, 2024a)) which provided an internal serum concentration in ng/L. The internal dose PODs were converted to a POD_{HED} using the modified Verner model described in Section 4.1.3.1.3 to calculate the dose that results in the same serum concentrations. Specifically, reverse dosimetry was performed by multiplying an internal dose POD by a model-predicted ratio of a standard exposure and the internal dose for that standard exposure. This expedited procedure can be performed because the human model is linear, that is, the ratio of external and internal dose is constant with dose. Additional details are provided below and in Table 4-8.

The PODs from the animal toxicological studies were derived by first converting the administered dose to an internal dose as described in Section 4.1.3.1.1. The rationale for the internal dosimetric selected for each endpoint is described in Appendix E.2 (U.S. EPA, 2024a). Because a toxicological endpoint of interest results from the presence of chemical at the organ-specific site of action, dose-response modeling is preferentially performed on internal doses rather than administered doses and assumes the internal dose metric is proportional to the target tissue dose. In addition, the non-linear elimination described in Wambaugh et al. (2013) requires conversion to an internal dose as the relationship between internal and external dose will not scale linearly. The internal doses were then modeled using the Benchmark Dose Software (BMDS) (see Appendix E, (U.S. EPA, 2024a)). If BMD modeling did not produce a viable model, a NOAEL or LOAEL approach was used consistent with EPA guidance (U.S. EPA, 2012a). The internal dose animal PODs were converted to a POD_{HED} using the model described in Section 4.1.3.1.3. Reverse dosimetry for the animal PODs used the ratio of standard exposure and internal dose as was applied to PODs from epidemiological data. For animal toxicological studies using the average concentration over the final week of the study (C_{last7}), the POD_{HED} is the human dose that would result in the same steady-state concentration in adults. When a concentration internal dose metric in the pup during lactation and/or gestation was selected, the POD_{HED} is the dose to the mother that results in the same average concentration in the fetus/infant over that period.

Table 4-8 displays the POD and estimated internal and POD_{HED}s for immune, developmental, cardiovascular (serum lipids), and hepatic endpoints from animal and/or human studies selected for the derivation of candidate RfDs.

Table 4-8. POD_{HED}s Considered for the Derivation of Candidate RfD Values

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Immunological Effects						
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations at age 5 and anti-tetanus antibody serum concentrations at age 7	$BMDL_{0.5\ SD}$	18.5 ng/mL	2.71×10^{-6}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 55% change in the effect size when controlling for PFOA; selected BMDL was based on a non-significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	29.9 ng/mL	5.21×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in poor quality of model fits; selected BMDL was based on a non-significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOS concentrations and anti-tetanus antibody concentrations at ages 7–12	$BMDL_{0.5\ SD}$	9.66 ng/mL	1.78×10^{-6}	BMDL based on non-significant regression parameter and resulted in a poor quality of model fit; BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations at age 5 and anti-diphtheria antibody serum concentrations at age 7	$BMDL_{0.5\ SD}$	12.5 ng/mL	1.83×10^{-6}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 36% change in the effect size when controlling for PFOA; selected BMDL was based on a significant regression parameter

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	20.0 ng/mL	3.48×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in comparable BMDLs though there was a 22% change in the effect size when controlling for PFOA; selected BMDL was based on a significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOS concentrations and anti-diphtheria antibody concentrations at ages 7–12	$BMDL_{0.5\ SD}$	5.61 ng/mL	1.03×10^{-6}	BMDL based on model with poor quality of fit; BMDL based on significant regression parameter; BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk
Decreased serum anti-rubella antibody concentration in children or adolescents	Granum et al. (2013) *Medium*	Human, male and female; PFOS concentrations in the mother at delivery and anti-rubella antibody concentrations at age 3	$BMDL_{0.5\ SD}$	1.6 ng/mL	2.79×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk; selected BMDL was based on a significant regression parameter
	Zhang et al. (2023) *Medium*	Human, male and female; PFOS concentrations and anti-rubella antibody concentrations at ages 12–19	$BMDL_{0.5\ SD}$	24.3 ng/mL	4.31×10^{-6}	Selected BMDL was based on a significant regression parameter; BMRs of ½ or 1 SD may not be reasonably good estimates of 5% and 10% extra risk
Decreased PFC response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, PNW 4 F_1 males	$BMDL_{1\ SD}$, Hill	1.8 mg/L $C_{avg\_pup\_gest\_lact}$	2.88×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and presented most protective

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
						BMDL associated with the effect in a sensitive lifestage; AICs from all models were comparable
Extramedullary Hematopoiesis in the Spleen	NTP (2019) *High*	Sprague-Dawley Rats, female, adults	$BMDL_{10RD}$, Multistage Degree 1	2.27 mg/L $C_{last7,avg}$	2.91×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and presented most protective BMDL; all BMDLs from adequate fitting models were comparable
	NTP (2019) *High*	Sprague-Dawley Rats, male, adults	$BMDL_{10RD}$, Logistic	9.59 mg/L $C_{last7,avg}$	1.23×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Developmental Effects						
Decreased Birth Weight	Chu et al. (2020) *High*	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	7.3 ng/mL	1.27×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on significant regression parameter
	Sagiv et al. (2018) *High*	Human, male and female; PFOS serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	41.0 ng/mL	6.00×10^{-6}	Selected BMDL based on non-significant regression parameter
	Starling et al. (2017) *High*	Human, male and female; PFOS serum concentrations in second and third trimesters	$BMDL_{5RD}$, Hybrid	5.7 ng/mL	9.26×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on non-significant regression parameter
	Wikström et al. (2020) *High*	Human, male and female; PFOS serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	7.7 ng/mL	1.13×10^{-6}	Selected BMDL based on significant regression parameter
	Darrow et al. (2013) *High*	Human, male and female, maternal PFOS serum	$BMDL_{5RD}$, Hybrid	17.4 ng/mL	2.51×10^{-6}	Modeled based on first prospective birth analysis (i.e., PFOS concentrations

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
		concentrations taken at time of enrollment in C8 project[d]				measured prior to pregnancy); selected BMDL based on significant regression parameter
	Yao et al. (2021) *High*	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	5.0 ng/L	8.70×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on non-significant regression parameter
Decreased Fetal Body Weight	Lee et al. (2015) *Medium*	CD-1 Mice, F_1 males and females (GD 17)	NOAEL[e] (0.5 mg/kg/day)	8.75×10^{-1} mg/L $C_{avg\_pup\_gest}$	3.40×10^{-4}	No models had adequate fit (residuals at BMD or control were greater than 2, or the BMDL was 3x lower than the lowest tested dose); NOAEL approach taken
Decreased Pup Body Weight	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 1)	$BMDL_{5RD}$, Exponential 3	14.7 mg/L $C_{avg\_pup\_gest}$	5.71×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 5)	$BMDL_{5RD}$, Polynomial Degree 6	2.30 mg/L $C_{avg\_pup\_gest\_lact}$	3.65×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Luebker et al. (2005a) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 1)	$BMDL_{5RD}$, Exponential 4	11.3 mg/L $C_{avg\_pup\_gest}$	4.39×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Decreased Pup Survival	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and female (PND 5)	NOAEL[e] (1 mg/kg/day)	13.0 mg/L $C_{avg\_pup\_gest\_lact}$	2.06×10^{-3}	No models had adequate fit (for all models, all model control response SD was 1.5x greater than actual response SD, and for most models, the calculated BMD was 3x lower than the lowest administered dose); NOAEL approach taken

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and female (PND 22)	NOAEL[e] (1 mg/kg/day)	17.3 mg/L $C_{avg\_pup\_gest\_lact}$	2.75×10^{-3}	No models had adequate fit (for all models, all model control response SD was 1.5x greater than actual response SD, and for most models, the calculated BMD was 3x lower than the lowest administered dose); NOAEL approach taken
Cardiovascular Effects (Serum Lipids)						
Increased Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	$BMDL_{5RD}$, Hybrid	9.34 ng/mL	1.20×10^{-6}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	$BMDL_{5RD}$, Hybrid	9.52 ng/mL	1.22×10^{-6}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Lin et al. (2019) *Medium*	Human, male and female, age 25 and older	$BMDL_{5RD}$, Linear	66.5 ng/mL	8.51×10^{-6}	BMDL based on analyses including individuals prescribed cholesterol medication and non-significant regression parameter
Hepatic Effects						
Elevated ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	56.8 ng/mL	7.27×10^{-6}	BMDL based on significant regression parameter
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	$BMDL_{5RD}$, Hybrid	15.1 ng/mL	1.94×10^{-6}	BMDL based on significant regression parameter
Increased Individual Cell	Butenhoff et al. (2012)/ Thomford (2002b)[f]	Sprague-Dawley Rats, females, adults	$BMDL_{10RD}$, Log-Logistic	27.0 mg/L $C_{last7,avg}$	3.45×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Necrosis in the Liver	*High*					lowest AIC among models with BMD/BMDL ratio < 3

Notes: ALT = alanine aminotransferase; AUC = area under the curve; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 0.5 SD from the control mean; $BMDL_{1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 1 SD from the control mean; $BMDL_{5RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response; $BMDL_{10RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit of a 10% change in response; $C_{avg\_pup\_gest}$ = average blood concentration during gestation; $C_{last7,avg}$ = average blood concentration over the last 7 days; F_1 = first generation; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFC = plaque forming cell; PNW = postnatal week; POD = point of departure; POD_{HED} = point of departure human equivalent dose; RfD = reference dose; SRBC = sheep red blood cell.

[a] See Appendix (U.S. EPA, 2024a) for additional details on BMD modeling.

[b] Supported by Grandjean et al. (2012); Grandjean et al. (2017a); Grandjean et al. (2017b).

[c] Maternal serum concentrations were taken either in the third trimester (32 weeks) or about two weeks after the expected term date.

[d] 99% of the pregnancies of participants in Darrow et al. (2013) were within 3 years of the serum PFOS measurement.

[e] No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.

[f] Butenhoff et al. (2012) and Thomford (2002b) reported the same data.

4.1.4.1 Hepatic Effects

Increased ALT in individuals aged 18 and older (Gallo et al., 2012) or 22 and older (Nian et al., 2019)

The POD for increased ALT in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E.1, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from adults aged 18 years and older (Nian et al., 2019; Gallo et al., 2012), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Individual Cell Necrosis in the Liver, Sprague-Dawley rats, females, $C_{last7,avg}$ (Butenhoff et al., 2012)

Increased incidence of individual cell necrosis in the liver was observed in female Sprague-Dawley Crl:CD(SD)IGS BR rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

4.1.4.2 Immune Effects

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 7 (Budtz-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 7 was derived by quantifying a benchmark dose (see Appendix E.1, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at age 5, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child is governed by the observed ratio between maternal serum and cord blood at delivery. Then the model is run through the 1-year breastfeeding period, where the exposure to the child is only through lactation, which is much greater than the exposure to the mother. After 1 year, the exposure to the child, relative to body weight, is set to the same value as the mother. The model provides predictions up to a child age of 5 years, when the serum concentrations used to

determine the POD were collected, and reverse dosimetry was used to determine the POD_{HED} that results in the POD serum concentration. Because of different growth curves used for male and female children used in the model, the model predicted slightly different (less than 5%) serum concentrations for each. The slightly lower HED in males was then selected as it was the most health protective.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 5 (Budtz-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 5 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother either in the third trimester (32 weeks) or about two weeks after the expected term date, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7, except that the model was only run until the maternal age of 25 years, when delivery occurs in the model. As the POD was based on maternal serum concentrations taken before and after birth, the time of delivery was chosen as an average of the two. Reverse dosimetry was performed on model-predicted maternal serum concentration at that time to calculate the POD_{HED}. This metric is independent of the sex of the child in the model.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at ages 7–12 (Timmermann et al., 2021)

The POD for decreased antibody production in children aged 7–12 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at ages 7–12, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7 (Budtz-Jørgensen and Grandjean, 2018), but the model was run until the median age of this cohort at blood collection, 9.9 years. Reverse dosimetry was used to calculate the POD_{HED} that resulted in a serum level equal to the POD at that age. Because different growth curves specific to male and female children were used in the model, the model predicted slightly different (less than 5%) serum concentrations for each sex. The lower HED was then selected as it was the most health protective.

Decreased Rubella antibody response in vaccinated adolescents at ages 12–19 (Zhang et al., 2023)

The POD for decreased antibody production in adolescents aged 12–19 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at ages 12–19, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For

this, the model was run similarly to the endpoint based on antibodies at age 7 (Budtz-Jørgensen and Grandjean, 2018), but the model was run until the median age of this cohort at blood collection, 15.5 years. Reverse dosimetry was used to calculate the POD_{HED} that resulted in a serum level equal to the POD at that age. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for them. The lower HED was then selected as it was the most health protective.

Decreased Rubella antibody response in vaccinated children at age 3 (Granum et al., 2013)

The POD for decreased antibody production at age 3 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother at delivery, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7, except that the model was only run until the maternal age of 25 years, when delivery occurs in the model. As the POD was based on maternal serum concentrations taken at the time of delivery. Reverse dosimetry was performed on model-predicted maternal serum concentration at that time to calculate the POD_{HED}. This metric was independent of the sex of the child in the model.

Decreased plaque forming cell (PFC) response to SRBC, C57BL/6 Mice, PNW 4 F_1 males, $C_{avg\_pup\_gest\_lact}$ (Zhong et al., 2016)

Decreased mean level of PFC response of splenic cells was observed in F_1 male C57BL/6 mice. Using the Wambaugh et al. (2013) model, daily exposure to PFOS through oral gavage was simulated from GD 1–GD 17 using female CD1 mice parameters (C57BL/6 mice parameters are not available for PFOS; Section 4.1.3.1). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). Continuous models were used to fit dose-response data. A benchmark response (BMR) of a change in the mean equal to 1 SD from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation and lactation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child is governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the 1-year breastfeeding period. The average serum concentration in the infant through gestation and lactation is determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. A male infant was used for this calculation to match the sex of the animals.

Extramedullary hematopoiesis in the spleen, Sprague-Dawley Rats, female and male, $C_{last7,avg}$ (NTP, 2019)

Increased incidence of extramedullary hematopoiesis in the spleen was observed in male and female Sprague-Dawley rats. Using the Wambaugh et al. (2013) model, daily exposure to PFOS through oral gavage was simulated for 28 days using Sprague-Dawley rat parameters (Section 4.1.3.1). The $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

4.1.4.3 Cardiovascular Effects

Increased total cholesterol in individuals aged 20–80, excluding individuals prescribed cholesterol medication (Dong et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from adults aged 20–80 years not prescribed cholesterol medication through the NHANES, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Increased total cholesterol in individuals aged 18 and older, excluding individuals prescribed cholesterol medication (Steenland et al., 2009)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from adults aged 18 years and older not prescribed cholesterol medication from the C8 study population, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day. Specifically, the POD_{HED} was calculated as the external dose (in mg/kg/day) that would result in a steady-state serum concentration equal to the internal serum POD (Section 4.1.3.2). This calculation was the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Increased total cholesterol in individuals aged 25 and older (Lin et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using BMDS (see Appendix E, (U.S. EPA, 2024a)) from the measured PFOS serum concentrations collected

in adults 25 years and older who were at high risk of developing type 2 diabetes and hyperlipidemia from the DPP and Outcomes Study (DPPOS), which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation was the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

4.1.4.4 Developmental Effects

Decreased birthweight using the mother's serum PFOS concentration collected in third trimester (Chu et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother in the third trimester (blood was collected within 3 days after delivery), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This calculation was performed similarly for each of the birthweight endpoints. The model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age. The model was stopped at a time to match the median gestational age of the cohort at sample time for samples taken during pregnancy, or at delivery (25 years maternal age) in the case of maternal samples at delivery or samples of cord blood. Reverse dosimetry was performed to calculate the POD_{HED} resulting in serum levels matching the POD at the model end time. For this study, maternal blood was drawn within a few days of the birth of the child, so delivery was chosen as the model end time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in the first and second trimesters (Sagiv et al., 2018)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother primarily in the first trimester (median gestational age of 9 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 9 weeks. The time after conception was calculated as the fraction of pregnancy competed after 9 weeks (9/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in second and third trimesters (Starling et al., 2017)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother in trimesters 2 and 3 (median gestational age of 27 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 27 weeks. The time after conception was calculated as the fraction of pregnancy completed after 27 weeks (27/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in first and second trimesters (Wikström et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother in the trimesters 1 and 2 (median gestational age of 10 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) study. The model was stopped at the median gestational age of this cohort, 10 weeks. The time after conception was calculated as the fraction of pregnancy completed at 10 weeks (10/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in third trimester (Yao et al., 2021)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother in the third trimester (blood was collected within 3 days of delivery, at hospital admittance), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This calculation was performed similarly for each of the birthweight endpoints. The model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was stopped at a time to match the median gestational age of the cohort at sample time for samples taken during pregnancy, or at delivery in the case of maternal samples at delivery or samples of cord blood. Reverse dosimetry was performed to calculate the POD_{HED} resulting in serum levels

matching the POD at the model end time. For these studies, maternal blood was drawn withing a few days of the birth of the child, so delivery was chosen as the model end time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected at enrollment into the C8 study (Darrow et al., 2013)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother prior to conception, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.2). This was performed as described for the Chu et al. (2020) study. In the selected cohort, blood samples were taken from women before conception. Therefore, the POD_{HED} was calculated based on a maternal age of 24.25 years, prior to any pharmacokinetic effects related to pregnancy. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time.

Decreased Fetal Body Weight, CD-1 Mice, F_1 males and females, $C_{avg\_pup\_gest}$ (Lee et al., 2015)

Decreased mean response of fetal body weight was observed in F_1 male and female CD-1 mice. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (see Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). The BMDS did not produce a model with adequate fit, so a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this endpoint, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Before birth, model predictions for male and female children are equivalent.

Decreased Pup Body Weight, Sprague-Dawley Rats, F_1 male and female (LD 5), $C_{avg\_pup\_gest\_lact}$ (Luebker et al., 2005b)

Decreased mean pup body weight relative to the litter at LD 5 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (see Appendix E, (U.S. EPA,

2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the entire 1-year breastfeeding period Then the model was run through the entire 1-year breastfeeding period because the lactational duration in humans that equates to lactational day 5 in rodents is unknown. Additionally, there is currently no mechanistic information to identify a specific window of susceptibility in lactation for this endpoint. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

Decreased Pup Body Weight, Sprague-Dawley Rats, F_1 male and female (LD 1), $C_{avg\_pup\_gest}$ (Luebker et al., 2005b; Luebker et al., 2005a)

Decreased mean pup body weight relative to the litter at LD 1 (the day of birth) was observed in F_1 male and female Sprague-Dawley rats in 1-generation and 2-generation reproductive studies. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2), and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (see Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Before birth, model predictions for male and female children are equivalent.

Decreased Pup Survival, Sprague-Dawley Rats, F_1 male and female (PND 5 and 22), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2003)

Decreased pup survival at PND 5 and PND 22 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) and a BMR of a change in the mean equal to 0.1 standard deviations from the control mean was provided for comparison purposes because decreased pup survival is a severe, frank effect (U.S.

EPA, 2012a) (see Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). The BMDS did not produce a model with adequate fit, so a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the entire 1-year breastfeeding period for both timepoints because the lactational duration in humans that equates to lactational day 5 in rodents is unknown. Additionally, there is currently no mechanistic information to identify a specific window of susceptibility in lactation for this endpoint. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

4.1.5 Derivation of Candidate Chronic Oral Reference Doses (RfDs)

Though multiple POD_{HED}s were derived for multiple health systems from both epidemiological and animal toxicological studies, EPA selected the POD_{HED}s with the greatest strength of evidence and the lowest risk of bias represented by *high* or *medium* confidence studies for candidate RfD derivation, as described below. For epidemiological studies, similar to the discussion of study selection factors in Section 4 and Section 4.1.1, EPA critically considered attributes for each POD_{HED} including timing of endpoint collection or measurement, uncertainties associated with modeling (see Appendix E (U.S. EPA, 2024a) and Table 4-8), and consideration of confounding. For animal toxicological studies, attributes considered included study confidence (i.e., *high* confidence studies were prioritized over *medium* confidence studies), amenability to benchmark dose modeling, study design, sensitive lifestages, and health effects observed after exposure in the lower dose range among the animal toxicological studies. As described in the subsections below, this examination of epidemiological and toxicological studies led to the exclusion of a number of studies from consideration for candidate RfD derivation. Health outcome- and study-specific considerations are discussed in Sections 4.1.5.1 (Hepatic) 4.1.5.2 (Immune) 4.1.5.3 (Cardiovascular), and 4.1.5.4 (Developmental).

Once studies and their corresponding POD_{HED}s were prioritized for candidate RfD derivation, EPA applied uncertainty factors (UFs) according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). Considerations for individual UFs differed between epidemiological and animal toxicological studies and are further described in Section 4.1.5.5. Presentation of the candidate RfDs for each health outcome is provided in Section 4.1.5.6.

4.1.5.1 Hepatic Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Nian et al., 2019; Gallo et al., 2012). EPA considered both studies as they represented the low-dose range of effects across hepatic endpoints and provided data from relatively large populations, including the U.S. population. Additionally, these studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. The two studies reported analyses examining different forms of confounding factors, sensitivity analyses excluding participants with lifestyle characteristics (e.g., excluding smokers, drinkers, medicine takers) impacting outcome assessment (Nian et al., 2019), and non-linear exposure-response relationships (Gallo et al., 2012). Both studies provided the necessary data for modeling.

One *high* confidence animal toxicological study was carried forward for candidate RfD determination (Butenhoff et al., 2012; Thomford, 2002b). This study was prioritized for candidate RfD development because it was determined to be a *high* confidence study, was amenable to BMD modeling, and was the only animal toxicological study with a chronic exposure duration that histopathologically examined the liver of animals treated with PFOS.

4.1.5.2 Immune Effects

Three *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). EPA considered all three studies as they represented the low-dose range of effects across immunological endpoints and provided data regarding sensitive populations (i.e., children) across three vaccine types. Although EPA derived POD_{HED}s for two time points reported by Budtz-Jørgensen and Grandjean (2018) (i.e., PFOS serum concentrations at age 5 and antibody concentrations at age 7; PFOS serum concentrations in the mother during the third trimester or approximately 2 weeks after the expected term date and antibody concentrations at age 5), EPA did not carry forward POD_{HED}s based on serum PFOS concentrations measured in the mother for candidate RfD derivation because of concerns surrounding potentially increased risk bias due to pregnancy-related hemodynamic effects. Similarly, EPA did not carry forward POD_{HED}s derived from Granum et al. (2013) because PFOS serum concentrations were measured in the mother at the time of delivery and therefore, this study also had potential for increased risk of bias due to pregnancy-related hemodynamic effects. EPA also derived candidate RfDs for both tetanus and diphtheria vaccine responses from Timmerman et al. (2021) for comparison to a second population of children. Zhang et al. (2023) was also selected for candidate RfD derivation because it provided results in adolescents from the U.S. population for a third vaccine type (i.e., rubella). Additionally, the BMDL derived from this study was based on a significant regression parameter. In total, five immunological POD_{HED}s from three epidemiological studies were carried forward for candidate RfD derivation.

Two animal toxicological studies, one *high* and one *medium* confidence, were carried forward for candidate RfD determination (NTP, 2019; Zhong et al., 2016). NTP (2019) is a *high* confidence study reporting the effect of extramedullary hematopoiesis of the spleen in both male and female rats, female rats being marginally more sensitive than males. This effect was accompanied by increased bone marrow hypocellularity, suggesting that PFOS disrupts hematopoiesis in the bone marrow. As extramedullary hematopoiesis was observed in a *high*

confidence study, in both sexes, and was amenable to BMD modeling, this endpoint was carried forward for candidate RfD derivation. The endpoint of reduced PFC response as reported by Zhong et al. (2016) was also selected for candidate RfD derivation because the effect was reported by multiple studies and represented effects in the low-dose range for immune effects reported by animal toxicological studies. In addition, Zhong et al. (2016) reported this effect in pups exposed to PFOS during gestation and therefore encompassed a sensitive population that is coherent with the developmental immunotoxicity observed in humans. For these reasons, EPA determined that both of these effects warranted candidate RfD derivation.

4.1.5.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Dong et al., 2019; Steenland et al., 2009). Of the three studies for which POD_{HED}s were derived, Dong et al. (2019) and Steenland et al. (2009) excluded individuals who were prescribed cholesterol medication, minimizing concerns surrounding confounding due to the medical intervention altering serum total cholesterol levels. This is in contrast to Lin et al. (2019) which did not control for individuals prescribed cholesterol medication and was therefore excluded from further consideration. Modeling of both Dong et al. (2019) and Steenland et al. (2009) resulted in POD_{HED}s with minimal risk of bias, representing both the general population and a high-exposure community, respectively and thus were both considered further for candidate RfD derivation.

4.1.5.4 Developmental Effects

Three *high* confidence epidemiological studies were carried forward for candidate RfD determination for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018; Darrow et al., 2013). Of the six epidemiological studies for which POD_{HED}s were derived, Darrow et al. (2013), Sagiv et al. (2018), and Wikström et al. (2020) assessed maternal PFOS serum concentrations either prior to conception or primarily in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Although Wikström et al. (2020) collected approximately 4% of samples during early weeks of the second trimester, sensitivity analyses showed no differences when trimester two samples were excluded. Additionally, these studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias and reflected two different study populations. Therefore, all three studies were considered further for candidate RfD derivation. The three excluded studies assessed PFOS concentrations in either umbilical cord blood or primarily during the second or third trimesters, increasing the uncertainty associated with the derived POD_{HED}s due to potential pregnancy-related hemodynamic effects, and as a result, were excluded from consideration for candidate RfD derivation (Yao et al., 2021; Chu et al., 2020; Starling et al., 2017).

One *medium* confidence animal toxicological study was carried forward for candidate RfD determination (Luebker et al., 2005b). The endpoint of reduced pup weight at LD 5 from this study was amenable to benchmark dose modeling (i.e., BMD modeling produced viable model fits), unlike the endpoints of decreased fetal weight reported by Lee et al. (2015) and decreased pup survival reported by Lau et al. (2003), which had NOAELs as the basis of the POD_{HED}s. Decreased pup weight at LD 5 was selected over the other time point reported by Luebker et al. (2005b) (i.e., LD 1) and decreased pup weight reported by Luebker et al. (2005a) (also LD 1)

because it was the most protective of the three POD_{HED}s, all of which were derived from BMDLs. The endpoint of decreased pup weight reported by Luebker et al. (2005b) encompassed a sensitive population and was coherent with the observed effect of decreased birth weight in humans and was therefore selected for candidate RfD derivation.

4.1.5.5 Application of Uncertainty Factors

To calculate the candidate RfD values, EPA applied UFs to the POD_{HED}s derived from selected epidemiological and animal toxicological studies (Table 4-9 and Table 4-10). UFs were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b).

Table 4-9. Uncertainty Factors for the Development of the Candidate Chronic RfD Values from Epidemiological Studies (U.S. EPA, 2002b)

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to effects observed in epidemiological studies as the study population is humans.
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1	A UF_S of 1 is applied when effects are observed in adult human populations that are assumed to have been exposed to a contaminant over the course of many years. A UF_S of 1 is applied for developmental effects because the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF_D	1	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterization of the contaminant's toxicity.
UF_C	10	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = composite uncertainty factors.

An interspecies UF (UF_A) of 1 was applied to POD_{HED}s derived from epidemiological studies because the dose-response information from these studies is directly relevant to humans. There is no need to account for uncertainty in extrapolating from laboratory animals to humans.

An intraspecies UF (UF_H) of 10 was applied to POD_{HED}s derived from epidemiological studies to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, lifestage, and health status) and extrinsic (lifestyle) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to quantitatively characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation UF (UF_L) of 1 was applied to POD_{HED}s derived from epidemiological studies because a BMDL is used as the basis for the POD_{HED} derivation. When

the POD type is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling.

A UF for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 1 was applied to POD_{HED}s derived from epidemiological studies. A UF_s of 1 was applied to the hepatic and cardiovascular endpoints because the effects were observed in adult populations that were assumed to have been exposed to PFOS over the course of many years. A UF_s of 1 was applied to the developmental endpoints because the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). A UF_S of 1 was also applied to the immune endpoints observed in children and adolescents because exposure is assumed to occur from gestation through childhood, when the response variable was measured. There is uncertainty regarding the critical window of exposure that results in these immune effects in children and adolescents. Therefore, EPA expects that any exposure during this period of development has the potential to impact this response (U.S. EPA, 1991). According to the WHO/International Programme on Chemical Safety (IPCS) *Immunotoxicity Guidance for Risk Assessment*, developmental immunotoxicity is assessed during the prenatal, neonatal, juvenile and adolescent life stages because immune system development occurs throughout these life stages and should be viewed differently in part due to increased susceptibility compared with the immune system of adults from a risk assessment perspective (IPCS, 2012).

A database UF (UF_D) of 1 was applied to account for deficiencies in the database for PFOS. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer-reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a large number of *medium* and *high* confidence epidemiological studies which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. Effects identified in developmental and multigenerational reproductive toxicity studies have been quantitatively considered in this assessment.

The composite UF applied to all epidemiological studies considered for candidate RfD derivation were the same value ($UF_C = 10$) (Table 4-9).

Increased uncertainty is associated with the use of animal toxicological studies as the basis of candidate RfDs. The composite UF applied to animal toxicological studies considered for candidate RfD derivation were either one of two values, depending on the duration of exposure (i.e., chronic vs. subchronic) or exposure window (e.g., gestational) (Table 4-10).

Table 4-10. Uncertainty Factors for the Development of the Candidate Chronic RfD Values From Animal Toxicological Studies (U.S. EPA, 2002b)

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied for the extrapolation from animal models to humans due to the implementation of a PK model for animal POD_{HED} derivation.

UF	Value	Justification
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1 or 10	A UF_S of 10 is applied for the extrapolation of subchronic-to-chronic exposure durations. A UF_S of 1 is applied to studies with chronic exposure durations or that encompass a developmental period (i.e., gestation). The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF_D	1	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various lifestages and populations and allow for a complete characterization of the contaminant's toxicity.
UF_C	30 or 300	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = composite uncertainty factors.

A UF_A of 3 was applied to POD_{HED}s derived from animal toxicological studies to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). The threefold factor is applied to account for toxicodynamic differences between the animals and humans. The HEDs were derived using a model that accounted for PK differences between animals and humans.

A UF_H of 10 was applied to POD_{HED}s derived from animal toxicological studies to account for variability in the responses within human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, lifestage, and health status) and extrinsic (lifestyle) factors can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A UF_L of 1 was applied to POD_{HED}s derived from animal toxicological studies because a BMDL was used as the basis for the POD_{HED} derivation. BMDLs were available for all animal toxicological endpoints and studies advanced for candidate RfD derivation.

A UF_S of 1 was applied to POD_{HED}s derived from chronic animal toxicological studies as well as animal toxicological studies that encompass a developmental period (i.e., gestation). A UF_s of 1 was applied to developmental endpoints because the developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). A UF_s of 10 was applied to POD_{HED}s derived from studies that implemented a less-than-chronic exposure duration because extrapolation is required to translate from a subchronic POD_{HED} to a chronic RfD.

A UF_D of 1 was applied to account for deficiencies in the database for PFOS. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer-reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and

developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a large number of *medium* and *high* confidence epidemiological studies which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. Effects identified in developmental and multigenerational reproductive toxicity studies have been quantitatively considered in this assessment.

In summary, the composite UF that was applied to candidate RfDs derived from all of the epidemiological studies were the same value (UF_C = 10) (Table 4-9). The composite UF that was applied to candidate RfDs derived from animal toxicological studies was either UF_C = 30 or 300 (Table 4-10). In all of these cases, the total uncertainty is well below the maximum recommended UF_C = 3,000 (U.S. EPA, 2002b).

4.1.5.6 Candidate RfDs

Table 4-11 shows the UFs applied to each candidate study to subsequently derive the candidate RfDs.

Table 4-11. Candidate Reference Doses (RfDs)

Endpoint	Reference, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_{TOT}	Candidate RfD[a] (mg/kg/day)
Immune Effects										
Decreased Serum Anti-Tetanus Antibody Concentration in Children	Budtz-Jørgensen and Grandjean (2018) *Medium*	Human, male and female, PFOS concentrations at age 5 and antibody concentrations at age 7	2.71×10^{-6}	1	10	1	1	1	10	$2.71 \times 10^{-7} = 3 \times 10^{-7}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOS and antibody concentrations at age 7–12	1.78×10^{-6}	1	10	1	1	1	10	$1.78 \times 10^{-7} = 2 \times 10^{-7}$
Decreased Serum Anti-Diphtheria Antibody Concentration in Children	Budtz-Jørgensen and Grandjean (2018) *Medium*	Human, male and female, PFOS concentrations at age 5 and antibody concentrations at age 7	1.83×10^{-6}	1	10	1	1	1	10	$1.83 \times 10^{-7} = 2 \times 10^{-7}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOS and antibody concentrations at age 7–12	1.03×10^{-6}	1	10	1	1	1	10	$1.03 \times 10^{-7} = 1 \times 10^{-7}$
Decreased Serum Anti-Rubella Antibody Concentration in Adolescents	Budtz-Jørgensen and Grandjean (2018) *Medium*	Human, male and female, PFOS and antibody concentrations at age 12–19	4.31×10^{-6}	1	10	1	1	1	10	$4.31 \times 10^{-7} = 4 \times 10^{-7}$
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, PNW 4 F_1 males	2.88×10^{-4}	3	10	1	1	1	30	$9.60 \times 10^{-6} = 1 \times 10^{-5}$
Extramedullary Hematopoiesis in the Spleen	NTP (2019) *High*	Sprague-Dawley rats, female, adults	2.91×10^{-4}	3	10	10	1	1	300	$9.70 \times 10^{-7} = 1 \times 10^{-6}$
Developmental Effects										
Decreased Birth Weight	Sagiv et al. (2018) *High*	Human, male and female, PFOS concentrations in first and second trimesters	6.00×10^{-6}	1	10	1	1	1	10	$6.00 \times 10^{-7} = 6 \times 10^{-7}$

Endpoint	Reference, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_{TOT}	Candidate RfD[a] (mg/kg/day)
	Wikström et al. (2020) *High*	Human, male and female, PFOS concentrations in first and second trimesters	1.13×10^{-6}	1	10	1	1	1	10	$1.13 \times 10^{-7} = 1 \times 10^{-7}$
	Darrow et al. (2013) *High*	Human, male and female, PFOS concentrations at time of enrollment[b]	2.51×10^{-6}	1	10	1	1	1	10	$2.51 \times 10^{-7} = 3 \times 10^{-7}$
Decreased Pup Body Weight	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 5)	3.65×10^{-4}	3	10	1	1	1	30	$1.22 \times 10^{-5} = 1 \times 10^{-5}$
Cardiovascular Effects										
Increased Serum Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, ages 20-80	1.20×10^{-6}	1	10	1	1	1	10	$1.20 \times 10^{-7} = 1 \times 10^{-7}$
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	1.22×10^{-6}	1	10	1	1	1	10	$1.22 \times 10^{-7} = 1 \times 10^{-7}$
Hepatic Effects										
Increased Serum ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	7.27×10^{-6}	1	10	1	1	1	10	$7.27 \times 10^{-7} = 7 \times 10^{-7}$
	Nian et al. (2019) *Medium*	Human, female, at age 22 and older	1.94×10^{-6}	1	10	1	1	1	10	$1.94 \times 10^{-7} = 2 \times 10^{-7}$
Individual Cell Necrosis in the Liver	Butenhoff et al. (2012)/Thomford (2002b)[c] *High*	Sprague-Dawley rats, females, adults	3.45×10^{-3}	3	10	1	1	1	30	$1.15 \times 10^{-4} = 1 \times 10^{-4}$

Notes: ALT = alanine transaminase; UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic extrapolation uncertainty factor; UF_L = extrapolation from a LOAEL to a NOAEL uncertainty factor; UF_{TOT} = composite uncertainty factor.

[a] RfDs were rounded to one significant figure.

[b] 99% of the pregnancies of participants in Darrow et al. (2013) were within 3 years of the serum PFOS measurement.

[c] Butenhoff et al. (2012) and Thomford (2002b) reported data from the same experiment.

4.1.6 RfD Selection

As presented in Section 4.1.5 (Table 4-11), EPA derived and considered multiple candidate RfDs across the four noncancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental). EPA derived candidate RfDs based on both epidemiological and animal toxicological studies. As depicted in Figure 4-3, the candidate RfDs derived from epidemiological studies were all within 1 order of magnitude of each other (10^{-6} to 10^{-7} mg/kg/day), regardless of endpoint, health outcome, or study population.

Candidate RfDs derived from animal toxicological studies were generally 2–3 orders of magnitude higher than candidate RfDs derived from epidemiological studies. However, EPA does not necessarily expect concordance between animal and epidemiological studies in terms of the adverse effect(s) observed or the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" (U.S. EPA, 1991). Additionally, for developmental effects, the guidance says that "the experimental animal data were generally predictive of adverse developmental effects in humans, but in some cases, the administered dose or exposure level required to achieve these adverse effects was much higher than the effective dose in humans" (U.S. EPA, 1991).

As shown in Table 4-11 and Figure 4-3, there is greater uncertainty associated with the use of animal toxicological studies as the basis of RfDs than human epidemiological studies. Though there are some uncertainties in the use of epidemiological studies for quantitative dose-response analyses (see Sections 5.1, 5.6, and 5.7), human data eliminate the uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species which are major uncertainties associated with the PFOS animal toxicological studies due to the half-life differences and sex-specific toxicokinetic differences in rodent species These uncertainties may explain, in part, the higher magnitude of candidate RfDs derived from animal toxicological studies compared to the candidate RfDs derived from epidemiological studies. Moreover, the human epidemiological studies also have greater relevance to human exposure than animal toxicological studies because they directly measure environmental or serum concentrations of PFOS. In accordance with EPA's current best practices for systematic review, "animal studies provide supporting evidence when adequate human studies are available, and they are considered to be the studies of primary interest when adequate human studies are not available" (U.S. EPA, 2022d). For these reasons, EPA determined that candidate RfDs based on animal toxicological studies would not be further considered for health outcome-specific RfD selection or overall RfD selection. See Section 5.2 for further comparisons between toxicity values derived from epidemiological and animal toxicological studies.

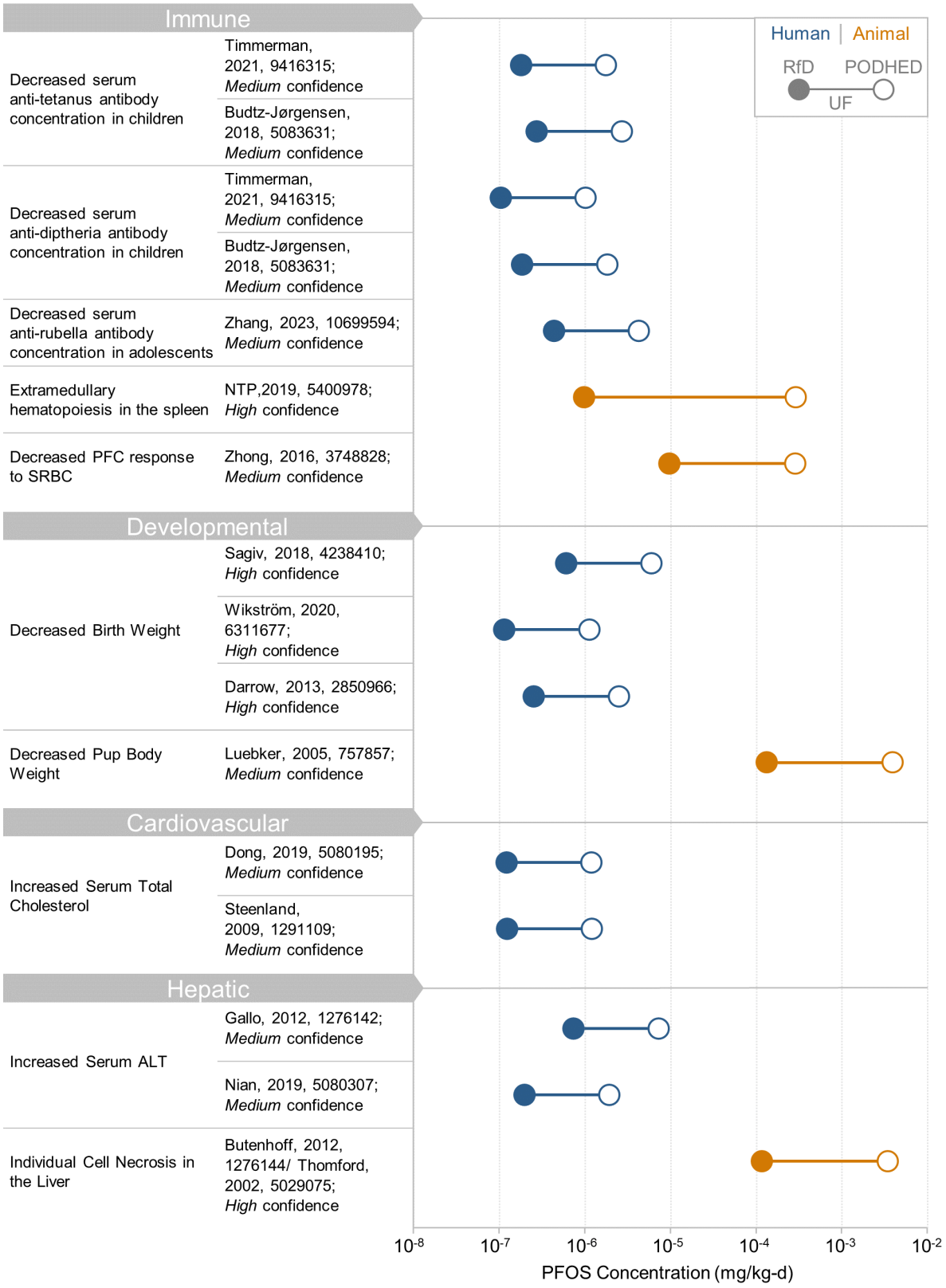


Figure 4-3. Comparison of Candidate RfDs Resulting from the Application of Uncertainty Factors to POD_{HED}s Derived from Epidemiological and Animal Toxicological Studies

As described in the subsections below, EPA selected amongst the candidate RfDs to identify an RfD representative of each of the four prioritized health outcomes (i.e., health outcome-specific RfDs), as well as an overall RfD that is protective of the effects of PFOS on all health outcomes and endpoints (Figure 4-4).

4.1.6.1 Health Outcome-Specific RfDs

At least two candidate RfDs were derived from epidemiological studies for each of the four prioritized noncancer health outcomes. EPA considered several factors when selecting health outcome-specific RfDs, including relevance of exposure or population characteristics to the general population, potential confounding factors, and characteristics of the modeled data. Health outcome- and study-specific considerations are discussed in Sections 4.1.6.1.1 (Hepatic), 4.1.6.1.2 (Immune), 4.1.6.1.3 (Cardiovascular), and 4.1.6.1.4 (Developmental), below.

4.1.6.1.1 Hepatic Effects

Two *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased ALT (Nian et al., 2019; Gallo et al., 2012). The larger study of PFOS and ALT in adults (Gallo et al., 2012) was conducted in over 30,000 adults from the C8 Study. The other study (Nian et al., 2019) examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project and observed significant increases in lognormal ALT per each ln-unit increase in PFOS, as well significant increases in odds ratios of elevated ALT. The candidate RfD for increased ALT from Nian et al. (2019) was ultimately selected as the health outcome-specific RfD for hepatic effects because PFOS was the predominating PFAS in this study which reduces concern about potential confounding by other PFAS in the population of interest. The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-4). Note that both candidate RfDs based on epidemiological studies for the hepatic outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.2 Immune Effects

Candidate RfDs were derived from three *medium* confidence epidemiological studies for the endpoint of decreased antibody production in response to various vaccinations in children (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). Candidate RfDs derived from Timmerman et al. (2021) were considered lower confidence candidate RfDs than those derived from Budtz-Jørgensen and Grandjean (2018). POD_{HED}s derived from Timmerman et al. (2021) were considered to have increased uncertainty compared with Budtz-Jørgensen and Grandjean (2018) due to two features of the latter study that strengthen the confidence in the POD_{HED}s: 1) the response reported by this study was more precise in that it reached statistical significance, and 2) the analysis considered co-exposures of other PFAS. Therefore, the candidate RfDs from Timmerman et al. (2021) were not considered for selection as the health outcome-specific RfD. Similarly, the candidate RfD derived from Zhang (2023) was also not considered since the analysis did not consider co-occurring PFAS and the resulting health outcome-specific RfD would be less protective.

The RfD for anti-diphtheria responses in 7-year-old Faroese children from Budtz-Jørgensen and Grandjean (2018) was ultimately selected as the basis for the health outcome-specific RfD for immune effects because the POD_{HED} were based on models with adequate quality of fit and

significant regression parameters, the analysis considered co-exposures of other PFAS and indicated minimal potential for confounding in the value of the POD_{HED} due to PFOA, and the response was more consistently observed across the two time points reported in the study between the two vaccine-specific responses reported by Budtz-Jørgensen and Grandjean (2018). The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-4). Note that all candidate RfDs based on epidemiological studies for the immune outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased total cholesterol (Dong et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Both of these studies excluded individuals prescribed cholesterol medication which minimizes concerns of confounding due to medical intervention. The candidate RfD for increased TC from Dong et al. (2019) was ultimately selected for the health outcome-specific RfD for cardiovascular effects as there is marginally increased confidence in the modeling from this study. Steenland et al. (2009) presented analyses using both PFOS and TC as categorical and continuous variables. The results using the natural log transformed TC and the natural log transformed PFOS were stated to fit the data slightly better than the ones using untransformed PFOS. However, the dramatically different changes in regression slopes between the two analyses by Steenland et al. (2009) resulting in different PODs raise concerns about the appropriateness of using the data for RfD derivation. Therefore, the resulting health outcome-specific RfD based on results from Dong et al. (2019) is 1×10^{-7} mg/kg/day (Figure 4-4). Note that the candidate RfDs for the cardiovascular outcome were the same.

4.1.6.1.4 Developmental Effects

Three *high* confidence epidemiological studies were considered for candidate RfD derivation for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018; Darrow et al., 2013). These candidate studies assessed maternal PFOS serum concentrations before birth (Darrow et al., 2013) or primarily in the first trimester (Wikström et al., 2020; Sagiv et al., 2018) minimizing concerns for bias due to pregnancy-related hemodynamic effects. All three studies were *high* confidence prospective cohort studies with many strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. Between these three studies, PFOS exposure concentrations observed in Wikström et al. (2020) are more comparable to current exposure levels in the United States and therefore may be more relevant to the general population than the candidate RfD derived from Sagiv et al. (2018) or Darrow et al., (2013). Additionally, the BMDL derived from Wikström et al. (2020) was based on a statistically significant regression parameter. For these reasons, the RfD for decreased birth weight from Wikström et al. (2020) was selected as the basis for the organ-specific RfD for developmental effects. The resulting health outcome-specific RfD is 1×10^{-7} mg/kg/day (Figure 4-4). Note that all three candidate RfDs based on epidemiological studies for the developmental outcome were within one order of magnitude of the selected health outcome-specific RfD.

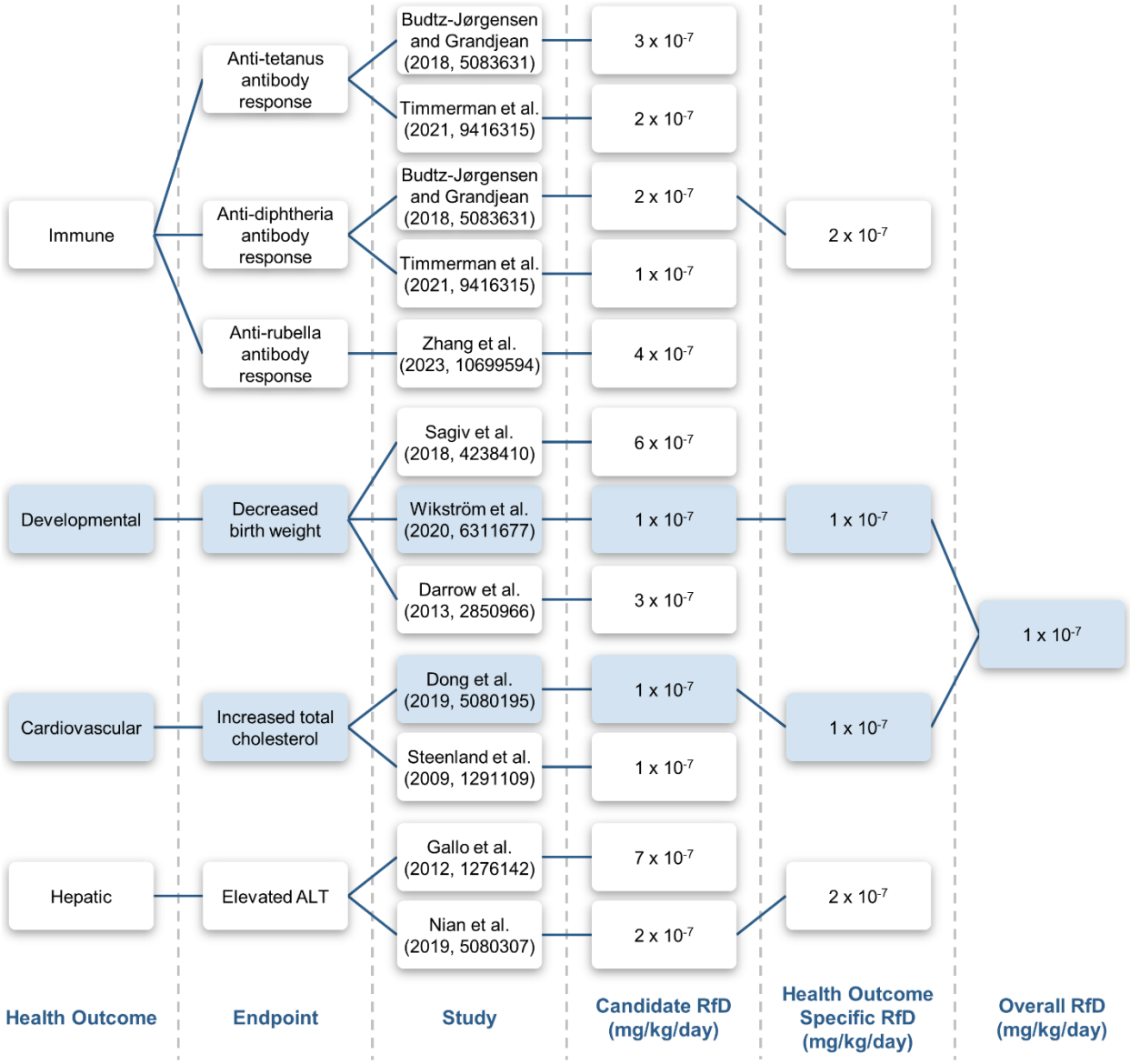


Figure 4-4. Schematic Depicting Selection of the Overall RfD for PFOS

4.1.6.2 Overall Noncancer RfD

The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure. In fact, candidate RfDs within the developmental and cardiovascular outcomes are the same value (i.e., 1×10^{-7} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOS of 1×10^{-7} mg/kg/day (Figure 4-4). The developmental and cardiovascular RfDs based on endpoints of decreased birth weight and increased total cholesterol, respectively, serve as co-critical effects for this RfD. Notably, the RfD is protective of effects that may occur in sensitive populations (i.e., infants and children; see Section 5.8), as well as immune and hepatic effects that may result from PFOS exposure. As one of the co-critical effects identified for PFOS is a developmental endpoint and can potentially result from a short-term exposure during critical

periods of development, EPA concludes that the overall RfD for PFOS is applicable to both short-term and chronic risk assessment scenarios.

The critical studies that serve as the basis of the RfD are all *medium* or *high* confidence epidemiological studies. The critical studies are supported by multiple other *medium* or *high* confidence studies in both humans and animal models and have health outcome databases for which EPA determined *evidence indicates* that oral PFOS exposure is associated with adverse effects. Additionally, the selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans.

4.2 Cancer

As described in the introduction of Section 3, there is evidence from both epidemiological and animal toxicological studies that oral PFOS exposure may result in adverse health effects across many health outcomes, including cancer (Section 3.5). In Section 3.5.5, EPA concluded that PFOS is *Likely to Be Carcinogenic to Humans* in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Therefore, the quantification of cancer effects was prioritized along with the four noncancer health outcomes that are described in Section 4.1. EPA considered only *high* or *medium* confidence human and animal toxicological studies for CSF derivation.

4.2.1 Study and Endpoint Selection

Human studies selected for CSF derivation reported all necessary analytical information (e.g., exposure distribution or variance) for the outcome of interest (any cancer). If available, *high* and *medium* confidence studies with exposures levels near the range of typical environmental human exposures, especially exposure levels comparable to human exposure in the general population, were preferred over studies reporting considerably higher exposure levels. Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to exposure levels that may be more relevant to the U.S. general population. Additionally, the most recent and comprehensive publication on a single study population was preferred over prior publications on the same or portions of the same population.

Preferred animal toxicological studies consisted of *medium* and *high* confidence studies with chronic exposure durations to capture potential latency of cancer effects. Studies with exposure durations during development (e.g., gestation) were also considered informative for assessing potential early lifestage susceptibility to cancer. Studies encompassing lower dose ranges were also preferred. These types of animal toxicological studies increase the confidence in the CSF relative to other animal toxicological studies because they are based on data with relatively low risk of bias, have sufficient study designs to observe the critical effects, and are associated with less uncertainty related to low-dose and exposure duration extrapolations.

4.2.1.1 Epidemiological Studies

The available epidemiology studies report elevated risk of liver, bladder, kidney, prostate, and breast cancers after chronic PFOS exposure in some studies, though limited evidence for some tumor types (i.e., liver and renal) and mixed results for other tumor types (i.e., bladder, prostate, breast) provide plausible but not definitively causal evidence of a relationship between PFOS

exposure and cancer outcomes from the epidemiological evidence alone. The animal chronic cancer bioassay provides additional support for carcinogenicity with the identification of multi-site tumorigenesis (liver and pancreas) in both male and female rats.

The limited renal or mixed results (breast, bladder, prostate) preclude definitive conclusions about the relationship between PFOS exposure and these cancer outcomes in humans and therefore limits the potential for quantitative assessment of these data. For example, Shearer et al., (2021) is a *medium* confidence study which suggests an association between PFOS and increased kidney cancer. However, it is the only study indicating an association for kidney cancer. Furthermore, the magnitude of the association between PFOS and kidney cancer was lower than that for PFOA and after adjustment for other PFAS, the adjusted OR for the highest quartile was relatively low in magnitude and not statistically significant. For these reasons, Shearer et al., 2021 was not considered for CSF derivation. Additionally, the breast cancer studies provide mixed evidence, with associations between PFOS and breast cancer observed in some studies, but only in specific groups of participants or for certain sub-types of breast cancer. Without plausible evidence for MOAs that inform these responses in specific populations, there is not strong support for quantitative analyses of these studies.

Recently published studies have provided additional evidence of an increased risk of liver cancer with PFOS exposure. Importantly, these data are concordant with the liver tumors observed in the published rodent studies (Butenhoff et al., 2012; Thomford, 2002a), providing cross-stream concordance for liver cancer which strengthens the weight of evidence for this endpoint. Results from publications considered in the 2016 PFOS HESD (U.S. EPA, 2016b), a *low* confidence occupational study (Alexander et al., 2003) and a *medium* confidence general population-based study (Eriksen et al., 2009), investigating associations between liver cancer and PFOS exposure reported non-significant associations, though these studies were considered imprecise (i.e., null results with wide confidence intervals). Recently, statistically significant increased risk of liver cancer has been reported in two additional studies, a *medium* confidence nested case-control study in the U.S. (Goodrich et al., 2022) and a *low* confidence general population study in China (Cao et al., 2022). Given the concordance of tumor site between these studies in humans and the available animal toxicological study, discussed further in Section 4.2.1.2, EPA considered liver cancer reported by Goodrich et al. (2022) for CSF derivation. EPA did not consider Cao et al. (2022) as there were several concerns with this study, including: the potential for selection bias due to lack of information on case recruitment and on source of healthy controls; uncertainties related to outcome assessment due to lack of liver cancer diagnosis detail; and potential for residual confounding because the list of confounders included in PFAS and liver cancer analyses was not provided. These concerns resulted in *low* confidence rating.

Goodrich et al. (2022), is a *medium* confidence study which reported on a small, nested case-control study of adults from the large Multiethnic Cohort (MEC) in California and Hawaii. The study examined incident non-viral hepatocellular carcinoma cases and individually matched controls (Goodrich et al., 2022). EPA identified several factors that also precluded use of Goodrich et al. (2022) from dose-response analyses. First, there was a lack of association observed in continuous analyses of PFOS exposure indicating a lack of dose-response. Thus, the study lacks a precise estimate of the slope needed for POD derivation. Second, the elevated risk in this study was observed only in analyses comparing participants with PFOS concentrations at or above the 85th percentile of PFOS (i.e., 54.9 μg/L). This indicates that only the highest

exposure group demonstrated a response, making 54.9 µg/L PFOS the LOAEL. With only a LOAEL from this dataset, EPA is unable to conduct a low-dose linear extrapolation or derive a CSF. Lastly, the elevated exposure level at which the response was observed in this study is outside the reported PFOS environmental human exposures ranges typical for U.S. and international populations. For example, the mean 90th percentile PFOS serum concentration from the 2017–2018 NHANES cycle was 11.5 µg/L. The small sample size for the study (50 cases and 50 controls) may have limited the study's sensitivity. For these reasons, Goodrich et al. (2022) was not selected for CSF derivation.

4.2.1.2 Animal Toxicological Studies

A single *high* confidence animal chronic cancer bioassay comprises the animal toxicological evidence database for the carcinogenicity of PFOS. This *high* confidence chronic cancer bioassay study, first published as an industry-sponsored report (Thomford, 2002b) and later published as a peer-reviewed journal article (Butenhoff et al., 2012) provides evidence of multisite tumorigenesis in male and female rats.

Hepatocellular tumors were observed in both male and female rats (Butenhoff et al., 2012). In males, there was a statistically significant increase in the incidence of hepatocellular adenomas in the highest dose group tested (20 ppm or approximately 1 mg/kg/day) and a significant trend of increased incidence with increasing PFOS dose. A similar response was observed in females, with the addition of one incidence of hepatocellular carcinoma in a rat from the highest dose group tested (20 ppm or approximately 1.25 mg/kg/day). As these tumors were observed in both sexes with similar sensitivity and since this effect is concordant with the associations between PFOS and liver cancer observed in humans, the endpoints of hepatocellular adenomas in male rats and hepatocellular adenomas or carcinomas in female rats were both selected for candidate CSF derivation.

Increased incidence of pancreatic islet cell tumors were also observed in male rats (Butenhoff et al., 2012). Though there were similar incidences of islet cell adenomas in control and PFOS-treated rats, there was a statistically significant trend of increased incidence of islet cell carcinomas with increasing PFOS dose. EPA additionally selected the incidence of pancreatic islet cell carcinomas in male rats for candidate CSF derivation as this is a malignant tumor and appears to be similar in sensitivity as the hepatocellular tumors observed in male and female rats. EPA also considered incidences of combined islet cell adenomas and carcinomas for quantitative analyses, the modeling for which is presented in Appendix E (U.S. EPA, 2024a) but was not selected for candidate CSF derivation because there was no dose-response relationship observed with the adenomas alone and combining the two tumor types resulted in a slight attenuation of the effect, evidenced by a loss of the statistically significant trend of response.

4.2.2 Candidate CSF Derivation

As described above, EPA did not identify epidemiological studies suitable for CSF derivation. However, EPA derived PODs and candidate CSFs for four endpoints reported by Thomford (2002b)/Butenhoff et al. (2012): hepatocellular adenomas in male rats; hepatocellular adenomas in female rats; combined hepatocellular adenomas and carcinomas in female rats; and pancreatic islet cell carcinomas in male rats (Table 4-12). As noted in Table 3-18, EPA expressed tumor incidence as the number of animals with reported tumors over the number of animals alive at the

time of first occurrence of the tumor. Expressing incidence in this way quantitatively eliminates animals that died prior to the PFOS treatment duration plausibly required to result in tumor formation in the critical study. For comparison purposes, EPA presents BMDLs derived using the number of animals in each dose group at the start of the study in Appendix E (U.S. EPA, 2024a). All BMDLs were derived using the BMDS 3.2 program.

Multistage models were used consistent with the longstanding practice of EPA to prefer multistage models to fit tumor dose-response data (U.S. EPA, 2005a) and a BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a). EPA selected the AUC averaged over the study duration (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, as the dose metric for modeling cancer endpoints. This is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the IRIS Handbook (U.S. EPA, 2022d), which recommend the cumulative dose received over a lifetime as the measure of exposure to a carcinogen when modeling chronic cancer effects. The BMDS produced a BMDL in mg/L. The animal POD was converted to a POD_{HED} by multiplying the POD by the human clearance value (Table 4-6). This POD_{HED} is equivalent to the constant exposure, per body weight, that would result in serum concentration equal to the POD at steady state. The CSF is then calculated by dividing the BMR of 10% by the POD_{HED}.

Table 4-12. Cancer Slope Factors Derived From Results Reported by Butenhoff et al. (2012)/Thomford (2002b)[a] in Sprague-Dawley Rats

Tumor Type	Sex	POD Type, Model	POD Internal Dose /Internal Dose Metric[b]	POD_{HED}	Candidate CSF (BMR/POD_{HED})	Notes on Modeling
Hepatocellular Adenomas	Male	$BMDL_{10}$ Multistage Degree 4 Model	25.6 mg/L (AUC normalized per day (AUC_{avg}))	3.28×10^{-3} mg /kg/day	30.5 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
Hepatocellular Adenomas	Female	$BMDL_{10}$ Multistage Degree 1 Model	21.8 mg/L (AUC normalized per day (AUC_{avg}))	2.79×10^{-3} mg /kg/day	35.8 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
Combined Hepatocellular Adenomas and Carcinomas	Female	$BMDL_{10}$ Multistage Degree 1 Model	19.8 mg/L (AUC normalized per day (AUC_{avg}))	2.53×10^{-3} mg /kg/day	39.5 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
Pancreatic Islet Cell Carcinomas	Male	$BMDL_{10}$ Multistage Degree 1 Model	26.1 mg/L (AUC normalized per day (AUC_{avg}))	3.34×10^{-3} mg /kg/day	29.9 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.

Notes: $BMDL_{10}$ = benchmark dose level corresponding to the 95% lower confidence limit of a 10% change.
[a] Butenhoff et al. (2012) and Thomford (2002b) reported data from the same experiment.
[b] See Appendix (U.S. EPA, 2024a) for additional details on benchmark dose modeling.

4.2.3 Overall CSF Selection

EPA selected the hepatocellular adenomas and carcinomas in female rats reported by Butenhoff et al. (2012)/Thomford (2002b) as the basis of the overall CSF for PFOS. This endpoint was selected because: 1) there is concordance between the observed hepatocellular tumors in rats with the liver cancer observed in human epidemiological studies; 2) the derived candidate CSF is representative of both malignant and benign tumors; 3) the endpoint is supported by the observation of hepatocellular adenomas in male rats; 4) there was a statistically significant increase in tumor incidence in the highest dose group; and 5) a statistically significant trend of increased incidence with increasing PFOS concentrations across dose groups. The resulting CSF is 39.5 $(mg/kg/day)^{-1}$.

4.2.4 Application of Age-Dependent Adjustment Factors

EPA's *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* require the consideration of applying age-dependent adjustment factors (ADAFs) to CSFs to address potential increased risk for cancer due to early lifestage susceptibility to chemical exposure (U.S. EPA, 2005a, b). ADAFs are only to be used for carcinogenic chemicals with a mutagenic MOA when chemical-specific data about early-life susceptibility are lacking. For carcinogens with any MOA, including mutagens and non-mutagens, but with available chemical-specific data for early-life exposure, those data should be used.

As described in Section 3.5.3.1.1, the limited number of in vivo and in vitro studies assessing mutagenicity following PFOS exposure were primarily negative. Therefore, EPA has determined that PFOS is unlikely to cause tumorigenesis via a mutagenic MOA. Given the lack of evidence of a mutagenic MOA, EPA does not recommend applying ADAFs when quantitatively determining the cancer risk for PFOS (U.S. EPA, 2011a).

Additionally, there is insufficient information available from epidemiological and animal toxicological studies to adequately determine whether PFOS exposure during early-life periods, per EPA's above-referenced supplemental guidance, may increase incidence or reduce latency for cancer compared with adult-only exposure. No current studies allow for comparisons of cancer incidence after early-life versus adult-only PFOS exposure.

5 Effects Characterization

5.1 Addressing Uncertainties in the Use of Epidemiological Studies for Quantitative Dose-Response Analyses

In the 2016 *Health Effects Support Document for Perfluorooctane Sulfonate* (PFOS) and Drinking Water Health Advisory (U.S. EPA, 2016a, b), the U.S. Environmental Protection Agency (EPA) qualitatively considered epidemiological studies as a supporting line of evidence but did not quantitatively consider them for point-of-departure (POD) derivation, citing the following as reasons to exclude the epidemiological data that were available at that time from quantitative analyses:

- Unexplained inconsistencies in the epidemiological database,
- The use of mean serum PFOS concentrations rather than estimates of exposure,
- Declining serum PFOS values in the U.S. general population over time (CDC, 2017),
- Uncertainties related to potential exposure to additional PFAS, telomer alcohols that metabolically break down into PFOS, and other bio-persistent contaminants, and
- Uncertainties related to the clinical significance of effects observed in epidemiological studies.

Since 2016, EPA has identified many additional epidemiology studies that have increased the database of information for PFOS (see Sections3.1.1, 3.4, and 3.5). Further, new tools that have facilitated the use of study quality evaluation as part of systematic review have enabled EPA to systematically assess studies in a way that includes consideration of confounding. As a result, EPA is now in a position to be able to quantitatively consider epidemiological studies of PFOS for POD derivation in this assessment.

In this assessment EPA has assessed the strength of epidemiological and animal evidence following current agency best practices for systematic review (U.S. EPA, 2022d), a process that was not followed in 2016. By performing an updated assessment using systematic review methods, EPA determined that four noncancer health outcomes and four epidemiological endpoints within these outcomes (i.e., decreased antibody response to vaccination in children, decreased birthweight, increased total cholesterol, and increased alanine aminotransferase (ALT)) have sufficient weight of evidence to consider quantitatively. Each endpoint quantified in this assessment has consistent evidence from multiple *medium* and/or *high* confidence epidemiological and animal toxicological studies supporting an association between PFOS exposure and the adverse effect. Each of the endpoints were also specifically supported by multiple *high* and/or *medium* confidence epidemiological studies with low risk of bias in different populations, including general and highly exposed populations. Many of these supporting studies have been published since 2016 and have strengthened the weight of evidence for this assessment.

As described in Section 4.1.1.34.1.3, EPA has improved upon the pharmacokinetic modeling approach used in 2016. Though there are challenges in estimations of human dosimetry from

measured or modeled serum concentrations (see Section 5.6.2), EPA has evaluated the available literature and developed a pharmacokinetic model that estimates PFOS exposure concentrations from the serum PFOS concentrations provided in epidemiological studies, which reduces uncertainties related to exposure estimations in humans. This new approach is supplemented with the uncertainty factor (UF) accounting for intraspecies variation of 10× applied to each POD_{HED}, which accounts for the sensitivities of specific populations, including those that may have increased susceptibility to PFOS toxicity due to differential toxicokinetics.

An additional source of uncertainty in using epidemiological data for POD derivation of chronic, nondevelopmental effects, is the documented decline in human serum PFOS levels over time, which raises concerns about whether one-time serum PFOS measurements are a good representation of lifetime peak exposure. Because of PFOS's long half-life in serum, however, one-time measurements likely reflect several years of exposure. Importantly, EPA considered multiple time periods when estimating PFOS exposure, ranging from the longest period with available data on PFOS serum levels within the U.S. population (1999–2018) to the shortest and most recent period (2017–2018) (see Appendix E, (U.S. EPA, 2024a)), when performing dose-response modeling of the ALT and TC endpoints in the epidemiological data. EPA selected PODs for these two endpoints using PFOS exposure estimates based on the serum PFOS data for 1999–2018, which is likely to capture the peak PFOS exposures in the United States that occurred in the 1990's (Dong et al., 2019; Nian et al., 2019; Gallo et al., 2012; Steenland et al., 2009). The modeling results show that the benchmark dose lower confidence limit (BMDL) estimates for increased TC derived using the longest range of exposure data (1999-2018) are consistently lower than those based on the 2017–2018 PFOS exposure data whereas for ALT, the BMDL estimates using data from the longest exposure period are consistently higher than those based on the 2017–2018 PFOS exposure data. Given these analyses, it appears that selection of one exposure time period over another does not predictably impact the modeling results. Therefore, for this assessment, EPA consistently selected the time periods more likely to capture peak PFOS exposures (e.g., 1999–2018) as the basis of BMDL estimates for all endpoints of interest (see Appendix E, (U.S. EPA, 2024a)).

It is plausible that observed associations between adverse health effects and PFOS exposure could be explained in part by confounding from other PFAS exposures, including the metabolism of precursor compounds to PFOS in the human body. However, mixture analysis remains an area of emerging research (Taylor et al., 2016), and there is no scientific consensus yet for the best approach to account for exposure by co-occurring PFAS. Additionally, multipollutant analyses from studies included in this assessment did not provide direct evidence that associations between exposure to PFOS and health effects are confounded by or are fully attributable to confounding by co-occurring PFAS. A detailed discussion of statical approaches for accounting for co-occurring PFAS and results from studies performing multipollutant analysis is provided in Section 5.1.1. For an extended review of the uncertainties associated with PFAS co-exposures, see *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

Additionally, there is uncertainty about the magnitude of the contribution of PFAS precursors to PFOS serum concentrations, especially as biotransformation efficiency appears to vary depending on the precursor of interest (Mcdonough et al., 2022; D'eon and Mabury, 2011; Vestergren et al., 2008). The contributions of PFAS precursors to serum concentrations also

varies between populations with differing PFAS exposure histories (i.e., individuals living at or near sites with AFFF use may have different precursor PFOS contributions than the general population).

In addition, some populations may be disproportionately exposed to other contaminants, such as polychlorobiphenyls and methylmercury. To address this, EPA quantified associations between PFOS serum concentrations and endpoints of interest in populations with varying exposure histories, including the general population and high-exposure communities. EPA observed associations for endpoints in populations known to have been predominantly exposed to PFOS (e.g., Isomers of C8 Health Project participants), reducing the uncertainty related to potential confounding of other contaminants, including PFAS precursor compounds. These sensitivity analyses are supportive of EPA's conclusions regarding the effects of PFOS reported across many epidemiological studies.

In this assessment, studies were not excluded from consideration based primarily on lack of or incomplete adjustments for potential confounders including socioeconomic status (SES) or race/ethnicity. A small number of studies examining PFAS serum levels across SES and racial/ethnic groups were identified. These studies (most with sampling from the early-mid 2000s) reported conflicting results regarding the relationship between race/ethnicity and serum PFOS concentrations, with studies differing depending on locations sampled, further stratification of results by age, cohort characteristics, etc. (Park et al., 2019c; Kato et al., 2014; Nelson et al., 2012; Calafat et al., 2007). EPA acknowledges that in observational epidemiological studies, potential residual confounding may result from complexities related to SES and racial/ethnic disparities. Additional racially and ethnically diverse studies in multiple U.S. communities are needed to fill this important data gap. Appendix D (U.S. EPA, 2024a) provides detailed information on the available epidemiological studies and identifies the study-specific confounding variables that were considered, such as SES.

Lastly, the potential uncertainty related to the clinical significance of effects observed in the PFOS epidemiological studies is sometimes cited for dismissing the epidemiological data quantitatively. However, as described in Section 4.1.1, the four selected critical effects (i.e., decreased antibody response to vaccination, increased serum ALT, increased TC, and decreased birthweight) are biologically significant effects and/or precursors to disease (e.g., CVD), which, according to agency guidance and methods, both warrant consideration as the basis of RfDs for PFOA (U.S. EPA, 2022d, 2005a, 2002b). EPA's *A Review of the Reference Dose and Reference Concentration Processes*, states that a reference dose (RfD) should be based on an adverse effect or a precursor to an adverse effect (e.g., increased risk of an adverse effect occurring) (U.S. EPA, 2002b). Also, at the individual level, the interpretation and impact of small magnitude changes in endpoints such as increased TC, increased ALT, decreased birth weight, and decreased antibody response to vaccination may be less clear. However, at the population level, even small magnitude changes in these effects will shift the distribution in the overall population and increase the number of individuals at risk for diseases, such as cardiovascular disease and liver disease(Gilbert and Weiss, 2006).

There are challenges associated with quantitative use of epidemiological data for risk assessment (Deener et al., 2018) as described above; however, improvements such as methodological advancements that minimize bias and confounding, strengthened methods to estimate and

measure exposure, and updated systematic review practices facilitate the use of epidemiological studies to quantitatively inform risk.

5.1.1 Uncertainty Due to Potential Confounding by Co-Occurring PFAS

5.1.1.1 PFAS Co-Exposure Statistical Approaches and Confounding Analysis

A potential source of uncertainty in epidemiologic studies examining associations between a particular PFAS and health outcomes is confounding by other co-occurring PFAS. In studies of PFOS, such confounding may occur if there are other PFAS that are moderately or highly correlated with PFOS, associated with the outcome of interest, and not on the causal pathway between PFOS and the outcome. If the association between co-occurring PFAS and the outcome is in the same direction as the association between PFOS and that outcome, the anticipated direction of bias resulting from not accounting for other PFAS would be away from the null. For an extended review of the uncertainties associated with PFAS co-exposures, see the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

Several statistical methods are currently used to estimate associations while accounting for potential confounding by co-occurring PFAS and other pollutants. One common approach is to include co-occurring PFAS as covariates in regression models. This approach allows for an estimation of the association between PFOS and the outcome of interest, adjusted for other covariates and the copollutants. Another approach is to screen large groups of exposures to identify which ones are most strongly related to the outcome, using methods such as principal components analysis, elastic net regression, and Bayesian kernel machine regression (BKMR). Each of these approaches has strengths and limitations. For example, when PFOS and the copollutants are highly correlated, then multipollutant models could be affected by multicollinearity or result in amplification bias, rather than reduce confounding bias compared with single-pollutant models (Weisskopf et al., 2018). Additionally, accounting for a variable in a multivariable regression model that is not a significant predictor of the response variable reduces the degrees of freedom and effectively dilutes the significance of the other exposure variables that are predictors of the response. The use of screening approaches, while effective at accounting for copollutants, can result in estimates that are not easily interpretable and make it difficult to differentiate the impact and contribution of individual PFAS (Meng et al., 2018). Mixture analysis is an emerging research area (Liu et al., 2022; Taylor et al., 2016), and there is no scientific consensus yet on the best approach for estimating independent effects of PFOS within complex PFAS mixtures.

In this assessment, the risk of bias due to confounding by co-occurring PFAS was considered as part of the study quality evaluation process. To further support the assessment, Section 5.1.1.2 below summarizes evidence from *high* and *medium* confidence studies that included at least one of the approaches described above (hereafter referred to collectively as "multipollutant models") to account for copollutants, in order to assesses the extent to which there may be confounding by other PFAS in studies reporting the associations between PFOS and birth weight.

5.1.1.2 Multipollutant Models of PFOS and Birth Weight

When assessing the associations between PFOS and a health effect of interest (e.g., decreased birth weight), there is concern for potential confounding by other PFAS when there is a strong correlation between the occurrence of PFOS and another PFAS and when the magnitude of the association between the co-exposure and the health effect is large.

To identify co-occurring PFAS with potential for confounding, Table 5-1 shows correlations between PFOS and other PFAS exposures in nine studies evaluating the association between exposure to PFOS and birth weight, each of which included mutually adjusted models. Four of these studies are *medium* confidence (Meng et al., 2018; Woods et al., 2017; Lenters et al., 2016; Robledo et al., 2015) and five are *high* confidence studies (Luo et al., 2021; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017). Moderately positive correlations (~0.6) between PFOS and PFOA were consistently observed in six of the seven studies that reported such information. Correlations between PFOS and other commonly examined PFAS, including PFNA (four studies), PFDA (four studies), and PFHxS (five studies), were less consistent than correlations with PFOA, ranging from weak (i.e., 0.0–0.3) to strong (i.e., 0.7–1.0). These results suggest that other PFAS may not consistently co-occur with PFOS.

Table 5-1. Correlation Coefficients Between PFOS and Other PFAS in Mutually Adjusted Studies

Reference	Study Setting	Correlations with PFOS			
		PFOA	PFNA	PFDA	PFHxS
Ashley-Martin et al. (2017)[a] *High*	Canada (10 cities)	0.59	–[b]	–	0.55
Luo et al. (2021)[a] *High*	Guangzhou, China	0.11	0.63	0.68	0.01
Manzano-Salgado et al. (2017a)[c] *High*	Gipuzkoa, Sabadell, and Valencia, Spain	NR	NR	NR	NR
Shoaff et al. (2018)[d] *High*	Cincinnati, Ohio, USA	0.60	–	–	–
Starling et al. (2017)[e] *High*	Colorado, USA	0.68	0.62	0.49	0.65
Lenters et al. (2016)[e] *Medium*	Greenland; Kharkiv, Ukraine; Warsaw, Poland	0.61	0.42	0.78	0.34
Meng et al. (2018)[d] *Medium*	Denmark	0.66	0.48	0.48	0.30
Robledo et al. (2015)[c] *Medium*	Michigan and Texas, USA	NR	NR	NR	NR
Woods et al. (2017)[f] *Medium*	Cincinnati, Ohio, USA	+[g]	+	+	+

Notes: NR = not reported.
Shaded cells indicate analytes for which a correlation with PFOA was not measured or reported.
[a] Pearson correlation of log10-transformed (Ashley-Martin et al., 2017) and ln-transformed (Luo et al., 2021) PFAS values.
[b] Analyte not measured.
[c] Correlation coefficients not reported.
[d] Pearson correlation of PFAS values, unclear if transformed prior to correlation analysis.
[e] Spearman rank correlation of PFAS values.
[f] Correlation coefficient type not specified.
[g] Correlations not reported numerically. Heat map of correlation coefficients (Figure S2, in Woods et al. (2017)) shows positive correlations between PFOS and PFOA, PFNA, PFHxS, and PFDA, ranging from about 0.6 to about 0.1, respectively.

Results from mutually adjusted models are summarized and compared in Table 5-2. The statistical approaches for accounting for PFAS co-exposures varied across the studies. Six studies included at least one additional PFAS as a predictor in ordinary least squares (OLS) regression models (Meng et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017; Robledo et al., 2015). Woods et al. (Woods et al., 2017) included multiple PFAS as predictors in a Bayesian hierarchical linear model. Three studies (Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016) used elastic net regression, and one study used BKMR (Luo et al., 2021). The impact of other PFAS adjustment on the association between PFOS and birth weight is evaluated by comparing the magnitude and direction of the effects from the single-PFOS model (when available) to those from mutually adjusted models.

Six studies provided results from both single and multipollutant models (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016). Multipollutant models in these six studies included PFOA but varied with respect to other PFAS considered (Table 5-2). Lenters et al. (2016) also adjusted for other types of chemicals (such as phthalates and organochlorides) in addition to several PFAS. Generally, the direction of effect estimates remained the same following adjustment for other PFAS, but precision was reduced. None of the studies that showed birth weight deficits in single-pollutant models reported greater magnitude or more precision of the association following statistical adjustment for other PFAS.

Three studies reported large inverse associations (range: −45 to −83 g) between PFOS and mean birth weight in single-pollutant (i.e., PFOS only) models (Luo et al., 2021; Meng et al., 2018; Lenters et al., 2016). In Luo et al. (2021), the association remained statistically significant in a BKMR model that included 11 other PFAS. In Meng et al. (2018), the association was slightly attenuated (from −45 to −38 g) and no longer statistically significant following adjustment for PFOA. Lenters et al. (2016) observed a nonsignificant inverse association between PFOS and reduced birth weight in single-pollutant models, but PFOS was not selected for inclusion in an elastic net regression model that included other pollutants. Manzano-Salgado et al. (2017a), Shoaff et al. (2018), and Starling (2017) reported null results in single and in multi-PFAS regression models. Additionally, Starling (2017) reported that PFOS was not selected for inclusion in an elastic net regression model. Although found in the minority of studies, the large inverse associations (range: −38 to −109 g) from two multipollutant OLS studies were comparable in magnitude to the single-pollutant models.

Three studies provided results only from multipollutant models (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015), thus making assessment of the impact of copollutants difficult. None of these studies reported statistically significant associations between PFOS and birth weight, and PFOS was not selected for the elastic net regression model in Woods et al. (2017), which reported on the same cohort as Shoaff et al. (2018), that included other endocrine-disrupting chemicals in addition to PFAS.

In summary, in the six studies that included both single and multipollutant models, associations were attenuated to various degrees while others were strengthened following adjustment for other PFAS (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016).Three additional studies presented results from multipollutant models only, making it difficult to determine the extent to which confounding by

other PFAS may have impacted the PFOS-birth weight associations (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015).

Considering all nine studies (8 different cohorts) together, it is challenging to draw conclusions about the extent of confounding by co-occurring PFAS, particularly given differences in modeling approaches, PFAS considered in the adjustment, and exposure contrasts used across studies. Additionally, these studies represented only a small fraction of the total number of studies examining associations between PFOS and birth weight and it is unclear whether their results are generalizable to the broader evidence base. Although it is an important source of uncertainty, there is no evidence in the entirety of the large evidence base that the observed associations between PFOS and birth weight deficits are fully attributable to confounding by co-occurring PFAS.

Similar conclusions can be drawn for other health outcomes. Budtz-Jorgensen (2018) evaluated the possibility of confounding across PFAS in analyses of decreased antibody response. The study reported significant decreases in the antibody response with elevated PFOS exposure, and there was no notable attenuation of the observed effects after estimates were adjusted for PFOA (see Section 3.4.2.1.1.1) (Budtz-Jørgensen and Grandjean, 2018). A limited number of studies performed co-exposure analyses for increased ALT and increased TC in adults. Lin et al. (2010) performed multipollutant modeling for the effects on serum ALT, but multipollutant modeling results for the association between PFOS exposure and ALT was not reported. Fan et al. (2020) examined cross-sectional associations between exposure to PFOS and increased TC in single- and multipollutant models in a sample of adults from NHANES (2012–2014). Exposure to PFOS was associated with significantly elevated TC in the single-pollutant model, but the association was no longer significant in multipollutant analyses. A significantly positive association was also observed for PFAS mixture and TC in WQS regression analyses (Fan et al., 2020).

Overall, there is no evidence that the consistently observed associations between exposures to PFOS and the four priority noncancer health outcomes are confounded or are fully attributable to confounding by co-occurring PFAS.

Table 5-2. Impact of Co-Exposure Adjustment on Estimated Change in Mean Birth Weight (Grams) per Unit Change (ng/mL) in PFOS Levels.

Reference	Single PFAS Model Result (95% CI)[a,b]	Multi-PFAS Model Result (95% CI)[a,b]	Elastic Net Regression Result[b]	Exposure Comparison	Effect of PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Ashley-Martin et al. (2017) *High*	NR	Girls: 94.31 (−76.30, 264.92) Boys: −11.15 (−174.26, 151.95)	–[c]	log_{10}-unit (ng/mL) increase	–	PFOA, PFHxS
Luo et al. (2021) *High*	−93.34 (−157.92, −28.75)	−109 (−215, −4)[d]	–	Single PFAS model: ln-unit (ng/mL) increase Multi-PFAS model: 75th vs. 25th percentile	Results not directly comparable due to different exposure comparisons, but both models showed large inverse associations	PFOA, PFBA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, 6:2 Cl-PFESA, 8:2 Cl-PFESA
Manzano-Salgado et al. (2017a) *High*	0.44 (−32.48, 33.36)	18.64 (−26.08, 63.36)	–	log_2-unit (ng/mL) increase	Strengthened (increased birth weight)	PFOA, PFNA, PFHxS
Shoaff et al. (2018) *High*	−0.06 (−0.16, 0.04)[e]	−0.06 (−0.26, 0.15)[e]	–	log_2-unit (ng/mL) increase	No change	PFOA, PFNA, PFHxS
Starling et al. (2017) *High*	−13.8 (−53.8, 26.3)	29.09 (−32.56, 90.75)	N/S	ln-unit (ng/mL) increase	Attenuated/changed direction	PFOA, PFNA, PFDA, PFHxS
Lenters et al. (2016) *Medium*	−68.84 (−152.90, 15.22)	–	N/S	2 SD ln-unit (ng/mL) increase	Attenuated	PFOA, PFNA, PFDA, PFHxS, PFHpA, PFUnDA, PFDoDA
Meng et al. (2018)[f] *Medium*	−45.2 (−76.8, −13.6)	−38.11 (−82.09, 5.88)	–	log_2-unit (ng/mL) increase	Slightly Attenuated	PFOA
Robledo et al. (2015)[g] *Medium*	NR	Girls: 14.16 (−81.83, 110.15) Boys: 37.51 (−73.45, 148.46)	–	1 SD ln-unit (ng/mL) increase	–	PFOA, PFDA, PFNA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH
Woods et al. (2017) *Medium*	NR	−9 (−53, 35)[h]	N/S	log_{10}-unit (ng/mL) increase	–	PFOA, PFNA, PFDA, PFHxS

Notes: NR = not reported; N/S = not sufficient.

5.2 Comparisons Between Toxicity Values Derived from Animal Toxicological Studies and Epidemiological Studies

As recommended by the SAB (U.S. EPA, 2022e), EPA derived candidate RfDs and CSFs for multiple health outcomes using data from both epidemiological and animal toxicological studies. Candidate RfDs from epidemiological and animal toxicological studies within a health outcome differed by approximately two to three orders of magnitude (see Figure 4-4), with epidemiological studies producing lower values. EPA does not necessarily expect concordance between animal and epidemiological studies in terms of the adverse effect(s) observed, as well as the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" (U.S. EPA, 1991). EPA further describes these factors in relation to this assessment below.

First, there are well-established differences in the toxicokinetics between humans and animal models such as rats and mice. As described in Section 3.3.1.4.5, PFOS half-life estimates vary considerably by species, being lowest in rodents (hours to days) and several orders of magnitude higher in humans (years). All candidate toxicity values based on animal toxicological studies were derived from studies conducted in rats or mice, adding a potential source of uncertainty related to toxicokinetic differences in these species compared with humans. To address this potential source of uncertainty, EPA utilized a pharmacokinetic (PK) model to estimate the internal dosimetry of each animal model and convert the values into predicted levels of human exposure that would result in the corresponding observed health effects. However, the outputs of these models are *estimates* and may not fully account for species-specific toxicokinetic differences, particularly differences in excretion. The application of uncertainty factors (i.e., UF_A) also may not precisely reflect animal-human toxicokinetic differences.

Second, candidate toxicity values derived from epidemiological studies are based on responses associated with actual environmental exposure levels, whereas animal toxicological studies are limited to the tested dose levels which are often several orders of magnitude higher than the ranges of exposure levels in humans. Extrapolation from relatively high experimental doses to environmental exposure levels introduces a potential source of uncertainty for toxicity values derived from animal toxicological studies; exposures at higher dose levels could result in different responses, perhaps due to differences in mechanisms activated, compared with responses to lower dose levels. One example of this is the difference between epidemiological and animal toxicological studies in the effect of PFOS exposure on serum lipid levels (i.e., potential nonmonotonic dose-response relationships that are not easily assessed in animal studies due to low dose levels needed to elicit the same response observed in humans).

Third, there may be differences in mechanistic responses between humans and animal models. One example of this is the PPARα response. It is unclear to what extent PPARα influences the responses to PFOS exposure observed in humans, though the rodent PPARα response may differ from those observed in humans (see Section 3.4.1.3.1). Mechanistic differences could influence dose-response relationships and subsequently result in differences between toxicity values derived from epidemiological and animal toxicological studies. There may be additional

mechanisms that differ between humans and animal models that could contribute to the magnitude of responses and doses required to elicit responses across species.

The factors described above represent some but not all potential contributors that may explain the differences between toxicity values derived from epidemiological and animal toxicological studies. In this assessment, EPA prioritized epidemiological studies of *medium* or *high* confidence for the selection of health outcome-specific and overall RfDs and CSFs (see Section 4.1.6). The use of human data to derive toxicity values removes uncertainties and assumptions about human relevance inherent in extrapolating from and interpreting animal toxicological data in quantitative risk assessment.

5.3 Updated Approach to Animal Toxicological RfD Derivation Compared with the 2016 PFOS HESD

For POD derivation in this assessment, EPA considered the studies identified in the recent literature searches and also re-examined the candidate RfDs derived in the 2016 PFOS Health Effects Support Document (HESD) (U.S. EPA, 2016b) and the animal toxicological studies and endpoints on which they were based. The updated approach used for hazard identification and dose response in the current assessment as compared with the 2016 PFOS HESD led to some differences between animal toxicological studies and endpoints used as the basis of candidate RfDs for each assessment. These updates and the resulting differences are further described below.

For the 2016 PFOS HESD, EPA did not use BMD modeling to derive PODs, and instead relied on the no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) approach for all candidate studies and endpoints (U.S. EPA, 2016b). The NOAEL/LOAEL approach allows for the incorporation of multiple endpoints from a single study to derive a single POD, if the endpoints have the same NOAEL and/or LOAEL. For example, in the 2016 PFOS HESD, EPA derived a candidate RfD based on the endpoints of increased ALT and increased blood urea nitrogen (BUN) reported by Seacat et al. (2003, 1290852), both of which shared a common POD (NOAEL). For the current assessment, EPA preferentially used BMD modeling to derive PODs because it allows for greater precision than the NOAEL/LOAEL approach and considers the entirety of the dose-response curve. This approach requires the consideration of endpoints on an individual basis and further examination of the weight of evidence for particular endpoints, as well as the dose-response relationship reported for each endpoint, in order to derive a BMDL. When considering an effect on a standalone basis rather than grouped with other effects occurring at the same exposure level, EPA sometimes determined the weight of evidence was not sufficient to consider an individual endpoint for POD derivation. For the current assessment, EPA used a systematic review approach consistent with the IRIS Handbook (U.S. EPA, 2022d) to consider the weight of evidence for both the health outcomes as well as for individual endpoints of interest when selecting endpoints and studies for dose-response modeling. In the case of the endpoints selected in the 2016 PFOS HESD from the Seacat et al. (2003) study, renal effects such as increased BUN were reevaluated and determined to have *evidence suggestive* of an association with PFOS exposure. As described in Section 4, in this assessment, EPA only derived PODs for endpoints from health outcomes with *evidence indicating* or *evidence demonstrating* an association with PFOS exposure.

Additionally, for the current assessment, EPA preferentially selected endpoints that were amenable to BMD modeling, had dose-dependent trends in responses, were supported by at least one other study in the available literature, and were direct/specific measures of toxicity for POD derivation. For some studies considered in the 2016 PFOS HESD and reevaluated during the current assessment, EPA attempted BMD modeling for specific endpoints but the efforts did not result in viable model fits. For the current assessment, EPA elected to derive a candidate RfD for hepatic effects based on histopathological lesions observed in the liver as reported by Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075) rather than serum ALT reported by Seacat et al. (2003, 1290852), as the Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075) studies were rated as *high* confidence (vs. the *medium* confidence Seacat et al. (2003, 1290852)), used a chronic study design (vs. the 14-week exposure used by Seacat et al. (2003, 1290852)), and histopathological lesions reflect direct damage to the liver whereas ALT is a less specific indicator of liver damage. In animal studies, evaluation of direct liver damage is possible, however in humans, it is difficult to obtain biopsy-confirmed histological data. Therefore, liver injury is typically assessed using serum biomarkers of hepatotoxicity (Costello et al., 2022).

For some health outcomes, new studies have been published since 2016 that improve upon the weight of evidence determined in the 2016 PFOS HESD. For example, in 2016, EPA did not derive a candidate RfD based on immune effects. Since that time, several *high* and *medium* confidence studies (both animal toxicological and epidemiological) have been published that increased the strength of evidence for this health outcome. As described in Section 3.4.2.4, *evidence indicates* that PFOS exposure is associated with immune effects and therefore, in this assessment, EPA derived candidate RfDs for the immune health outcome.

For transparency, EPA has provided a comparison of studies and endpoints used to derive candidate RfDs for both the 2016 PFOS HESD and the present assessment in Table 5-3.

Table 5-3. Comparison of Candidate RfDs Derived from Animal Toxicological Studies for Priority Health Outcomes[a]

Studies and Effects Used in 2016 for Candidate RfD Derivation[b]	Studies and Effects Used in 2024 for Candidate RfD Derivation
Immune	
NA	Zhong et al. (2016), *medium* confidence – decreased pup PFC response to SRBC NTP (2019), *high* confidence – extramedullary hematopoiesis in the spleen
Developmental	
Luebker et al. (2005b) *medium* confidence – decreased pup body weight Luebker et al. (2005a), *medium* confidence – decreased pup survival Lau et al. (2003), *medium* confidence – decreased pup survival	Luebker et al. (2005b), *medium* confidence – decreased pup body weight
Hepatic	
Seacat et al. (2003), *medium* confidence – increased ALT (and increased BUN)	Butenhoff et al. (2012)/Thomford (2002b), *high* confidence – individual cell necrosis in the liver

Notes: RfD = reference dose; NA = not applicable; PFC = plaque forming cell; SRBC = sheep red blood cell; NTP = National Toxicology Program; ALT = alanine aminotransferase; BUN = blood urea nitrogen.

[a] Note that candidate RfDs for the fourth priority noncancer health outcome (i.e., cardiovascular) are not presented in this table because candidate RfDs based on animal toxicological studies representing this health outcome were not derived in the 2016 PFOS HESD or the current assessment.
[b] Candidate RfDs from the 2016 PFOS HESD that correspond to nonprioritized health outcomes (e.g., nervous) are not presented here.

5.4 Reevaluation of the PFOS Carcinogenicity Database

In November 2021, EPA published the draft *Proposed Approaches to the Derivation of a Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* for review by the SAB PFAS Review Panel (U.S. EPA, 2021b). As part of the review process, EPA charged the SAB panel with providing comment on the rationale and conclusion for the PFOS cancer classification. Prior to SAB review, EPA had concluded that the weight of evidence supported the determination of PFOS as having *Suggestive Evidence of Carcinogenicity*, similar to the conclusions of the 2016 PFOS HESD (U.S. EPA, 2016b), which was, in part, because no new animal toxicological studies had been published since publication of the 2016 PFOS HESD and the new epidemiological literature published up until 2021 continued to provide mixed results.

As part of the final report, the SAB noted, "[s]everal new studies have been published that warrant further evaluation to determine whether the "likely" designation is appropriate" for PFOS and requested that the agency provide an "explicit description of why the available data for PFOS do not meet the EPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005) criterion for the higher designation as 'likely carcinogenic'" (U.S. EPA, 2022e). The SAB recommended EPA reevaluate several aspects of the carcinogenicity database for PFOS to confirm or update the draft *Proposed Approaches* conclusion that PFOS has *Suggestive Evidence of Carcinogenic Potential*, including epidemiological studies reporting kidney cancer (i.e., Shearer et al. (2021) and Li et al. (2022)), mechanistic data (e.g., Benninghoff et al. (2012)), and conclusions about animal toxicological data in rats (i.e., Butenhoff et al. (2012)). EPA has reevaluated these aspects of the database and relevant discussions of the recommended studies are provided in Section 3.5.

Upon reassessment of the PFOS carcinogenicity database, including the epidemiological, animal toxicological, and mechanistic databases, the agency has determined the available data for PFOS surpass many of the descriptions for *Suggestive Evidence of Carcinogenic Potential* according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and meet the descriptions for *Likely to Be Carcinogenic to Humans,* as described in Section 3.5.5. This conclusion was based on four independent factors. First, EPA considered the SAB's request that EPA "reevaluate the 2012 Butenhoff study" (U.S. EPA, 2022e). After reviewing the available data, as described in Sections 3.5.2, 3.5.5, and below in this subsection, EPA subsequently agreed with the SAB that the agency's prior "interpretation of the hepatocellular carcinoma data from the Butenhoff (2012b) study in the 2016 PFOS HESD is overly conservative in dismissing the appearance of a dose-response relationship for this endpoint, particularly in females" (U.S. EPA, 2022e). Second, as requested by the SAB, and following agency methodology (U.S. EPA, 2022d), EPA incorporated syntheses of mechanistic literature, which served as the basis of EPA's conclusions that multiple, potentially human-relevant MOAs may contribute to the hepatocellular tumors reported in PFOS toxicological studies of rats (see Section 3.5.4.2). This conclusion aligned with the SAB's comments that "multiple MOAs may be operative" in the reported hepatocellular tumorigenesis and that "the rodent liver tumors caused by PFOS do not appear to be PPAR−α

dependent," (U.S. EPA, 2022e). Third, EPA considered the SAB's comment that there were inconsistencies between EPA's draft conclusions and "the California EPA conclusions based on the same human, animal, and mechanistic evidence presented in the EPA PFOS document," leading the EPA to re-review the CalEPA's *draft Public Health Goals for PFOA and PFOS* technical document (CalEPA, 2021) and identify data indicating the occurrence of tumorigenesis in a second tumor site in male rats (i.e., pancreatic islet cell tumors) (U.S. EPA, 2022e). Fourth, EPA identified new supporting epidemiological literature resulting from the SAB's recommendation that EPA update the literature search prior to finalization of the toxicity assessments for PFOA and PFOS (U.S. EPA, 2022e). This new epidemiological literature included two studies reporting increased risk of hepatocellular carcinoma associated with increased PFOS exposure in humans (Cao et al., 2022; Goodrich et al., 2022), which provided concordant evidence between one of the tumor types and sites observed in the available animal toxicological study. This concordance further supports the potential human relevance of the hepatocellular tumors observed in animal toxicological studies of PFOS.

More specifically, the examples for which the PFOS database exceeds the *Suggestive Evidence* descriptions outlined in the *Guidelines for Carcinogen Risk Assessment* include:

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans;'
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;
- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion; and
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend." (U.S. EPA, 2005a).

The strongest evidence for the carcinogenicity of PFOS is from one chronic animal bioassay which presents findings surpassing several of these criteria (Butenhoff et al., 2012; Thomford, 2002b). The Thomford/Butenhoff et al. (Butenhoff et al., 2012; 2002b) study is a *high* confidence study that observed statistically significant increases at individual dose levels and/or statistically significant trends in two tumor types and in one or more sexes, even with the relatively low dose levels used. The background incidence of these tumor types was low or negligible. As described in Section 3.5.4.2, EPA determined that these tumor types are potentially relevant to humans.

In the initial draft of this toxicity assessment published for SAB review (i.e., the *Proposed Approaches* document) (U.S. EPA, 2021b), as well as the 2016 PFOS HESD (U.S. EPA, 2016b), EPA relied upon the tumor incidences provided in Butenhoff et al. (2012), which is the peer-reviewed manuscript of an industry report – Thomford (2002b). Upon further review of the results presented in the Thomford (2002b) report prior to finalization of this assessment, the agency identified two factors that limited previous qualitative and quantitative interpretations of the data: 1) the Butenhoff et al. (2012) study reported combined incidences of neoplastic lesions in the control and high-dose groups (males and females) from the interim time point (52 weeks of dietary exposure; n = 10) and terminal time point (104 weeks of dietary exposure; n = 50); and

2) the Butenhoff et al. (2012) study did not report incidences for pancreatic islet cell neoplasms. The first factor resulted in statistical dilution of tumor incidence in the high-dose group as many of the tumor types observed in the study, including hepatocellular neoplasms, were not reported until approximately 70 weeks of treatment or later. Therefore, EPA conducted a re-analysis that excluded animals sacrificed at the interim time point from statistical analyses as it was biologically implausible for the 10 animals from the interim time point to have presented with neoplasms. As a result of this reanalysis, EPA agreed with the SAB that the original analysis was "overly conservative in dismissing the appearance of a dose-response relationship for this endpoint, particularly in females" (U.S. EPA, 2022e).

The second factor prevented EPA from previously identifying the statistically significant trend in a second tumor site/type (pancreatic islet cell carcinomas) observed in the chronic cancer bioassay. As a result of identifying the second tumor site/type and updating the conclusions regarding hepatocellular tumors in females, the EPA concluded that PFOS met an additional characteristic for the designation of *Likely to Be Carcinogenic to Humans:* "an agent that has tested positive in animal experiments in more than one species, **sex**, strain, **site**, or exposure route, with or without evidence of carcinogenicity in humans" (emphasis added) (U.S. EPA, 2005a).

Overall, the Thomford/Butenhoff et al. (2012; 2002b) report, along with plausible associations between PFOS exposure and carcinogenicity reported in epidemiological studies, particularly for hepatocellular carcinoma, provide substantive evidence that PFOS exceeds the designation of *Suggestive Evidence of Carcinogenic Potential* and is consistent with *Likely Evidence of Carcinogenic Potential in Humans* (see Section 3.5.5 for more information on the *Likely* determination). See Table 5-4 below for specific details on how PFOS exceeds the examples supporting the *Suggestive Evidence of Carcinogenic Potential* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

After reviewing the examples of the descriptor *Carcinogenic to Humans*, EPA has determined that at this time, the evidence supporting the carcinogenicity of PFOS does not warrant a descriptor exceeding *Likely to Be Carcinogenic to Humans*. The *Guidelines* indicate that a chemical agent can be deemed *Carcinogenic to Humans* if it meets <u>all</u> the following conditions:

- "there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and
- there is extensive evidence of carcinogenicity in animals, and
- the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and
- there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information" (U.S. EPA, 2005a).

As discussed in Section 3.5.5, convincing epidemiological evidence supporting a causal association between human exposure to PFOS and cancer is currently lacking. Additionally, though the available evidence indicates that there are positive associations between PFOS and multiple cancer types, there is uncertainty regarding the identification of carcinogenic modes of

action (MOAs) and associated key precursor events for PFOS in animals. See Table 5-4 below for specific details on how PFOS does not align with the examples supporting the *Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 5-4. Comparison of the PFOS Carcinogenicity Database with Cancer Descriptors as Outlined in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

Comparison of Evidence for Suggestive and Carcinogenic Cancer Descriptors	
Suggestive Evidence of Carcinogenic Potential	
"A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "*Likely to Be Carcinogenic* to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system" (U.S. EPA, 2005a)	**PFOS data exceed this description**. Observed statistically significant increases in hepatic tumors in rats (adenomas in males and adenomas and carcinomas in females) at the high dose and a statistically significant trend overall in both sexes. Concordant evidence of increased risk of hepatocellular carcinoma from two human epidemiological studies. Observed statistically significant trend of increased incidence of pancreatic islet cell tumors in male rats.
"A small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed." (U.S. EPA, 2005a)	**This description is not applicable to the tumor types observed after PFOS exposure.**
"Evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships)." (U.S. EPA, 2005a)	**PFOS data exceed this description**. The animal study from which carcinogenicity data are available was determined to be *high* confidence during study quality evaluation.
"A statistically significant increase at one dose only, but no significant response at the other doses and no overall trend." (U.S. EPA, 2005a)	**PFOS data exceed this description**. Observed statistically significant increases in hepatic tumors (adenomas in males and adenomas and carcinomas in females) at the high dose and a statistically significant trend overall. Also observed statistically significant trend of increased pancreatic islet cell carcinomas with increasing dose.
Carcinogenic to Humans	
This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.	**PFOS data are not consistent with this description.** There is evidence of a plausible association between PFOS exposure and cancer in humans, however, the database is limited, there is uncertainty regarding the potential confounding of other PFAS, and there is limited mechanistic information that could contribute to the determination of a causal relationship. The database would benefit from large *high* confidence cohort studies in independent populations.
Or, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* of the following conditions are met:	
There is strong evidence of an association between human exposure and either cancer or the key precursor	**PFOS data are not consistent with this description.** There is evidence of an association between human exposure and cancer, however, there is limited mechanistic

Comparison of Evidence for Suggestive and Carcinogenic Cancer Descriptors	
events of the agent's MOA but not enough for a causal association.	information that could contribute to the determination of a causal relationship.
There is extensive evidence of carcinogenicity in animals.	**PFOS data are not consistent with this description.** Only one chronic cancer bioassay is available for PFOS. The database would benefit from *high* confidence chronic studies in other species and/or strains.
The mode(s) of carcinogenic action and associated key precursor events have been identified in animals.	**PFOS data are not consistent with this description.** A definitive MOA has not been identified for each of the PFOS-induced tumor types identified in rats.
There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information.	**PFOS data are not consistent with this description.** The animal database does not provide significant clarity on the MOA(s) of PFOS in animals.

Notes: MOA = mode of action.

5.5 Health Outcomes with Evidence Integration Judgments of *Evidence Suggests* Bordering on *Evidence Indicates*

EPA evaluated 16 noncancer health outcomes as part of this assessment. In accordance with recommendations from the SAB (U.S. EPA, 2022e) and the IRIS Handbook (U.S. EPA, 2022d), for both quantitative and qualitative analyses in the final assessment, EPA prioritized health outcomes with either *evidence demonstrating* or *evidence indicating* associations between PFOS exposure and adverse health effects. Health outcomes reaching these tiers of judgment were the hepatic, immune, developmental, cardiovascular, and cancer outcomes. Some other health outcomes were determined to have *evidence suggestive* of associations between PFOS and adverse health effects as well as some characteristics associated with the *evidence indicates* tier, and EPA made judgments on these health outcomes as described below.

For PFOS, two health outcomes that had characteristics of both *evidence suggests* and *evidence indicates* were the endocrine and nervous system outcomes. Endpoints relevant to these two health outcomes had been previously considered for POD derivation in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b). However, upon further examination using the protocols for evidence integration outlined in Appendix A (U.S. EPA, 2024a) and Section 2.1.5, EPA concluded that the available epidemiological and animal toxicological evidence did not meet the criteria recommended for subsequent quantitative dose-response analyses. Although these health outcomes were not prioritized in the current assessment, based on the available data, EPA concluded that PFOS exposure may cause adverse endocrine or nervous system effects.

Epidemiological studies published since the 2016 PFOS HESD considered for evidence integration for adverse endocrine effects include *high* and *medium* confidence studies, though EPA determined that there was *slight evidence* to suggest human endocrine toxicity, including associations between PFOS exposure and thyroid disease. The available evidence supports the relationship between PFOS exposure and thyroid stimulating hormone (TSH) in children and, to a lesser extent, adults. However, similar to what was concluded in the 2016 PFOS HESD, evidence supporting adverse endocrine effects was inconsistent among epidemiological studies.

Animal toxicological studies considered for evidence integration consisted of 13 *high* or *medium* confidence studies. The animal evidence for an association between PFOS exposure and effects on the endocrine system was considered *moderate*, based on observed disruptions of normal thyroid function (i.e., decreased free thyroxine (T4), total T4 and total triiodothyronine (T3)). In addition, reductions in hormones associated with the hypothalamic-pituitary-adrenal axis were observed, although the corresponding histopathological data was inconsistent. Overall, the available human and animal evidence was *suggestive* but not *indicative* of adverse endocrine effects due to PFOS exposure. Therefore, EPA did not prioritize this outcome for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of endocrine evidence synthesis and integration.

Similar endocrine effects are observed among the family of PFAS chemicals. For example, the thyroid was identified as a target for oral exposure to PFBS (U.S. EPA, 2021d). Additionally, the final IRIS Toxicological Reviews for both PFBA (U.S. EPA, 2022c) and PFHxA (U.S. EPA, 2023) concluded that the available *evidence indicates* that the observed thyroid effects were likely due to PFBA and PFHxA exposure, respectively. Given the similarities across PFAS, these findings support potential associations between PFOS and adverse endocrine effects.

There was also *slight* evidence from epidemiological studies published since the 2016 PFOS HESD that supported a relationship between PFOS exposure and adverse nervous system effects, but study results were mostly mixed or limited. For example, studies evaluating neurodevelopmental, neuropsychological, and cognitive outcomes were limited with only one study supporting an adverse effect of PFOS exposure on hearing (Li, 2020). Although multiple studies examining associations between PFOS and ADHD were available, only one study reported a significant relationship between PFOS and ADHD (Lenters et al., 2019). There was an indication of a potential relationship between PFOS and autistic behaviors or ASD diagnosis in some studies (Shin et al., 2020; Oulhote et al., 2016; Braun et al., 2014), however there were methodology concerns associated with these studies. Animal studies considered for evidence integration suggest a relationship between PFOS exposure and nervous system effects, specifically in relation to learning and memory and neurotransmitter concentrations. Although there is *moderate* evidence to support adverse effects on the nervous system following exposure to PFOS from animal toxicological studies, EPA concluded there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. Overall, the available human and animal evidence was *suggestive* but not *indicative* of adverse nervous system effects due to PFOS exposure. Therefore, EPA did not prioritize this outcome for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of endocrine evidence synthesis and integration.

As the databases for endocrine and nervous system outcomes were *suggestive* of human health effects resulting from PFOS exposure, they were not prioritized during the updated literature reviews conducted in February 2022 and 2023. However, EPA acknowledges that future studies of these currently "borderline" associations could impact the strength of the association and the weight of evidence for these health outcomes. The currently available studies indicate the potential for endocrine and nervous system effects after PFOS exposure. Studies on endocrine and nervous system health outcomes represent two important research needs.

5.6 Challenges and Uncertainty in Modeling

5.6.1 Modeling of Animal Internal Dosimetry

There are several limitations and uncertainties associated with using pharmacokinetic models in general and estimating animal internal dosimetry. In this assessment, EPA utilized the Wambaugh et al. (2013) animal internal dosimetry model because it had availability of model parameters across almost all species of interest, agreement with out-of-sample datasets (see Appendix F, (U.S. EPA, 2024a)), and flexibility to implement life-course modeling (see Section 4.1.3.1). However, there were some limitations to this approach.

First, posterior parameter distributions summarized in Table 4-3 for each sex/species combination were determined using a single study. Therefore, uncertainty in these parameters represents only uncertainty in fitting that single study; any variability between studies or differences in study design were not accounted for in the uncertainty of these parameters. Second, issues with parameter identifiability for some sex/species combinations resulted in substantial uncertainty for some parameters. For example, filtrate volume (Vfil) represents a parameter with poor identifiability when determined using only serum data due to lack of sensitivity to serum concentrations (see Appendix F, (U.S. EPA, 2024a)). Measurements in additional matrices, such as urine, would help inform this parameter and reduce the uncertainty reflected in the wide credible intervals of the posterior distribution. These parameters with wide posterior CIs represent parameters that are not sensitive to the concentration-time datasets on which the model was trained (see Appendix, (U.S. EPA, 2024a)). However, these uncertain model parameters will not impact the median prediction used for BMD modeling and simply demonstrate that the available data are unable to identify all parameters across every species over the range of doses used for model calibration. Finally, the model is only parameterized using adult, single dose, PFOS study designs. Gestational and lactational PK modeling parameters were later identified from numerous sources (Table 4-5) to allow for the modeling of these lifestages with a more detailed description of the life-course modeling in Section 4.1.3.1.3.

The Wambaugh et al. (2013) model fit the selected PFOS developmental study data well, though there are several limitations to using this method to model developmental lifestages. First, perinatal fetal concentrations assume instantaneous equilibration across the placenta and do not account for the possibility of active transporters mediating distribution to the fetus. Second, clearance in the pup during lactation is assumed to be a first-order process governed by a single half-life. At low doses, this assumption is in line with adult clearance, but it is unclear how physiological changes during development impact the infant half-life. Finally, PFOS concentrations in breast milk are assumed to partition passively from the maternal blood. This assumption does not account for the presence of active transport in the mammary gland or time-course changes for PFOS uptake to the milk. Despite these limitations, the incorporation of model parameters related to developmental lifestages is a significant improvement over the model used in the 2016 PFOS HESD which did not implement life-course modeling (U.S. EPA, 2016b).

5.6.2 Modeling of Human Dosimetry

Uncertainties may stem from efforts to model human dosimetry. One limitation is that the clearance parameter, which is a function of the measured half-life and V_d values, is difficult to estimate in the human general population. Specifically for PFOS, the measurement of half-life is

hindered by slow excretion and ongoing exposure. Additionally, it is unclear whether some of the variability in measured half-life values reflects actual variability in the population, as opposed to uncertainty in the measurement of the value. There is also a lack of reported V_d values in humans because this parameter requires knowledge of the total dose or exposure. V_d values are difficult to determine from environmental exposures, and only one reported value is available (Thompson et al., 2010b).

In the Verner et al. (2016) model, half-life, V_d, and hence clearance values are assumed to be constant across ages and sexes. The excretion of PFOS in children and infants is not well understood. The ontogeny of renal transporters, age-dependent changes in overall renal function, and the amount of protein binding (especially in serum) could all play a role in PFOS excretion and could vary between children and adults. It is even difficult to predict the overall direction of change in excretion in children (higher or lower than in adults) without a clear understanding of these age-dependent differences. V_d is also expected to be different in children. Children have a higher body water content, which results in a greater distribution of hydrophilic chemicals to tissues compared with blood in neonates and infants compared with adults (Fernandez et al., 2011). This behavior is well known for pharmaceuticals, but PFOS is unlike most pharmaceuticals in that it undergoes extensive protein interaction, such that its distribution in the body is driven primarily by protein binding and active transport. Hence, it is difficult to infer the degree to which increased body water content will impact the distribution of PFOS.

The updated half-life value was developed based upon a review of recent literature (see Section 3.3.1.4.5). Many half-life values have been reported for the clearance of PFOS in humans (see Appendix B, (U.S. EPA, 2024a)). The slow excretion of PFOS requires measurement of a small change in serum concentration over a long time; the difficulties associated with making these measurements may represent one reason for the variance in reported values. Another challenge is the ubiquity of PFOS exposure. Ongoing exposure will result in a positive bias in observed half-life values if not considered (Russell et al., 2015). In studies that calculate the half-life in a population with greatly decreased PFOS exposures, typically due to the end of occupational exposure or the introduction of drinking water filtration, the amount of bias due to continuing exposure will be related to the ratio of the prior and ongoing exposure. That is, for a given ongoing exposure, a higher prior exposure may be less likely to overestimate half-life compared with a lower prior exposure. However, a half-life value determined from a population with very high exposure may not be informative of the half-life in typical exposure scenarios because of non-linearities in PK that may occur due to the saturation of PFAS-protein interactions. This will likely take the form of an under-estimation of the half-life that is relevant to lower levels, which are more representative of the general population, due to saturation of renal resorption and increased urinary clearance in the study population.

Because the derivation of the V_d for PFOS relied on the value for PFOA, it is important to consider alternate values for V_d for PFOA. For PFOA, the V_d calculation depended on the half-life. Thompson et al. (2010a) used 2.3 years, which was estimated within their population. If EPA chosen half-life of 2.7 years was used instead, the V_d for PFOA would be 200 mL/kg, which results in a PFOS value of 271 mL/kg. EPA did not update the V_d values based on the updated half-life because the value of 2.3 years was calculated based on the same data as the V_d and this half-life may be more representative of that population at that specific time. Gomis et al. (2017) also calculated V_d by taking the average of reported animal and human values and

estimated values of 235 mL/kg for PFOS. This calculation included the value from Thompson et al. (2010a) and did not include additional values derived from human data. This average value shows that the value from Thompson et al. (2010a), which was selected based on the fact that it was derived only from human and nonhuman primate data, is reasonable.

Lastly, the description of breastfeeding in the updated Verner et al. (2016) model relied on a number of assumptions: that infants were exclusively breastfed for 1 year, that there was a constant relationship between maternal serum and breastmilk PFOS concentrations, and that weaning was an immediate process with the infant transitioning from a fully breastmilk diet to the background exposure at 1 year. This is a relatively long duration of breastfeeding, only 27% of children in the United States are being breastfed at 1 year of age (CDC, 2013). Along with using the 95th percentile of breastmilk consumption, this provides a scenario of high but realistic lactational exposure. Lactational exposure to the infant is much greater than background exposure so the scenario of long breastfeeding is a conservative approach and will result in a lower POD_{HED} than a scenario with earlier weaning. Children in the United States are very unlikely to be exclusively breastfed for up to 1 year, and this approach does not account for potential PFOS exposure via the introduction of solid foods. However, since lactational exposure is much greater than exposure after weaning, a breastfeeding scenario that does not account for potential PFOS exposure from introduction of infants to solid foods is not expected to introduce substantial error.

5.6.3 Approach of Estimating a Benchmark Dose from a Regression Coefficient

EPA identified epidemiological studies that reported associations between PFOS exposure and response variables as regression coefficients. Since such a regression coefficient is associated with a change in the biological response variable, it is biologically meaningful and can therefore be used for POD derivation. EPA modeled these regression coefficients using the same approach used to model studies that reported measured response variables. The SAB PFAS Review Panel agreed with this approach, stating, "it would seem straightforward to apply the same methodology to derive the beta-coefficients ("re-expressed," if necessary, in units of per ng/mL) for antibody responses to vaccines and other health-effect-specific endpoints. Such a coefficient could then be used for deriving PODs" (U.S. EPA, 2022e). When modeling regression coefficients that were reported per log-transformed units of exposure, EPA used the SAB's recommended approach and re-expressed the reported β coefficients in units of per ng/mL (see Appendix E, (U.S. EPA, 2024a)). Sensitivity analyses to evaluate the potential impact of re-expression in a hybrid approach when modeling hepatic and serum lipid studies for PFOS showed little impact on BMDLs (see Appendix E, (U.S. EPA, 2024a)).

To evaluate this potential uncertainty in BMDLs derived based on regression coefficients, EPA obtained the measured dose-response data across exposure deciles from Steenland et al. (2009) (kindly provided to EPA on June 30, 2022 via email communication with the corresponding study author) and conducted sensitivity analyses to compare BMDs produced by the reported regression coefficients with the measured response variable (i.e., mean total cholesterol and odds ratios of elevated total cholesterol). These analyses are presented in detail in Appendix E (U.S. EPA, 2024a).

For PFOS, $BMDL_5$ values estimated using the regression coefficient and using the measured response variable were 9.52 ng/L and 26.39 ng/L, respectively. The two BMDL estimates from the two approaches are within an order of magnitude, less than a threefold difference. The RfD allows for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. Therefore, EPA is confident in its use of regression coefficients, re-expressed or not, as the basis of POD_{HEDS}.

5.7 Human Dosimetry Models: Consideration of Alternate Modeling Approaches

Physiologically based pharmacokinetic (PBPK) models are typically preferred over a one-compartment approach because they can provide individual tissue information and have a one-to-one correspondence with the biological system that can be used to incorporate additional features of pharmacokinetics, including tissue-specific internal dosimetry and local metabolism. In addition, though PBPK models are more complex than one-compartment models, many of the additional parameters are chemical-independent and have widely accepted values. Even some of the chemical-dependent values can be extrapolated from animal toxicological studies when parameterizing a model for humans, for which data are typically scarcer.

The decision to select a non-physiologically based model as opposed to one of the PBPK models was influenced in part by past issues identified during evaluation of the application of PBPK models to other PFAS for the purpose of risk assessment. During the process of adapting a published PBPK model for EPA needs, models are subjected to an extensive EPA internal QA review. During initial review of the Loccisano family of models (Loccisano et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011), an unusual implementation of PFOS plasma binding appeared to introduce a mass balance error. Because of the stated goal of minimizing new model development (see Section 4.1.3.2), EPA did not pursue resolution of the discrepancies, which would have required modifications to one of these models for application in this assessment.

A new publication describing a developmental PBPK model in rats and humans was also evaluated for this effort (Chou and Lin, 2021). This model used the in vitro extrapolation that was previously developed by Worley et al. (2017b) for PFOA as an initial point for parameter optimization for PFOS. The complex nature of this renal model, with processes for resorption, secretion, and passive diffusion presented multiple competing options for parameterization based on the available human data. Specifically, the set of available model parameters can take numerous values that fit the human observations equally well. However, when the model is applied within similar conditions to the human observations, predicting the exact values of the parameters may not impact the model's ability to predict the targeted biomarkers (i.e., human milk, fetal serum, and maternal serum). For our purposes, it was not clear whether the exposure and internal doses that needed modeling would be within the bounds of the doses used to parameterize the Chou et al. (2021) model.

Because of the previous issues that EPA encountered for other PFAS when implementing PBPK models, the known issue with the Loccisano model and the models based upon it, and the concerns about application of the Chou et al. (2021) model outside its original parameterization space, EPA concluded that a one-compartment model was the strongest approach to predict blood (or serum/plasma) concentrations. Serum/plasma is a good biomarker for exposure,

because a major proportion of the PFOS in the body is found in serum/plasma due to albumin binding (Forsthuber et al., 2020). There were no other specific tissues that were considered essential to describe the dosimetry of PFOS. A full PBPK model can predict serum concentrations equally well, but with many more parameters, many of which are difficult to predict for PFOS due to parameter identifiability issues. PFOS presents an unusually high barrier in this regard because much of its PK is dependent on the interaction between PFOS and proteins in the form of binding (Forsthuber et al., 2020) and active transport (Zhao et al., 2017). These protein interactions are more difficult to extrapolate from animal toxicological studies to humans than PK that is dependent on blood flow and passive diffusion.

The two one-compartment approaches identified in the literature for PFOA was the model of Verner et al. (2016, 3299692) and the model developed by the Minnesota Department of Health (MDH model) (Goeden et al., 2019), which was published as a PFOA model, but has been applied to other PFAS, including PFOS (Goeden et al., 2019). These two models are structurally very similar, with a single compartment each for mother and child, first-order excretion from those compartments, and a similar methodology for describing lactational transfer from mother to child. The following paragraphs describe the slight differences in model implementations, but it is first worth emphasizing the similarity in the two approaches. The overall agreement in approach between the two models supports its validity for the task of human health risk assessment for PFOS.

One advantage of the Verner model is that it explicitly models the mother from birth through the end of breastfeeding. The MDH model, however, is limited to predictions for the time period after the birth of the child with maternal levels set to an initial steady-state level. An explicit description of maternal blood levels allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy, as has been observed for serum PFOS in serial samples from pregnant women (Glynn et al., 2012). This decrease occurs due to the relatively rapid increase in body weight during pregnancy (compared with the years preceding pregnancy) and the increase in blood volume that occurs to support fetal growth (Sibai and Frangieh, 1995). Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Another distinction of the Verner model is that it is written in terms of rates of change in mass rather than concentrations, as in the MDH model. This approach includes the effect of dilution of PFOS during childhood growth, without the need for an explicit term in the equations. Not accounting for growth will result in the overprediction of serum concentration in individuals exposed during growth. Despite this, PFOS concentration in infants at any specific time is driven more by recent lactational exposure than by earlier exposure (either during pregnancy or early breastfeeding), which tends to minimize the impact of growth dilution. Additionally, this structural consideration best matches the approach taken in our animal model, presenting a harmonized approach. These structural considerations favor the application of the updated Verner model over the MDH model.

EPA evaluated two other factors that were present in the MDH model: the application of a scaling factor to increase the V_d in children and the treatment of exposure as a drinking water intake rather than a constant exposure relative to body weight. After testing these features within the updated Verner model structure, EPA determined that neither of these features were

appropriate for this assessment, primarily because they did not meaningfully improve the comparison of model predictions to validation data.

In the MDH model, V_d in children starts at 2.4 times the adult V_d and decreases relatively quickly to 1.5 times the adults V_d between 6 and 12 months, reaching the adult level at 10 years of age. These scaling values originated from measurements of body water content relative to weight compared with the adult value. There is no chemical-specific information to suggest that V_d is larger in children compared with adults for PFOS. However, it is generally accepted in pharmaceutical research that hydrophilic chemicals have greater V_d in children (Batchelor and Marriott, 2015), which is attributed to increased body water. Still, PFOS is amphiphilic, not simply hydrophilic, and its distribution is driven by interactions with binding proteins and transporters, not by passive diffusion with body water. While it is plausible that V_d is larger in children, it is unknown to what degree.

Since increased V_d in children is plausible, but it is neither supported nor contradicted by direct evidence, EPA evaluated the effect of variable V_d by implementing this change in the updated Verner model and comparing the results with constant and variable V_d (see Appendix F, (U.S. EPA, 2024a)). This resulted in reduced predictions of serum concentrations, primarily during their peak in early childhood. The model with variable V_d did not decrease the root mean squared error compared with the model with constant V_d. Because the model with constant V_d had better performance and was an overall simpler solution, EPA did not implement variable V_d in the application of the model for POD_{HED} calculation.

The other key difference between the MDH model and the updated Verner model is that instead of constant exposure relative to body weight, exposure in the MDH model was based on drinking water consumption, which is greater relative to body weight in young children compared with adults. Drinking water consumption is also greater in lactating women. To evaluate the potential impact of calculating a drinking water concentration directly, bypassing the RfD step, EPA implemented drinking water consumption in the modified Verner model (see Appendix F, (U.S. EPA, 2024a)). EPA evaluated this decision for PFOA and PFOS together because the choice of units used for human exposure represents a substantial difference in risk assessment methodology. For reasons explained below, EPA ultimately decided to continue to calculate an RfD in terms of constant exposure, with a maximum contaminant level goal (MCLG) calculated thereafter using lifestage specific drinking water consumption values.

When comparing exposure based on drinking water consumption to the traditional RfD approach, the impact on the serum concentrations predicted by the updated Verner model differed between PFOA and PFOS. For PFOA, the predicted serum concentration in the child was qualitatively similar, with the main effect seen in overprediction of timepoints that occur later in childhood. These timepoints are more susceptible to changes in exposure as early childhood exposure is dominated by lactational exposure. Lactational exposure is slightly increased in this scenario, because of increased drinking water consumption during lactation. However, the main source of PFOA or PFOS in breastmilk in the model with exposure based on drinking water consumption is that which accumulated over the mother's life prior to childbirth, not that which was consumed during lactation. For PFOS, the increased exposure predicted based on children's water intake results in much greater levels in later childhood compared with the model with constant exposure relative to body weight. Use of water ingestion rates to adjust the dose in the Verner model fails to match the decrease in PFOS concentration present in the

reported data with multiple timepoints and overestimates the value for the Norwegian Mother, Father, and Child Cohort Study (MoBa) cohort with a single timepoint. There was a much greater effect on PFOS model results relative to PFOA, but in both cases model performance, as quantified by root mean squared error, was superior with constant exposure compared with exposure based on drinking water consumption. This comparison suggests that incorporating variations in drinking water exposure in this way is not appropriate for the updated Verner model.

In addition to the comparison with reported data, EPA's decision to use the Verner model was also considered in the context of the effect on the derivation of MCLGs under SDWA. The epidemiological endpoints can be placed into three categories based on the age of the individuals at the time of exposure measurement: adults, children, and pregnant women. Because increased drinking water exposure is only applied to children and lactating women, the group of endpoints in children are the only ones that would be affected. While the RfD estimated using the updated Verner model assumed constant exposure, the MCLG based on noncancer effects or for nonlinear carcinogens is an algebraic calculation that incorporates the RfD, RSC, and drinking water intake. The drinking water intake used for this type of MCLG calculation would be chosen based on the target population relevant to the exposure interval used in the critical study and/or timing of exposure measurement and the response variable that serves as the basis of the RfD. Therefore, even if the RfD does not incorporate increased drinking water intake in certain lifestages, the subsequent MCLG calculation does take this into account. Furthermore, derivation of an RfD is useful for general assessment of risk and not limited to drinking water exposure.

For these reasons and based on EPA's analyses presented in Appendix F (U.S. EPA, 2024a), EPA determined that the updated Verner model was the most appropriate available model structure for POD_{HED} calculation for PFOS. Specifically, the EPA concluded that the determination that assuming V_d in children equal to the adult values and calculating a RfD assuming a constant dose (mg/kg/day) were appropriate for this assessment.

5.8 Sensitive Populations

Some populations may be more susceptible to the potential adverse health effects of toxic substances such as PFOS. These potentially susceptible populations include populations exhibiting a more severe response than others despite similar PFOS exposure due to increased biological sensitivity, as well as populations exhibiting a more severe response due to higher PFOS exposure and/or exposure to other chemicals or nonchemical stressors. Populations with greater biological sensitivity may include pregnant women and their developing fetuses, children, adolescents, lactating women, the elderly, and people with certain underlying medical conditions (see Section 5.8.1). Populations that could exhibit a greater response to PFOS exposure due to higher exposures to PFOS or other chemicals include communities overburdened by chemical exposures or nonchemical stressors such as communities with environmental justice concerns (see Section 5.8.2).

The potential health effects after PFOS exposure have been evaluated in some sensitive populations (e.g., pregnant women, children) and a small number of studies have assessed differences in exposure to PFOS across populations to assess whether racial/ethnic or socioeconomic differences are associated with greater PFOS exposure. However, the available research on PFOS's potential impacts on sensitive populations is limited and more research is

needed. Health effects differences in sensitivity to PFOS exposure have not allowed for the identification or characterization of all potentially sensitive subpopulations. This lack of knowledge about susceptibility to PFOS represents a potential source of uncertainty in the assessment of PFOS.

5.8.1 Fetuses, Infants, Children

One of the more well-studied sensitive populations to PFOS exposure is developing fetuses, infants, and children. Both animal toxicological and epidemiological data suggest that the developing fetus is particularly sensitive to PFOS-induced toxicity. As described in Sections 0 and 3.4.2.1, results of some epidemiological studies indicate an association between PFOS exposure during pregnancy and/or early childhood and adverse outcomes such as decreased birth weight and decreased antibody response to vaccinations. The available animal toxicological data lend support to these findings; as described in Section 3.4.4.2, numerous studies in rodents report effects similar to those seen in humans (e.g., decreased body weights in offspring exposed to PFOS during gestation). Additionally, PFOS exposure during certain lifestages or exposure windows (e.g., prenatal or early postnatal exposure windows) may be more consequential than others. For example, as described in Appendix C (U.S. EPA, 2024a), Grasty et al. (2005; 2003) identified GD 19–21 as a critical exposure window for neonatal lung development and subsequent neonatal mortality in rats. These potentially different effects in different populations and/or exposure windows have not been fully characterized. More research is needed to fully understand the specific critical windows of exposure during development.

With respect to the decreased antibody production endpoint, children who have autoimmune diseases (e.g., juvenile arthritis) or are taking medications that weaken the immune system would be expected to mount a relatively low antibody response compared to other children and would therefore represent potentially susceptible populations for PFOS exposure. There are also concerns about declines in vaccination status (Bramer et al., 2020; Smith et al., 2011) for children overall, and the possibility that diseases which are considered eradicated (such as diphtheria or tetanus) could return to the United States (Hotez, 2019). As noted by Dietert et al. (2010), the risks of developing infectious diseases may increase if immunosuppression occurs in the developing immune system.

5.8.2 Other Susceptible Populations

As noted in the SAB PFAS review panel's final report (U.S. EPA, 2022e), there is uncertainty about whether there are susceptible populations, such as certain racial/ethnic groups, that might be more sensitive to the health effects of PFOS exposure because of either greater biological sensitivity or higher exposure to PFOS and/or other environmental chemicals. Although some studies have evaluated differences in PFAS exposure levels across SES and racial/ethnic groups (see Section 5.1), studies of differential health effects incidence and PFOS exposure are limited. To fully address equity and environmental justice concerns about PFOS, these data gaps regarding differential exposure and health effects after PFOS exposure need to be addressed. In the development of the proposed PFAS NPDWR, EPA conducted an analysis to evaluate potential environmental justice impacts of the proposed regulation (see Chapter 8 of the *Economic Analysis for the Final Per- and Polyfluoroalkyl Substances National Primary Drinking Water Regulation* (U.S. EPA, 2024b)). EPA acknowledges that exposure to PFOS, and PFAS in general, may have a disproportionate impact on certain communities (e.g., low SES

communities; Tribal communities; minority communities; communities in the vicinity of areas of historical PFOS manufacturing and/or contamination) and that studies of these communities are high priority research needs.

6 References

3M. (2002). Environmental, health, safety, and regulatory (EHSR) profile of perfluorobutane sulfonate (PFBS). http://multimedia.3m.com/mws/media/172303O/ehsr-profile-of-perfluorobutane-sulfonate-pfbs.pdf

Abbott, BD; Wolf, CJ; Das, KP; Zehr, RD; Schmid, JE; Lindstrom, AB, et al. (2009). Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPAR alpha) in the mouse. Reproductive Toxicology 27: 258-265. http://dx.doi.org/10.1016/j.reprotox.2008.05.061

Abdallah, MA; Wemken, N; Drage, DS; Tlustos, C; Cellarius, C; Cleere, K, et al. (2020). Concentrations of perfluoroalkyl substances in human milk from Ireland: Implications for adult and nursing infant exposure. Chemosphere 246: 125724. http://dx.doi.org/10.1016/j.chemosphere.2019.125724

Abdullah Soheimi, SS; Abdul Rahman, A; Abd Latip, N; Ibrahim, E; Sheikh Abdul Kadir, SH. (2021). Understanding the impact of perfluorinated compounds on cardiovascular diseases and their risk factors: A meta-analysis study [Review]. International Journal of Environmental Research and Public Health 18: 8345. http://dx.doi.org/10.3390/ijerph18168345

Abraham, K; Mielke, H; Fromme, H; Völkel, W; Menzel, J; Peiser, M, et al. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Archives of Toxicology 94: 2131-2147.

Ahrens, L. (2011). Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate [Review]. Journal of Environmental Monitoring 13: 20-31. http://dx.doi.org/10.1039/c0em00373e

Ait Bamai, Y; Goudarzi, H; Araki, A; Okada, E; Kashino, I; Miyashita, C; Kishi, R. (2020). Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: The Hokkaido study on environment and children's health. Environment International 143: 105979.

Alexander, BH; Olsen, GW. (2007). Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Annals of Epidemiology 17: 471-478. http://dx.doi.org/10.1016/j.annepidem.2007.01.036

Alexander, BH; Olsen, GW; Burris, JM; Mandel, JH; Mandel, JS. (2003). Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occupational and Environmental Medicine 60: 722-729. http://dx.doi.org/10.1136/oem.60.10.722

Alkhalawi, E; Kasper-Sonnenberg, M; Wilhelm, M; Völkel, W; Wittsiepe, J. (2016). Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the Duisburg Birth Cohort. Journal of Toxicology and Environmental Health, Part A: Current Issues 79: 1041-1049.

Ammitzbøll, C; Börnsen, L; Petersen, ER; Oturai, AB; Søndergaard, HB; Grandjean, P; Sellebjerg, F. (2019). Perfluorinated substances, risk factors for multiple sclerosis and cellular immune activation. Journal of Neuroimmunology 330: 90-95.

Anand-Ivell, R; Cohen, A; Nørgaard-Pedersen, B; Jönsson, BAG; Bonde, JP; Hougaard, DM, et al. (2018). Amniotic fluid INSL3 measured during the critical time window in human

pregnancy relates to cryptorchidism, hypospadias, and phthalate load: a large case-control study. Frontiers in Physiology 9: 406.
Andersen, CS; Fei, C; Gamborg, M; Nohr, EA; Sørensen, TI; Olsen, J. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. American Journal of Epidemiology 172: 1230-1237. http://dx.doi.org/10.1093/aje/kwq289
Andersen, ME; Butenhoff, JL; Chang, SC; Farrar, DG; Kennedy, GL; Lau, C, et al. (2008). Perfluoroalkyl acids and related chemistries--toxicokinetics and modes of action. Toxicological Sciences 102: 3-14. http://dx.doi.org/10.1093/toxsci/kfm270
Andersen, ME; Clewell, HJ; Tan, YM; Butenhoff, JL; Olsen, GW. (2006). Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys--probing the determinants of long plasma half-lives. Toxicology 227: 156-164. http://dx.doi.org/10.1016/j.tox.2006.08.004
Apelberg, BJ; Goldman, LR; Calafat, AM; Herbstman, JB; Kuklenyik, Z; Heidler, J, et al. (2007a). Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environmental Science and Technology 41: 3891-3897. http://dx.doi.org/10.1021/es0700911
Apelberg, BJ; Witter, FR; Herbstman, JB; Calafat, AM; Halden, RU; Needham, LL; Goldman, LR. (2007b). Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115: 1670-1676. http://dx.doi.org/10.1289/ehp.10334
Arbuckle, T; Kubwabo, C; Walker, M; Davis, K; Lalonde, K; Kosarac, I, et al. (2013). Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. International Journal of Hygiene and Environmental Health 216: 184-194.
Argus Research Laboratories. (2000). Support: Oral (stomach tube) developmental toxicity study of PFOS in rabbits with attachments and cover letter dated 0425000. (EPA/OTS; Doc #8EHQ-0800-0373).
Armstrong, LE; Guo, GL. (2019). Understanding environmental contaminants' direct effects on non-alcoholic fatty liver disease progression. Current Environmental Health Reports 6: 95–104. http://dx.doi.org/10.1007/s40572-019-00231-x
Ashby, J; Brady, A; Elcombe, CR; Elliott, BM; Ishmael, J; Odum, J, et al. (1994). Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis [Review]. Human & Experimental Toxicology 13: S1-S117. http://dx.doi.org/10.1177/096032719401300201
Ashley-Martin, J; Dodds, L; Arbuckle, TE; Bouchard, MF; Fisher, M; Morriset, AS, et al. (2017). Maternal concentrations of perfluoroalkyl substances and fetal markers of metabolic function and birth weight. American Journal of Epidemiology 185: 185-193.
ATSDR. (2021). Toxicological profile for perfluoroalkyls [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://dx.doi.org/10.15620/cdc:59198
ATSDR (Agency for Toxic Substances and Disease Registry). (2022). PFAS exposure assessments final report: Findings across ten exposure assessment sites. Atlanta, GA: U.S. Department of Health and Human Services. https://www.atsdr.cdc.gov/pfas/docs/PFAS-EA-Final-Report-508.pdf
Attanasio, R. (2019). Sex differences in the association between perfluoroalkyl acids and liver function in US adolescents: Analyses of NHANES 2013-2016. Environmental Pollution 254: 113061.

Averina, M; Brox, J; Huber, S; Furberg, AS. (2021). Exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Fit Futures study. Environmental Research 195: 110740. http://dx.doi.org/10.1016/j.envres.2021.110740

Averina, M; Brox, J; Huber, S; Furberg, AS; Sørensen, M. (2019). Serum perfluoroalkyl substances (PFAS) and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern Norway. Environmental Research 169: 114-121.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bonefeld-Jørgensen, EC, et al. (2016). Perfluoroalkyl acids in maternal serum and indices of fetal growth: The Aarhus Birth Cohort. Environmental Health Perspectives 124: 848-854.

Bae, J; Kim, S; Schisterman, EF; Boyd Barr, D; Buck Louis, GM. (2015). Maternal and paternal serum concentrations of perfluoroalkyl and polyfluoroalkyl substances and the secondary sex ratio. Chemosphere 133: 31-40. http://dx.doi.org/10.1016/j.chemosphere.2015.03.015

Bagley, BD; Chang, SC; Ehresman, DJ; Eveland, A; Zitzow, JD; Parker, GA, et al. (2017). Perfluorooctane Sulfonate-Induced Hepatic Steatosis in Male Sprague Dawley Rats Is Not Attenuated by Dietary Choline Supplementation. Toxicological Sciences 160: 284-298. http://dx.doi.org/10.1093/toxsci/kfx185

Balk, FGP; Winkens Pütz, K; Ribbenstedt, A; Gomis, MI; Filipovic, M; Cousins, IT. (2019). Children's exposure to perfluoroalkyl acids - a modelling approach. Environmental Science: Processes & Impacts 21: 1875-1886. http://dx.doi.org/10.1039/c9em00323a

Bangma, J; Eaves, LA; Oldenburg, K; Reiner, JL; Manuck, T; Fry, RC. (2020). Identifying Risk Factors for Levels of Per- and Polyfluoroalkyl Substances (PFAS) in the Placenta in a High-Risk Pregnancy Cohort in North Carolina. Environmental Science and Technology 54: 8158-8166.

Bao, WW; Qian, ZM; Geiger, SD; Liu, E; Liu, Y; Wang, SQ, et al. (2017). Gender-specific associations between serum isomers of perfluoroalkyl substances and blood pressure among Chinese: Isomers of C8 Health Project in China. Science of the Total Environment 607-608: 1304-1312.

Bassler, J; Ducatman, A; Elliott, M; Wen, S; Wahlang, B; Barnett, J; Cave, MC. (2019). Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. Environmental Pollution 247: 1055-1063. http://dx.doi.org/10.1016/j.envpol.2019.01.064

Batchelor, HK; Marriott, JF. (2015). Paediatric pharmacokinetics: key considerations. British Journal of Clinical Pharmacology 79: 395-404. http://dx.doi.org/10.1111/bcp.12267

Beach, SA; Newsted, JL; Coady, K; Giesy, JP. (2006). Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS) [Review]. Reviews of Environmental Contamination and Toxicology 186: 133-174. http://dx.doi.org/10.1007/0-387-32883-1_5

Beck, IH; Timmermann, CAG; Nielsen, F; Schoeters, G; Jøhnk, C; Kyhl, HB, et al. (2019). Association between prenatal exposure to perfluoroalkyl substances and asthma in 5-year-old children in the Odense Child Cohort. Environmental Health: A Global Access Science Source 18: 97.

Beesoon, S; Martin, JW. (2015). Isomer-Specific Binding Affinity of Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) to Serum Proteins. Environmental Science and Technology 49: 5722-5731.

Beggs, KM; Mcgreal, S. R.; Mccarthy, A; Gunewardena, S; Lampe, JN; Lau, C; Apte, U. (2016). The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and

perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicology and Applied Pharmacology 304: 18-29. http://dx.doi.org/10.1016/j.taap.2016.05.001

Behr, AC; Kwiatkowski, A; Ståhlman, M; Schmidt, FF; Luckert, C; Braeuning, A; Buhrke, T. (2020a). Impairment of bile acid metabolism by perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in human HepaRG hepatoma cells. Archives of Toxicology 94: 1673-1686. http://dx.doi.org/10.1007/s00204-020-02732-3

Behr, AC; Plinsch, C; Braeuning, A; Buhrke, T. (2020b). Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). Toxicology In Vitro 62: 104700. http://dx.doi.org/10.1016/j.tiv.2019.104700

Bell, EM; Yeung, EH; Ma, W; Kannan, K; Sundaram, R; Smarr, MM; Buck Louis, GM. (2018). Concentrations of endocrine disrupting chemicals in newborn blood spots and infant outcomes in the upstate KIDS study. Environment International 121: 232-239.

Benninghoff, AD; Orner, GA; Buchner, CH; Hendricks, JD; Duffy, AM; Williams, DE. (2012). Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout. Toxicological Sciences 125: 69-78. http://dx.doi.org/10.1093/toxsci/kfr267

Benskin, JP; Muir, DCG; Scott, BF; Spencer, C; De Silva, AO; Kylin, H, et al. (2012). Perfluoroalkyl Acids in the Atlantic and Canadian Arctic Oceans. Environmental Science and Technology 46: 5815-5823. http://dx.doi.org/10.1021/es300578x

Bernstein, AS; Kapraun, DF; Schlosser, PM. (2021). A Model Template Approach for Rapid Evaluation and Application of Physiologically Based Pharmacokinetic Models for Use in Human Health Risk Assessments: A Case Study on Per- and Polyfluoroalkyl Substances. Toxicological Sciences 182: 215–228. http://dx.doi.org/10.1093/toxsci/kfab063

Berntsen, HF; Bølling, AK; Bjørklund, CG; Zimmer, K; Ropstad, E; Zienolddiny, S, et al. (2018). Decreased macrophage phagocytic function due to xenobiotic exposures in vitro, difference in sensitivity between various macrophage models. Food and Chemical Toxicology 112: 86-96. http://dx.doi.org/10.1016/j.fct.2017.12.024

Bijland, S; Rensen, PCN; Pieterman, EJ; Maas, ACE; van Der Hoorn, JW; van Erk, MJ, et al. (2011). Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. Toxicological Sciences 123: 290-303. http://dx.doi.org/10.1093/toxsci/kfr142

Bjerregaard-Olesen, C; Bach, CC; Long, M; Wielsøe, M; Bech, BH; Henriksen, TB, et al. (2019). Associations of Fetal Growth Outcomes with Measures of the Combined Xenoestrogenic Activity of Maternal Serum Perfluorinated Alkyl Acids in Danish Pregnant Women. Environmental Health Perspectives 127: 17006.

Bjork, JA; Wallace, KB. (2009). Structure-Activity Relationships and Human Relevance for Perfluoroalkyl Acid-Induced Transcriptional Activation of Peroxisome Proliferation in Liver Cell Cultures. Toxicological Sciences 111: 89-99. http://dx.doi.org/10.1093/toxsci/kfp093

Bjorke-Monsen, AL; Varsi, K; Averina, M; Brox, J; Huber, S. (2020). Perfluoroalkyl substances (PFASs) and mercury in never-pregnant women of fertile age: association with fish consumption and unfavorable lipid profile. 3: 277-284. http://dx.doi.org/10.1136/bmjnph-2020-000131

Blanc, M; Rüegg, J; Scherbak, N; Keiter, SH. (2019). Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (ZF-L) cell line and in zebrafish embryos. Aquatic Toxicology 215: 105272. http://dx.doi.org/10.1016/j.aquatox.2019.105272

Blomberg, AJ; Shih, YH; Messerlian, C; Jørgensen, LH; Weihe, P; Grandjean, P. (2021). Early-life associations between per- and polyfluoroalkyl substances and serum lipids in a longitudinal birth cohort. Environmental Research 200: 111400. http://dx.doi.org/10.1016/j.envres.2021.111400

Bogdanska, J; Borg, D; Sundström, M; Bergström, U; Halldin, K; Abedi-Valugerdi, M, et al. (2011). Tissue distribution of ^{35}S-labelled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose. Toxicology 284: 54-62. http://dx.doi.org/10.1016/j.tox.2011.03.014

Bonefeld-Jorgensen, EC; Long, M; Bossi, R; Ayotte, P; Asmund, G; Krüger, T, et al. (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. Environmental Health: A Global Access Science Source 10: 88. http://dx.doi.org/10.1186/1476-069X-10-88

Bonefeld-Jørgensen, EC; Long, M; Fredslund, SO; Bossi, R; Olsen, J. (2014). Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort. Cancer Causes and Control 25: 1439-1448. http://dx.doi.org/10.1007/s10552-014-0446-7

Bonner-Weir, S; Sullivan, BA; Weir, GC. (2015). Human Islet Morphology Revisited: Human and Rodent Islets Are Not So Different After All. Journal of Histochemistry and Cytochemistry 63: 604-612. http://dx.doi.org/10.1369/0022155415570969

Boone, L; Meyer, D; Cusick, P; Ennulat, D; Bolliger, AP; Everds, N, et al. (2005). Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies [Review]. Veterinary Clinical Pathology 34: 182-188. http://dx.doi.org/10.1111/j.1939-165X.2005.tb00041.x

Borg, D; Bogdanska, J; Sundström, M; Nobel, S; Håkansson, H; Bergman, Å, et al. (2010). Tissue distribution of (35)S-labelled perfluorooctane sulfonate (PFOS) in C57Bl/6 mice following late gestational exposure. Reproductive Toxicology 30: 558-565. http://dx.doi.org/10.1016/j.reprotox.2010.07.004

Bramer, CA; Kimmins, LM; Swanson, R; Kuo, J; Vranesich, P; Jacques-Carroll, LA; Shen, AK. (2020). Decline in Child Vaccination Coverage During the COVID-19 Pandemic - Michigan Care Improvement Registry, May 2016-May 2020. MMWR Morbidity and Mortality Weekly Report 69: 630-631. http://dx.doi.org/10.15585/mmwr.mm6920e1

Braun, JM; Kalkbrenner, AE; Just, AC; Yolton, K; Calafat, AM; Sjödin, A, et al. (2014). Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environmental Health Perspectives 122: 513-520.

Brochot, C; Casas, M; Manzano-Salgado, C; Zeman, FA; Schettgen, T; Vrijheid, M; Bois, FY. (2019). Prediction of maternal and foetal exposures to perfluoroalkyl compounds in a Spanish birth cohort using toxicokinetic modelling. Toxicology and Applied Pharmacology 379: 114640.

Buck Louis, GM; Sapra, KJ; Barr, DB; Lu, Z; Sundaram, R. (2016). Preconception perfluoroalkyl and polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. Reproductive Toxicology 65: 11-17.

Buck Louis, GM; Zhai, S; Smarr, MM; Grewal, J; Zhang, C; Grantz, KL, et al. (2018). Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environment International 119: 515-526.

Buck, RC; Franklin, J; Berger, U; Conder, JM; Cousins, IT; de Voogt, P, et al. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins [Review]. Integrated Environmental Assessment and Management 7: 513-541. http://dx.doi.org/10.1002/ieam.258

Budtz-Jørgensen, E; Grandjean, P. (2018). Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS ONE 13: e0205388. http://dx.doi.org/10.1371/journal.pone.0205388

Bulka, CM; Avula, V; Fry, RC. (2021). Associations of exposure to perfluoroalkyl substances individually and in mixtures with persistent infections: Recent findings from NHANES 1999-2016. Environmental Pollution 275: 116619. http://dx.doi.org/10.1016/j.envpol.2021.116619

Buser, MC; Scinicariello, F. (2016). Perfluoroalkyl substances and food allergies in adolescents. Environment International 88: 74-79.

Butenhoff, JL; Chang, SC; Olsen, GW; Thomford, PJ. (2012). Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague Dawley rats. Toxicology 293: 1-15. http://dx.doi.org/10.1016/j.tox.2012.01.003

Butenhoff, JL; Ehresman, DJ; Chang, SC; Parker, GA; Stump, DG. (2009). Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: developmental neurotoxicity. Reproductive Toxicology 27: 319-330.

Butenhoff, JL; Kennedy, GL; Hinderliter, PM; Lieder, PH; Jung, R; Hansen, KJ, et al. (2004). Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. Toxicological Sciences 82: 394-406. http://dx.doi.org/10.1093/toxsci/kfh302

Calafat, AM; Kato, K; Hubbard, K; Jia, T; Botelho, JC; Wong, LY. (2019). Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013-2014 National Health and Nutrition Examination Survey. Environment International 131: 105048. http://dx.doi.org/10.1016/j.envint.2019.105048

Calafat, AM; Wong, LY; Kuklenyik, Z; Reidy, JA; Needham, LL. (2007). Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environmental Health Perspectives 115: 1596-1602. http://dx.doi.org/10.1289/ehp.10598

CalEPA. (2021). Public Health Goals: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water (First Public Review Draft ed.). California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Branch. https://oehha.ca.gov/sites/default/files/media/downloads/crnr/pfoapfosphgdraft061021.pdf

Callan, AC; Rotander, A; Thompson, K; Heyworth, J; Mueller, JF; Odland, JØ; Hinwood, AL. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Science of the Total Environment 569-570: 1107-1113.

Canova, C; Barbieri, G; Zare Jeddi, M; Gion, M; Fabricio, A; Daprà, F, et al. (2020). Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environment International 145: 106117. http://dx.doi.org/10.1016/j.envint.2020.106117

Canova, C; Di Nisio, A; Barbieri, G; Russo, F; Fletcher, T; Batzella, E, et al. (2021). PFAS Concentrations and Cardiometabolic Traits in Highly Exposed Children and Adolescents.

International Journal of Environmental Research and Public Health 18. http://dx.doi.org/10.3390/ijerph182412881

Cao, L; Guo, Y; Chen, Y; Hong, J; Wu, J; Hangbiao, J. (2022). Per-/polyfluoroalkyl substance concentrations in human serum and their associations with liver cancer. Chemosphere 296: 134083. http://dx.doi.org/10.1016/j.chemosphere.2022.134083

Cao, T; Qu, A; Li, Z; Wang, W; Liu, R; Wang, X, et al. (2021). The relationship between maternal perfluoroalkylated substances exposure and low birth weight of offspring: a systematic review and meta-analysis. Environmental Science and Pollution Research 28: 67053-67065. http://dx.doi.org/10.1007/s11356-021-15061-4

Cao, W; Liu, X; Liu, X; Zhou, Y; Zhang, X; Tian, H, et al. (2018). Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a Chinese birth cohort. Environment International 116: 197-205.

Cao, Y; Ng, C. (2021). Absorption, distribution, and toxicity of per- and polyfluoroalkyl substances (PFAS) in the brain: a review [Review]. Environmental Science: Processes & Impacts 23: 1623-1640. http://dx.doi.org/10.1039/d1em00228g

Cardenas, A; Hivert, MF; Gold, DR; Hauser, R; Kleinman, KP; Lin, PD, et al. (2019). Associations of perfluoroalkyl and polyfluoroalkyl substances with incident diabetes and microvascular disease. Diabetes Care 42: 1824-1832.

Cariou, R; Veyrand, B; Yamada, A; Berrebi, A; Zalko, D; Durand, S, et al. (2015). Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environment International 84: 71-81.

Carmichael, S; Abrams, B; Selvin, S. (1997). The pattern of maternal weight gain in women with good pregnancy outcomes. American Journal of Public Health 87: 1984-1988. http://dx.doi.org/10.2105/AJPH.87.12.1984

CDC. (2013). Breastfeeding report card : United States 2013. Atlanta, GA.

CDC. (2017). Fourth national report on human exposure to environmental chemicals, updated tables, January 2017, volume one. Atlanta, GA. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf

CDC. (2021). Mortality in the United States, 2020. (Data Brief No. 427). Atlanta, GA: National Center for Health Statistics. https://www.cdc.gov/nchs/products/databriefs/db427.htm

Çelik, A; Eke, D; Ekinci, SY; Yıldırım, S. (2013). The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: single cell gel electrophoresis and micronucleus test. Food and Chemical Toxicology 53: 249-255. http://dx.doi.org/10.1016/j.fct.2012.11.054

Chang, CJ; Barr, DB; Ryan, PB; Panuwet, P; Smarr, MM; Liu, K, et al. (2022). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. Environment International 158: 106964. http://dx.doi.org/10.1016/j.envint.2021.106964

Chang, S; Allen, BC; Andres, KL; Ehresman, DJ; Falvo, R; Provencher, A, et al. (2017). Evaluation of serum lipid, thyroid, and hepatic clinical chemistries in association with serum perfluorooctanesulfonate (PFOS) in cynomolgus monkeys after oral dosing with potassium PFOS. Toxicological Sciences 156: 387-401.

Chang, SC; Ehresman, DJ; Bjork, JA; Wallace, KB; Parker, GA; Stump, DG; Butenhoff, JL. (2009). Gestational and lactational exposure to potassium perfluorooctanesulfonate

(K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression. Reproductive Toxicology 27: 387-399. http://dx.doi.org/10.1016/j.reprotox.2009.01.005

Chang, SC; Noker, PE; Gorman, GS; Gibson, SJ; Hart, JA; Ehresman, DJ; Butenhoff, JL. (2012). Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reproductive Toxicology 33: 428-440. http://dx.doi.org/10.1016/j.reprotox.2011.07.002

Charlton, CL; Lai, FY; Dover, DC. (2016). How to determine protective immunity in the post-vaccine era. 12: 903-906. http://dx.doi.org/10.1080/21645515.2015.1128600

Château-Degat, ML; Pereg, D; Dallaire, R; Ayotte, P; Dery, S; Dewailly, E. (2010). Effects of perfluorooctanesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec). Environmental Research 110: 710-717. http://dx.doi.org/10.1016/j.envres.2010.07.003

Chen, A; Jandarov, R; Zhou, L; Calafat, AM; Zhang, G; Urbina, EM, et al. (2019). Association of perfluoroalkyl substances exposure with cardiometabolic traits in an island population of the eastern Adriatic coast of Croatia. Science of the Total Environment 683: 29-36.

Chen, F; Yin, S; Kelly, BC; Liu, W. (2017a). Isomer-specific transplacental transfer of perfluoroalkyl acids: Results from a survey of paired maternal, cord sera, and placentas. Environmental Science and Technology 51: 5756-5763.

Chen, J; Liu, X; Zhong, Y. (2020). Interleukin-17A: The Key Cytokine in Neurodegenerative Diseases [Review]. Frontiers in Aging Neuroscience 12: 566922. http://dx.doi.org/10.3389/fnagi.2020.566922

Chen, J; Tanguay, RL; Tal, TL; Gai, Z; Ma, X; Bai, C, et al. (2014). Early life perfluorooctanesulphonic acid (PFOS) exposure impairs zebrafish organogenesis. Aquatic Toxicology 150: 124-132. http://dx.doi.org/10.1016/j.aquatox.2014.03.005

Chen, L; Tong, C; Huo, X; Zhang, J; Tian, Y. (2021). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and birth outcomes: A longitudinal cohort with repeated measurements. Chemosphere 267: 128899. http://dx.doi.org/10.1016/j.chemosphere.2020.128899

Chen, MH; Ha, EH; Wen, TW; Su, YN; Lien, GW; Chen, CY, et al. (2012a). Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS ONE 7: e42474. http://dx.doi.org/10.1371/journal.pone.0042474

Chen, MH; Ng, S; Hsieh, CJ; Lin, CC; Hsieh, WS; Chen, PC. (2017b). The impact of prenatal perfluoroalkyl substances exposure on neonatal and child growth. Science of the Total Environment 607-608: 669-675.

Chen, Q; Huang, R; Hua, L; Guo, Y; Huang, L; Zhao, Y, et al. (2018). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: A prospective birth cohort study. Environmental Health: A Global Access Science Source 17: 1-12.

Chen, T; Zhang, L; Yue, JQ; Lv, ZQ; Xia, W; Wan, YJ, et al. (2012b). Prenatal PFOS exposure induces oxidative stress and apoptosis in the lung of rat off-spring. Reproductive Toxicology 33: 538-545. http://dx.doi.org/10.1016/j.reprotox.2011.03.003

Chen, YM; Guo, LH. (2009). Fluorescence study on site-specific binding of perfluoroalkyl acids to human serum albumin. Archives of Toxicology 83: 255-261. http://dx.doi.org/10.1007/s00204-008-0359-x

Cheng, J; Lv, S; Nie, S; Liu, J; Tong, S; Kang, N, et al. (2016). Chronic perfluorooctane sulfonate (PFOS) exposure induces hepatic steatosis in zebrafish. Aquatic Toxicology 176: 45-52. http://dx.doi.org/10.1016/j.aquatox.2016.04.013

Cheng, W; Yu, Z; Feng, L; Wang, Y. (2013). Perfluorooctane sulfonate (PFOS) induced embryotoxicity and disruption of cardiogenesis. Toxicology In Vitro 27: 1503-1512. http://dx.doi.org/10.1016/j.tiv.2013.03.014

Chou, WC; Lin, Z. (2019). Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats, monkeys, and humans: Development and performance verification. Environment International 129: 408-422. http://dx.doi.org/10.1016/j.envint.2019.03.058

Chou, WC; Lin, Z. (2021). Development of a Gestational and Lactational Physiologically Based Pharmacokinetic (PBPK) Model for Perfluorooctane Sulfonate (PFOS) in Rats and Humans and Its Implications in the Derivation of Health-Based Toxicity Values. Environmental Health Perspectives 129: 37004. http://dx.doi.org/10.1289/EHP7671

Christensen, KY; Raymond, M; Meiman, J. (2019). Perfluoroalkyl substances and metabolic syndrome. International Journal of Hygiene and Environmental Health 222: 147-153.

Christensen, KY; Raymond, M; Thompson, BA; Anderson, HA. (2016). Perfluoroalkyl substances in older male anglers in Wisconsin. Environment International 91: 312-318.

Chu, C; Zhou, Y; Li, QQ; Bloom, MS; Lin, S; Yu, YJ, et al. (2020). Are perfluorooctane sulfonate alternatives safer? New insights from a birth cohort study. Environment International 135: 105365.

Cichoz-Lach, H; Michalak, A. (2014). Oxidative stress as a crucial factor in liver diseases [Review]. World Journal of Gastroenterology 20: 8082-8091. http://dx.doi.org/10.3748/wjg.v20.i25.8082

Cifone, MA. (1999). Unscheduled DNA synthesis in rat liver primary cell cultures with PFOS. Final report. (Covance Study No. 207840447). Vienna, VA: Covance Laboratories.

Cobb, BA. (2020). The history of IgG glycosylation and where we are now [Review]. Glycobiology 30: 202-213. http://dx.doi.org/10.1093/glycob/cwz065

Cohn, BA; La Merrill, MA; Krigbaum, NY; Wang, M; Park, JS; Petreas, M, et al. (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reproductive Toxicology 92: 112-119.

Cong, J; Chu, C; Li, QQ; Zhou, Y; Qian, ZM; Geiger, SD, et al. (2021). Associations of perfluorooctane sulfonate alternatives and serum lipids in Chinese adults. Environment International 155: 106596. http://dx.doi.org/10.1016/j.envint.2021.106596

Conley, JM; Lambright, CS; Evans, N; Medlock-Kakaley, E; Hill, D; McCord, J, et al. (2022). Developmental toxicity of Nafion byproduct 2 (NBP2) in the Sprague-Dawley rat with comparisons to hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) and perfluorooctane sulfonate (PFOS). Environment International 160: 107056. http://dx.doi.org/10.1016/j.envint.2021.107056

Cope, HA; Blake, BE; Love, C; McCord, J; Elmore, SA; Harvey, JB, et al. (2021). Latent, sex-specific metabolic health effects in CD-1 mouse offspring exposed to PFOA or HFPO-DA (GenX) during gestation. Emerging Contaminants 7: 219-235. http://dx.doi.org/10.1016/j.emcon.2021.10.004

Corley, RA; Mendrala, AL; Smith, FA; Staats, DA; Gargas, ML; Conolly, RB, et al. (1990). Development of a physiologically based pharmacokinetic model for chloroform. Toxicology and Applied Pharmacology 103: 512-527.

Corsini, E; Avogadro, A; Galbiati, V; Dell'Agli, M; Marinovich, M; Galli, CL; Germolec, DR. (2011). In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). Toxicology and Applied Pharmacology 250: 108-116. http://dx.doi.org/10.1016/j.taap.2010.11.004

Corsini, E; Sangiovanni, E; Avogadro, A; Galbiati, V; Viviani, B; Marinovich, M, et al. (2012). In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicology and Applied Pharmacology 258: 248-255. http://dx.doi.org/10.1016/j.taap.2011.11.004

Corton, JC; Cunningham, ML; Hummer, BT; Lau, C; Meek, B; Peters, JM, et al. (2014). Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study [Review]. Critical Reviews in Toxicology 44: 1-49. http://dx.doi.org/10.3109/10408444.2013.835784

Corton, JC; Peters, JM; Klaunig, JE. (2018). The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions [Review]. Archives of Toxicology 92: 83-119. http://dx.doi.org/10.1007/s00204-017-2094-7

Costa, G; Sartori, S; Consonni, D. (2009). Thirty years of medical surveillance in perfluorooctanoic acid production workers. Journal of Occupational and Environmental Medicine 51: 364-372. http://dx.doi.org/10.1097/JOM.0b013e3181965d80

Costa, O; Iñiguez, C; Manzano-Salgado, CB; Amiano, P; Murcia, M; Casas, M, et al. (2019). First-trimester maternal concentrations of polyfluoroalkyl substances and fetal growth throughout pregnancy. Environment International 130: 104830. http://dx.doi.org/10.1016/j.envint.2019.05.024

Costello, E; Rock, S; Stratakis, N; Eckel, SP; Walker, DI; Valvi, D, et al. (2022). Exposure to per- and Polyfluoroalkyl Substances and Markers of Liver Injury: A Systematic Review and Meta-Analysis [Review]. Environmental Health Perspectives 130: 46001. http://dx.doi.org/10.1289/EHP10092

Cui, L; Zhou, QF; Liao, CY; Fu, JJ; Jiang, GB. (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Archives of Environmental Contamination and Toxicology 56: 338-349. http://dx.doi.org/10.1007/s00244-008-9194-6

Cui, R; Zhang, H; Guo, X; Cui, Q; Wang, J; Dai, J. (2015a). Proteomic analysis of cell proliferation in a human hepatic cell line (HL-7702) induced by perfluorooctane sulfonate using iTRAQ. Journal of Hazardous Materials 299: 361-370. http://dx.doi.org/10.1016/j.jhazmat.2015.06.031

Cui, Y; Liu, W; Xie, W; Yu, W; Wang, C; Chen, H. (2015b). Investigation of the Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) on Apoptosis and Cell Cycle in a Zebrafish (Danio rerio) Liver Cell Line. International Journal of Environmental Research and Public Health 12: 15673-15682. http://dx.doi.org/10.3390/ijerph121215012

Cui, Y; Lv, S; Liu, J; Nie, S; Chen, J; Dong, Q, et al. (2017). Chronic perfluorooctanesulfonic acid exposure disrupts lipid metabolism in zebrafish. Human & Experimental Toxicology 36: 207-217. http://dx.doi.org/10.1177/0960327116646615

Curran, I; Hierlihy, SL; Liston, V; Pantazopoulos, P; Nunnikhoven, A; Tittlemier, S, et al. (2008). Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS). Journal of Toxicology and Environmental Health, Part A: Current Issues 71: 1526-1541. http://dx.doi.org/10.1080/15287390802361763

Cutland, CL; Lackritz, EM; Mallett-Moore, T; Bardají, A; Chandrasekaran, R; Lahariya, C, et al. (2017). Low birth weight: Case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. Vaccine 35: 6492-6500. http://dx.doi.org/10.1016/j.vaccine.2017.01.049

D'Agostino, RB; Vasan, RS; Pencina, MJ; Wolf, PA; Cobain, M; Massaro, JM; Kannel, WB. (2008). General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 117: 743-753. http://dx.doi.org/10.1161/CIRCULATIONAHA.107.699579

D'Alessandro, ML; Ellis, DA; Carter, JA; Stock, NL; March, RE. (2013). Competitive binding of aqueous perfluoroctanesulfonic acid and ibuprofen with bovine serum albumin studied by electrospray ionization mass spectrometry. International Journal of Mass Spectrometry 345: 28-36. http://dx.doi.org/10.1016/j.ijms.2012.12.012

D'eon, JC; Mabury, SA. (2011). Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? [Review]. Environmental Science & Technology 45: 7974-7984. http://dx.doi.org/10.1021/es200171y

Dalla Zuanna, T; Savitz, DA; Barbieri, G; Pitter, G; Zare Jeddi, M; Daprà, F, et al. (2021). The association between perfluoroalkyl substances and lipid profile in exposed pregnant women in the Veneto region, Italy. Ecotoxicology and Environmental Safety 209: 111805. http://dx.doi.org/10.1016/j.ecoenv.2020.111805

Dalsager, L; Christensen, N; Halekoh, U; Timmermann, CAG; Nielsen, F; Kyhl, HB, et al. (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. Environment International 149: 106395. http://dx.doi.org/10.1016/j.envint.2021.106395

Dalsager, L; Christensen, N; Husby, S; Kyhl, H; Nielsen, F; Høst, A, et al. (2016). Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environment International 96: 58-64.

Dang, Y; Wang, F; Liu, C. (2018). Real-time PCR array to study the effects of chemicals on the growth hormone/insulin-like growth factors (GH/IGFs) axis of zebrafish embryos/larvae. Chemosphere 207: 365-376. http://dx.doi.org/10.1016/j.chemosphere.2018.05.102

Dangudubiyyam, SV; Mishra, JS; Song, R; Kumar, S. (2022). Maternal PFOS exposure during rat pregnancy causes hypersensitivity to angiotensin II and attenuation of endothelium-dependent vasodilation in the uterine arteries. Biology of Reproduction 107: 1072-1083. http://dx.doi.org/10.1093/biolre/ioac141

Daniels, SR; Greer, FR; Nutr, C. (2008). Lipid screening and cardiovascular health in childhood. Pediatrics 122: 198-208. http://dx.doi.org/10.1542/peds.2008-1349

Darrow, LA; Howards, PP; Winquist, A; Steenland, K. (2014). PFOA and PFOS serum levels and miscarriage risk. Epidemiology 25: 505-512. http://dx.doi.org/10.1097/EDE.0000000000000103

Darrow, LA; Stein, CR; Steenland, K. (2013). Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environmental Health Perspectives 121: 1207-1213. http://dx.doi.org/10.1289/ehp.1206372

Das, KP; Wood, CR; Lin, MT; Starkov, AA; Lau, C; Wallace, KB, et al. (2017). Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378: 37-52. http://dx.doi.org/10.1016/j.tox.2016.12.007

Dassuncao, C; Hu, XC; Nielsen, F; Weihe, P; Grandjean, P; Sunderland, EM. (2018). Shifting Global Exposures to Poly- and Perfluoroalkyl Substances (PFASs) Evident in Longitudinal Birth Cohorts from a Seafood-Consuming Population. Environmental Science and Technology 52: 3738-3747.

Daugherty, A; Tall, AR; Daemen, MJA; Falk, E; Fisher, EA; García-Cardeña, G, et al. (2017). Recommendation on design, execution, and reporting of animal atherosclerosis studies: A scientific statement from the American Heart Association [Review]. Arteriosclerosis, Thrombosis, and Vascular Biology 37: e131-e157. http://dx.doi.org/10.1161/ATV.0000000000000062

Davies, B; Morris, T. (1993). Physiological parameters in laboratory animals and humans [Review]. Pharmaceutical Research 10: 1093-1095. http://dx.doi.org/10.1023/A:1018943613122

De Boo, HA; Harding, JE. (2006). The developmental origins of adult disease (Barker) hypothesis [Review]. Australian and New Zealand Journal of Obstetrics and Gynaecology 46: 4-14. http://dx.doi.org/10.1111/j.1479-828X.2006.00506.x

de Cock, M; de Boer, MR; Lamoree, M; Legler, J; van De Bor, M. (2014). First Year Growth in Relation to Prenatal Exposure to Endocrine Disruptors - A Dutch Prospective Cohort Study. International Journal of Environmental Research and Public Health 11: 7001-7021.

de Cock, M; De Boer, MR; Lamoree, M; Legler, J; Van De Bor, M. (2016). Prenatal exposure to endocrine disrupting chemicals and birth weight-A prospective cohort study. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 51: 178-185.

Deener, KC; Sacks, JD; Kirrane, EF; Glenn, BS; Gwinn, MR; Bateson, TF; Burke, TA. (2018). Epidemiology: a foundation of environmental decision making [Review]. Journal of Exposure Science & Environmental Epidemiology 28: 515-521. http://dx.doi.org/10.1038/s41370-018-0059-4

Deji, Z; Liu, P; Wang, X; Zhang, X; Luo, Y; Huang, Z. (2021). Association between maternal exposure to perfluoroalkyl and polyfluoroalkyl substances and risks of adverse pregnancy outcomes: A systematic review and meta-analysis [Review]. Science of the Total Environment 783: 146984. http://dx.doi.org/10.1016/j.scitotenv.2021.146984

Dewey, KG; Heinig, MJ; Nommsen, LA. (1993). Maternal weight-loss patterns during prolonged lactation. American Journal of Clinical Nutrition 58: 162-166.

Dewitt, JC; Blossom, SJ; Schaider, LA. (2019). Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence [Review]. Journal of Exposure Science and Environmental Epidemiology 29: 148-156. http://dx.doi.org/10.1038/s41370-018-0097-y

DeWitt, JC; Germolec, DR; Luebke, RW; Johnson, VJ. (2016). Associating changes in the immune system with clinical diseases for interpretation in risk assessment. Current

Protocols in Toxicology 67: 1.1-18.11.22. http://dx.doi.org/10.1002/0471140856.tx1801s67

Dewitt, JC; Keil, DE. (2017). Current issues in developmental immunotoxicity. In G Parker (Ed.), Immunopathology in Toxicology and Drug Development: Volume 1, Immunobiology, Investigative Techniques, and Special Studies (pp. 601-618). Totowa, NJ: Humana Press. http://dx.doi.org/10.1007/978-3-319-47377-2_13

Dietert, RR; Dewitt, JC; Germolec, DR; Zelikoff, JT. (2010). Breaking patterns of environmentally influenced disease for health risk reduction: Immune perspectives [Review]. Environmental Health Perspectives 118: 1091-1099. http://dx.doi.org/10.1289/ehp.1001971

Dinglasan-Panlilio, MJ; Prakash, SS; Baker, JE. (2014). Perfluorinated compounds in the surface waters of Puget Sound, Washington and Clayoquot and Barkley Sounds, British Columbia. Marine Pollution Bulletin 78: 173-180. http://dx.doi.org/10.1016/j.marpolbul.2013.10.046

Dixon, LJ; Barnes, M; Tang, H; Pritchard, MT; Nagy, LE. (2013). Kupffer cells in the liver [Review]. Comprehensive Physiology 3: 785-797. http://dx.doi.org/10.1002/cphy.c120026

Domazet, SL; Grøntved, A; Timmermann, AG; Nielsen, F; Jensen, TK. (2016). Longitudinal associations of exposure to perfluoroalkylated substances in childhood and adolescence and indicators of adiposity and glucose metabolism 6 and 12 years later: The European Youth Heart Study. Diabetes Care 39: 1745-1751.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Koponen, J, et al. (2019). Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environment International 124: 58-65.

Dong, GH; Liu, MM; Wang, D; Zheng, L; Liang, ZF; Jin, YH. (2011). Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. Archives of Toxicology 85: 1235-1244. http://dx.doi.org/10.1007/s00204-011-0661-x

Dong, GH; Tung, KY; Tsai, CH; Liu, MM; Wang, D; Liu, W, et al. (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environmental Health Perspectives 121: 507-513, 513e501-508. http://dx.doi.org/10.1289/ehp.1205351

Dong, GH; Zhang, YH; Zheng, L; Liu, W; Jin, YH; He, QC. (2009). Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. Archives of Toxicology 83: 805-815. http://dx.doi.org/10.1007/s00204-009-0424-0

Dong, H; Curran, I; Williams, A; Bondy, G; Yauk, CL; Wade, MG. (2016). Hepatic miRNA profiles and thyroid hormone homeostasis in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS). Environmental Toxicology and Pharmacology 41: 201-210. http://dx.doi.org/10.1016/j.etap.2015.12.009

Dong, Z; Wang, H; Yu, YY; Li, YB; Naidu, R; Liu, Y. (2019). Using 2003-2014 U.S. NHANES data to determine the associations between per- and polyfluoroalkyl substances and cholesterol: Trend and implications. Ecotoxicology and Environmental Safety 173: 461-468.

Du, J; Wang, S; You, H; Jiang, R; Zhuang, C; Zhang, X. (2014). Developmental toxicity and DNA damage to zebrafish induced by perfluorooctane sulfonate in the presence of ZnO

nanoparticles. Environmental Toxicology 31: 360-371. http://dx.doi.org/10.1002/tox.22050

Dubois, M; Pattou, F; Kerr-Conte, J; Gmyr, V; Vandewalle, B; Desreumaux, P, et al. (2000). Expression of peroxisome proliferator-activated receptor gamma (PPARgamma) in normal human pancreatic islet cells. Diabetologia 43: 1165-1169. http://dx.doi.org/10.1007/s001250051508

Ducatman, A; Tan, Y; Nadeau, B; Steenland, K. (2023). Perfluorooctanoic Acid (PFOA) Exposure and Abnormal Alanine Aminotransferase: Using Clinical Consensus Cutoffs Compared to Statistical Cutoffs for Abnormal Values. Toxics 11: 449. http://dx.doi.org/10.3390/toxics11050449

Ducatman, A; Zhang, J; Fan, H. (2015). Prostate-specific antigen and perfluoroalkyl acids in the C8 health study population. Journal of Occupational and Environmental Medicine 57: 111-114.

Dzierlenga, M, .W.; Crawford, L, .; Longnecker, M, .P. (2020a). Birth weight and perfluorooctane sulfonic acid: a random-effects meta-regression analysis. Environmental Epidemiology 4: e095. http://dx.doi.org/10.1097/EE9.0000000000000095

Dzierlenga, MW; Allen, BC; Clewell, HJ; Longnecker, MP. (2020b). Pharmacokinetic bias analysis of an association between clinical thyroid disease and two perfluoroalkyl substances. Environment International 141: 105784. http://dx.doi.org/10.1016/j.envint.2020.105784

Dzierlenga, MW; Allen, BC; Ward, PL; Clewell, HJ; Longnecker, MP. (2019). A model of functional thyroid disease status over the lifetime. PLoS ONE 14: e0219769. http://dx.doi.org/10.1371/journal.pone.0219769

Dzierlenga, MW; Moreau, M; Song, G; Mallick, P; Ward, PL; Campbell, JL, et al. (2020c). Quantitative bias analysis of the association between subclinical thyroid disease and two perfluoroalkyl substances in a single study. Environmental Research 182: 109017. http://dx.doi.org/10.1016/j.envres.2019.109017

Ebert, A; Allendorf, F; Berger, U; Goss, KU; Ulrich, N. (2020). Membrane/water partitioning and permeabilities of perfluoroalkyl acids and four of their alternatives and the effects on toxicokinetic behavior. Environmental Science and Technology 54: 5051-5061.

Ehresman, DJ; Froehlich, JW; Olsen, GW; Chang, SC; Butenhoff, JL. (2007). Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. Environmental Research 103: 176-184. http://dx.doi.org/10.1016/j.envres.2006.06.008

Eibl, G; Wente, MN; Reber, HA; Hines, OJ. (2001). Peroxisome proliferator-activated receptor gamma induces pancreatic cancer cell apoptosis. Biochemical and Biophysical Research Communications 287: 522-529. http://dx.doi.org/10.1006/bbrc.2001.5619

Eick, SM; Demicco, E; Valeri, L; Woodruff, TJ; Morello-Frosch, R; Hom Thepaksorn, EK, et al. (2020). Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. Environmental Health 19: 100-100. http://dx.doi.org/10.1186/s12940-020-00654-2

Eke, D; Çelik, A. (2016). Curcumin prevents perfluorooctane sulfonate-induced genotoxicity and oxidative DNA damage in rat peripheral blood. Drug and Chemical Toxicology 39: 97-103. http://dx.doi.org/10.3109/01480545.2015.1041601

Eke, D; Çelik, A; Yilmaz, MB; Aras, N; Kocatürk Sel, S; Alptekin, D. (2017). Apoptotic gene expression profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective role of curcumin. International Journal of Biological Macromolecules 104: 515-520. http://dx.doi.org/10.1016/j.ijbiomac.2017.06.075

Elcombe, CR; Elcombe, BM; Foster, JR; Chang, SC; Ehresman, DJ; Butenhoff, JL. (2012a). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. Toxicology 293: 16-29. http://dx.doi.org/10.1016/j.tox.2011.12.014

Elcombe, CR; Elcombe, BM; Foster, JR; Chang, SC; Ehresman, DJ; Noker, PE; Butenhoff, JL. (2012b). Evaluation of hepatic and thyroid responses in male Sprague Dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. Toxicology 293: 30-40. http://dx.doi.org/10.1016/j.tox.2011.12.015

Elcombe, CR; Peffer, RC; Wolf, DC; Bailey, J; Bars, R; Bell, D, et al. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator [Review]. Critical Reviews in Toxicology 44: 64-82. http://dx.doi.org/10.3109/10408444.2013.835786

EMEA. (2008). Non-clinical guideline on drug induced hepatotoxicity. (Doc. Ref. EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003355.pdf

EMEA. (2010). Reflection paper on non-clinical evaluation of drug-induced liver injury (DILI). (EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094591.pdf

Ericson, I; Gómez, M; Nadal, M; van Bavel, B; Lindström, G; Domingo, JL. (2007). Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: a pilot study. Environment International 33: 616-623. http://dx.doi.org/10.1016/j.envint.2007.01.003

Eriksen, KT; Raaschou-Nielsen, O; Mclaughlin, JK; Lipworth, L; Tjønneland, A; Overvad, K; Sørensen, M. (2013). Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PLoS ONE 8: e56969. http://dx.doi.org/10.1371/journal.pone.0056969

Eriksen, KT; Sørensen, M; Mclaughlin, JK; Lipworth, L; Tjønneland, A; Overvad, K; Raaschou-Nielsen, O. (2009). Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. Journal of the National Cancer Institute 101: 605-609. http://dx.doi.org/10.1093/jnci/djp041

Erol, E; Kumar, LS; Cline, GW; Shulman, GI; Kelly, DP; Binas, B. (2004). Liver fatty acid binding protein is required for high rates of hepatic fatty acid oxidation but not for the action of PPARalpha in fasting mice. FASEB Journal 18: 347-349. http://dx.doi.org/10.1096/fj.03-0330fje

Eryasa, B; Grandjean, P; Nielsen, F; Valvi, D; Zmirou-Navier, D; Sunderland, E, et al. (2019). Physico-chemical properties and gestational diabetes predict transplacental transfer and partitioning of perfluoroalkyl substances. Environment International 130: 104874.

Espindola-Santos, AD; Meyer, A; Dabkiewicz, VE; Câmara, VDM; Asmus, CIR, F. (2021). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonic acid in pregnant women: Maternal predictors and associations with birth outcomes in the PIPA Project. Journal of Obstetrics and Gynaecology Research 47: 3107-3118. http://dx.doi.org/10.1111/jog.14883

Fàbrega, F; Kumar, V; Benfenati, E; Schuhmacher, M; Domingo, JL; Nadal, M. (2015). Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. Toxicological and Environmental Chemistry 97: 814-827. http://dx.doi.org/10.1080/02772248.2015.1060976

Fàbrega, F; Kumar, V; Schuhmacher, M; Domingo, JL; Nadal, M. (2014). PBPK modeling for PFOS and PFOA: validation with human experimental data. Toxicology Letters 230: 244-251. http://dx.doi.org/10.1016/j.toxlet.2014.01.007

Fai Tse, WK; Li, JW; Kwan Tse, AC; Chan, TF; Hin Ho, JC; Sun Wu, RS, et al. (2016). Fatty liver disease induced by perfluorooctane sulfonate: Novel insight from transcriptome analysis. Chemosphere 159: 166-177. http://dx.doi.org/10.1016/j.chemosphere.2016.05.060

Falkner, B; Gidding, SS; Baker-Smith, CM; Brady, TM; Flynn, JT; Malle, LM, et al. (2023). Pediatric Primary Hypertension: An Underrecognized Condition: A Scientific Statement From the American Heart Association [Review]. Hypertension 80: e101-e111. http://dx.doi.org/10.1161/HYP.0000000000000228

Fan, H; Ducatman, A; Zhang, J. (2014). Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population. Environmental Research 135: 70-75.

Fan, Y; Lu, C; Li, X; Xu, Q; Zhang, Y; Yang, X, et al. (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environmental Pollution 266 Pt 2: 115138. http://dx.doi.org/10.1016/j.envpol.2020.115138

FDA. (2002). Guidance for industry: immunotoxicology evaluation of investigational new drugs.

FDA. (2009). Drug-induced liver injury: Premarketing clinical evaluation. (Docket no. FDA-2008-D-0128). Rockville, MD: U.S. Department of Health and Human Services, Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/drug-induced-liver-injury-premarketing-clinical-evaluation

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2008a). Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental Health Perspectives 116: 1391-1395. http://dx.doi.org/10.1289/ehp.11277

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2010a). Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scandinavian Journal of Work, Environment and Health 36: 413-421. http://dx.doi.org/10.5271/sjweh.2908

Fei, C; Mclaughlin, JK; Lipworth, L; Olsen, J. (2010b). Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environmental Research 110: 773-777. http://dx.doi.org/10.1016/j.envres.2010.08.004

Fei, C; Mclaughlin, JK; Tarone, RE; Olsen, J. (2007). Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort. Environmental Health Perspectives 115: 1677-1682. http://dx.doi.org/10.1289/ehp.10506

Fei, C; Mclaughlin, JK; Tarone, RE; Olsen, J. (2008b). Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. American Journal of Epidemiology 168: 66-72. http://dx.doi.org/10.1093/aje/kwn095

Felter, SP; Foreman, JE; Boobis, A; Corton, JC; Doi, AM; Flowers, L, et al. (2018). Human relevance of rodent liver tumors: key insights from a toxicology forum workshop on nongenotoxic modes of action. Regulatory Toxicology and Pharmacology 92: 1-7. http://dx.doi.org/https://doi.org/10.1016/j.yrtph.2017.11.003

Fernandez, E, .; Perez, R, .; Hernandez, A, .; Tejada, P, .; Arteta, M, .; Ramos, J, .T. (2011). Factors and Mechanisms for Pharmacokinetic Differences between Pediatric Population and Adults. Pharmaceutics 3: 53-72. http://dx.doi.org/10.3390/pharmaceutics3010053

Fisher, M; Arbuckle, TE; Wade, M; Haines, DA. (2013). Do perfluoroalkyl substances affect metabolic function and plasma lipids?--Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environmental Research 121: 95-103. http://dx.doi.org/10.1016/j.envres.2012.11.006

Fitz-Simon, N; Fletcher, T; Luster, MI; Steenland, K; Calafat, AM; Kato, K; Armstrong, B. (2013). Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology 24: 569-576. http://dx.doi.org/10.1097/EDE.0b013e31829443ee

Fletcher, T; Galloway, TS; Melzer, D; Holcroft, P; Cipelli, R; Pilling, LC, et al. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environment International 57-58: 2-10. http://dx.doi.org/10.1016/j.envint.2013.03.008

Florentin, A; Deblonde, T; Diguio, N; Hautemaniere, A; Hartemann, P. (2011). Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: cytotoxicity but no genotoxicity? International Journal of Hygiene and Environmental Health 214: 493-499. http://dx.doi.org/10.1016/j.ijheh.2011.05.010

Forsthuber, M; Kaiser, AM; Granitzer, S; Hassl, I; Hengstschläger, M; Stangl, H; Gundacker, C. (2020). Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environment International 137: 105324.

Fragki, S; Dirven, H; Fletcher, T; Grasl-Kraupp, B; Bjerve Gützkow, K; Hoogenboom, R, et al. (2021). Systemic PFOS and PFOA exposure and disturbed lipid homeostasis in humans: what do we know and what not? Critical Reviews in Toxicology141-164. http://dx.doi.org/10.1080/10408444.2021.1888073

Franco, ME; Fernandez-Luna, MT; Ramirez, AJ; Lavado, R. (2020a). Metabolomic-based assessment reveals dysregulation of lipid profiles in human liver cells exposed to environmental obesogens. Toxicology and Applied Pharmacology 398: 115009. http://dx.doi.org/10.1016/j.taap.2020.115009

Franco, ME; Sutherland, GE; Fernandez-Luna, MT; Lavado, R. (2020b). Altered expression and activity of phase I and II biotransformation enzymes in human liver cells by perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Toxicology 430: 152339. http://dx.doi.org/10.1016/j.tox.2019.152339

Frescas, D; Valenti, L; Accili, D. (2005). Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. Journal of Biological Chemistry 280: 20589-20595. http://dx.doi.org/10.1074/jbc.M412357200

Frisbee, SJ; Shankar, A; Knox, SS; Steenland, K; Savitz, DA; Fletcher, T; Ducatman, AM. (2010). Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Archives of Pediatrics and Adolescent Medicine 164: 860-869. http://dx.doi.org/10.1001/archpediatrics.2010.163

Fry, K; Power, MC. (2017). Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environmental Health: A Global Access Science Source 16: 105.

Fu, J; Gao, Y; Cui, L; Wang, T; Liang, Y; Qu, G, et al. (2016). Occurrence, temporal trends, and half-lives of perfluoroalkyl acids (PFAAs) in occupational workers in China. Scientific Reports 6: 38039.

Fu, SH; Chien, MW; Hsu, CY; Liu, YW; Sytwu, HK. (2020). Interplay between Cytokine Circuitry and Transcriptional Regulation Shaping Helper T Cell Pathogenicity and Plasticity in Inflammatory Bowel Disease [Review]. International Journal of Molecular Sciences 21. http://dx.doi.org/10.3390/ijms21093379

Fuentes, S; Colomina, MT; Rodriguez, J; Vicens, P; Domingo, JL. (2006). Interactions in developmental toxicology: concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. Toxicology Letters 164: 81-89. http://dx.doi.org/10.1016/j.toxlet.2005.11.013

Galazka, A; Kardymowicz, B. (1989). Immunity against diphtheria in adults in Poland. Epidemiology and Infection 103: 587-593. http://dx.doi.org/10.1017/s0950268800030983

Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. (1993). The immunological basis for immunization module 2 : Diphtheria. (WHO/EPI/Gen/93.11-18). Galazka, AM; Milstien, JB; Robertson, SE; Cutts, FT. http://apps.who.int/iris/bitstream/handle/10665/58891/WHO-EPI-GEN-93.12-mod2-eng.pdf?sequence=38&isAllowed=y

Gallo, V; Leonardi, G; Genser, B; Lopez-Espinosa, MJ; Frisbee, SJ; Karlsson, L, et al. (2012). Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environmental Health Perspectives 120: 655-660. http://dx.doi.org/10.1289/ehp.1104436

Gao, K, e; Zhuang, T; Liu, X; Fu, J; Zhang, J; Fu, J, ie, et al. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. Environmental Science and Technology 53: 6529-6538.

Gao, X; Ni, W; Zhu, S; Wu, Y; Cui, Y; Ma, J, et al. (2021). Per- and polyfluoroalkyl substances exposure during pregnancy and adverse pregnancy and birth outcomes: A systematic review and meta-analysis. Environmental Research 201: 111632. http://dx.doi.org/10.1016/j.envres.2021.111632

Gao, Y; Fu, J; Cao, H; Wang, Y; Zhang, A; Liang, Y, et al. (2015). Differential Accumulation and Elimination Behavior of Perfluoroalkyl Acid Isomers in Occupational Workers in a Manufactory in China. Environmental Science and Technology 49: 6953-6962.

Gardener, H; Sun, Q; Grandjean, P. (2021). PFAS concentration during pregnancy in relation to cardiometabolic health and birth outcomes. Environmental Research 192: 110287. http://dx.doi.org/10.1016/j.envres.2020.110287

Gaylord, A; Berger, KI; Naidu, M; Attina, TM; Gilbert, J; Koshy, TT, et al. (2019). Serum perfluoroalkyl substances and lung function in adolescents exposed to the World Trade Center disaster. Environmental Research 172: 266-272.

Gaylord, A; Trasande, L; Kannan, K; Thomas, KM; Lee, S; Liu, M; Levine, J. (2020). Persistent organic pollutant exposure and celiac disease: A pilot study. Environmental Research 186: 109439.

Geiger, SD; Xiao, J; Ducatman, A; Frisbee, S; Innes, K; Shankar, A. (2014a). The association between PFOA, PFOS and serum lipid levels in adolescents. Chemosphere 98: 78-83. http://dx.doi.org/10.1016/j.chemosphere.2013.10.005

Geiger, SD; Xiao, J; Shankar, A. (2014b). No association between perfluoroalkyl chemicals and hypertension in children. Integrated Blood Pressure Control 7: 1-7. http://dx.doi.org/10.2147/IBPC.S47660

Gennings, C; Wolk, A; Hakansson, N; Lindh, C; Bornehag, CG. (2020). Contrasting prenatal nutrition and environmental exposures in association with birth weight and cognitive function in children at 7 years. 3: 162-171. http://dx.doi.org/10.1136/bmjnph-2020-000099

Genuis, SJ; Birkholz, D; Ralitsch, M; Thibault, N. (2010). Human detoxification of perfluorinated compounds. Public Health 124: 367-375. http://dx.doi.org/10.1016/j.puhe.2010.03.002

Genuis, SJ; Liu, Y; Genuis, QI; Martin, JW. (2014). Phlebotomy treatment for elimination of perfluoroalkyl acids in a highly exposed family: a retrospective case-series. PLoS ONE 9: e114295.

Getz, GS; Reardon, CA. (2012). Animal models of atherosclerosis [Review]. Arteriosclerosis Thrombosis and Vascular Biology 32: 1104-1115. http://dx.doi.org/10.1161/ATVBAHA.111.237693

Ghisari, M; Long, M; Røge, DM; Olsen, J; Bonefeld-Jørgensen, EC. (2017). Polymorphism in xenobiotic and estrogen metabolizing genes, exposure to perfluorinated compounds and subsequent breast cancer risk: A nested case-control study in the Danish National Birth Cohort. Environmental Research 154: 325-333.

Gilbert, SG; Weiss, B. (2006). A rationale for lowering the blood lead action level from 10 to 2 microg/dL [Review]. NeuroToxicology 27: 693-701. http://dx.doi.org/10.1016/j.neuro.2006.06.008

Giménez-Bastida, JA; Surma, M; Zieliński, H. (2015). In vitro evaluation of the cytotoxicity and modulation of mechanisms associated with inflammation induced by perfluorooctanesulfonate and perfluorooctanoic acid in human colon myofibroblasts CCD-18Co. Toxicology In Vitro 29: 1683-1691. http://dx.doi.org/10.1016/j.tiv.2015.07.001

Girardi, P; Merler, E. (2019). A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Environmental Research 179: 108743.

Glassmeyer, ST; Furlong, ET; Kolpin, DW; Batt, AL; Benson, R; Boone, JS, et al. (2017). Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States. Science of the Total Environment 581-582: 909-922. http://dx.doi.org/10.1016/j.scitotenv.2016.12.004

Gleason, JA; Post, GB; Fagliano, JA. (2015). Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environmental Research 136: 8-14. http://dx.doi.org/10.1016/j.envres.2014.10.004

Gluckman, PD; Hanson, MA; Cooper, C; Thornburg, KL. (2008). Effect of in utero and early-life conditions on adult health and disease. New England Journal of Medicine 359: 61-73. http://dx.doi.org/10.1056/NEJMra0708473

Glynn, A; Berger, U; Bignert, A; Ullah, S; Aune, M; Lignell, S; Darnerud, PO. (2012). Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environmental Science and Technology 46: 9071-9079. http://dx.doi.org/10.1021/es301168c

Goeden, HM; Greene, CW; Jacobus, JA. (2019). A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. Journal of Exposure Science and Environmental Epidemiology 29: 183-195. http://dx.doi.org/10.1038/s41370-018-0110-5

Goff, DC; Lloyd-Jones, DM; Bennett, G; Coady, S; D'Agostino, RB; Gibbons, R, et al. (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 129: S49-S73. http://dx.doi.org/10.1161/01.cir.0000437741.48606.98

Gogola, J; Hoffmann, M; Nimpsz, S; Ptak, A. (2020a). Disruption of 17β-estradiol secretion by persistent organic pollutants present in human follicular fluid is dependent on the potential of ovarian granulosa tumor cell lines to metabolize estrogen. Molecular and Cellular Endocrinology 503: 110698. http://dx.doi.org/10.1016/j.mce.2019.110698

Gogola, J; Hoffmann, M; Ptak, A. (2019). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of granulosa tumor spheroids via GPR30 and IGF1R but not via the classic estrogen receptors. Chemosphere 217: 100-110. http://dx.doi.org/10.1016/j.chemosphere.2018.11.018

Gogola, J; Hoffmann, M; Ptak, A. (2020b). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate IGF1 secretion by adult ovarian granulosa cell tumor spheroids and thereby increase proliferation of non-cancer ovarian granulosa cells. Toxicology In Vitro 65: 104769. http://dx.doi.org/10.1016/j.tiv.2020.104769

Goldenthal, EI; Jessup, DC; Geil, RB; Mehring, JS. (1979). Ninety-Day Subacute Rhesus Monkey Toxicity Study. (Study No. 137-087). Mattawan, MI: International Research and Development Corporation. http://internal-pdf://0747719523/1529_3M-EPA-00070027_native.pdf

Gomis, MI; Vestergren, R; Macleod, M; Mueller, JF; Cousins, IT. (2017). Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling. Environment International 108: 92-102. http://dx.doi.org/10.1016/j.envint.2017.08.002

Goodrich, JA; Walker, D; Lin, X; Wang, H; Lim, T; Mcconnell, R, et al. (2022). Exposure to perfluoroalkyl substances and risk of hepatocellular carcinoma in a multiethnic cohort. 4: 100550. http://dx.doi.org/10.1016/j.jhepr.2022.100550

Gorrochategui, E; Pérez-Albaladejo, E; Casas, J; Lacorte, S; Porte, C. (2014). Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. Toxicology and Applied Pharmacology 277: 124-130. http://dx.doi.org/10.1016/j.taap.2014.03.012

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Chen, CJ; Ito, S, et al. (2017). Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environment International 104: 132-138.

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Kobayashi, S; Chen, CJ, et al. (2016). Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environment International 94: 124-132.

Govarts, E; Iszatt, N; Trnovec, T; de Cock, M; Eggesbø, M; Palkovicova Murinova, L, et al. (2018). Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven European birth cohorts. Environment International 115: 267-278.

Govarts, E; Remy, S; Bruckers, L; Den Hond, E; Sioen, I; Nelen, V, et al. (2016). Combined effects of prenatal exposures to environmental chemicals on birth weight. International Journal of Environmental Research and Public Health 13: n/a.

Governini, L; Guerranti, C; De Leo, V; Boschi, L; Luddi, A; Gori, M, et al. (2015). Chromosomal aneuploidies and DNA fragmentation of human spermatozoa from patients exposed to perfluorinated compounds. Andrologia 47: 1012-1019. http://dx.doi.org/10.1111/and.12371

Graber, JM; Alexander, C; Laumbach, RJ; Black, K; Strickland, PO; Georgopoulos, PG, et al. (2019). Per and polyfluoroalkyl substances (PFAS) blood levels after contamination of a community water supply and comparison with 2013-2014 NHANES. Journal of Exposure Science and Environmental Epidemiology 29: 172-182.

Grandjean, P; Andersen, EW; Budtz-Jørgensen, E; Nielsen, F; Mølbak, K; Weihe, P; Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA: Journal of the American Medical Association 307: 391-397. http://dx.doi.org/10.1001/jama.2011.2034

Grandjean, P; Budtz-Jørgensen, E. (2013). Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environmental Health: A Global Access Science Source 12: 35. http://dx.doi.org/10.1186/1476-069X-12-35

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Budtz-Jørgensen, E. (2017a). Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. Environmental Health Perspectives 125: 077018.

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Timmermann, A; Budtz-Jørgensen, E. (2017b). Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. Journal of Immunotoxicology 14: 188-195.

Grandjean, P; Timmermann, CAG; Kruse, M; Nielsen, F; Vinholt, PJ; Boding, L, et al. (2020). Severity of COVID-19 at elevated exposure to perfluorinated alkylates. PLoS ONE 15: e0244815. http://dx.doi.org/10.1371/journal.pone.0244815

Granum, B; Haug, LS; Namork, E; Stølevik, SB; Thomsen, C; Aaberge, IS, et al. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. Journal of Immunotoxicology 10: 373-379. http://dx.doi.org/10.3109/1547691X.2012.755580

Grasty, RC; Bjork, JA; Wallace, KB; Lau, CS; Rogers, JM. (2005). Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the perinatal rat. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 74: 405-416. http://dx.doi.org/10.1002/bdrb.20059

Grasty, RC; Wolf, DC; Grey, BE; Lau, CS; Rogers, JM. (2003). Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-

Dawley rat. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 68: 465-471. http://dx.doi.org/10.1002/bdrb.10046

Grasty, RC; Wolf, DC; Grey, BE; Lau, CS; Rogers, JM. (2006). Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat. Birth defects research (Part B) (vol 68, pg 465, 2003) [Erratum]. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 77: 86. http://dx.doi.org/10.1002/bdrb.20069

Grice, MM; Alexander, BH; Hoffbeck, R; Kampa, DM. (2007). Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. Journal of Occupational and Environmental Medicine 49: 722-729. http://dx.doi.org/10.1097/JOM.0b013e3180582043

Gross, RS; Ghassabian, A; Vandyousefi, S; Messito, MJ; Gao, C; Kannan, K; Trasande, L. (2020). Persistent organic pollutants exposure in newborn dried blood spots and infant weight status: A case-control study of low-income Hispanic mother-infant pairs. Environmental Pollution 267: 115427. http://dx.doi.org/10.1016/j.envpol.2020.115427

Gundacker, C; Graf-Rohrmeister, K; Gencik, M; Hengstschläger, M; Holoman, K; Rosa, P, et al. (2021). Gene variants determine placental transfer of perfluoroalkyl substances (PFAS), mercury (Hg) and lead (Pb), and birth outcome: Findings from the Ummuki Bratislava-Vienna study. Frontiers in Genetics 12: 664946. http://dx.doi.org/10.3389/fgene.2021.664946

Guyatt, HL; Snow, RW. (2004). Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clinical Microbiology Reviews 17: 760-769. http://dx.doi.org/10.1128/cmr.17.4.760-769.2004

Gyllenhammar, I; Benskin, JP; Sandblom, O; Berger, U; Ahrens, L; Lignell, S, et al. (2018a). Perfluoroalkyl acids (PFAAs) in serum from 2-4-month-old infants: Influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. Environmental Science and Technology 52: 7101-7110.

Gyllenhammar, I; Diderholm, B; Gustafsson, J; Berger, U; Ridefelt, P; Benskin, JP, et al. (2018b). Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environment International 111: 191-199.

Gyllenhammar, I; lynn, A; Lignell, S; Aune, M; Cantillana, T; Fridén, U; Lampa, E. (2017). Evaluation of the relationship between maternal body burden of POPs during pregnancy and lactation period and the health of their children. (2215-17-008). Swedish Environmental Protection Agency.

Hack, M; Flannery, DJ; Schluchter, M; Cartar, L; Borawski, E; Klein, N. (2002). Outcomes in young adulthood for very-low-birth-weight infants. New England Journal of Medicine 346: 149-157. http://dx.doi.org/10.1056/NEJMoa010856

Hack, M; Klein, NK; Taylor, HG. (1995). Long-term developmental outcomes of low birth weight infants. The Future of Children 5: 176-196.

Hagenaars, A; Vergauwen, L; De Coen, W; Knapen, D. (2011). Structure-activity relationship assessment of four perfluorinated chemicals using a prolonged zebrafish early life stage test. Chemosphere 82: 764-772. http://dx.doi.org/10.1016/j.chemosphere.2010.10.076

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A, et al. (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicologic Pathology 40: 971-994. http://dx.doi.org/10.1177/0192623312448935

Hamm, MP; Cherry, NM; Chan, E; Martin, JW; Burstyn, I. (2010). Maternal exposure to perfluorinated acids and fetal growth. Journal of Exposure Science and Environmental Epidemiology 20: 589-597. http://dx.doi.org/10.1038/jes.2009.57

Han, R; Hu, M; Zhong, Q; Wan, C; Liu, L; Li, F, et al. (2018a). Perfluorooctane sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-κB/TNF-α-mediated pathway. Chemosphere 191: 1056-1064.

Han, R; Zhang, F; Wan, C; Liu, L; Zhong, Q; Ding, W. (2018b). Effect of perfluorooctane sulphonate-induced Kupffer cell activation on hepatocyte proliferation through the NF-κB/TNF-α/IL-6-dependent pathway. Chemosphere 200: 283-294.

Han, X; Meng, L; Zhang, G; Li, Y; Shi, Y; Zhang, Q; Jiang, G. (2021). Exposure to novel and legacy per- and polyfluoroalkyl substances (PFASs) and associations with type 2 diabetes: A case-control study in East China. Environment International 156: 106637. http://dx.doi.org/10.1016/j.envint.2021.106637

Hanahan, D. (2022). Hallmarks of cancer: New dimensions [Review]. Cancer Discovery 12: 31-46. http://dx.doi.org/10.1158/2159-8290.CD-21-1059

Hanahan, D; Weinberg, RA. (2000). The hallmarks of cancer [Review]. Cell 100: 57-70. http://dx.doi.org/10.1016/S0092-8674(00)81683-9

Hanahan, D; Weinberg, RA. (2011). Hallmarks of cancer: The next generation [Review]. Cell 144: 646-674. http://dx.doi.org/10.1016/j.cell.2011.02.013

Harada, KH; Hashida, S; Kaneko, T; Takenaka, K; Minata, M; Inoue, K, et al. (2007). Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans. Environmental Toxicology and Pharmacology 24: 134-139. http://dx.doi.org/10.1016/j.etap.2007.04.003

Hardell, E; Kärrman, A; van Bavel, B; Bao, J; Carlberg, M; Hardell, L. (2014). Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63: 35-39. http://dx.doi.org/10.1016/j.envint.2013.10.005

Haug, LS; Huber, S; Becher, G; Thomsen, C. (2011). Characterisation of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. Environment International 37: 687-693. http://dx.doi.org/10.1016/j.envint.2011.01.011

He, X; Liu, Y; Xu, B; Gu, L; Tang, W. (2018). PFOA is associated with diabetes and metabolic alteration in US men: National Health and Nutrition Examination Survey 2003-2012. Science of the Total Environment 625: 566-574.

Henry, ND; Fair, PA. (2013). Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid. Journal of Applied Toxicology 33: 265-272. http://dx.doi.org/10.1002/jat.1736

Hessel, EVS; Tonk, ECM; Bos, PMJ; Van Loveren, H; Piersma, AH. (2015). Developmental immunotoxicity of chemicals in rodents and its possible regulatory impact [Review]. Critical Reviews in Toxicology 45: 68-82. http://dx.doi.org/10.3109/10408444.2014.959163

Hill, AB. (1965). The environment and disease: Association or causation? Proceedings of the Royal Society of Medicine 58: 295-300.

Hjermitslev, MH; Long, M; Wielsøe, M; Bonefeld-Jørgensen, EC. (2019). Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. Science of the Total Environment 698: 134118.

Honda-Kohmo, K; Hutcheson, R; Innes, KE; Conway, BN. (2019). Perfluoroalkyl substances are inversely associated with coronary heart disease in adults with diabetes. Journal of Diabetes and its Complications 33: 407-412.

Hotez, P. (2019). America and Europe's new normal: the return of vaccine-preventable diseases. 85: 912-914. http://dx.doi.org/10.1038/s41390-019-0354-3

Hu, X; Li, S; Cirillo, PM; Krigbaum, NY; Tran, V; Jones, DP; Cohn, BA. (2019a). Metabolome wide association study of serum poly and perfluoroalkyl substances (PFASs) in pregnancy and early postpartum. Reproductive Toxicology 87: 70-78. http://dx.doi.org/10.1016/j.reprotox.2019.05.058

Hu, XDC; Tokranov, AK; Liddie, J; Zhang, XM; Grandjean, P; Hart, JE, et al. (2019b). Tap Water Contributions to Plasma Concentrations of Poly- and Perfluoroalkyl Substances (PFAS) in a Nationwide Prospective Cohort of U.S. Women. Environmental Health Perspectives 127: 67006. http://dx.doi.org/10.1289/EHP4093

Hu, XZ; Hu, DC. (2009). Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. Archives of Toxicology 83: 851-861. http://dx.doi.org/10.1007/s00204-009-0441-z

Huang, H; Yu, K; Zeng, X; Chen, Q; Liu, Q; Zhao, Y, et al. (2020). Association between prenatal exposure to perfluoroalkyl substances and respiratory tract infections in preschool children. Environmental Research 191: 110156. http://dx.doi.org/10.1016/j.envres.2020.110156

Huang, JS; Borensztajn, J; Reddy, JK. (2011). Hepatic lipid metabolism. In SPS Monga (Ed.), Molecular pathology of liver diseases (pp. 133–146). Boston, MA: Springer. http://dx.doi.org/10.1007/978-1-4419-7107-4_10

Huang, M; Jiao, J; Zhuang, P; Chen, X; Wang, J; Zhang, Y. (2018). Serum polyfluoroalkyl chemicals are associated with risk of cardiovascular diseases in national US population. Environment International 119: 37-46.

Huang, MC; Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA, et al. (2019). Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female Hsd:Sprague Dawley SD rats after intravenous and gavage administration. Toxicology Reports 6: 645-655.

Huang, MC; Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA, et al. (2021). Corrigendum to "Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female Hsd:Sprague Dawley SD rats after intravenous and gavage administration" [Toxicol. Rep. 6 (2019) 645-655] [Erratum]. Toxicology Reports 8: 365. http://dx.doi.org/10.1016/j.toxrep.2021.02.001

Huang, Q; Zhang, J; Peng, S; Du, M; Ow, S; Pu, H, et al. (2014). Proteomic analysis of perfluorooctane sulfonate-induced apoptosis in human hepatic cells using the iTRAQ technique. Journal of Applied Toxicology 34: 1342-1351. http://dx.doi.org/10.1002/jat.2963

Huck, I; Beggs, K; Apte, U. (2018). Paradoxical protective effect of perfluorooctanesulfonic acid against high-fat diet-induced hepatic steatosis in mice. International Journal of Toxicology 37: 383-392. http://dx.doi.org/10.1177/1091581818790934

Humblet, O; Diaz-Ramirez, LG; Balmes, JR; Pinney, SM; Hiatt, RA. (2014). Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008).

Environmental Health Perspectives 122: 1129-1133. http://dx.doi.org/10.1289/ehp.1306606

Hunter, P. (2012). The inflammation theory of disease. The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. EMBO Reports 13: 968-970. http://dx.doi.org/10.1038/embor.2012.142

Huo, X; Zhang, L; Huang, R; Feng, L; Wang, W; Zhang, J; Cohort, SB. (2020). Perfluoroalkyl substances exposure in early pregnancy and preterm birth in singleton pregnancies: a prospective cohort study. Environmental Health: A Global Access Science Source 19: 60.

Hurley, S; Goldberg, D; Wang, M; Park, JS; Petreas, M; Bernstein, L, et al. (2018). Breast cancer risk and serum levels of per- and poly-fluoroalkyl substances: a case-control study nested in the California Teachers Study. Environmental Health: A Global Access Science Source 17: 83.

Hutcheson, R; Innes, K; Conway, B. (2020). Perfluoroalkyl substances and likelihood of stroke in persons with and without diabetes. Diabetes and Vascular Disease Research 17: 1-8.

IARC. (2019). Preamble to the IARC Monographs. In IARC Monographs on the Identification of Carcinogenic Hazards to Humans. Lyon, France. https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf

Impinen, A; Longnecker, MP; Nygaard, UC; London, SJ; Ferguson, KK; Haug, LS; Granum, B. (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. Environment International 124: 462-472.

Impinen, A; Nygaard, UC; Lødrup Carlsen, KC; Mowinckel, P; Carlsen, KH; Haug, LS; Granum, B. (2018). Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environmental Research 160: 518-523.

Innes, KE; Wimsatt, JH; Frisbee, S; Ducatman, AM. (2014). Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer 14: 45. http://dx.doi.org/10.1186/1471-2407-14-45

Ioannou, GN; Boyko, EJ; Lee, SP. (2006a). The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. American Journal of Gastroenterology 101: 76-82. http://dx.doi.org/10.1111/j.1572-0241.2005.00341.x

Ioannou, GN; Weiss, NS; Boyko, EJ; Mozaffarian, D; Lee, SP. (2006b). Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. Hepatology 43: 1145-1151. http://dx.doi.org/10.1002/hep.21171

IPCS. (2012). Harmonization project document no. 10: Guidance for immunotoxicity risk assessment for chemicals. Geneva, Switzerland: World Health Organization. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Ishida, K; Tsuyama, Y; Sanoh, S; Ohta, S; Kotake, Y. (2017). Perfluorooctane sulfonate induces neuronal vulnerability by decreasing GluR2 expression. Archives of Toxicology 91: 885-895.

Ishigame, H; Kakuta, S; Nagai, T; Kadoki, M; Nambu, A; Komiyama, Y, et al. (2009). Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30: 108-119. http://dx.doi.org/10.1016/j.immuni.2008.11.009

Itoh, H; Harada, KH; Kasuga, Y; Yokoyama, S; Onuma, H; Nishimura, H, et al. (2021). Serum perfluoroalkyl substances and breast cancer risk in Japanese women: A case-control study. Science of the Total Environment 800: 149316. http://dx.doi.org/10.1016/j.scitotenv.2021.149316

ITRC (Interstate Technology & Regulatory Council). (2020). PFAS technical and regulatory guidance document and fact sheets [Website]. https://pfas-1.itrcweb.org/

Iwabuchi, K; Senzaki, N; Mazawa, D; Sato, I; Hara, M; Ueda, F, et al. (2017). Tissue toxicokinetics of perfluoro compounds with single and chronic low doses in male rats. Journal of Toxicological Sciences 42: 301-317.

Jackson-Browne, MS; Eliot, M; Patti, M; Spanier, AJ; Braun, JM. (2020). PFAS (per- and polyfluoroalkyl substances) and asthma in young children: NHANES 2013-2014. International Journal of Hygiene and Environmental Health 229: 113565.

Jacquet, N; Maire, MA; Landkocz, Y; Vasseur, P. (2012). Carcinogenic potency of perfluorooctane sulfonate (PFOS) on Syrian hamster embryo (SHE) cells. Archives of Toxicology 86: 305-314. http://dx.doi.org/10.1007/s00204-011-0752-8

Jain, RB. (2019). Concentration of selected liver enzymes across the stages of glomerular function: The associations with PFOA and PFOS. Heliyon 5: e02168.

Jain, RB. (2020a). Impact of the co-occurrence of obesity with diabetes, anemia, hypertension, and albuminuria on concentrations of selected perfluoroalkyl acids. Environmental Pollution 266 Pt. 2: 115207.

Jain, RB. (2020b). Variabilities in concentrations of selected perfluoroalkyl acids among normotensives and hypertensives across various stages of glomerular function. Archives of Environmental and Occupational Health 76: 1-11.

Jain, RB; Ducatman, A. (2018). Associations between lipid/lipoprotein levels and perfluoroalkyl substances among US children aged 6-11 years. Environmental Pollution 243: 1-8.

Jain, RB; Ducatman, A. (2019a). Perfluoroalkyl acids serum concentrations and their relationship to biomarkers of renal failure: Serum and urine albumin, creatinine, and albumin creatinine ratios across the spectrum of glomerular function among US adults. Environmental Research 174: 143-151.

Jain, RB; Ducatman, A. (2019b). Roles of gender and obesity in defining correlations between perfluoroalkyl substances and lipid/lipoproteins. Science of the Total Environment 653: 74-81.

Jain, RB; Ducatman, A. (2019c). Selective associations of recent low concentrations of perfluoroalkyl substances with liver function biomarkers: nhanes 2011 to 2014 data on us adults aged ≥20 years. Journal of Occupational and Environmental Medicine 61: 293-302.

Jain, RB; Ducatman, A. (2020). Associations between apolipoprotein B and selected perfluoroalkyl substances among diabetics and nondiabetics. Environmental Science and Pollution Research International 2020: 13819-13828. http://dx.doi.org/10.1007/s11356-020-11593-3

JAMA. (2002). JAMA patient page. Low birth weight. JAMA: Journal of the American Medical Association 287: 270.

Jantzen, CE; Annunziato, KA; Bugel, SM; Cooper, KR. (2016a). PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. Aquatic Toxicology 175: 160-170. http://dx.doi.org/10.1016/j.aquatox.2016.03.026

Jantzen, CE; Annunziato, KM; Cooper, KR. (2016b). Behavioral, morphometric, and gene expression effects in adult zebrafish (Danio rerio) embryonically exposed to PFOA, PFOS, and PFNA. Aquatic Toxicology 180: 123-130. http://dx.doi.org/10.1016/j.aquatox.2016.09.011

Jarvis, AL; Justice, JR; Elias, MC; Schnitker, B; Gallagher, K. (2021). Perfluorooctane Sulfonate in US Ambient Surface Waters: A Review of Occurrence in Aquatic Environments and Comparison to Global Concentrations. Environmental Toxicology and Chemistry 40: 2425-2442. http://dx.doi.org/10.1002/etc.5147

Jensen, RC; Andersen, MS; Larsen, PV; Glintborg, D; Dalgård, C; Timmermann, CAG, et al. (2020). Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study. Environmental Health Perspectives 128: 77001.

Jensen, TK; Andersen, LB; Kyhl, HB; Nielsen, F; Christesen, HT; Grandjean, P. (2015). Association between Perfluorinated Compound Exposure and Miscarriage in Danish Pregnant Women. PLoS ONE 10: e0123496.

Ji, J; Song, L; Wang, J; Yang, Z; Yan, H; Li, T, et al. (2021). Association between urinary per- and poly-fluoroalkyl substances and COVID-19 susceptibility. Environment International 153: 106524. http://dx.doi.org/10.1016/j.envint.2021.106524

Jiang, W; Zhang, Y; Zhu, L; Deng, J. (2014). Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environment International 64: 40-47.

Jin, H; Zhang, Y; Jiang, W; Zhu, L; Martin, JW. (2016). Isomer-Specific Distribution of Perfluoroalkyl Substances in Blood. Environmental Science and Technology 50: 7808-7815.

Jin, R; Mcconnell, R; Catherine, C; Xu, S; Walker, DI; Stratakis, N, et al. (2020). Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environment International 134: 105220.

Johanson, CE. (1979). Distribution of fluid between extracellular and intracellular compartments in the heart, lungs, liver and spleen of neonatal rats. 36: 282-289. http://dx.doi.org/10.1159/000241241

Johnson, JD; Gibson, SJ; Ober, RE. (1984). Cholestyramine-enhanced fecal elimination of c-14 in rats after administration of ammonium [c-14] perfluorooctanoate or potassium [c-14]perfluorooctanesulfonate. Fundamental and Applied Toxicology 4: 972-976. http://dx.doi.org/10.1016/0272-0590(84)90235-5

Jusko, TA; Oktapodas, M; Palkovičová Murinová, L; Babinská, K; Babjaková, J; Verner, MA, et al. (2016). Demographic, reproductive, and dietary determinants of perfluorooctane sulfonic (PFOS) and perfluorooctanoic acid (PFOA) concentrations in human colostrum. Environmental Science and Technology 50: 7152-7162. http://dx.doi.org/10.1021/acs.est.6b00195

Kang, H; Lee, HK; Moon, HB; Kim, S; Lee, J; Ha, M, et al. (2018). Perfluoroalkyl acids in serum of Korean children: Occurrences, related sources, and associated health outcomes. Science of the Total Environment 645: 958-965.

Kang, Q; Gao, F; Zhang, X; Wang, L; Liu, J; Fu, M, et al. (2020). Nontargeted identification of per- and polyfluoroalkyl substances in human follicular fluid and their blood-follicle transfer. Environment International 139: 105686.

Kapraun, DF; Zurlinden, TJ; Verner, M-A; Chiang, C; Dzierlenga, MW; Carlson, LM, et al. (2022). A Generic Pharmacokinetic Model for Quantifying Mother-to-Offspring Transfer of Lipophilic Persistent Environmental Chemicals. Toxicological Sciences.

Karakas-Celik, S; Aras, N. (2014). An in-vitro investigation of the effect of perfluorooctane sulphonate on cell lines of embryonic origin. Molecular Biology Reports 41: 3755-3759. http://dx.doi.org/10.1007/s11033-014-3240-4

Kärrman, A; Ericson, I; van Bavel, B; Darnerud, PO; Aune, M; Glynn, A, et al. (2007). Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. Environmental Health Perspectives 115: 226-230. http://dx.doi.org/10.1289/ehp.9491

Kasarala, G; Tillmann, HL. (2016). Standard liver tests. Clinical Liver Disease 8: 13-18. http://dx.doi.org/10.1002/cld.562

Kashino, I; Sasaki, S; Okada, E; Matsuura, H; Goudarzi, H; Miyashita, C, et al. (2020). Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environment International 136: 105355.

Kato, K; Wong, LY; Chen, A; Dunbar, C; Webster, GM; Lanphear, BP; Calafat, AM. (2014). Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environmental Science and Technology 48: 9600-9608.

Kavlock, RJ; Allen, BC; Faustman, EM; Kimmel, CA. (1995). Dose-response assessments for developmental toxicity. IV. Benchmark doses for fetal weight changes. Toxicological Sciences 26: 211-222. http://dx.doi.org/10.1006/faat.1995.1092

Kawamoto, K; Oashi, T; Oami, K; Liu, W; Jin, YH; Saito, N, et al. (2010). Perfluorooctanoic acid (PFOA) but not perfluorooctane sulfonate (PFOS) showed DNA damage in comet assay on Paramecium caudatum. Journal of Toxicological Sciences 35: 835-841. http://dx.doi.org/10.2131/jts.35.835

Keil, DE; Mehlmann, T; Butterworth, L; Peden-Adams, MM. (2008). Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicological Sciences 103: 77-85. http://dx.doi.org/10.1093/toxsci/kfn015

Kerstner-Wood, C; Coward, L; Gorman, G; Southern Research Institute. (2003). Protein binding of perfluorobutane sulfonate, perfluorohexane sulfonate, perfluorooctane sulfonate and perfluorooctanoate to plasma (human, rat, and monkey), and various human-derived plasma protein fractions. Study ID 9921.7 [TSCA Submission]. (8EHQ-04-15845A; 88040000364). St. Paul, MN: 3M Company.

Khalil, N; Ducatman, AM; Sinari, S; Billheimer, D; Hu, C; Littau, S; Burgess, JL. (2020). Per- and polyfluoroalkyl substance and cardio metabolic markers in firefighters. Journal of Occupational and Environmental Medicine 62: 1076-1081. http://dx.doi.org/10.1097/JOM.0000000000002062

Khalil, N; Ebert, JR; Honda, M; Lee, M; Nahhas, RW; Koskela, A, et al. (2018). Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8-12 year old children: A pilot study. Environmental Research 160: 314-321.

Khansari, MR; Yousefsani, BS; Kobarfard, F; Faizi, M; Pourahmad, J. (2017). In vitro toxicity of perfluorooctane sulfonate on rat liver hepatocytes: probability of distructive binding to CYP 2E1 and involvement of cellular proteolysis. Environmental Science and Pollution Research 24: 23382-23388. http://dx.doi.org/10.1007/s11356-017-9908-2

Khazaee, M; Guardian, MGE; Aga, DS; Ng, CA. (2019). Impacts of sex and exposure duration on gene expression in zebrafish following perfluorooctane sulfonate (PFOS) exposure. Environmental Toxicology and Chemistry 39: 437-449. http://dx.doi.org/10.1002/etc.4628

Kielsen, K; Shamim, Z; Ryder, LP; Nielsen, F; Grandjean, P; Budtz-Jørgensen, E; Heilmann, C. (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. Journal of Immunotoxicology 13: 270-273. http://dx.doi.org/10.3109/1547691X.2015.1067259

Kim, HC; Nam, CM; Jee, SH; Han, KH; Oh, DK; Suh, I. (2004). Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. British Medical Journal (Clinical Research Edition) 328: 983. http://dx.doi.org/10.1136/bmj.38050.593634.63

Kim, S; Choi, K; Ji, K; Seo, J; Kho, Y; Park, J, et al. (2011a). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environmental Science and Technology 45: 7465-7472. http://dx.doi.org/10.1021/es202408a

Kim, SJ; Heo, SH; Lee, DS; Hwang, IG; Lee, YB; Cho, HY. (2016). Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. Food and Chemical Toxicology 97: 243-255.

Kim, SK; Lee, KT; Kang, CS; Tao, L; Kannan, K; Kim, KR, et al. (2011b). Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environmental Pollution 159: 169-174. http://dx.doi.org/10.1016/j.envpol.2010.09.008

Kim, WR; Flamm, SL; Di Bisceglie, AM; Bodenheimer, HC. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47: 1363-1370. http://dx.doi.org/10.1002/hep.22109

Kingsley, SL; Walker, DI; Calafat, AM; Chen, A; Papandonatos, GD; Xu, Y, et al. (2019). Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 15: 95. http://dx.doi.org/10.1007/s11306-019-1560-z

Kishi, R; Nakajima, T; Goudarzi, H; Kobayashi, S; Sasaki, S; Okada, E, et al. (2015). The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: the hokkaido study. Environmental Health Perspectives 123: 1038-1045.

Klaunig, JE; Babich, MA; Baetcke, KP; Cook, JC; Corton, JC; David, RM, et al. (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance [Review]. Critical Reviews in Toxicology 33: 655-780. http://dx.doi.org/10.1080/713608372

Knox, SS; Jackson, T; Javins, B; Frisbee, SJ; Shankar, A; Ducatman, AM. (2011). Implications of early menopause in women exposed to perfluorocarbons. Journal of Clinical Endocrinology and Metabolism 96: 1747-1753. http://dx.doi.org/10.1210/jc.2010-2401

Kobayashi, S; Azumi, K; Goudarzi, H; Araki, A; Miyashita, C; Kobayashi, S, et al. (2017). Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: The Hokkaido Study. Journal of Exposure Science and Environmental Epidemiology 27: 251-259.

Kobayashi, S; Sata, F; Goudarzi, H; Araki, A; Miyashita, C; Sasaki, S, et al. (2021). Associations among perfluorooctanesulfonic/perfluorooctanoic acid levels, nuclear receptor gene

polymorphisms, and lipid levels in pregnant women in the Hokkaido study. Scientific Reports 11: 9994. http://dx.doi.org/10.1038/s41598-021-89285-2

Kobayashi, S; Sata, F; Ikeda-Araki, A; Miyashita, C; Goudarzi, H; Iwasaki, Y, et al. (2022). Relationships between maternal perfluoroalkyl substance levels, polymorphisms of receptor genes, and adverse birth outcomes in the Hokkaido birth cohort study, Japan. Reproductive Toxicology 107: 112-122. http://dx.doi.org/10.1016/j.reprotox.2021.12.004

Koshy, TT; Attina, TM; Ghassabian, A; Gilbert, J; Burdine, LK; Marmor, M, et al. (2017). Serum perfluoroalkyl substances and cardiometabolic consequences in adolescents exposed to the World Trade Center disaster and a matched comparison group. Environment International 109: 128-135.

Koskela, A; Ducatman, A; Schousboe, JT; Nahhas, RW; Khalil, N. (2022). Perfluoroalkyl Substances and Abdominal Aortic Calcification. Journal of Occupational and Environmental Medicine. http://dx.doi.org/10.1097/JOM.0000000000002479

Kotlarz, N; Mccord, J; Collier, D; Lea, CS; Strynar, M; Lindstrom, AB, et al. (2020). Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. Environmental Health Perspectives 128: 77005.

Kuczmarski, RJ; Ogden, CL; Guo, SS; Grummer-Strawn, LM; Flegal, KM; Mei, Z, et al. (2002). 2000 CDC growth charts for the United States: Methods and development. (Vital and Health Statistics: Series 11, No. 246). Hyattsville, MD: National Center for Health Statistics. https://www.cdc.gov/nchs/data/series/sr_11/sr11_246.pdf

Kuklenyik, Z; Reich, JA; Tully, JS; Needham, LL; Calafat, AM. (2004). Automated solid-phase extraction and measurement of perfluorinated organic acids and amides in human serum and milk. Environmental Science and Technology 38: 3698-3704. http://dx.doi.org/10.1021/es040332u

Kummu, M; Sieppi, E; Koponen, J; Laatio, L; Vã¤Hã¤Kangas, K; Kiviranta, H, et al. (2015). Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. Placenta 36: 1185-1191.

Kvalem, HE; Nygaard, UC; Lødrup Carlsen, KC; Carlsen, KH; Haug, LS; Granum, B. (2020). Perfluoroalkyl substances, airways infections, allergy and asthma related health outcomes - Implications of gender, exposure period and study design. Environment International 134: 105259.

Kwiatkowski, CF; Andrews, DQ; Birnbaum, LS; Bruton, TA; Dewitt, JC; Knappe, DRU, et al. (2020). Scientific Basis for Managing PFAS as a Chemical Class. Environmental Science & Technology Letters 7: 532-543. http://dx.doi.org/10.1021/acs.estlett.0c00255

Kwo, PY; Cohen, SM; Lim, JK. (2017). ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. American Journal of Gastroenterology 112: 18-35. http://dx.doi.org/10.1038/ajg.2016.517

Kwon, EJ; Shin, JS; Kim, BM; Shah-Kulkarni, S; Park, H; Kho, YL, et al. (2016). Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. Journal of Occupational and Environmental Medicine 58: e198-e205.

Lai, KP; Lee, JC; Wan, HT; Li, JW; Wong, AY; Chan, TF, et al. (2017a). Effects of in Utero PFOS Exposure on Transcriptome, Lipidome, and Function of Mouse Testis. Environmental Science and Technology 51: 8782-8794.

Lai, KP; Li, JW; Cheung, A; Li, R; Billah, MB; Chan, TF; Wong, CK. (2017b). Transcriptome sequencing reveals prenatal PFOS exposure on liver disorders. Environmental Pollution 223: 416-425.

Lai, KP; Ng, AH; Wan, HT; Wong, AY; Leung, CC; Li, R; Wong, CK. (2018). Dietary exposure to the environmental chemical, pfos on the diversity of gut microbiota, associated with the development of metabolic syndrome. Frontiers in Microbiology 9: 2552.

Laptook, AR; O'Shea, TM; Shankaran, S; Bhaskar, B; Network, NN. (2005). Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents. Pediatrics 115: 673-680. http://dx.doi.org/10.1542/peds.2004-0667

Larroque, B; Bertrais, S; Czernichow, P; Léger, J. (2001). School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study. Pediatrics 108: 111-115. http://dx.doi.org/10.1542/peds.108.1.111

Lau, C; Thibodeaux, JR; Hanson, RG; Rogers, JM; Grey, BE; Stanton, ME, et al. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicological Sciences 74: 382-392.

Lauritzen, HB; Larose, TL; Øien, T; Sandanger, TM; Odland, JØ; van de Bor, M; Jacobsen, GW. (2017). Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatric Research 81: 33-42.

Leary, DB; Takazawa, M; Kannan, K; Khalil, N. (2020). Perfluoroalkyl substances and metabolic syndrome in firefighters a pilot study. Journal of Occupational and Environmental Medicine 62: 52-57. http://dx.doi.org/10.1097/JOM.0000000000001756

Lee, CK; Kang, SG; Lee, JT; Lee, SW; Kim, JH; Kim, DH, et al. (2015). Effects of perfluorooctane sulfuric acid on placental PRL-family hormone production and fetal growth retardation in mice. Molecular and Cellular Endocrinology 401: 165-172.

Lee, ES; Han, S; Oh, JE. (2016). Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reproductive Toxicology 59: 53-59.

Lee, J; Lee, S; Choi, YA, e; Jin, M; Kim, YY; Kang, BC, et al. (2018a). Perfluorooctane sulfonate exacerbates mast cell-mediated allergic inflammation by the release of histamine. Molecular and Cellular Toxicology 14: 173-181. http://dx.doi.org/10.1007/s13273-018-0019-z

Lee, JS; Ward, WO; Liu, J; Ren, H; Vallanat, B; Delker, D; Corton, JC. (2011). Hepatic xenobiotic metabolizing enzyme and transporter gene expression through the life stages of the mouse. PLoS ONE 6: e24381. http://dx.doi.org/10.1371/journal.pone.0024381

Lee, JW; Choi, K; Park, K; Seong, C; Yu, SD; Kim, P. (2020). Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: A review [Review]. Science of the Total Environment 707: 135334. http://dx.doi.org/10.1016/j.scitotenv.2019.135334

Lee, S; Kim, S; Park, J; Kim, HJ; Choi, G; Choi, S, et al. (2017). Perfluoroalkyl substances (PFASs) in breast milk from Korea: Time-course trends, influencing factors, and infant exposure. Science of the Total Environment 612: 286-292.

Lee, TH; Kim, WR; Benson, JT; Therneau, TM; Melton, LJ. (2008). Serum aminotransferase activity and mortality risk in a United States community. Hepatology 47: 880-887. http://dx.doi.org/10.1002/hep.22090

Lee, YA; Kim, JH; Jung, HW; Lim, YH; Bae, S; Kho, Y, et al. (2018b). The serum concentrations of perfluoroalkyl compounds were inversely associated with growth parameters in 2-year old children. Science of the Total Environment 628-629: 226-232.

Lee, YJ; Kim, MK; Bae, J; Yang, JH. (2013). Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere 90: 1603-1609.

Lefebvre, DE; Curran, I; Armstrong, C; Coady, L; Parenteau, M; Liston, V, et al. (2008). Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague-Dawley rats. Journal of Toxicology and Environmental Health, Part A: Current Issues 71: 1516-1525. http://dx.doi.org/10.1080/15287390802391943

Lehmann, GM; Verner, MA; Luukinen, B; Henning, C; Assimon, SA; Lakind, JS, et al. (2014). Improving the risk assessment of lipophilic persistent environmental chemicals in breast milk [Review]. Critical Reviews in Toxicology 44: 600-617. http://dx.doi.org/10.3109/10408444.2014.926306

Lehner, R; Quiroga, AD. (2016). Chapter 5: Fatty acid handling in mammalian cells. In ND Ridgway; RS McLeod (Eds.), Biochemistry of lipids, lipoproteins and membranes (6th ed., pp. 149-184). Amsterdam, Netherlands: Elsevier. http://dx.doi.org/10.1016/b978-0-444-63438-2.00005-5

Lenters, V; Iszatt, N; Forns, J; Čechová, E; Kočan, A; Legler, J, et al. (2019). Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: A multi-pollutant analysis of a Norwegian birth cohort. Environment International 125: 33-42.

Lenters, V; Portengen, L; Rignell-Hydbom, A; Jönsson, BA; Lindh, CH; Piersma, AH, et al. (2016). Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environmental Health Perspectives 124: 365-372.

Leung, YK; Ouyang, B; Niu, L; Xie, C; Ying, J; Medvedovic, M, et al. (2018). Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics 13: 290-300. http://dx.doi.org/10.1080/15592294.2018.1445901

Li, H; Hammarstrand, S; Midberg, B; Xu, Y; Li, Y; Olsson, DS, et al. (2022). Cancer incidence in a Swedish cohort with high exposure to perfluoroalkyl substances in drinking water. Environmental Research 204: 112217. http://dx.doi.org/10.1016/j.envres.2021.112217

Li, J; Cai, D; Chu, C; Li, QQ; Zhou, Y; Hu, LW, et al. (2020a). Transplacental Transfer of Per- and Polyfluoroalkyl Substances (PFASs): Differences between Preterm and Full-Term Deliveries and Associations with Placental Transporter mRNA Expression. Environmental Science and Technology 54: 5062-5070.

Li, J; Quan, X; Lei, S; Huang, Z; Wang, Q; Xu, P. (2021a). PFOS inhibited normal functional development of placenta cells via PPARγ signaling. Biomedicines 9: 677. http://dx.doi.org/10.3390/biomedicines9060677

Li, J; Quan, XJ; Chen, G; Hong, JW; Wang, Q; Xu, LL, et al. (2020b). PFOS-induced placental cell growth inhibition is partially mediated by lncRNA H19 through interacting with miR-19a and miR-19b. Chemosphere 261: 127640. http://dx.doi.org/10.1016/j.chemosphere.2020.127640

Li, L; Li, X; Chen, X; Chen, Y; Liu, J; Chen, F, et al. (2018a). Perfluorooctane sulfonate impairs rat Leydig cell development during puberty. Chemosphere 190: 43-53. http://dx.doi.org/10.1016/j.chemosphere.2017.09.116

Li, M; Zeng, XW; Qian, ZM; Vaughn, MG; Sauvé, S; Paul, G, et al. (2017). Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environment International 102: 1-8.

Li, MC. (2020). Serum Per- and Polyfluoroalkyl Substances Are Associated with Increased Hearing Impairment: A Re-Analysis of the National Health and Nutrition Examination Survey Data. International Journal of Environmental Research and Public Health 17.

Li, N; Liu, Y; Papandonatos, GD; Calafat, AM; Eaton, CB; Kelsey, KT, et al. (2021b). Gestational and childhood exposure to per- and polyfluoroalkyl substances and cardiometabolic risk at age 12 years. Environment International 147: 106344. http://dx.doi.org/10.1016/j.envint.2020.106344

Li, R; Guo, C; Lin, X; Chan, TF; Lai, KP; Chen, J. (2020c). Integrative omics analyses uncover the mechanism underlying the immunotoxicity of perfluorooctanesulfonate in human lymphocytes. Chemosphere 256: 127062. http://dx.doi.org/10.1016/j.chemosphere.2020.127062

Li, X; Li, T; Wang, Z; Wei, J; Liu, J; Zhang, Y; Zhao, Z. (2021c). Distribution of perfluorooctane sulfonate in mice and its effect on liver lipidomic. Talanta 226: 122150. http://dx.doi.org/10.1016/j.talanta.2021.122150

Li, X; Ye, L; Ge, Y; Yuan, K; Zhang, Y; Liang, Y, et al. (2016). In utero perfluorooctane sulfonate exposure causes low body weights of fetal rats: A mechanism study. Placenta 39: 125-133.

Li, Y; Barregard, L; Xu, Y; Scott, K; Pineda, D; Lindh, CH, et al. (2020d). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environmental Health: A Global Access Science Source 19: 33.

Li, Y; Fletcher, T; Mucs, D; Scott, K; Lindh, CH; Tallving, P; Jakobsson, K. (2018b). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occupational and Environmental Medicine 75: 46-51.

Li, Y; Oliver, DP; Kookana, RS. (2018c). A critical analysis of published data to discern the role of soil and sediment properties in determining sorption of per and polyfluoroalkyl substances (PFASs) [Review]. Science of the Total Environment 628-629: 110-120. http://dx.doi.org/10.1016/j.scitotenv.2018.01.167

Liang, X; Xie, G; Wu, X; Su, M; Yang, B. (2019). Effect of prenatal PFOS exposure on liver cell function in neonatal mice. Environmental Science and Pollution Research 26: 18240-18246.

Liao, S; Yao, W; Cheang, I; Tang, X; Yin, T; Lu, X, et al. (2020). Association between perfluoroalkyl acids and the prevalence of hypertension among US adults. Ecotoxicology and Environmental Safety 196: 110589.

Liao, Y; Dong, S; Kiyama, R; Cai, P; Liu, L; Shen, H. (2013). Flos lonicerae extracts and chlorogenic acid protect human umbilical vein endothelial cells from the toxic damage of perfluorooctane sulphonate. Inflammation 36: 767-779. http://dx.doi.org/10.1007/s10753-013-9603-5

Liew, Z; Luo, J; Nohr, EA; Bech, BH; Bossi, R; Arah, OA; Olsen, J. (2020). Maternal Plasma Perfluoroalkyl Substances and Miscarriage: A Nested Case-Control Study in the Danish National Birth Cohort. Environmental Health Perspectives 128: 47007.

Lin, CY; Chen, PC; Lo, SC; Torng, PL; Sung, FC; Su, TC. (2016). The association of carotid intima-media thickness with serum Level of perfluorinated chemicals and endothelium-platelet microparticles in adolescents and young adults. Environment International 94: 292-299.

Lin, CY; Lee, HL; Hwang, YT; Su, TC. (2020a). The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. Environmental Research 182: 109064.

Lin, CY; Lin, LY; Chiang, CK; Wang, WJ; Su, YN; Hung, KY; Chen, PC. (2010). Investigation of the Associations Between Low-Dose Serum Perfluorinated Chemicals and Liver Enzymes in US Adults. American Journal of Gastroenterology 105: 1354-1363. http://dx.doi.org/10.1038/ajg.2009.707

Lin, CY; Lin, LY; Wen, TW; Lien, GW; Chien, KL; Hsu, SH, et al. (2013). Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. International Journal of Cardiology 168: 3309-3316.

Lin, HW; Feng, HX; Chen, L; Yuan, XJ; Tan, Z. (2020b). Maternal exposure to environmental endocrine disruptors during pregnancy is associated with pediatric germ cell tumors. Nagoya Journal of Medical Science 82: 323-333.

Lin, P; Cardenas, A; Hauser, R; Gold, DR; Kleinman, K; Hivert, MF, et al. (2019). Per- and polyfluoroalkyl substances and blood lipid levels in pre-diabetic adults-longitudinal analysis of the diabetes prevention program outcomes study. Environment International 129: 343-353.

Lin, PD; Cardenas, A; Hauser, R; Gold, DR; Kleinman, KP; Hivert, MF, et al. (2020c). Per- and polyfluoroalkyl substances and blood pressure in pre-diabetic adults-cross-sectional and longitudinal analyses of the diabetes prevention program outcomes study. Environment International 137: 105573.

Lin, TW; Chen, MK; Lin, CC; Chen, MH; Tsai, MS; Chan, DC, et al. (2020d). Association between exposure to perfluoroalkyl substances and metabolic syndrome and related outcomes among older residents living near a Science Park in Taiwan. International Journal of Hygiene and Environmental Health 230: 113607. http://dx.doi.org/10.1016/j.ijheh.2020.113607

Lind, DV; Priskorn, L; Lassen, TH; Nielsen, F; Kyhl, HB; Kristensen, DM, et al. (2017a). Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reproductive Toxicology 68: 200-206.

Lind, PM; Salihovic, S; van Bavel, B; Lind, L. (2017b). Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environmental Research 152: 157-164.

Lindstrom, AB; Strynar, MJ; Libelo, EL. (2011). Polyfluorinated compounds: past, present, and future [Review]. Environmental Science and Technology 45: 7954-7961. http://dx.doi.org/10.1021/es2011622

Lira, PI; Ashworth, A; Morris, SS. (1996). Low birth weight and morbidity from diarrhea and respiratory infection in northeast Brazil. Journal of Pediatrics 128: 497-504. http://dx.doi.org/10.1016/s0022-3476(96)70360-8

Litton Bionetics. (1979). Mutagenicity evaluation of T-2014 CoC in the ames salmonella/microsome plate test. Final report. Beverly Hills, CA: Litton Bionetics, Inc.

Liu, CY; Chen, PC; Lien, PC; Liao, YP. (2018a). Prenatal perfluorooctyl sulfonate exposure and Alu DNA hypomethylation in cord blood. International Journal of Environmental Research and Public Health 15: 1066. http://dx.doi.org/10.3390/ijerph15061066

Liu, D; Liu, NY; Chen, LT; Shao, Y; Shi, XM; Zhu, DY. (2020a). Perfluorooctane sulfonate induced toxicity in embryonic stem cell-derived cardiomyocytes via inhibiting autophagy-lysosome pathway. Toxicology In Vitro 69: 104988. http://dx.doi.org/10.1016/j.tiv.2020.104988

Liu, G; Dhana, K; Furtado, JD; Rood, J; Zong, G; Liang, L, et al. (2018b). Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Medicine 15: e1002502.

Liu, G; Zhang, B; Hu, Y; Rood, J; Liang, L; Qi, L, et al. (2020b). Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environmental Health: A Global Access Science Source 19: 5.

Liu, H; Chen, Q; Lei, L; Zhou, W; Huang, L; Zhang, J; Chen, D. (2018c). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. Environmental Pollution 235: 446-452. http://dx.doi.org/10.1016/j.envpol.2017.12.095

Liu, HS; Wen, LL; Chu, PL; Lin, CY. (2018d). Association among total serum isomers of perfluorinated chemicals, glucose homeostasis, lipid profiles, serum protein and metabolic syndrome in adults: NHANES, 2013-2014. Environmental Pollution 232: 73-79.

Liu, J; Li, J; Liu, Y; Chan, HM; Zhao, Y; Cai, Z; Wu, Y. (2011). Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environment International 37: 1206-1212. http://dx.doi.org/10.1016/j.envint.2011.05.001

Liu, J; Liu, S; Huang, Z; Fu, Y; Fei, J; Liu, X; He, Z. (2020c). Associations between the serum levels of PFOS/PFOA and IgG N-glycosylation in adult or children. Environmental Pollution 265: 114285. http://dx.doi.org/10.1016/j.envpol.2020.114285

Liu, L; Liu, W; Song, J; Yu, H; Jin, Y; Oami, K, et al. (2009). A comparative study on oxidative damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages. Journal of Toxicological Sciences 34: 245-254. http://dx.doi.org/10.2131/jts.34.245

Liu, M; Zhang, G; Meng, L; Han, X; Li, Y; Shi, Y, et al. (2021). Associations between novel and legacy per- and polyfluoroalkyl substances in human serum and thyroid cancer: A case and healthy population in Shandong Province, East China. Environmental Science & Technology. http://dx.doi.org/10.1021/acs.est.1c02850

Liu, QS; Hao, F; Sun, Z; Long, Y; Zhou, Q; Jiang, G. (2018e). Perfluorohexadecanoic acid increases paracellular permeability in endothelial cells through the activation of plasma kallikrein-kinin system. Chemosphere 190: 191-200. http://dx.doi.org/10.1016/j.chemosphere.2017.10.002

Liu, QS; Sun, Y; Qu, G; Long, Y; Zhao, X; Zhang, A, et al. (2017a). Structure-Dependent Hematological Effects of Per- and Polyfluoroalkyl Substances on Activation of Plasma Kallikrein-Kinin System Cascade. Environmental Science & Technology 51: 10173-10183. http://dx.doi.org/10.1021/acs.est.7b02055

Liu, SH; Kuiper, JR; Chen, Y; Feuerstahler, L; Teresi, J; Buckley, JP. (2022). Developing an exposure burden score for chemical mixtures using item response theory, with applications to PFAS mixtures. Environmental Health Perspectives 130: 117001. http://dx.doi.org/10.1289/EHP10125

Liu, X; Chen, D; Wang, B; Xu, F; Pang, Y; Zhang, L, et al. (2020d). Does Low Maternal Exposure to Per- and Polyfluoroalkyl Substances Elevate the Risk of Spontaneous Preterm Birth? A Nested Case-Control Study in China. Environmental Science and Technology 54: 8259-8268.

Liu, Y; Cao, Z; Zong, W; Liu, R. (2017b). Interaction rule and mechanism of perfluoroalkyl sulfonates containing different carbon chains with human serum albumin. RSC Advances 7: 24781-24788.

Liu, Z; Que, S; Xu, J; Peng, T. (2014). Alanine aminotransferase-old biomarker and new concept: a review. International Journal of Medical Sciences 11: 925-935. http://dx.doi.org/10.7150/ijms.8951

Lloyd-Jones, DM; Huffman, MD; Karmali, KN; Sanghavi, DM; Wright, JS; Pelser, C, et al. (2017). Estimating Longitudinal Risks and Benefits From Cardiovascular Preventive Therapies Among Medicare Patients: The Million Hearts Longitudinal ASCVD Risk Assessment Tool: A Special Report From the American Heart Association and American College of Cardiology [Review]. Circulation 135: e793-e813. http://dx.doi.org/10.1161/CIR.0000000000000467

Loccisano, AE; Campbell, JL; Butenhoff, JL; Andersen, ME; Clewell, HJ. (2012a). Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33: 468-490. http://dx.doi.org/10.1016/j.reprotox.2011.07.003

Loccisano, AE; Campbell, JL, Jr; Andersen, ME; Clewell, HJ, III. (2011). Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology and Pharmacology 59: 157-175. http://dx.doi.org/10.1016/j.yrtph.2010.12.004

Loccisano, AE; Campbell, JL, Jr; Butenhoff, JL; Andersen, ME; Clewell, HJ, III. (2012b). Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33: 452-467. http://dx.doi.org/10.1016/j.reprotox.2011.04.006

Loccisano, AE; Longnecker, MP; Campbell, JL, Jr; Andersen, ME; Clewell, HJ, III. (2013). Development of pbpk models for pfoa and pfos for human pregnancy and lactation life stages. Journal of Toxicology and Environmental Health, Part A: Current Issues 76: 25-57. http://dx.doi.org/10.1080/15287394.2012.722523

Looker, C; Luster, MI; Calafat, AM; Johnson, VJ; Burleson, GR; Burleson, FG; Fletcher, T. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicological Sciences 138: 76-88. http://dx.doi.org/10.1093/toxsci/kft269

Lopez-Espinosa, MJ; Carrizosa, C; Luster, MI; Margolick, JB; Costa, O; Leonardi, GS; Fletcher, T. (2021). Perfluoroalkyl substances and immune cell counts in adults from the Mid-Ohio Valley (USA). Environment International 156: 106599. http://dx.doi.org/10.1016/j.envint.2021.106599

Lorber, M; Eaglesham, GE; Hobson, P; Toms, LM; Mueller, JF; Thompson, JS. (2015). The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. Chemosphere 118: 170-177. http://dx.doi.org/10.1016/j.chemosphere.2014.07.093

Loret de Mola, C; de França, GV; Quevedo Lde, A; Horta, BL. (2014). Low birth weight, preterm birth and small for gestational age association with adult depression: systematic review and meta-analysis. British Journal of Psychiatry 205: 340-347. http://dx.doi.org/10.1192/bjp.bp.113.139014

Louisse, J; Rijkers, D; Stoopen, G; Janssen, A; Staats, M; Hoogenboom, R, et al. (2020). Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorononanoic acid (PFNA) increase triglyceride levels and decrease cholesterogenic gene expression in human HepaRG liver cells. Archives of Toxicology 94: 3137-3155. http://dx.doi.org/10.1007/s00204-020-02808-0

Lu, L; Xu, L; Kang, T; Cheng, S. (2012). Investigation of DNA damage treated with perfluorooctane sulfonate (PFOS) on ZrO2/DDAB active nano-order film. Biosensors and Bioelectronics 35: 180-185. http://dx.doi.org/10.1016/j.bios.2012.02.043

Luebker, DJ; Case, MT; York, RG; Moore, JA; Hansen, KJ; Butenhoff, JL. (2005a). Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 215: 126-148.

Luebker, DJ; Hansen, KJ; Bass, NM; Butenhoff, JL; Seacat, AM. (2002). Interactions of fluorochemicals with rat liver fatty acid-binding protein. Toxicology 176: 175-185. http://dx.doi.org/10.1016/S0300-483X(02)00081-1

Luebker, DJ; York, RG; Hansen, KJ; Moore, JA; Butenhoff, JL. (2005b). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology 215: 149-169.

Luo, D; Wu, W; Pan, Y; Du, B; Shen, M; Zeng, L. (2021). Associations of prenatal exposure to per- and polyfluoroalkyl substances with the neonatal birth size and hormones in the growth hormone/insulin-like growth factor axis. Environmental Science & Technology 55: 11859-11873. http://dx.doi.org/10.1021/acs.est.1c02670

Luster, MI; Johnson, VJ; Yucesoy, B; Simeonova, PP. (2005). Biomarkers to assess potential developmental immunotoxicity in children. Toxicology and Applied Pharmacology 206: 229-236. http://dx.doi.org/10.1016/j.taap.2005.02.010

Lv, QY; Wan, B; Guo, LH; Yang, Y; Ren, XM; Zhang, H. (2015). In vivo immunotoxicity of perfluorooctane sulfonate in BALB/c mice: Identification of T-cell receptor and calcium-mediated signaling pathway disruption through gene expression profiling of the spleen. Chemico-Biological Interactions 240: 84-93. http://dx.doi.org/10.1016/j.cbi.2015.07.015

Lv, Z; Wu, W; Ge, S; Jia, R; Lin, T; Yuan, Y, et al. (2018). Naringin protects against perfluorooctane sulfonate-induced liver injury by modulating NRF2 and NF-κB in mice. International Immunopharmacology 65: 140-147. http://dx.doi.org/10.1016/j.intimp.2018.09.019

Ma, S; Xu, C; Ma, J; Wang, Z; Zhang, Y; Shu, Y; Mo, X. (2019). Association between perfluoroalkyl substance concentrations and blood pressure in adolescents. Environmental Pollution 254: 112971.

Macgillivray, DM; Kollmann, TR. (2014). The role of environmental factors in modulating immune responses in early life [Review]. Frontiers in Immunology 5: 434. http://dx.doi.org/10.3389/fimmu.2014.00434

Maekawa, R; Ito, R; Iwasaki, Y; Saito, K; Akutsu, K; Takatori, S, et al. (2017). Evidence of exposure to chemicals and heavy metals during pregnancy in Japanese women. Reproductive Medicine and Biology 16: 337-348.

Maestri, L; Negri, S; Ferrari, M; Ghittori, S; Fabris, F; Danesino, P; Imbriani, M. (2006). Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Communications in Mass Spectrometry 20: 2728-2734. http://dx.doi.org/10.1002/rcm.2661

Maharjan, CK; Ear, PH; Tran, CG; Howe, JR; Chandrasekharan, C; Quelle, DE. (2021). Pancreatic neuroendocrine tumors: Molecular mechanisms and therapeutic targets. Cancers 13. http://dx.doi.org/10.3390/cancers13205117

Maisonet, M; Terrell, ML; Mcgeehin, MA; Christensen, KY; Holmes, A; Calafat, AM; Marcus, M. (2012). Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environmental Health Perspectives 120: 1432-1437. http://dx.doi.org/10.1289/ehp.1003096

Malhotra, P; Gill, RK; Saksena, S; Alrefai, WA. (2020). Disturbances in Cholesterol Homeostasis and Non-alcoholic Fatty Liver Diseases [Review]. Frontiers in Medicine 7: 467. http://dx.doi.org/10.3389/fmed.2020.00467

Mamsen, LS; Björvang, RD; Mucs, D; Vinnars, MT; Papadogiannakis, N; Lindh, CH, et al. (2019). Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environment International 124: 482-492.

Mamsen, LS; Jönsson, BAG; Lindh, CH; Olesen, RH; Larsen, A; Ernst, E, et al. (2017). Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Science of the Total Environment 596-597: 97-105.

Mancini, FR; Cano-Sancho, G; Gambaretti, J; Marchand, P; Boutron-Ruault, MC; Severi, G, et al. (2020). Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort. International Journal of Cancer 146: 917-928.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D, et al. (2017a). Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environment International 108: 278-284.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D, et al. (2017b). Prenatal exposure to perfluoroalkyl substances and cardiometabolic risk in children from the Spanish INMA birth cohort study. Environmental Health Perspectives 125: 097018.

Manzano-Salgado, CB; Granum, B; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Gascón, M, et al. (2019). Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in children from a Spanish birth cohort study. International Journal of Hygiene and Environmental Health 222: 945-954.

Maras, M; Vanparys, C; Muylle, F; Robbens, J; Berger, U; Barber, JL, et al. (2006). Estrogen-like properties of fluorotelomer alcohols as revealed by mcf-7 breast cancer cell proliferation. Environmental Health Perspectives 114: 100-105. http://dx.doi.org/10.1289/ehp.8149

Marks, KJ; Cutler, AJ; Jeddy, Z; Northstone, K; Kato, K; Hartman, TJ. (2019). Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. International Journal of Hygiene and Environmental Health 222: 889-895.

Martin, MT; Brennan, RJ; Hu, W; Ayanoglu, E; Lau, C; Ren, H, et al. (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. Toxicological Sciences 97: 595-613. http://dx.doi.org/10.1093/toxsci/kfm065

Mathiesen, UL; Franzén, LE; Frydén, A; Foberg, U; Bodemar, G. (1999). The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. Scandinavian Journal of Gastroenterology 34: 85-91. http://dx.doi.org/10.1080/00365529950172880

Matilla-Santander, N; Valvi, D; Lopez-Espinosa, MJ; Manzano-Salgado, CB; Ballester, F; Ibarluzea, J, et al. (2017). Exposure to Perfluoroalkyl Substances and Metabolic Outcomes in Pregnant Women: Evidence from the Spanish INMA Birth Cohorts. Environmental Health Perspectives 125: 117004.

Mattsson, K; Rignell-Hydbom, A; Holmberg, S; Thelin, A; Jönsson, BA; Lindh, CH, et al. (2015). Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environmental Research 142: 148-154.

Mcdonough, CA; Li, W; Bischel, HN; De Silva, AO; Dewitt, JC. (2022). Widening the Lens on PFASs: Direct Human Exposure to Perfluoroalkyl Acid Precursors (pre-PFAAs). Environmental Science & Technology 56: 6004-6013. http://dx.doi.org/10.1021/acs.est.2c00254

McIntire, DD; Bloom, SL; Casey, BM; Leveno, KJ. (1999). Birth weight in relation to morbidity and mortality among newborn infants. New England Journal of Medicine 340: 1234-1238.

Mecchi, MS. (1999). Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with PFOS. Final report. (Covance Study No. 20784-0-409). Vienna, VA: Covance Laboratories.

Meng, Q; Inoue, K; Ritz, B; Olsen, J; Liew, Z. (2018). Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the danish national birth cohort. International Journal of Environmental Research and Public Health 15: 1832.

Mi, X; Yang, YQ; Zeeshan, M; Wang, ZB; Zeng, XY; Zhou, Y, et al. (2020). Serum levels of per- and polyfluoroalkyl substances alternatives and blood pressure by sex status: Isomers of C8 health project in China. Chemosphere 261: 127691.

Minatoya, M; Itoh, S; Miyashita, C; Araki, A; Sasaki, S; Miura, R, et al. (2017). Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environmental Research 156: 175-182.

Mitro, SD; Sagiv, SK; Fleisch, AF; Jaacks, LM; Williams, PL; Rifas-Shiman, SL, et al. (2020). Pregnancy per- and polyfluoroalkyl substance concentrations and postpartum health in project viva: A prospective cohort. Journal of Clinical Endocrinology and Metabolism 105: e3415–e3426.

Miura, R; Araki, A; Miyashita, C; Kobayashi, S; Kobayashi, S; Wang, SL, et al. (2018). An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: The Hokkaido study. Environment International 115: 21-28. http://dx.doi.org/10.1016/j.envint.2018.03.004

Mobacke, I; Lind, L; Dunder, L; Salihovic, S; Lind, PM. (2018). Circulating levels of perfluoroalkyl substances and left ventricular geometry of the heart in the elderly. Environment International 115: 295-300.

Mogensen, UB; Grandjean, P; Heilmann, C; Nielsen, F; Weihe, P; Budtz-Jørgensen, E. (2015a). Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. Environmental Health: A Global Access Science Source 14: 47.

Mogensen, UB; Grandjean, P; Nielsen, F; Weihe, P; Budtz-Jørgensen, E. (2015b). Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates. Environmental Science and Technology 49: 10466-10473.

Mondal, D; Weldon, RH; Armstrong, BG; Gibson, LJ; Lopez-Espinosa, MJ; Shin, HM; Fletcher, T. (2014). Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environmental Health Perspectives 122: 187-192.

Monnier, P; Martinet, C; Pontis, J; Stancheva, I; Ait-Si-Ali, S; Dandolo, L. (2013). H19 lncRNA controls gene expression of the Imprinted Gene Network by recruiting MBD1. Proceedings of the National Academy of Sciences of the United States of America 110: 20693-20698. http://dx.doi.org/10.1073/pnas.1310201110

Monroy, R; Morrison, K; Teo, K; Atkinson, S; Kubwabo, C; Stewart, B; Foster, WG. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental Research 108: 56-62. http://dx.doi.org/10.1016/j.envres.2008.06.001

Mora, AM; Fleisch, AF; Rifas-Shiman, SL; Woo Baidal, JA; Pardo, L; Webster, TF, et al. (2018). Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine aminotransferase levels. Environment International 111: 1-13.

Mora, S. (2016). Nonfasting for Routine Lipid Testing: From Evidence to Action. JAMA Internal Medicine 176: 1005-1006. http://dx.doi.org/10.1001/jamainternmed.2016.1979

Mordenti, J; Chen, SA; Moore, JA; Ferraiolo, BL; Green, JD. (1991). Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. Pharmaceutical Research 8: 1351–1359. http://dx.doi.org/10.1023/a:1015836720294

Mshaty, A; Haijima, A; Takatsuru, Y; Ninomiya, A; Yajima, H; Kokubo, M, et al. (2020). Neurotoxic effects of lactational exposure to perfluorooctane sulfonate on learning and memory in adult male mouse. Food and Chemical Toxicology 145: 111710.

Murli, H. (1996). Mutagenicity test on T-6295 in an in vivo mouse micronucleus assay. Final report. (CHV Study No. 17403-0-455). Vienna, VA: Corning Hazleton.

Murli, H. (1999). Chromosomal aberrations in human whole blood lymphocytes with PFOS. Final report. (Covance Study No. 2784-0-499; EPA-AR-226-0131). Vienna, VA: Covance Laboratories.

Myers, LP. (2018). Clinical immunotoxicology. In JC DeWitt; CE Rockwell; CC Bowman (Eds.), Immunotoxicity testing: Methods and protocols (2nd ed., pp. 15–26). Totowa, NJ: Humana Press. http://dx.doi.org/10.1007/978-1-4939-8549-4_2

Nakayama, S; Strynar, MJ; Helfant, L; Egeghy, P; Ye, X; Lindstrom, AB. (2007). Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. Environmental Science and Technology 41: 5271-5276. http://dx.doi.org/10.1021/es070792y

NASEM. (2021). Review of U.S. EPA's ORD staff handbook for developing IRIS assessments: 2020 version. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/26289

NCBI. (2022). PubChem: Compound summary: Perfluorooctanoic acid. Available online at https://pubchem.ncbi.nlm.nih.gov/compound/Perfluorooctanoic-acid (accessed April 21, 2022).

NCHS. (2019). Health, United States – Data Finder. 2019: Table 23. Hyattsville, MD. https://www.cdc.gov/nchs/hus/contents2019.htm

Negri, E; Metruccio, F; Guercio, V; Tosti, L; Benfenati, E; Bonzi, R, et al. (2017). Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data [Review]. Critical Reviews in Toxicology 47: 482-508. http://dx.doi.org/10.1080/10408444.2016.1271972

Nelson, JW; Hatch, EE; Webster, TF. (2010). Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General US Population. Environmental Health Perspectives 118: 197-202. http://dx.doi.org/10.1289/ehp.0901165

Nelson, JW; Scammell, MK; Hatch, EE; Webster, TF. (2012). Social disparities in exposures to bisphenol A and polyfluoroalkyl chemicals: a cross-sectional study within NHANES 2003-2006. Environmental Health 11: 10. http://dx.doi.org/10.1186/1476-069X-11-10

Ngueta, G; Longnecker, MP; Yoon, M; Ruark, CD; Clewell, HJ; Andersen, ME; Verner, MA. (2017). Quantitative bias analysis of a reported association between perfluoroalkyl substances (PFAS) and endometriosis: The influence of oral contraceptive use. Environment International 104: 118-121. http://dx.doi.org/10.1016/j.envint.2017.03.023

Nguyen, HC; Qadura, M; Singh, KK. (2020a). Role of the Fatty Acid Binding Proteins in Cardiovascular Diseases: A Systematic Review [Review]. 9. http://dx.doi.org/10.3390/jcm9113390

Nguyen, TMH; Bräunig, J; Thompson, K; Thompson, J; Kabiri, S; Navarro, DA, et al. (2020b). Influences of Chemical Properties, Soil Properties, and Solution pH on Soil-Water Partitioning Coefficients of Per- and Polyfluoroalkyl Substances (PFASs). Environmental Science & Technology 54: 15883-15892. http://dx.doi.org/10.1021/acs.est.0c05705

Nian, M; Li, QQ; Bloom, M; Qian, ZM; Syberg, KM; Vaughn, MG, et al. (2019). Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. Environmental Research 172: 81-88.

NJDWQI. (2018). Health-based maximum contaminant level support document: Perfluorooctane sulfonate (PFOS): Appendix A. https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf

NLM. (2022). ChemIDplus: Perfluorooctane sulfonic acid. Available online at https://chem.nlm.nih.gov/chemidplus/name/pfos

Noker, PE; Gorman, G. (2003). A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey. (U.S. EPA docket AR-226-1356). Washington, D.C.: U.S. Environmental Protection Agency.

NTP. (2016a). NTP Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS).

NTP. (2016b). NTP Monograph: Immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). Research Triangle Park, NC: U.S. Department of Health and Human Services, Office of Health Assessment and Translation. https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf

NTP. (2019). NTP technical report on the toxicity studies of perfluoroalkyl sulfonates (perfluorobutane sulfonic acid, perfluorohexane sulfonate potassium salt, and perfluorooctane sulfonic acid) administered by gavage to Sprague Dawley (Hsd:Sprague Dawley SD) rats. (Toxicity Report 96). Research Triangle Park, NC.

NTP. (2020a). NTP historical controls report all routes and vehicles Harlan Sprague-Dawley rats [NTP]. Research Triangle Park, NC.

https://ntp.niehs.nih.gov/ntp/historical_controls/ntp2000_2020/r_hcrpt_allrte20201200.pdf

NTP. (2020b). NTP technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague Dawley (Hsd:Sprague Dawley SD) rats [NTP]. (Technical Report 598). Research Triangle Park, NC.

OECD. (2018). Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). (ENV/JM/MONO(2018)7). http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/

Oh, JH; Kim, EY; Choi, YH; Nam, TJ. (2017). Negative regulation of ERK1/2 by PI3K is required for the protective effects of Pyropia yezoensis peptide against perfluorooctane sulfonate-induced endoplasmic reticulum stress. Molecular Medicine Reports 15: 2583-2587. http://dx.doi.org/10.3892/mmr.2017.6285

Ojo, AF; Peng, C; Ng, JC. (2020). Combined effects and toxicological interactions of perfluoroalkyl and polyfluoroalkyl substances mixtures in human liver cells (HepG2). Environmental Pollution 263: 114182. http://dx.doi.org/10.1016/j.envpol.2020.114182

Okada, E; Sasaki, S; Kashino, I; Matsuura, H; Miyashita, C; Kobayashi, S, et al. (2014). Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environment International 65: 127-134. http://dx.doi.org/10.1016/j.envint.2014.01.007

Olsen, G, .W., B.,urlew, M.,.M.; Burris, J, .M.; Mandel, J, .H. (2001a). A Longitudinal Analysis of Serum Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997 and 2000 Fluorochemical Medical Surveillance Program. Final Report. (Epidemiology, 220-3W-05). St. Paul, MN: 3M Company. https://www.ag.state.mn.us/Office/Cases/3M/docs/PTX/PTX1799.pdf

Olsen, GW; Burlew, MM; Burris, JM; Mandel, JH. (2001b). A cross-sectional analysis of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee participants of the 2000 antwerp and decatur fluorochemical medical surveillance program. Final report. St. Paul, MN: 3M Company.

Olsen, GW; Burris, JM; Burlew, MM; Mandel, JH. (2003). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. Journal of Occupational and Environmental Medicine 45: 260-270. http://dx.doi.org/10.1097/01.jom.0000052958.59271.10

Olsen, GW; Ehresman, DJ; Buehrer, BD; Gibson, BA; Butenhoff, JL; Zobel, LR. (2012). Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. Journal of Occupational and Environmental Medicine 54: 974-983.

Olsen, GW; Hansen, KJ; Clemen, LA; Burris, JM; Mandel, JH. (2001c). Identification of Fluorochemicals in Human Tissue. (U.S. Environmental Protection Agency Administrative Record 226-1030a022). 3M.

Omoike, OE; Pack, RP; Mamudu, HM; Liu, Y; Strasser, S; Zheng, S, et al. (2020). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environmental Research 196: 110361. http://dx.doi.org/10.1016/j.envres.2020.110361

Omoike, OE; Pack, RP; Mamudu, HM; Liu, Y; Wang, L. (2021). A cross-sectional study of the association between perfluorinated chemical exposure and cancers related to deregulation of estrogen receptors. Environmental Research 196: 110329. http://dx.doi.org/10.1016/j.envres.2020.110329

Ong, KK; Dunger, DB. (2002). Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Practice and Research: Clinical Endocrinology and Metabolism 16: 191-207. http://dx.doi.org/10.1053/beem.2002.0195

Oppi, S; Lüscher, TF; Stein, S. (2019). Mouse models for atherosclerosis research- Which is my line? [Review]. Frontiers in Cardiovascular Medicine 6: 46. http://dx.doi.org/10.3389/fcvm.2019.00046

Oshida, K; Vasani, N; Jones, C; Moore, T; Hester, S; Nesnow, S, et al. (2015a). Identification of chemical modulators of the constitutive activated receptor (CAR) in a gene expression compendium. Nuclear Receptor Signaling 13: e002. http://dx.doi.org/10.1621/nrs.13002

Oshida, K; Vasani, N; Thomas, RS; Applegate, D; Rosen, M; Abbott, B, et al. (2015b). Identification of modulators of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) in a mouse liver gene expression compendium. PLoS ONE 10: e0112655. http://dx.doi.org/10.1371/journal.pone.0112655

Osmond, C; Barker, DJ. (2000). Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women [Review]. Environmental Health Perspectives 108: 545-553. http://dx.doi.org/10.2307/3454545

Osorio-Yáñez, C; Sanchez-Guerra, M; Cardenas, A; Lin, PID; Hauser, R; Gold, DR, et al. (2021). Per- and polyfluoroalkyl substances and calcifications of the coronary and aortic arteries in adults with prediabetes: Results from the diabetes prevention program outcomes study. Environment International 151: 106446. http://dx.doi.org/10.1016/j.envint.2021.106446

OSTP. (2023). Per- and polyfluoroalkyl substances (PFAS) report. National Science and Technology Council, Joint Subcommittee on Environment, Innovation, and Public Health Per- and Polyfluoroalkyl Substances Strategy Team. https://www.whitehouse.gov/wp-content/uploads/2023/03/OSTP-March-2023-PFAS-Report.pdf?trk=public_post_comment-text

Osuna, CE; Grandjean, P; Weihe, P; El-Fawal, HA. (2014). Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicological Sciences 142: 158-166. http://dx.doi.org/10.1093/toxsci/kfu163

Ou, Y; Zeng, X; Lin, S; Bloom, MS; Han, F; Xiao, X, et al. (2021). Gestational exposure to perfluoroalkyl substances and congenital heart defects: A nested case-control pilot study. Environment International 154: 106567. http://dx.doi.org/10.1016/j.envint.2021.106567

Ouidir, M; Mendola, P; Louis, GMB; Kannan, K; Zhang, C; Tekola-Ayele, F. (2020). Concentrations of persistent organic pollutants in maternal plasma and epigenome-wide placental DNA methylation. Clinical Epigenetics 12: 103. http://dx.doi.org/10.1186/s13148-020-00894-6

Oulhote, Y; Shamim, Z; Kielsen, K; Weihe, P; Grandjean, P; Ryder, LP; Heilmann, C. (2017). Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reproductive Toxicology 68: 207-214. http://dx.doi.org/10.1016/j.reprotox.2016.08.001

Oulhote, Y; Steuerwald, U; Debes, F; Weihe, P; Grandjean, P. (2016). Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances [Review]. Environment International 97: 237-245.

Pan, Y; Zhu, Y; Zheng, T; Cui, Q; Buka, SL; Zhang, B, et al. (2017). Novel Chlorinated Polyfluorinated Ether Sulfonates and Legacy Per-/Polyfluoroalkyl Substances: Placental Transfer and Relationship with Serum Albumin and Glomerular Filtration Rate. Environmental Science and Technology 51: 634-644.

Papadopoulou, E; Stratakis, N; Basagaña, X; Brantsæter, AL; Casas, M; Fossati, S, et al. (2021). Prenatal and postnatal exposure to PFAS and cardiometabolic factors and inflammation status in children from six European cohorts. Environment International 157: 106853. http://dx.doi.org/10.1016/j.envint.2021.106853

Park, JH; Choi, J; Jun, DW; Han, SW; Yeo, YH; Nguyen, MH. (2019a). Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study. Journal of Clinical Medicine 8. http://dx.doi.org/10.3390/jcm8091445

Park, MH; Gutiérrez-García, AK; Choudhury, M. (2019b). Mono-(2-ethylhexyl) phthalate aggravates inflammatory response via sirtuin regulation and inflammasome activation in RAW 264.7 cells. Chemical Research in Toxicology 32: 935-942. http://dx.doi.org/10.1021/acs.chemrestox.9b00101

Park, SK; Peng, Q; Ding, N; Mukherjee, B; Harlow, SD. (2019c). Determinants of per- and polyfluoroalkyl substances (PFAS) in midlife women: Evidence of racial/ethnic and geographic differences in PFAS exposure. Environmental Research 175: 186-199. http://dx.doi.org/10.1016/j.envres.2019.05.028

Peden-Adams, MM; Keller, JM; Eudaly, JG; Berger, J; Gilkeson, GS; Keil, DE. (2008). Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicological Sciences 104: 144-154. http://dx.doi.org/10.1093/toxsci/kfn059

Pennings, JLA; Jennen, DGJ; Nygaard, UC; Namork, E; Haug, LS; van Loveren, H; Granum, B. (2016). Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. Journal of Immunotoxicology 13: 173-180. http://dx.doi.org/10.3109/1547691X.2015.1029147

Pérez, F; Nadal, M; Navarro-Ortega, A; Fàbrega, F; Domingo, JL; Barceló, D; Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. Environment International 59: 354-362.

Perng, We; Rifas-Shiman, SL; Kramer, MS; Haugaard, LK; Oken, E; Gillman, MW; Belfort, MB. (2016). Early Weight Gain, Linear Growth, and Mid-Childhood Blood Pressure A Prospective Study in Project Viva. Hypertension 67: 301-308. http://dx.doi.org/10.1161/HYPERTENSIONAHA.115.06635

Pierozan, P; Cattani, D; Karlsson, O. (2020). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) induce epigenetic alterations and promote human breast cell carcinogenesis in vitro. Archives of Toxicology 94: 3893-3906. http://dx.doi.org/10.1007/s00204-020-02848-6

Pierozan, P; Karlsson, O. (2018). PFOS induces proliferation, cell-cycle progression, and malignant phenotype in human breast epithelial cells. Archives of Toxicology 92: 705-716. http://dx.doi.org/10.1007/s00204-017-2077-8

Pilkerton, CS; Hobbs, GR; Lilly, C; Knox, SS. (2018). Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. PLoS ONE 13: e0203330.

Pitter, G; Zare Jeddi, M; Barbieri, G; Gion, M; Fabricio, ASC; Daprà, F, et al. (2020). Perfluoroalkyl substances are associated with elevated blood pressure and hypertension in highly exposed young adults. Environmental Health: A Global Access Science Source 19: 102. http://dx.doi.org/10.1186/s12940-020-00656-0

Pizzurro, DM; Seeley, M; Kerper, LE; Beck, BD. (2019). Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria [Review]. Regulatory Toxicology and Pharmacology 106: 239-250.

Podder, A, .; Sadmani, A, .; Reinhart, D, .; Chang, N, . B.; Goel, R, . (2021). Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: A transboundary review of their occurrences and toxicity effects. Journal of Hazardous Materials 419: 126361. http://dx.doi.org/10.1016/j.jhazmat.2021.126361

Poothong, S; Thomsen, C; Padilla-Sanchez, JA; Papadopoulou, E; Haug, LS. (2017). Distribution of novel and well-known poly- and perfluoroalkyl substances (PFASs) in human serum, plasma, and whole blood. Environmental Science and Technology 51: 13388-13396.

Popovic, M; Zaja, R; Fent, K; Smital, T. (2014). Interaction of environmental contaminants with zebrafish organic anion transporting polypeptide, Oatp1d1 (Slco1d1). Toxicology and Applied Pharmacology 280: 149-158. http://dx.doi.org/10.1016/j.taap.2014.07.015

Porpora, MG; Lucchini, R; Abballe, A; Ingelido, AM; Valentini, S; Fuggetta, E, et al. (2013). Placental transfer of persistent organic pollutants: a preliminary study on mother-newborn pairs. International Journal of Environmental Research and Public Health 10: 699-711.

Portier, K; Tolson, JK; Roberts, SM. (2007). Body weight distributions for risk assessment. Risk Analysis 27: 11-26. http://dx.doi.org/10.1111/j.1539-6924.2006.00856.x

Predieri, B; Iughetti, L; Guerranti, C; Bruzzi, P; Perra, G; Focardi, SE. (2015). High Levels of Perfluorooctane Sulfonate in Children at the Onset of Diabetes. International Journal of Endocrinology 2015: 234358.

Pritchard, JA. (1965). Changes in the blood volume during pregnancy and delivery [Review]. Anesthesiology 26: 393-399. http://dx.doi.org/10.1097/00000542-196507000-00004

Qazi, MR; Abedi, MR; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2010). Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. International Immunopharmacology 10: 1420-1427. http://dx.doi.org/10.1016/j.intimp.2010.08.009

Qazi, MR; Bogdanska, J; Butenhoff, JL; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2009a). High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 262: 207-214. http://dx.doi.org/10.1016/j.tox.2009.06.010

Qazi, MR; Nelson, BD; Depierre, JW; Abedi-Valugerdi, M. (2012). High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. Food and Chemical Toxicology 50: 2955-2963. http://dx.doi.org/10.1016/j.fct.2012.06.023

Qazi, MR; Xia, Z; Bogdanska, J; Chang, SC; Ehresman, DJ; Butenhoff, JL, et al. (2009b). The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose

phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). Toxicology 260: 68-76. http://dx.doi.org/10.1016/j.tox.2009.03.009

Qian, Y; Ducatman, A; Ward, R; Leonard, S; Bukowski, V; Lan Guo, N, et al. (2010). Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. Journal of Toxicology and Environmental Health, Part A: Current Issues 73: 819-836. http://dx.doi.org/10.1080/15287391003689317

Qin, W; Ren, X; Zhao, L; Guo, L. (2022). Exposure to perfluorooctane sulfonate reduced cell viability and insulin release capacity of β cells. Journal of Environmental Sciences 115: 162-172. http://dx.doi.org/10.1016/j.jes.2021.07.004

Qiu, L; Zhang, X; Zhang, X; Zhang, Y; Gu, J; Chen, M, et al. (2013). Sertoli cell is a potential target for perfluorooctane sulfonate-induced reproductive dysfunction in male mice. Toxicological Sciences 135: 229-240.

Rainieri, S; Conlledo, N; Langerholc, T; Madorran, E; Sala, M; Barranco, A. (2017). Toxic effects of perfluorinated compounds at human cellular level and on a model vertebrate. Food and Chemical Toxicology 104: 14-25. http://dx.doi.org/10.1016/j.fct.2017.02.041

Rantakokko, P; Männistö, V; Airaksinen, R; Koponen, J; Viluksela, M; Kiviranta, H; Pihlajamäki, J. (2015). Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: A cohort study. Environmental Health: A Global Access Science Source 14: 79.

Rao, MS; Reddy, JK. (1996). Hepatocarcinogenesis of peroxisome proliferators [Review]. In JK Reddy; T Suga; GP Mannaerts; PB Lazarow; S Subramani (Eds.), Annals of the New York Academy of Sciences, vol 804 (pp. 573-587). New York, NY: New York Academy of Sciences. http://dx.doi.org/10.1111/j.1749-6632.1996.tb18646.x

Remucal, CK. (2019). Spatial and temporal variability of perfluoroalkyl substances in the Laurentian Great Lakes [Review]. Environmental Science: Processes & Impacts 21: 1816-1834. http://dx.doi.org/10.1039/c9em00265k

Reyes, L; Mañalich, R. (2005). Long-term consequences of low birth weight [Review]. Kidney International Supplement 68: S107-S111. http://dx.doi.org/10.1111/j.1523-1755.2005.09718.x

Risnes, KR; Vatten, LJ; Baker, JL; Jameson, K; Sovio, U; Kajantie, E, et al. (2011). Birthweight and mortality in adulthood: a systematic review and meta-analysis [Review]. International Journal of Epidemiology 40: 647-661. http://dx.doi.org/10.1093/ije/dyq267

Robinson, MW; Harmon, C; O'Farrelly, C. (2016). Liver immunology and its role in inflammation and homeostasis [Review]. Cellular and Molecular Immunology 13: 267-276. http://dx.doi.org/10.1038/cmi.2016.3

Robledo, CA; Yeung, E; Mendola, P; Sundaram, R; Maisog, J; Sweeney, AM, et al. (2015). Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environmental Health Perspectives 123: 88-94. http://dx.doi.org/10.1289/ehp.1308016

Roduit, R; Morin, J; Massé, F; Segall, L; Roche, E; Newgard, CB, et al. (2000). Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta -cell. Journal of Biological Chemistry 275: 35799-35806. http://dx.doi.org/10.1074/jbc.M006001200

Rogers, JM; Ellis-Hutchings, RG; Grey, BE; Zucker, RM; Norwood, J, Jr; Grace, CE, et al. (2014). Elevated blood pressure in offspring of rats exposed to diverse chemicals during pregnancy. Toxicological Sciences 137: 436-446. http://dx.doi.org/10.1093/toxsci/kft248

Rokoff, LB; Rifas-Shiman, SL; Coull, BA; Cardenas, A; Calafat, AM; Ye, X, et al. (2018). Cumulative exposure to environmental pollutants during early pregnancy and reduced fetal growth: the Project Viva cohort. Environmental Health: A Global Access Science Source 17: 19.

Rooney, JP; Oshida, K; Kumar, R; Baldwin, WS; Corton, JC. (2019). Chemical Activation of the Constitutive Androstane Receptor Leads to Activation of Oxidant-Induced Nrf2. Toxicological Sciences 167: 172-189. http://dx.doi.org/10.1093/toxsci/kfy231

Rosen, MB; Das, KP; Rooney, J; Abbott, B; Lau, C; Corton, JC. (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387: 95-107. http://dx.doi.org/10.1016/j.tox.2017.05.013

Rosen, MB; Das, KP; Wood, CR; Wolf, CJ; Abbott, BD; Lau, C. (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology 308: 129-137. http://dx.doi.org/10.1016/j.tox.2013.03.011

Rosen, MB; Schmid, JE; Das, KP; Wood, CR; Zehr, RD; Lau, C. (2009). Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid. Reproductive Toxicology 27: 278-288. http://dx.doi.org/10.1016/j.reprotox.2009.01.007

Rosen, MB; Schmid, JR; Corton, JC; Zehr, RD; Das, KP; Abbott, BD; Lau, C. (2010). Gene expression profiling in wild-type and PPAR alpha-null mice exposed to perfluorooctane sulfonate reveals PPAR alpha-independent effects. PPAR Research 2010: 794739. http://dx.doi.org/10.1155/2010/794739

Rosenmai, AK; Ahrens, L; le Godec, T; Lundqvist, J; Oskarsson, A. (2018). Relationship between peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl substances in HepG2 cells. Journal of Applied Toxicology 38: 219-226. http://dx.doi.org/10.1002/jat.3515

Rotander, A; Toms, LM; Aylward, L; Kay, M; Mueller, JF. (2015). Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environment International 82: 28-34.

Roth, K; Yang, Z; Agarwal, M; Liu, W; Peng, Z; Long, Z, e, et al. (2021). Exposure to a mixture of legacy, alternative, and replacement per- and polyfluoroalkyl substances (PFAS) results in sex-dependent modulation of cholesterol metabolism and liver injury. Environment International 157: 106843. http://dx.doi.org/10.1016/j.envint.2021.106843

Ruark, CD; Song, G; Yoon, M; Verner, MA; Andersen, ME; Clewell, HJ; Longnecker, MP. (2017). Quantitative bias analysis for epidemiological associations of perfluoroalkyl substance serum concentrations and early onset of menopause. Environment International 99: 245-254. http://dx.doi.org/10.1016/j.envint.2016.11.030

Ruhl, CE; Everhart, JE. (2009). Elevated Serum Alanine Aminotransferase and gamma-Glutamyltransferase and Mortality in the United States Population. Gastroenterology 136: 477-485. http://dx.doi.org/10.1053/j.gastro.2008.10.052

Ruhl, CE; Everhart, JE. (2013). The Association of Low Serum Alanine Aminotransferase Activity With Mortality in the US Population. American Journal of Epidemiology 178: 1702-1711. http://dx.doi.org/10.1093/aje/kwt209

Rusch, GM. (1979). An Acute Inhalation Toxicity Study of T-2307 CoC in the Rat. (3M-EPA-0029829). Rusch, GM.

Russell, MH; Waterland, RL; Wong, F. (2015). Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid (PFOA). Chemosphere 129: 210-216. http://dx.doi.org/10.1016/j.chemosphere.2014.07.061

Sagiv, SK; Rifas-Shiman, SL; Fleisch, AF; Webster, TF; Calafat, AM; Ye, X, et al. (2018). Early Pregnancy Perfluoroalkyl Substance Plasma Concentrations and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? American Journal of Epidemiology 187: 793-802.

Salihovic, S; Stubleski, J; Kärrman, A; Larsson, A; Fall, T; Lind, L; Lind, PM. (2018). Changes in markers of liver function in relation to changes in perfluoroalkyl substances - A longitudinal study. Environment International 117: 196-203.

Salvalaglio, M; Muscionico, I; Cavallotti, C. (2010). Determination of energies and sites of binding of PFOA and PFOS to human serum albumin. Journal of Physical Chemistry B 114: 14860-14874. http://dx.doi.org/10.1021/jp106584b

Sanchez Garcia, D; Sjödin, M; Hellstrandh, M; Norinder, U; Nikiforova, V; Lindberg, J, et al. (2018). Cellular accumulation and lipid binding of perfluorinated alkylated substances (PFASs) - A comparison with lysosomotropic drugs. Chemico-Biological Interactions 281: 1-10.

Schiller, JS; Lucas, JW; Ward, BW; Peregoy, JA. (2012). Summary health statistics for U.S. adults: National Health Interview Survey, 2010. (DHHS Publication No. (PHS) 2012–1580). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_252.pdf

Schlezinger, JJ; Puckett, H; Oliver, J; Nielsen, G; Heiger-Bernays, W; Webster, S. (2020). Perfluorooctanoic acid activates multiple nuclear receptor pathways and skews expression of genes regulating cholesterol homeostasis in liver of humanized PPARα mice fed an American diet. Toxicology and Applied Pharmacology 405: 115204. http://dx.doi.org/10.1016/j.taap.2020.115204

Seacat, AM; Thomford, PJ; Hansen, KJ; Clemen, LA; Eldridge, SR; Elcombe, CR; Butenhoff, JL. (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats [Erratum]. Toxicology 183: 117-131.

Seacat, AM; Thomford, PJ; Hansen, KJ; Olsen, GW; Case, MT; Butenhoff, JL. (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicological Sciences 68: 249-264.

Selgrade, MK. (2007). Immunotoxicity: The risk is real [Review]. Toxicological Sciences 100: 328-332. http://dx.doi.org/10.1093/toxsci/kfm244

Seo, SH; Son, MH; Choi, SD; Lee, DH; Chang, YS. (2018). Influence of exposure to perfluoroalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects. Environment International 113: 149-161.

Shah, D. (2009). Healthy worker effect phenomenon. Indian Journal of Occupational and Environmental Medicine 13: 77-79. http://dx.doi.org/10.4103/0019-5278.55123

Shan, G; Wang, Z, hi; Zhou, L; Du, P, in; Luo, X; Wu, Q; Zhu, L. (2016). Impacts of daily intakes on the isomeric profiles of perfluoroalkyl substances (PFASs) in human serum. Environment International 89-90: 62-70. http://dx.doi.org/10.1016/j.envint.2016.01.002

Shan, G; Ye, M; Zhu, B; Zhu, L. (2013). Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. Chemosphere 93: 2101-2107. http://dx.doi.org/10.1016/j.chemosphere.2013.07.054

Shearer, JJ; Callahan, CL; Calafat, AM; Huang, WY; Jones, RR; Sabbisetti, VS, et al. (2021). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. Journal of the National Cancer Institute 113: 580-587. http://dx.doi.org/10.1093/jnci/djaa143

Shen, M; Xiao, Y; Huang, Y; Jing, D; Su, J; Luo, D, et al. (2022). Perfluoroalkyl substances are linked to incident chronic spontaneous urticaria: A nested case-control study. Chemosphere 287: 132358. http://dx.doi.org/10.1016/j.chemosphere.2021.132358

Sheng, N; Cui, R; Wang, J; Guo, Y; Wang, J; Dai, J. (2018). Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid binding protein. Archives of Toxicology 92: 359-369. http://dx.doi.org/10.1007/s00204-017-2055-1

Sheng, N; Wang, J; Guo, Y; Wang, J; Dai, J. (2020). Interactions of perfluorooctanesulfonate and 6:2 chlorinated polyfluorinated ether sulfonate with human serum albumin: A comparative study. Chemical Research in Toxicology 33: 1478-1486.

Shi, Y; Yang, L; Li, J; Lai, J; Wang, Y; Zhao, Y; Wu, Y. (2017). Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 169: 396-402.

Shih, YH; Blomberg, AJ; Bind, MA; Holm, D; Nielsen, F; Heilmann, C, et al. (2021). Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. Journal of Immunotoxicology 18: 85-92. http://dx.doi.org/10.1080/1547691X.2021.1922957

Shin, HM; Bennett, DH; Calafat, AM; Tancredi, D; Hertz-Picciotto, I. (2020). Modeled prenatal exposure to per- and polyfluoroalkyl substances in association with child autism spectrum disorder: A case-control study. Environmental Research 186: 109514.

Shinwell, ES; Shlomo, M. (2003). Measured length of normal term infants changes over the first two days of life. Journal of Pediatric Endocrinology & Metabolism 16: 537-540. http://dx.doi.org/10.1515/jpem.2003.16.4.537

Shipley, JM; Hurst, CH; Tanaka, SS; Deroos, FL; Butenhoff, JL; Seacat, AM; Waxman, DJ. (2004). Trans-activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based chemicals. Toxicological Sciences 80: 151-160. http://dx.doi.org/10.1093/toxsci/kfh130

Shoaff, J; Papandonatos, GD; Calafat, AM; Chen, A; Lanphear, BP; Ehrlich, S, et al. (2018). Prenatal exposure to perfluoroalkyl substances: Infant birth weight and early life growth. Environmental Epidemiology 2: e010.

Sibai, BM; Frangieh, A. (1995). Maternal adaptation to pregnancy. Current Opinion in Obstetrics and Gynecology 7: 420-426. http://dx.doi.org/10.1097/00001703-199512000-00003

Simmon, VF. (1978). In-vitro microbiological mutagenicity assays of 3M Company compounds T-2247 CoC and T-2248 CoC. Final report. (SRI Project LSC-442-016). Menlo Park, CA: SRI International.

Singer, AB; Whitworth, KW; Haug, LS; Sabaredzovic, A; Impinen, A; Papadopoulou, E; Longnecker, MP. (2018). Menstrual cycle characteristics as determinants of plasma concentrations of perfluoroalkyl substances (PFASs) in the Norwegian Mother and Child

Cohort (MoBa study). Environmental Research 166: 78-85. http://dx.doi.org/10.1016/j.envres.2018.05.019

Sinisalu, L; Sen, P; Salihović, S; Virtanen, SM; Hyöty, H; Ilonen, J, et al. (2020). Early-life exposure to perfluorinated alkyl substances modulates lipid metabolism in progression to celiac disease. Environmental Research 188: 109864. http://dx.doi.org/10.1016/j.envres.2020.109864

Sinisalu, L; Yeung, LWY; Wang, J; Pan, Y; Dai, J; Hyötyläinen, T. (2021). Prenatal exposure to poly-/per-fluoroalkyl substances is associated with alteration of lipid profiles in cord-blood. Metabolomics 17: 103. http://dx.doi.org/10.1007/s11306-021-01853-9

Skendzel, LP. (1996). Rubella immunity. Defining the level of protective antibody [Review]. American Journal of Clinical Pathology 106: 170-174. http://dx.doi.org/10.1093/ajcp/106.2.170

Skuladottir, M; Ramel, A; Rytter, D; Haug, LS; Sabaredzovic, A; Bech, BH, et al. (2015). Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environmental Research 143: 33-38.

Smit, LA; Lenters, V; Høyer, BB; Lindh, CH; Pedersen, HS; Liermontova, I, et al. (2015). Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 70: 653-660.

Smith, CJ; Ryckman, KK; Barnabei, VM; Howard, BV; Isasi, CR; Sarto, GE, et al. (2016a). The impact of birth weight on cardiovascular disease risk in the Women's Health Initiative. Nutrition, Metabolism & Cardiovascular Diseases 26: 239-245. http://dx.doi.org/10.1016/j.numecd.2015.10.015

Smith, MT; Guyton, KZ; Gibbons, CF; Fritz, JM; Portier, CJ; Rusyn, I, et al. (2016b). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis [Review]. Environmental Health Perspectives 124: 713-721. http://dx.doi.org/10.1289/ehp.1509912

Smith, MT; Guyton, KZ; Kleinstreuer, N; Borrel, A; Cardenas, A; Chiu, WA, et al. (2020). The Key Characteristics of Carcinogens: Relationship to the Hallmarks of Cancer, Relevant Biomarkers, and Assays to Measure Them [Editorial]. Cancer Epidemiology Biomarkers and Prevention. http://dx.doi.org/10.1158/1055-9965.EPI-19-1346

Smith, PJ; Humiston, SG; Marcuse, EK; Zhao, Z; Dorell, CG; Howes, C; Hibbs, B. (2011). Parental delay or refusal of vaccine doses, childhood vaccination coverage at 24 months of age, and the Health Belief Model. 126: 135-146. http://dx.doi.org/10.1177/00333549111260S215

Smithwick, M; Norstrom, RJ; Mabury, SA; Solomon, K; Evans, TJ; Stirling, I, et al. (2006). Temporal trends of perfluoroalkyl contaminants in polar bears (Ursus maritimus) from two locations in the North American Arctic, 1972-2002. Environmental Science and Technology 40: 1139-1143. http://dx.doi.org/10.1021/es051750h

Song, M, i-Kyung; Cho, Y, oon; Jeong, S, eung-Chan; Ryu, J, ae-Chun. (2016). Analysis of gene expression changes in relation to hepatotoxicity induced by perfluorinated chemicals in a human hepatoma cell line. Toxicology and Environmental Health Sciences 8: 114–127. http://dx.doi.org/10.1007/s13530-016-0269-x

Sonkar, R; Kay, MK; Choudhury, M. (2019). PFOS modulates interactive epigenetic regulation in first-trimester human trophoblast cell line HTR-8/SVneo. Chemical Research in Toxicology 32: 2016-2027. http://dx.doi.org/10.1021/acs.chemrestox.9b00198