April 2024
EPA Document No. 815R24008

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

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

Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

EPA Document Number: 815R24008

April 2024

Disclaimer

This document has been reviewed in accordance with U.S. Environmental Protection Agency (EPA) policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Contents

Disclaimer ..... **i**
Contents ..... **ii**
Figures ..... **vii**
Tables ..... **xiv**
Acronyms and Abbreviations ..... **xxvii**
Appendix A. Systematic Review Protocol for Updated PFOA Toxicity Assessment ... **A-1**
A.1 Overview of Background Information and Systematic Review Protocol A-2
A.1.1 Summary of Chemical Identity and Occurrence Information A-2
A.1.2 Problem Formulation A-3
A.1.3 Overall Objective and Specific Aims A-5
A.1.4 Populations, Exposures, Comparators, and Outcomes (PECO) Criteria A-6
A.1.5 Literature Search A-10
A.1.6 Literature Screening Process to Target Dose-Response Studies and PK Models A-23
A.1.7 Study Quality Evaluation Overview A-55
A.1.8 Data Extraction for Epidemiological Studies A-101
A.1.9 Data Extraction for Animal Toxicological Studies A-110
A.1.10 Evidence Synthesis and Integration A-115
A.1.11 Dose-Response Assessment: Selecting Studies and Quantitative Analysis A-121
A.2 Meta-Analysis Table A-131
A.3 Studies Identified In Supplemental Literature Search Assessment Literature Cut-Off Date A-139
A.4 Studies Identified After Assessment Literature Searches A-156
Appendix B. Detailed Toxicokinetics **B-1**
B.1 Absorption B-1
B.1.1 Cellular Uptake B-1
B.1.2 Oral Exposure B-2
B.1.3 Inhalation Exposure B-3
B.1.4 Dermal Exposure B-3
B.1.5 Developmental Exposure B-4
B.1.6 Bioavailability B-4
B.2 Distribution B-5
B.2.1 Protein Binding B-6
B.2.2 Subcellular Distribution B-8
B.2.3 Tissue Distribution B-9
B.2.4 Distribution During Reproduction and Development B-23
B.2.5 Volume of Distribution Data B-48

B.3 Metabolism B-56
B.4 Excretion B-57
B.4.1 Urinary and Fecal Excretion B-57
B.4.2 Physiological and Mechanistic Factors Impacting Excretion B-64
B.4.3 Maternal Elimination Through Lactation and Fetal Partitioning B-71
B.4.4 Other Routes of Elimination B-73
B.4.5 Half-life Data B-74
Appendix C. Nonpriority Health System Evidence Synthesis and Integration C-1
C.1 Reproductive C-1
C.1.1 Human Evidence Study Quality Evaluation and Synthesis C-1
C.1.2 Animal Evidence Study Quality Evaluation and Synthesis C-15
C.1.3 Mechanistic Evidence Synthesis C-28
C.1.4 Evidence Integration C-29
C.2 Endocrine C-47
C.2.1 Human Evidence Study Quality Evaluation and Synthesis C-47
C.2.2 Animal Evidence Study Quality Evaluation and Synthesis C-54
C.2.3 Mechanistic Evidence C-63
C.2.4 Evidence Integration C-64
C.3 Metabolic/Systemic C-70
C.3.1 Human Evidence Study Quality Evaluation and Synthesis C-70
C.3.2 Animal Evidence Study Quality Evaluation and Synthesis C-88
C.3.3 Mechanistic Evidence C-99
C.3.4 Evidence Integration C-100
C.4 Nervous C-108
C.4.1 Human Evidence Study Quality Evaluation and Synthesis C-108
C.4.2 Animal Evidence Study Quality Evaluation and Synthesis C-116
C.4.3 Mechanistic Evidence C-119
C.4.4 Evidence Integration C-120
C.5 Renal C-129
C.5.1 Human Evidence Study Quality Evaluation and Synthesis C-129
C.5.2 Animal Evidence Study Quality Evaluation and Synthesis C-134
C.5.3 Mechanistic Evidence C-143
C.5.4 Evidence Integration C-143
C.6 Hematological C-150
C.6.1 Human Evidence Study Quality Evaluation and Synthesis C-150
C.6.2 Animal Evidence Study Quality Evaluation and Synthesis C-153
C.6.3 Mechanistic Evidence C-156
C.6.4 Evidence Integration C-157
C.7 Respiratory C-160
C.7.1 Human Evidence Study Quality Evaluation and Synthesis C-160
C.7.2 Animal Evidence Study Quality Evaluation and Synthesis C-162

C.7.3 Mechanistic Evidence C-166
C.7.4 Evidence Integration C-166
C.8 Musculoskeletal C-170
C.8.1 Human Evidence Study Quality Evaluation and Synthesis C-170
C.8.2 Animal Evidence Study Quality Evaluation and Synthesis C-173
C.8.3 Mechanistic Evidence C-174
C.8.4 Evidence Integration C-175
C.9 Gastrointestinal C-179
C.9.1 Human Evidence Study Quality Evaluation and Synthesis C-179
C.9.2 Animal Evidence Study Quality Evaluation and Synthesis C-181
C.9.3 Mechanistic Evidence C-184
C.9.4 Evidence Integration C-184
C.10 Dental C-188
C.10.1 Human Evidence Study Quality Evaluation and Synthesis C-188
C.10.2 Animal Evidence Study Quality Evaluation and Synthesis C-190
C.10.3 Mechanistic Evidence C-190
C.10.4 Evidence Integration C-190
C.11 Ocular C-192
C.11.1 Human Evidence Study Quality Evaluation and Synthesis C-192
C.11.2 Animal Evidence Study Quality Evaluation and Synthesis C-193
C.11.3 Mechanistic Evidence C-194
C.11.4 Evidence Integration C-195
C.12 Dermal C-197
C.12.1 Human Evidence Study Quality Evaluation and Synthesis C-197
C.12.2 Animal Evidence Study Quality Evaluation and Synthesis C-198
C.12.3 Mechanistic Evidence C-199
C.12.4 Evidence Integration C-200
Appendix D. Detailed Information from Epidemiology Studies D-1
D.1 Developmental D-1
D.2 Reproductive D-57
D.2.1 Male D-57
D.2.2 Female D-65
D.3 Hepatic D-75
D.4 Immune D-89
D.5 Cardiovascular D-131
D.5.1 Cardiovascular Endpoints D-131
D.5.2 Serum Lipids D-152
D.6 Endocrine D-181
D.7 Metabolic/Systemic D-190
D.8 Nervous D-209
D.9 Renal D-234

D.10 Hematological D-247
D.11 Respiratory D-249
D.12 Musculoskeletal D-251
D.13 Gastrointestinal D-257
D.14 Ocular D-259
D.15 Dermal D-260
D.16 Cancer D-261
Appendix E. Benchmark Dose Modeling E-1
E.1 Epidemiology Studies E-1
E.1.1 Modeling Results for Immunotoxicity E-3
E.1.2 Modeling Results for Decreased Birthweight E-24
E.1.3 Modeling Results for Liver Toxicity E-31
E.1.4 Modeling Results for Increased Cholesterol E-44
E.1.5 Modeling Results for Cancer E-55
E.2 Toxicology Studies E-62
E.2.1 Butenhoff et al. (2012) E-62
E.2.2 Dewitt et al. (2008) E-65
E.2.3 Lau et al. (2006) E-68
E.2.4 Li et al. (2018a) E-75
E.2.5 Loveless et al. (2008) E-75
E.2.6 NTP (2020) E-81
E.2.7 Song et al. (2018a) E-107
Appendix F. Pharmacokinetic Modeling F-1
F.1 Animal Pharmacokinetic Model F-1
F.1.1 Comparison of Fits to Training Datasets Used in Wambaugh et al. (2013) F-1
F.1.2 Visual Inspection of Test Datasets Not Used for Initial Fitting F-4
F.1.3 Consideration of Hinderliter et al. (2006b) in the Animal Model F-8
F.2 Human Model Validation F-10
Appendix G. Relative Source Contribution G-1
G.1 Background G-1
G.2 Literature Review G-2
G.2.1 Systematic Review G-2
G.2.2 Evidence Mapping G-3
G.3 Summary of Potential PFOA Sources G-4
G.3.1 Dietary Sources G-4
G.3.2 Consumer Product Uses G-9
G.3.3 Indoor Dust G-10
G.3.4 Ambient Air G-10
G.3.5 Other Exposure Considerations G-11
G.4 Recommended Relative Source Contribution G-11

References **R-1**

Figures

Figure A-1. Overview of Study Quality Evaluation Approach A-55

Figure B-1. Summary of PFOA Absorption Studies B-1

Figure B-2. Summary of PFOA Distribution Studies B-5

Figure B-3. Summary of PFOA Metabolism Studies B-56

Figure B-4. Summary of PFOA Excretion Studies.....B-57

Figure B-5. Localization of Transport Proteins B-65

Figure C-1. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Male Reproductive Effects.....C-4

Figure C-2. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects C-9

Figure C-3. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects (Continued).....C-10

Figure C-4. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects (Continued).....C-11

Figure C-5. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Reproductive Effects.....C-16

Figure C-6. Sperm Counts in Rodents Following Exposure to PFOA (Logarithmic Scale) C-19

Figure C-7. Percent Change in Male Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA.....C-22

Figure C-8. Percent Change in Female Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA.....C-24

Figure C-9. Summary of Mechanistic Studies of PFOA and Reproductive Effects.....C-29

Figure C-10. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Endocrine Effects C-49

Figure C-11. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Endocrine Effects (Continued).....C-50

Figure C-12. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Endocrine Effects.....C-54

Figure C-13. Percent Change in Endocrine Organ Weights Relative to Controls in Rodents Following Exposure to PFOA[a].....C-56

Figure C-14. Percent Change in Thyroid and Thyroid-Related Hormone Levels of Male and Female Rats Exposed to PFOA for 28 Days as Reported by NTP (2019a)[a,b].....C-58

Figure C-15. Endocrine Organ Histopathology in Rodents Following Exposure to PFOA (Logarithmic Scale)....C-63

Figure C-16. Summary of Mechanistic Studies of PFOA and Endocrine Effects....C-64

Figure C-17. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects....C-73

Figure C-18. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects (Continued)....C-74

Figure C-19. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects (Continued)....C-75

Figure C-20. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Metabolic Effects....C-89

Figure C-21. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Systemic Effects....C-91

Figure C-22. Effects on Survival in Rodents Following Exposure to PFOA (Logarithmic Scale)....C-92

Figure C-23. Effects on Body Weight in Rodents Following Exposure to PFOA (Logarithmic Scale)....C-95

Figure C-24. Effects on Body Weight in Rodents Following Developmental Exposure to PFOA (Logarithmic Scale)....C-97

Figure C-25. Effects on Food Consumption in Rodents Following Exposure to PFOA....C-99

Figure C-26. Summary of Mechanistic Studies of PFOA and Metabolic Effects....C-99

Figure C-27. Summary of Mechanistic Studies of PFOA and Systemic Effects....C-100

Figure C-28. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Neurological Effects....C-110

Figure C-29. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Neurological Effects (Continued)....C-111

Figure C-30 Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Nervous Effects....C-117

Figure C-31. Summary of Mechanistic Studies of PFOA and Nervous Effects....C-120

Figure C-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Renal Effects....C-131

Figure C-33. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Renal Effects....C-135

Figure C-34. Absolute Kidney Weights in Rodents Following Exposure to PFOA (Logarithmic Scale)....C-136

Figure C-35. Percent Change in Relative Kidney Weights of Male Rats Following Exposure to PFOA C-138

Figure C-36. Percent Change in Relative Kidney Weights of Female Rodents Following Exposure to PFOA C-140

Figure C-37. Summary of Mechanistic Studies of PFOA and Renal Effects C-143

Figure C-38. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hematological Effects C-152

Figure C-39. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hematological Effects C-154

Figure C-40. Hematological Effects in Male and Female Sprague-Dawley Rats Dosed with PFOA for 28 Days as Reported by NTP (2019a) C-155

Figure C-41. Summary of Mechanistic Studies of PFOA and Hematological Effects C-157

Figure C-42. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Respiratory Effects C-161

Figure C-43. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Respiratory Effects C-163

Figure C-44. Incidence of Nonneoplastic Nasal Lesions in Male and Female Sprague-Dawley Rats Following 28-day Oral Exposure to PFOA, as Reported by NTP (2019a) C-165

Figure C-45. Summary of Mechanistic Studies of PFOA and Respiratory Effects C-166

Figure C-46. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Musculoskeletal Effects C-172

Figure C-47. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Musculoskeletal Effects C-174

Figure C-48. Summary of Mechanistic Studies of PFOA and Musculoskeletal Effects C-175

Figure C-49. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Gastrointestinal Effects C-181

Figure C-50. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Gastrointestinal Effects C-182

Figure C-51. Gastrointestinal Effects in Rodents and Nonhuman Primates Following Exposure to PFOA (Logarithmic Scale) C-183

Figure C-52. Summary of Mechanistic Studies of PFOA and Gastrointestinal Effects C-184

Figure C-53. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Dental Effects C-189

Figure C-54. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Ocular Effects C-193

Figure C-55. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Ocular Effects C-194

Figure C-56. Summary of Mechanistic Studies of PFOA and Ocular Effects C-195

Figure C-57. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Dermal Effects C-198

Figure C-58. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Dermal Effects C-199

Figure C-59. Summary of Mechanistic Studies of PFOA and Dermal Effects C-200

Figure E-1. Difference in population tail probabilities resulting from a one standard deviation shift in the mean from a standard normal distribution, illustrating the theoretical basis for a baseline BMR of 1 SD E-6

Figure E-2. Difference in Population Tail Probabilities Resulting From a ½ Standard Deviation Shift in the Mean From an Estimation of the Distribution of Log_2(Tetanus Antibody Concentrations at Age 7 Years) E-8

Figure E-3. Regression Coefficients and 95% CIs Between the Log of the RCC ORs and Serum PFOA Concentrations Using Data From Shearer et al. (2021): Adjusted (glst) and Unadjusted (vwls) for OR Dependence E-59

Figure E-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA with BMR 4% Extra Risk (Butenhoff et al., 2012).....E-65

Figure E-5. Plot of Mean Response by Dose with Fitted Curve for the Polynomial Degree 4 Model for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Dewitt et al., 2008) E-67

Figure E-6. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 5 Model for Time to Eye Opening in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006) E-71

Figure E-7. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Dichotomous Hill Model for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008).....E-77

Figure E-8. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008).....E-79

Figure E-9. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 3 Model for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008).....E-81

Figure E-10. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Gamma Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-83

Figure E-11. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-85

Figure E-12. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020).....E-86

Figure E-13. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-88

Figure E-14. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-89

Figure E-15. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020).....E-91

Figure E-16. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-93

Figure E-17. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-94

Figure E-18. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020).....E-95

Figure E-19. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020).....E-97

Figure E-20. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) ..E-99

Figure E-21. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020).....E-100

Figure E-22. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-102

Figure E-23. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-105

Figure E-24. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Pup Survival using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a) E-109

Figure E-25. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 2 Model for Pup Survival using $C_{avg,pup,gest}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a) E-111

Figure E-26. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Pup Survival using $C_{avg,pup,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a) E-113

Figure E-27. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Pup Survival using $C_{max,pup,gest}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a) E-115

Figure E-28. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Offspring Survival using $C_{max,pup,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a) E-117

Figure F-1. Experimentally Observed Serum Concentrations (Lou et al., 2009) and Median Predictions for a Single Oral Dose of 1, 10, or 60 mg/kg PFOA to Female CD1 Mice F-1

Figure F-2. Experimentally Observed Serum Concentrations (Kemper, 2003) and Median Prediction for a Single IV Dose of 1 mg/kg or an Oral Dose of 0.1, 1, 5, or 25 mg/kg PFOA to Male Sprague-Dawley Rats F-2

Figure F-3. Experimentally Observed Serum Concentrations (Kemper, 2003) and Median Prediction for a Single IV Dose of 1 mg/kg or a Single Oral Dose of 0.1, 1, 5, or 15 mg/kg PFOA to Female Sprague-Dawley Rats[a] F-2

Figure F-4. Model Prediction Summary for PFOA Training Data F-3

Figure F-5. PFOA Sensitivity Coefficients of the Adult Model and Developmental Model F-4

Figure F-6. Experimentally Observed Serum Concentrations (Dzierlenga et al., 2019) and Median Predictions for a Single IV Dose of 6 mg/kg or a Single Oral Dose of 6, 12, or 45 mg/kg PFOA to Male Sprague-Dawley Rats F-5

Figure F-7. Experimentally Observed Serum Concentrations (Dzierlenga et al., 2019) and Median Predictions for a Single IV Dose of 40 mg/kg or a Single Oral Dose of 40, 80, or 320 mg/kg PFOA to Female Sprague-Dawley Rats[a,b] F-5

Figure F-8. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA (Kim et al., 2016b) or an IV Dose of 20 mg/kg PFOA (Kudo et al., 2002) to Male Sprague-Dawley Rats F-6

Figure F-9. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA (Kim et al., 2016b) or an IV Dose of 20 mg/kg PFOA (Kudo et al., 2002) to Female Sprague-Dawley Rats[a] F-6

Figure F-10. Observed and Predicted PFOA Plasma Concentration in Female Sprague-Dawley Rats Following Perinatal, Lactational, and Post-weaning Exposure During Study 1 of NTP (2020)[a,b] F-7

Figure F-11. Observed and Predicted PFOA Plasma Concentrations in Male Sprague-Dawley Rats Following Perinatal, Lactational, and Post-weaning Exposure During Study 2 of NTP (2020)[a,b] F-7

Figure F-12. Model Prediction Summary for PFOA Test Data F-8

Figure F-13. Model Prediction Summary for PFOA Data from Hinderliter et al. (2006b) F-9

Figure F-14. Model Comparison F-10

Figure F-15. Predicted Child Serum Levels Compared with Reported Values F-11

Figure F-16. Comparison of Predicted and Observed Child Serum Concentration F-12

Figure F-17. Sensitivity Coefficients F-13

Figure F-18. Predicted Child Serum Levels Compared with Reported Values with Increased Volume of Distribution in Children as Implemented in the Minnesota Department of Health Model F-14

Figure F-19. Predicted Child Serum Levels Compared with Reported Values with Constant Volume of Distribution and Variable Exposure Based on Drinking Water Intake. F-15

Figure G-1. Application of the Exposure Decision Tree (U.S. EPA, 2000) for PFOA G-12

Tables

Table A-1. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects from Exposure to PFOA and PFOS..... A-7

Table A-2. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Absorption, Distribution, Metabolism, and/or Excretion (ADME) Studies A-8

Table A-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Mechanistic Studies A-9

Table A-4. Search String for April 2019 Database Searches..... A-11

Table A-5. Search String for September 2020, February 2022, and February 2023 Database Searches..... A-12

Table A-6. Key Epidemiological Studies of Priority Health Outcomes Identified from the 2016 PFOA Health Effects Support Document A-16

Table A-7. Key Animal Toxicological Studies Identified from the 2016 PFOA Health Effects Support Document A-20

Table A-8. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects from Exposure to PFOA and PFOS..... A-23

Table A-9. DistillerSR Form for Title/Abstract Screening..... A-25

Table A-10. SWIFT-Active Form for Title/Abstract Screening..... A-27

Table A-11. Supplemental Tags for Title/Abstract and Full-Text Screening..... A-27

Table A-12. Mechanistic Study Categories Considered as Supplemental..... A-28

Table A-13. DistillerSR Form for Full-Text Screening..... A-30

Table A-14. Health Effect Categories Considered for Epidemiological Studies..... A-33

Table A-15. Litstream Forms for ADME Screening and Light Data Extraction..... A-37

Table A-16. litstream Forms for Mechanistic Screening and Light Data Extraction A-47

Table A-17. Possible Domain Scores for Study Quality Evaluation A-56

Table A-18. Overall Study Confidence Classifications A-56

Table A-19. Study Quality Evaluation Considerations for Participant Selection..... A-58

Table A-20. Study Quality Evaluation Considerations for Exposure Measurement A-60

Table A-21. Study Quality Evaluation Considerations for PFAS-Specific Exposure Measurement A-63

Table A-22. Study Quality Evaluation Considerations for Outcome Ascertainment..... A-65

Table A-23. Study Quality Evaluation Considerations for Confounding..... A-67

Table A-24. Study Quality Evaluation Considerations for Analysis A-69

Table A-25. Study Quality Evaluation Considerations for Selective Reporting A-71

Table A-26. Study Quality Evaluation Considerations for Study Sensitivity A-72

Table A-27. Study Quality Evaluation Considerations for Overall Study Confidence – Epidemiological Studies A-73

Table A-28. Study Evaluation Considerations for Reporting Quality A-75

Table A-29. Study Quality Evaluation Considerations for Selection and Performance – Allocation A-78

Table A-30. Study Quality Evaluation Considerations for Selection and Performance – Observational Bias/Blinding A-80

Table A-31. Study Quality Evaluation Considerations for Confounding/Variable Control..... A-84

Table A-32. Study Quality Evaluation Considerations for Selective Reporting and Attrition – Reporting and Attrition Bias A-86

Table A-33. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Chemical Administration and Characterization A-88

Table A-34. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Exposure Timing, Frequency, and Duration A-91

Table A-35. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Endpoint Sensitivity and Specificity A-93

Table A-36. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Results Presentation A-96

Table A-37. Study Quality Evaluation Considerations for Overall Study Confidence – Animal Toxicological Studies A-98

Table A-38. DistillerSR Form Fields for Data Extraction of Epidemiological Studies A-101

Table A-39. Epidemiological Study Design Definitions A-108

Table A-40. HAWC Form Fields for Data Extraction of Animal Toxicological Studies A-110

Table A-41. Framework for Strength-of-Evidence Judgments for Epidemiological Studies[a] A-116

Table A-42. Framework for Strength-of-Evidence Judgments for Animal Toxicological Studies[a] A-117

Table A-43. Evidence Integration Judgments for Characterizing Potential Human Health Effects in the Evidence Integration[a] A-118

Table A-44. Attributes used to evaluate studies for derivation of toxicity values (adapted from ORD Staff Handbook for Developing IRIS Assessments Table 7-2). A-123

Table A-45. Epidemiologic Meta-Analysis Studies Identified from Literature Review A-131

Table A-46. Animal Toxicity Meta-Analysis Studies Identified From Literature Review A-138

Table A-47. Epidemiological Studies Identified After 2022 Updated Literature Search (Published or Identified Between February 2022 and February 2023)..... A-141

Table A-48. Animal Toxicity Studies Identified After Updated Literature Review (Published or Identified After February 2022)..... A-154

Table A-49. Epidemiological Studies Identified After 2023 Updated Literature Search (Published or Identified After February 2023)..... A-156

Table B-1. Cellular Accumulation and Retention Relative to Lipophilicity and Phospholipophilicity as Reported by Sanchez Garcia et al. (2018) B-2

Table B-2. PFOA Parameters From Toxicokinetic Studies Informing Bioavailability in Sprague-Dawley Rats.....B-4

Table B-3. Dissociation Constants of Binding Between PFOA and Albumin as Reported by Han et al. (2003).....B-6

Table B-4. Tissue Distribution of PFOA in Wistar Rats After Exposure via Gavage for 28 Days as Reported by Ylinen et al. (1990) B-13

Table B-5. Distribution of PFOA in Male Sprague-Dawley Rats After a Single Oral Gavage Dose[a] as Reported by Kemper et al. (2003) B-15

Table B-6. Distribution of PFOA in Female Sprague-Dawley Rats After a Single Oral Gavage Dose[a] as Reported by Kemper et al. (2003).....B-16

Table B-7. Distribution of PFOA in Male C57BL/6 Mice Following Exposure to [^{14}C]PFOA for 1, 3, or 5 days in Feed[a] as Reported by Bogdanska et al. (2020)...B-19

Table B-8. PFOA Concentrations in Wild-type and PPARα-null Male Mice Exposed to PFOA by Gavage for Four Weeks[a] as Reported by Minata et al. (2010) B-22

Table B-9. PFOA Concentrations in Human Cord Blood, Maternal Blood, and Transplacental Transfer Ratios (RCM).....B-28

Table B-10. PFOA Concentrations in Human Maternal Blood, Cord Blood, Placenta and Amniotic Fluid Across Studies B-32

Table B-11. Summary of Studies Evaluating PFOA Concentrations in Maternal Serum, Breast Milk, and Infant Serum.....B-37

Table B-12. Percent Change in PFOA Ratios in Maternal Serum to Breast Milk and Breast Milk to Infant Serum by Infant Age in Humans as Reported by Mondal et al. (2014) B-39

Table B-13. Percent Change in PFOA Serum Concentration by Exclusive, Mixed or No Breastfeeding per Month in Humans as Reported by Mogensen et al. (2015b) B-39

Table B-14. Maternal Plasma PFOA Levels in Sprague-Dawley Rats During Gestation and Lactation[a] as Reported by Hinderliter et al. (2005).....B-40

Table B-15. Placenta, Amniotic Fluid, and Embryo/Fetus PFOA Concentrations in Sprague-Dawley Rats[a] as Reported by Hinderliter et al. (2005).....B-41

Table B-16. Fetus/Pup PFOA Concentration in Sprague-Dawley Rats During Gestation and Lactation[a] as Reported by Hinderliter et al. (2005).....B-41

Table B-17. Maternal Milk PFOA Concentration in Sprague-Dawley Rat During Lactation[a] as Reported by Hinderliter et al. (2005).....B-41

Table B-18. Plasma PFOA Concentrations in Postweaning Sprague-Dawley Rats[a] as Reported by Han (2003).....B-42

Table B-19. Plasma PFOA Concentrations in Male Sprague-Dawley Rats at 2 and 24 Hours After Oral Gavage as Reported by Hinderliter et al. (2006b).....B-42

Table B-20. Plasma PFOA Concentrations in Female Sprague-Dawley Rats at 2 and 24 Hours After Oral Gavage as Reported by Hinderliter et al. (2006b).....B-43

Table B-21. Select Fluids and Tissues PFOA Concentrations in CD-1 Mice During Gestation and Lactation[a] as Reported by Fenton et al. (2009).....B-45

Table B-22. Serum, Liver, and Brain PFOA Concentration in Female CD-1 Mouse Pups After GD 10−17 Exposure[a] as Reported by Macon et al. (2011).....B-46

Table B-23. Serum PFOA Concentrations in Female CD-1 Mouse Pups After GD 10−17 Exposure as Reported by Macon et al. (2011).....B-47

Table B-24. Serum PFOA Concentration in CD-1 Mice Over Three Generations[a] as Reported by White et al. (2011).....B-47

Table B-25. Maternal Serum, Amniotic Fluid, and Whole Embryo PFOA Concentrations in CD-1 Mice Exposed During Gestation Day 1.5–17.5 as Reported by Blake et al. (2020).....B-48

Table B-26. Summary of PFOA Volume of Distribution Values Assigned in Human Studies.....B-50

Table B-27. PFOA Volume of Distribution in Serum of FVB/NJcl Mice as Reported by Fujii et al. (2015).....B-51

Table B-28. Summary of PFOA Volume of Distribution Calculations in Rats.....B-53

Table B-29. Urine PFOA Concentrations in Male and Female Sprague-Dawley Rats, 24 Hours After Oral Gavage[a] as Reported by Hinderliter et al. (2006b).....B-60

Table B-30.Cumulative Percent [^{14}C]PFOA Excreted in Urine and Feces by Male and Female CD Rats[a] as Reported by Hundley et al. (2006).....B-62

Table B-31. Percentage of Dose Excreted in Urine and Feces of Male and Female Sprague-Dawley Rats Exposed to [^{14}C]PFOA via Oral Gavage as Reported by Kemper (2003).....B-62

Table B-32. Cumulative Percent [^{14}C]PFOA Excreted in Urine and Feces in Mouse, Hamster, and Rabbit[a] as Reported by Hundley et al. (2006).....B-63

Table B-33. Kinetic Parameters of Perfluorinated Carboxylate Transport by OAT1, OAT3, and OATP1a1 as Reported by Weaver et al. (2010) B-68

Table B-34. Estimated Percentage of the Sum of PFOS, PFNA, and PFOA in Excreta and Serum of Male and Female Wistar Rats[a] as Reported by Gao et al. (2015) B-74

Table B-35. Summary of PFOA Concentration in Blood and Urine in Relation to Half-life Values in Humans B-80

Table B-36. Summary of Human PFOA Half-Life Values B-82

Table B-37. PK Parameters in Male Sprague-Dawley Rats Following Administration of PFOA as Reported by Kemper et al. (2003) B-85

Table B-38. PK Parameters in Female Sprague-Dawley Rats Following Administration of PFOA as Reported by Kemper et al. (2003) B-86

Table B-39. PK Parameters in Male and Female Sprague-Dawley Rats Following Oral and IV Administration of PFOA as Reported by Kim et al. (2016b) B-87

Table B-40. Summary of Animal PFOA Half-life Values Identified in the Literature Review B-89

Table C-1. Evidence Profile Table for PFOA Reproductive Effects in Males C-31

Table C-2. Evidence Profile Table for PFOA Reproductive Effects in Females C-39

Table C-3. Associations Between PFOA Exposure and Thyroid and Thyroid-Related Hormone Effects in Rodents and Nonhuman Primates C-59

Table C-4. Associations Between PFOA Exposure and Adrenocortical Hormone Effects in Rodents C-61

Table C-5. Evidence Profile Table for PFOA Endocrine Effects C-66

Table C-6. Evidence Profile Table for PFOA Systemic and Metabolic Effects C-102

Table C-7. Evidence Profile Table for PFOA Nervous System Effects C-122

Table C-8. Incidences of Nonneoplastic Lesions in the Kidneys of Female Sprague-Dawley Rats as Reported by NTP (2020) C-141

Table C-9. Evidence Profile Table for PFOA Renal Effects C-145

Table C-10. Evidence Profile Table for PFOA Hematological Effects C-158

Table C-11. Incidences of Nonneoplastic Pulmonary Lesions in Male Rats as Reported by Butenhoff et al. (2012) C-164

Table C-12. Evidence Profile Table for PFOA Respiratory Effects C-168

Table C-13. Evidence Profile Table for PFOA Musculoskeletal Effects C-177

Table C-14. Evidence Profile Table for PFOA Gastrointestinal Effects C-186

Table C-15. Evidence profile table for PFOA Dental Effects C-191

Table C-16. Evidence Profile Table for PFOA Ocular Effects C-196

Table C-17. Evidence Profile Table for PFOA Dermal Effects C-201

Table D-1. Associations Between PFOA Exposure and Developmental Effects in Recent Epidemiological Studies..... D-1

Table D-2. Associations Between PFOA Exposure and Male Reproductive Effects in Recent Epidemiologic Studies D-57

Table D-3. Associations Between PFOA Exposure and Female Reproductive Health Effects in Female Children and Adolescents D-65

Table D-4. Associations Between PFOA Exposure and Female Reproductive Health Effects in Pregnant Women D-69

Table D-5. Associations Between PFOA Exposure and Female Reproductive Health Effects in Nonpregnant Adult Women..... D-72

Table D-6. Associations Between PFOA Exposure and Hepatic Effects in Epidemiology Studies D-75

Table D-7. Associations Between PFOA Exposure and Vaccine Response in Recent Epidemiological Studies..... D-89

Table D-8. Associations Between PFOA Exposure and Infectious Disease in Recent Epidemiological Studies..... D-102

Table D-9. Associations Between PFOA Exposure and Asthma in Recent Epidemiologic Studies D-109

Table D-10. Associations Between PFOA Exposure and Allergies in Recent Epidemiologic Studies D-118

Table D-11. Associations Between PFOA Exposure and Eczema in Recent Epidemiologic Studies D-124

Table D-12. Associations Between PFOA Exposure and Autoimmune Health Effects in Recent Epidemiologic Studies D-127

Table D-13. Associations Between PFOA Exposure and Cardiovascular Effects in Recent Epidemiological Studies..... D-131

Table D-14. Associations Between PFOA Exposure and Serum Lipid Effects in Recent Epidemiologic Studies D-152

Table D-15. Associations Between PFOA Exposure and Endocrine Effects in Recent Epidemiologic Studies D-181

Table D-16. Associations Between PFOA Exposure and Metabolic Effects in Recent Epidemiologic Studies D-190

Table D-17. Associations Between PFOA Exposure and Neurological Effects in Recent Epidemiologic Studies D-209

Table D-18. Associations Between PFOA Exposure and Renal Effects in Recent Epidemiologic Studies D-234

Table D-19. Associations Between PFOA Exposure and Hematological Effects in Recent Epidemiologic Studies D-247

Table D-20. Associations Between PFOA Exposure and Respiratory Effects in Recent Epidemiologic Studies D-249

Table D-21. Associations Between PFOA Exposure and Musculoskeletal Health Effects in Recent Epidemiologic Studies D-251

Table D-22. Associations Between PFOA Exposure and Gastrointestinal Health Effects in Recent Epidemiologic Studies D-257

Table D-23. Associations Between PFOA Exposure and Dental Health Effects in Recent Epidemiologic Studies D-258

Table D-24. Associations Between PFOA Exposure and Ocular Effects in Recent Epidemiologic Studies D-259

Table D-25. Associations Between PFOA Exposure and Dermal Health Effects in Recent Epidemiologic Studies D-260

Table D-26. Associations Between PFOA Exposure and Cancer in Recent Epidemiologic Studies D-261

Table E-1. Summary of Modeling Approaches for POD Derivation From Epidemiological Studies E-1

Table E-2. Results Specific to the Slope From the Linear Analyses of PFOA Measured at Age 5 Years and Log_2(Tetanus Antibody Concentrations) Measured at Age 7 Years From Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b] E-3

Table E-3. BMDs and BMDLs for Effect of PFOA at Age 5 Years on Anti-Tetanus Antibody Concentrations at Age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a BMR of ½ SD Change in Log_2(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_2(Tetanus Antibodies Concentration) E-8

Table E-4. BMDs and BMDLs for Effect of Serum PFOA in Children on Anti-Tetanus Antibody Concentrations Using a BMR of ½ SD Change in Log_{10}(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_{10}(Tetanus Antibodies Concentration) Timmerman et al. (2021) E-9

Table E-5. BMDLs for Effect of PFOA on Anti-Tetanus Antibody Concentrations Using a BMR of ½ SD E-10

Table E-6. Results of the Linear Analyses of PFOA Measured Perinatally and Tetanus Antibodies Measured at Age 5 Years From Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b] E-11

Table E-7. BMDs and BMDLs for Effect of PFOA Measured Perinatally and Anti-Tetanus Antibody Concentrations at Age 5 Years (Budtz-Jørgensen and Grandjean, 2018a).....E-13

Table E-8. Results Specific to the Slope From the Linear Analyses of PFOA Measured at Age 5 Years and Log_2(Diphtheria Antibodies) Measured at Age 7 Years From Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b].....E-14

Table E-9. BMDs and BMDLs for Effect of PFOA at Age 5 Years on Anti-Diphtheria Antibody Concentrations at Age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a BMR of ½ SD Change in Log_2(Diphtheria Antibodies Concentration) and a BMR of 1 SD Log_2(Diphtheria Antibodies Concentration) .E-16

Table E-10. Results of the Analyses of PFOA Measured Perinatally and Diphtheria Antibodies Measured at Age 5 Years From Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b] E-17

Table E-11. BMDs and BMDLs for Effect of PFOA Measured Perinatally and Anti-Diphtheria Antibody Concentrations at Age 5 Years (Budtz-Jørgensen and Grandjean, 2018a) E-19

Table E-12. BMDs and BMDLs for Effect of PFOA on Anti- Diphtheria Antibody Concentrations (Timmermann et al., 2021) Using a BMR of ½ SD Change in log_{10}(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_{10}(Diphtheria Antibodies Concentration).....E-20

Table E-13. BMDLs for Effect of PFOA on Anti-Diphtheria Antibody Concentrations Using a BMR of ½ SD E-21

Table E-14. Levels of Rubella Vaccine-Induced Antibodies at the Age of 3 Years (Adapted From Table 3 in Granum et al. (2013)) E-21

Table E-15. BMDs and BMDLs for Effect of Maternal Serum PFOA on Anti-Rubella Antibody Concentrations in Children Using a BMR of ½ SD Change in Rubella Antibodies Concentration and a BMR of 1 SD Change in Rubella Antibodies Concentration (Granum et al., 2013).....E-23

Table E-16. BMDs and BMDLs for Effect of PFOA on Decreased Birth Weight, by Using Percentage (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight, or Alternative Study-Specific Tail Probability.....E-29

Table E-17. BMDs and BMDLs for Effect of PFOA on Decreased Birth Weight by Background Exposure, Using the Exact Percentage of the Population (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight, or Alternative Tail Probability E-30

Table E-18. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Nian et al. (2019) E-32

Table E-19. NHANES Mean and Standard Deviation of Ln(ALT) (ln IU/L) and Mean PFOA (Ln ng/mL).....E-33

Table E-20. Prevalence of Elevated ALT E-34

Table E-21. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Gallo et al. (2012) E-35

Table E-22. Odds Ratios for Elevated ALT by Decile of PFOA Serum Concentrations (ng/mL) From Gallo et al. (2012) E-36

Table E-23. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted Mean PFOA Serum Concentration E-38

Table E-24. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Mean PFOA Serum Concentration ... E-39

Table E-25. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted Median PFOA Serum Concentration E-40

Table E-26. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Median PFOA Serum Concentration E-41

Table E-27. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Darrow et al. (2016) E-42

Table E-28. Dose-Response Modeling Data for Increased Mean ALT Concentration in Darrow et al. (2016) E-42

Table E-29. Summary of Benchmark Dose Modeling Results for Increased Mean ALT Concentrations in Darrow et al. (2016) E-43

Table E-30. Linear Regression Results for Ln(ALT) by Quintiles of Serum PFOA Concentration in Darrow et al. (2016) E-44

Table E-31. BMDLs for Effect of PFOA on Serum ALT Using a BMR of 5% E-44

Table E-32. NHANES Mean and Standard Deviation of TC (mg/dL) and Mean PFOA (ng/mL) E-46

Table E-33. BMDs and BMDLs for Effect of PFOA on Increased Cholesterol in Dong et al. (2019) E-47

Table E-34. NHANES Mean and Standard Deviation of Ln(TC) (Ln(mg/dL)) and Mean Ln(PFOA) (Ln(ng/mL)) E-48

Table E-35. BMD and BMDL for Effect of PFOA on Increased Cholesterol in Steenland et al. (2009) E-48

Table E-36. Regression Results for Serum Total Cholesterol by Deciles of Serum PFOA from Steenland et al. (2009) E-49

Table E-37. Summary of Benchmark Dose Modeling Results for Increased Mean Serum Total Cholesterol in Steenland et al. (2009) E-50

Table E-38. Odds Ratios for Elevated Serum Total Cholesterol by Quartiles of Serum PFOA From Steenland et al. (2009).....E-51

Table E-39. Summary of Benchmark Dose Modeling Results for Elevated Total Cholesterol in Steenland et al. (2009).....E-52

Table E-40. Adjusted Mean Differences in Serum Total Cholesterol by Quartiles of Serum PFOA (ng/mL) From Lin et al. (2019).....E-53

Table E-41. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol From Lin et al. (2019).....E-54

Table E-42. BMDLs for Effect of PFOA on Serum Total Cholesterol Using a BMR of 5% ...E-55

Table E-43. ORs for the Association Between PFOA Serum Concentrations and RCC in Shearer et al. (2021) and Data Used for CSF Calculations.....E-58

Table E-44. Internal CSF Calculations for Shearer et al. (2021).....E-58

Table E-45. ORs for the Association Between PFOA Serum Concentrations and RCC in Vieira et al. (2013) and Data Used for CSF Calculations.....E-60

Table E-46. Internal CSF Calculations for Vieira et al. (2013).....E-60

Table E-47. CSF Calculations Pooling Dose Response for Shearer et al. (2021) and Vieira et al. (2013) Studies.....E-61

Table E-48. Dose-Response Modeling Data for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA (Butenhoff et al., 2012).....E-62

Table E-49. Summary of Benchmark Dose Modeling Results for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA (Butenhoff et al., 2012).....E-64

Table E-50. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-Specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Dewitt et al., 2008).....E-66

Table E-51. Summary of Benchmark Dose Modeling Results for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Nonconstant Variance) (Dewitt et al., 2008).....E-66

Table E-52. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA (Dewitt et al., 2008).....E-67

Table E-53. Dose-Response Modeling Data for Time to Eye Opening in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006).....E-68

Table E-54. Summary of Benchmark Dose Modeling Results for Time to Eye Opening Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Nonconstant Variance) (Lau et al., 2006).....E-70

Table E-55. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006) E-71

Table E-56. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006) E-72

Table E-57. Dose-Response Modeling Data for Pup Body Weight in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006) E-73

Table E-58. Summary of Benchmark Dose Modeling Results for Pup Weight Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Nonconstant Variance) (Lau et al., 2006) E-74

Table E-59. Dose-Response Modeling Data for Fetal Body Weight in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Li et al., 2018a) E-75

Table E-60. Dose-Response Modeling Data for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008) E-76

Table E-61. Summary of Benchmark Dose Modeling Results for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008) E-76

Table E-62. Dose-Response Modeling Data for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008) E-78

Table E-63. Summary of Benchmark Dose Modeling Results for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008) E-78

Table E-64. Dose-Response Modeling Data for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008) E-79

Table E-65. Summary of Benchmark Dose Modeling Results for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Constant Variance) (Loveless et al., 2008) E-80

Table E-66. Dose-Response Modeling Data for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-81

Table E-67. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-82

Table E-68. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-84

Table E-69. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-85

Table E-70. Dose-Response Modeling Data for Necrosis in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-87

Table E-71. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-87

Table E-72. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-89

Table E-73. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-90

Table E-74. Dose-Response Modeling Data for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-91

Table E-75. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-92

Table E-76. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-93

Table E-77. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-95

Table E-78. Dose-Response Modeling Data for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-96

Table E-79. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-97

Table E-80. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-98

Table E-81. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-99

Table E-82. Dose-Response Modeling Data for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-101

Table E-83. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-101

Table E-84. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-103

Table E-85. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-103

Table E-86. Dose-Response Modeling Data for Pancreatic Acinar Cell Adenoma or Adenocarcinoma (Combined) in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020) E-104

Table E-87. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020) E-105

Table E-88. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020) E-106

Table E-89. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020) E-106

Table E-90. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Song et al., 2018a) E-107

Table E-91. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (constant variance) (Song et al., 2018a) E-108

Table E-92. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,gest}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a) E-110

Table E-93. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,lact}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a) E-112

Table E-94. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{max,pup,gest,}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a) E-114

Table E-95. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{max,pup,lact,}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a) E-116

Table F-1. Root Mean Squared Error Comparison Between the Baseline Model (as Applied in the Main Risk Assessment) and Alternative Models with Features Inspired by the MDH Model. F-13

Table G-1. Summary of EPA National Freshwater Fish Tissue Monitoring Results for PFOA G-6

Acronyms and Abbreviations

17-OHP	17-hydroxyprogesterone
ABC	ATP-binding cassette transporter
aBMD	areal bone mineral density
ACD	anterior chamber depth
ACE	America's Children and the Environment
ACTH	adrenocorticotropic hormone
ADHD	attention deficit hyperactivity disorder
ADME	absorption, distribution, metabolism, and excretion
AGD	anogenital distance
AIC	Akaike information criterion
AMH	anti-Müllerian hormone
ANOVA	analysis of variance
APFO	ammonium perfluorooctanoate
apoB	apolipoprotein B
aPPT	activated partial thromboplastin time
ASD	autism spectrum disorder
ASQ	Ages and Stages Questionnaire
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
AUMC	area under the first moment curve
β	regression coefficients
BBB	blood-brain barrier
BCRP	breast cancer resistance protein
BD	bolus dose
BDI	Beck Depression Inventory
BDI-II	Beck Depression Inventory-II
BMC	bone mineral content
BMD	benchmark dose
BMDL	lower limit of benchmark dose
$BMDL_{0.5SD}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean
$BMDL_{1SD}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean
$BMDL_4$	lower bound on the dose level corresponding to the 95% lower confidence limit for a 4% change in the response
$BMDL_5$	lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level
$BMDL_{10}$	lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BSID-II	Bayley Scales of Infant Development

BUN	blood urea nitrogen
BW	body weight
$C_{avg,pup,gest}$	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
$C_{7,avg}$	average concentration over final week of study
CalEPA	California Environmental Protection Agency
CAR	constitutive androstane receptor
C-F	carbon-fluorine
CH	congenital hypothyroidism
CHARGE	Childhood Autism Risk from Genetics and Environment
CHECK	Children's Health and Environmental Chemicals in Korea
CHEF	Children's Health and the Environment in the Faroes
CHO	Chinese hamster ovary
CI	confidence interval
CKD	chronic kidney disease
CL	post-dosing clearance
CL_R	renal clearance
C_{max}	maximum blood concentration
$C_{max,dam}$	maximum maternal concentration during gestation
$C_{max,pup,gest}$	maximum fetal concentration during gestation
$C_{max,pup,lact}$	maximum pup concentration during lactation
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CSF	cancer slope factor
CVD	cardiovascular disease
DFI	deoxyribonucleic acid fragmentation index
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DPP	Diabetes Prevention Program
dU	diurnal urinary
E	embryonic day
EFSA	European Food Safety Authority
eGFR	estimated glomerular filtration rate
eNT	equilibrative nucleoside transporter
EPA	U.S. Environmental Protection Agency
ES3	estrone-3-sulfate
F_1	first generation
F_2	second generation
FDA	U.S. Food and Drug Administration
FEV1	forced expiratory volume in one second
FR	folate receptor

FSH	follicle stimulating hormone
FT3	free triiodothyronine
FTI	free thyroxine index
FTOH	fluorotelomer alcohols
FVC	forced vital capacity
FXR	farnesoid X receptor
GD	gestation day
GM	geometric mean
GSD	geometric standard deviation
Hb	hemoglobin
HDL	high-density-lipoprotein
HED	human equivalent dose
HEK 293	human embryonic kidney cells
HERO	Health and Environmental Research Online
HESD	health effects support document
HFD	high fat diets
HHRA	human health risk assessment
HOMA-B	Homeostatic Model Assessment of Beta-Cell Function
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HOME	Health Outcome Measures of the Environment
HPA	hypothalamic-pituitary-adrenal
HPLC/MS	high-performance liquid chromatography mass spectrometry
HUMIS	Norwegian Human Milk Study
IBD	inflammatory bowel disease
IC_{50}	median inhibiting concentration
ID	intellectual disability
INUENDO	Biopersistent Organochlorines in Diet and Human Fertility
i.p.	intraperitoneal
IQ	intelligence quotient
IQR	interquartile range
IRIS	Integrated Risk Information System
IUFD	intrauterine fetal death
IV	intravenous
IVD	*in vitro* digestion method
K_d	disassociation constant
$K_{mem/w}$	membrane/water partition coefficients
K_{oc}	organic carbon-water partitioning coefficient
LD	lactation day
LDL	low-density lipoprotein
L-FABP	liver fatty acid binding protein
LFD	low fat diets
LH	luteinizing hormone
LIFE	Longitudinal Investigation of Fertility and the Environment Study
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
MCDI	MacArthur Communicative Development Inventories for Infants
MCLG	Maximum Contaminant Level Goal
MDH	Minnesota Department of Health

MDI	Mental Development Index
MDR1	p-glycoprotein
MeSH	medical subject headings
Mg/kg-day	milligrams per kilogram per day
MLR	mixed linear regression
MOA	mode of action
MoBA	Norwegian Mother, Father, and Child Cohort Study
M/P	milk/plasma
MRL	minimum reporting level
mRNA	messenger ribonucleic acid
MRP	multi-drug resistance-associated protein
MPAH	2-(N-methyl-PFOSA) acetate
MS	multiple sclerosis
NCI	National Cancer Institute
NEPSY-II	neuropsychological tests
NHANES	National Health and Examination Survey
NICHD	U.S. National Institute of Child Health and Human Development
NJDEP	New Jersey Department of Environmental Protection
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOAEC	no observed adverse effect concentration
NPDWR	national primary drinking water regulation
NTCP	sodium-taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OATs	organic anion transporters
OATPs	organic anion transporting polypeptides
OCC	Odense Child Cohort
OCISS	Ohio Cancer Incidence Surveillance System
OECD	Organisation for Economic Co-operation and Development
OR	Odds Ratio
ORD	Office of Research and Development
P_0	parental generation
PBET	physiologically based extraction test
PBPK	physiologically-based pharmacokinetic
PCBs	polychlorinated biphenyls
PECO	Populations, Exposures, Comparator, and Outcomes
PEF	peak expiratory flow rate
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluorocarboxylates
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFSA	perfluoroalkanesulfonic acid
P_{ion}	passive anionic permeability
PFUnDA	perfluoroundecanoic acid
PK	pharmacokinetic

PLCO	Prostate, Lung, Colorectal, and Ovarian Screening Trial
PND	postnatal day
PNW	postnatal week
POD	point-of-departure
POD_{HED}	point-of-departure human equivalent dose
POPUP	Persistent Organic Pollutants in Uppsala Primiparas
PPARα	proliferator-activated receptor alpha
PXR	pregnane X receptor
Q_1	quantile 1
Q_2	quantile 2
Q_3	quantile 3
Q_4	quantile 4
QA	quality assurance
RCM	ratio of cord blood to maternal blood concentrations
RFC	reduce folate carrier
RfD	reference dose
RIS	Research Information System
ROBINS-I	Risk of Bias in Nonrandomized Studies of Interventions
R_{PM}	ratio of placental:maternal concentrations
RSC	relative source contribution
SAB	Science Advisory Board
SE	standard errors
SERT	serotonin transporter
SES	socioeconomic status
SD	standard deviation
SDQ	Strengths and Difficulties Questionnaire
SDWA	Safe Drinking Water Act
SRBC	serum sheep red blood cells
SMBCS	Shanghai Minhang Birth Cohort Study
SWAN	Study of Women's Health Across the Nation
T3	triiodothyronine
T4	thyroxine
TA	thyroid antibody
TC	total cholesterol
TDS	Total Diet Study
TgAB	thyroblobulin antibodies
TiAb	title-abstract
T_{max}	maximum plasma concentration
TPoAb	thyroid peroxidase antibody
TRR	total reactive residues
TSH	thyroid stimulating hormone
TTE	transplacental transfer efficiencies
TTR	transthyretin
UBM	unified BARGE method
UCMR 3	third Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
V_1	volume of central distribution
V_2	volume of peripheral distibution
V_d	volume of distribution
V_{dss}	volume of distribution at steady state
VI	visual impairment
VLDL	very low-density lipoproteins
VMWM	Virtual Morris Water Maze
WBHGB	whole blood hemoglobin
WCST	Wisconsin Card Sorting Test

WHO	World Health Organization
WIAT-II	Wechsler Individual Achievement Test-II
WVCR	West Virginia Cancer Registry

Appendix A. Systematic Review Protocol for Updated PFOA Toxicity Assessment

Per- and polyfluoroalkyl substances (PFAS) refers to a large group of fluorinated anthropogenic chemicals that includes perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and thousands of other chemicals. The universe of environmentally relevant PFAS, including parent chemicals, metabolites, and degradants, is greater than 12,000 compounds (https://comptox.epa.gov/dashboard/chemical-lists/PFASMASTER). The 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). The number of PFAS used globally in commercial products at the time of the drafting of this document is approximately 250 substances (Buck et al., 2021).

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). 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). The chemical structures of PFAS enable 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 some PFAS extremely persistent in the human body and the environment (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.

To understand and address the complexities associated with PFAS, the U.S. Environmental Protection Agency (EPA) is developing human health toxicity assessments for individual PFAS, in addition to other components of the broader PFAS action plan underway at EPA (https://www.epa.gov/pfas/epas-pfas-action-plan). The updated toxicity assessment that was developed for PFOA according to the scope and methods outlined in this protocol builds upon several other assessments, including the *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2016c) (hereafter referred to as the 2016 PFOA HESD) and *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), which was released to the public for review by the Science Advisory Board (SAB) in November 2021.

This protocol describes the methods used for conducting the systematic reviews and dose-response analyses for the assessment of PFOA (*Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts*, (U.S. EPA, 2024b)) and has been updated in response to comments from the SAB. It should be noted that PFOA and PFOS underwent some steps of systematic review (e.g., literature searches) concurrently.

A.1 Overview of Background Information and Systematic Review Protocol

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, 2022b, 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, 2022a). 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).

The Safe Drinking Water Act (SDWA) regulatory process enables EPA to receive comments and feedback on this systematic review protocol, including through SAB early input and via the public comment period associated with rule proposal. This protocol has been updated based on SAB recommendations to improve the clarity and transparency of the methods descriptions. It now includes information about additional data sources and how they were evaluated and expands the application of systematic review through dose-response analysis.

A.1.1 Summary of Chemical Identity and Occurrence Information

This section summarizes more detailed sections on these topics from the *Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts* (hereafter referred to as the Toxicity Assessment, (U.S. EPA, 2024b)) and is provided for context. Please refer to the Toxicity Assessment (U.S. EPA, 2024b) for more detailed information about chemical identity, physical-chemical properties, and occurrence.

A.1.1.1 Chemical Identity

The systematic review described by this protocol 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 a perfluorinated aliphatic carboxylic acid. It is a strong acid that is generally present in solution as the perfluorooctanoate anion. PFOA is water soluble and mobile in water, with an estimated log organic carbon-water partitioning coefficient (log K_{oc}) of 2.06. 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 dissipation is by advection, dispersion, and sorption to particulate matter. PFOA has low volatility in ionized form but can absorb particles and be deposited on the ground and into water bodies. It can be transported long distances in air or water, as evidenced by detections of PFOA in arctic media and biota including polar bears, ocean-going birds, and fish found in remote areas (Lindstrom et al., 2011; Smithwick et al., 2006).

A.1.1.2 Occurrence Summary

Key PFOA occurrence information is summarized below. More detail is provided in Chapter 1 of the Toxicity Assessment (U.S. EPA, 2024b).

A.1.1.2.1 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 analyzed serum samples representative of the U.S. general population; however, blood levels of PFOA dropped 60% to 80% between 1999 and 2014, presumably due to reductions in its commercial usage in the United States.

A.1.1.2.2 Occurrence in Water

PFOA is one of the dominant PFAS detected in ambient water, along with PFOS (Remucal, 2019; Dinglasan-Panlilio et al., 2014; Zareitalabad et al., 2013; Benskin et al., 2012; Ahrens, 2011; Nakayama et al., 2007).

Data from the third Unregulated Contaminant Monitoring Rule (UCMR 3), collected from 2013–2015, are currently the best available nationally representative finished water occurrence information for PFOA (U.S. EPA, 2023a, 2021a, 2017). UCMR 3 analyzed 36,972 samples from 4,920 PWSs for PFOA. The minimum reporting level (MRL)[1] for PFOA was 0.02 µg/L. A total of 379 samples from 117 PWSs had detections of PFOA (i.e., greater than or equal to the MRL). PFOA concentrations for these detections ranged from 0.02 µg/L (the MRL) to 0.349 µg/L (median concentration of 0.03 µg/L; 90th percentile concentration of 0.07 µg/L).

A.1.2 Problem Formulation

As described in the Toxicity Assessment (U.S. EPA, 2024b), EPA conducted this updated assessment of PFOA (including all isomers 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)) to support derivation of chronic cancer and noncancer toxicity values for PFOA. This problem formulation section will describe the key considerations and scope of the assessment, which were informed in part by EPA's past human health assessments of PFOA (2016 PFOA HESD and 2021 *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water*) as well as ongoing and final EPA assessments of other PFAS (e.g., perfluorobutanoic acid (PFBA) and draft

[1] The reporting level is the threshold at or above which a contaminant's presence or concentration is officially quantitated. In the case of many of EPA's nation-wide drinking water studies, the selected reporting level is known officially as the MRL. The MRL for each contaminant in each study is set at a level that EPA believes can be achieved with specified confidence by a broad spectrum of capable laboratories across the nation (U.S. EPA, 2021d).

perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) IRIS assessments (U.S. EPA, 2020b)).

The 2016 PFOA HESD identified several adverse health outcomes associated with PFOA exposure based on results from animal toxicological and epidemiological studies, including: developmental effects (e.g., decreased birth weight, accelerated puberty, skeletal variations); cancer (e.g., testicular, kidney); liver effects (e.g., tissue damage); immune effects (e.g., antibody production and immunity); thyroid effects (e.g., hypothyroidism); and other effects (e.g., cholesterol changes). It concluded that there was "suggestive evidence of carcinogenic potential" for PFOA. EPA's 2021 *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) evaluated associations between PFOA and all cancer and noncancer health outcomes. After reviewing that draft scoping assessment, the SAB recommended that the scope be narrowed to focus on the five priority health outcomes that have the strongest weight of evidence (immune, developmental, hepatic, cardiovascular, and cancer), most of which were also identified in the conclusions from the 2016 PFOA HESD. Therefore, the current assessment provides a comprehensive systematic review of all health effects literature published through February 2022 for these five health outcomes. Mechanistic data for these health outcomes were also synthesized. For other health outcomes beyond the five priority outcomes, the current assessment summarizes the health effects literature published prior to 2016 and includes a systematic review of the health effects literature published from 2016−2020.

The *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments* outlines key science issues relevant to PFAS in general (U.S. EPA, 2020b), many of which are relevant to PFOA. They include: toxicokinetic differences across species and sexes; human relevance of effects in animals that involve peroxisome proliferator-activated receptor-alpha (PPARα); potential confounding by other PFAS exposures in epidemiology studies; and toxicological relevance of changes in certain hepatic endpoints in rodents. Differences in PFOA toxicokinetics across species and sexes were accounted for in the PFOA-specific animal and human pharmacokinetic models (see Toxicity Assessment, (U.S. EPA, 2024b)). The human relevance of effects in animals that involve PPARα was investigated in the mechanistic syntheses of the five priority health outcomes (see Toxicity Assessment, (U.S. EPA, 2024b)). Potential confounding by other PFAS (and other co-occurring contaminants) in epidemiology studies was considered as part of the confounding domain during study quality evaluations and was discussed in Section 5 of the Toxicity Assessment (U.S. EPA, 2024b). Specifically, if a study did not account for potential confounding with other co-occurring PFAS in its statistical analyses, then the maximum quality rating this domain could receive was *adequate*. Concerns about potential confounding by other PFAS were limited when there was evidence that exposure was predominantly PFOA-based (such as in certain occupational or high-exposure studies) and the potential for co-exposure was minimal, or the correlations between co-exposures were small. The toxicological relevance of changes in certain hepatic endpoints in rodents was accounted for by incorporating the Hall (Hall et al., 2012) criteria into the animal hepatic synthesis and hazard conclusions.

An additional key science issue that EPA has encountered for PFAS toxicity assessments is a general lack of data on human and ecological toxicity. For PFOA, this is less of an issue as there

has been substantial research and publication of both epidemiological and animal toxicological studies.

A.1.3 Overall Objective and Specific Aims

A.1.3.1 Objective

The primary objective of this toxicity assessment for PFOA is to support derivation of chronic cancer and noncancer toxicity values for PFOA, as well as update the cancer descriptor for PFOA, if warranted. EPA also considered potential pathways of exposure and derived a relative source contribution (RSC) specific to the final RfD for PFOA. The toxicity values, cancer classification, and RSC derived in this assessment build upon the work completed 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) and the 2016 PFOA HESD (U.S. EPA, 2016c).

A.1.3.2 Specific Aims

The specific aims of the PFOA toxicity assessment document are 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 of the Toxicity Assessment (U.S. EPA, 2024b); Appendix A and Appendix B).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b); Appendix A).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b)).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b)).
- Describe and document the data from all epidemiological studies and animal toxicological studies that were considered for POD derivation (Section 3 of the Toxicity Assessment (U.S. EPA, 2024b)).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b)) – 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).

- 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 priority health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer) (Section 3 of the Toxicity Assessment (U.S. EPA, 2024b)).
- 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 outcomes (Section 3 of the Toxicity Assessment (U.S. EPA, 2024b)).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b)).
- Determine the cancer classification for PFOS using a weight-of-evidence approach (Section 3 of the Toxicity Assessment (U.S. EPA, 2024b)).
- 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 of the Toxicity Assessment (U.S. EPA, 2024b)).
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation (Section 4 of the Toxicity Assessment (U.S. EPA, 2024b)).
- Derive candidate RfDs (Section 4.1 of the Toxicity Assessment (U.S. EPA, 2024b)) and CSFs (Section 4.2 of the Toxicity Assessment (U.S. EPA, 2024b)), select the final RfD (Section 4.1.6 of the Toxicity Assessment (U.S. EPA, 2024b)) and CSF (Section 4.2.3 of the Toxicity Assessment (U.S. EPA, 2024b)) for PFOA, and describe the rationale.
- Characterize hazards (e.g., uncertainties, data gaps) (Sections 3, 4, and 5 of the Toxicity Assessment (U.S. EPA, 2024b)).

A.1.4 Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

This section describes the PECO criteria that were developed and used for this assessment.[2] As described in the IRIS Handbook (U.S. EPA, 2022c), the PECO criteria provide the framework for literature search strategies and are the inclusion/exclusion criteria by which literature search results will be screened for relevancy to identify epidemiological and animal toxicological evidence that addresses the aims of the assessment. For the PFOA assessment, the PECO criteria were used to screen results of the literature searches to identify and prioritize the dose-response literature and studies containing pharmacokinetic (PK) or PBPK models. For studies captured in the 2019 and 2020 literature searches, the PECO criteria were used to screen and categorize

[2] *Notes*: Although this appendix and its accompanying Toxicity Assessment (U.S. EPA, 2024b) pertain to PFOA, the PECO criteria also cover PFOS because the literature searching and screening were performed concurrently for PFOA and PFOS.

("tag") studies of PFOA absorption, distribution, metabolism, and excretion (ADME) and studies with mechanistic data for further evaluation using ADME- and mechanistic-specific PECO criteria. ADME, mechanistic, and other supplemental studies captured in the 2022 and 2023 literature searches were not tagged or considered further in this assessment.

Table A-1 describes the PECO criteria used to screen the results of the literature search (the literature search is described in Section A.1.5 of this appendix). ADME- and mechanistic-specific PECO criteria are outlined in Table A-2 and Table A-3, respectively.

Table A-1. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects from Exposure to PFOA and PFOS

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, *in utero*, lactation, peripubertal, and adult stages). In vitro/cell studies or in silico/modeling toxicity studies should be tagged as supplemental.
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including but not limited to**: PFOA (*Chemical Abstracts Service (CAS) number 335-67-1*). Other names: perfluorooctanoate; perfluorooctanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic-acid, pentadecafluoro- Relevant Salts of PFOA: ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluorooctanoate PFOS (*CAS number 1763-23-1*). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, Heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid Relevant Salts of PFOS: lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate **Human:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, or unknown/multiple routes will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." **Animal:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, injection or unknown/multiple routes, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." Studies involving exposures to mixtures will be included only if they include exposure to PFOA or PFOS alone. Studies with less than 28 d of dosing, with the exception of reproductive, developmental, immune and neurological health outcome studies, should be tagged as supplemental.
Comparator	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcome	All health outcomes (both cancer and noncancer).
PBPK Models	Studies describing physiologically based pharmacokinetic (PBPK) models will be included.

Epidemiological, animal toxicological, and *in vitro* studies tagged as containing potentially relevant ADME data were further screened using ADME-focused PECO criteria (Table A-2). Key information from each study meeting the ADME-focused PECO criteria was extracted using ICF's litstream™ software.

Table A-2. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Absorption, Distribution, Metabolism, and/or Excretion (ADME) Studies

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations): whole organism, tissues, individual cells, or biomolecules. **Animal:** Select non-human mammalian animal species: only non-human primates, rats, and mice (whole organism, tissues, individual cells, or biomolecules) of any lifestage (preconception, in utero, lactation, peripubertal, and adult stages).
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including** in vitro, in vivo (by various routes of exposure), and ex vivo. In silico studies will also be included if the model system can be linked to a PECO-relevant species. PFOA (CAS number 335-67-1). Other names: perfluorooctanoate, perfluorooctanoic acid, perfluoroctanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroheptanecarboxylic acid, octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluoroctanoate PFOS (CAS number 1763-23-1). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctane sulfonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid, heptadecafluorooctanesulfonic acid, lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate, ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate
Comparator	Any comparison that informs PFOA or PFOS (1) absorption by the oral, inhalation, or dermal route of exposure, (2) distribution across biological compartments, (3) metabolism, and/or (4) excretion.
Outcome	Any examination of PFOA and/or PFOS (1) absorption of dose through gastrointestinal (GI) tract, lungs, or skin, (2) distribution across biological compartments, (3) metabolism, and/or (4) excretion. Studies describing PK models for PFOA and/or PFOS will be included. Information and terms that are typically found in relevant ADME/PK modeling studies include the following: **Absorption:** Bioavailability; absorption rate(s); uptake rates; tissue location of absorption (e.g., stomach vs. intestine, nasal vs. lung); blood:air partition coefficient (PC); irritant/respiratory depression; overall mass transfer coefficient; gas-phase diffusivity; gas-phase mass transfer coefficient; liquid- (or tissue-) phase mass transfer coefficient; deposition fraction; retained fractions; computational fluid (airway) dynamics. **Distribution:** Volume of distribution (V_d) and parameters that determine V_d, including blood: tissue PCs (especially for the target or a surrogate tissue) or lipophilicity; tissue burdens; storage tissues or tissue components (e.g., serum binding proteins) and the binding coefficients; transporters (active and passive). Note: PFOA/PFOS are not metabolized so we are not expecting studies that focus on metabolites. The terms below are general terms associated with metabolism. **Metabolism:** Metabolic/biotransformation pathway(s); enzymes involved; metabolic rate; maximum rate of transport (V_{max}), Michaelis constant (K_m); ; metabolic induction; metabolic inhibition, Ki; metabolic saturation/nonlinearity; key organs involved in metabolism; key

PECO Element	Inclusion Criteria
	metabolites (if any)/pathways; metabolites measured; species-, inter-individual-, and/or age-related differences in enzyme activity or expression ("ontogeny"); site-specific activation (may be toxicologically significant, but little systemic impact); cofactor (e.g., glutathione) depletion. **Excretion:** Route(s)/pathway(s) of excretion for parent and metabolites; urine, fecal, exhalation, hair, sweat, lactation; elimination rate(s); mechanism(s) of excretion (e.g., passive diffusion, active transport).

Notes: ADME = absorption, distribution, metabolism, and/or excretion; CAS = Chemical Abstracts Service; PK = pharmacokinetic.

Epidemiological and animal toxicological studies that were tagged as containing potentially relevant mechanistic data were further screened using mechanistic-focused PECO criteria (Table A-3). Studies meeting the mechanistic-focused PECO criteria underwent a light extraction of key study information using ICF's litstream™ software.

Table A-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for Mechanistic Studies

PECO Element	Evidence
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Select mammals (i.e., non-human primates and rodents (i.e., rats, mice, rabbits, guinea pigs, other rodent models) and fish (i.e., zebrafish) of any lifestage (preconception, in utero, lactation, peripubertal, and adult stages). Ex vivo, in vitro, in silico: Cultures of human or animal cells from relevant animal models (primary, immortalized, transformed), organ slices, organotypic culture, in vitro molecular or biochemical assay systems. In silico modeling data if it informs PFOA/PFOS MOA.
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including** in vitro, in vivo (by various routes of exposure), and ex vivo. In silico studies will also be included if the model system can be linked to a PECO-relevant species. PFOA (CAS number 335-67-1). Other names: perfluorooctanoate, perfluorooctanoic acid, perfluoroctanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroheptanecarboxylic acid, octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluoroctanoate PFOS (CAS number 1763-23-1). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctane sulfonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid, heptadecafluorooctanesulfonic acid, lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate, ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate
Comparator	**Human:** Comparison to group with no exposure or lower exposure. **Animal:** ex vivo, in vitro, in silico: Comparison to an appropriate vehicle or no treatment control.
Outcome	Any mechanistic data related to the MOA of PFOA/PFOS toxicity. This may include molecular initiating events with PFOA/PFOS or downstream key events that inform the MOA or adverse outcome pathway linking PFOA/PFOS exposure to disease.

Notes: CAS = Chemical Abstracts Service; MOA = mode of action.

A.1.5 Literature Search

EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this updated toxicity assessment based on three data streams: 1) literature published from 2013 through 2019 and then updated in the course of this review (i.e., through February 6, 2023) identified via literature searches 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, and studies submitted through public comment), and 3) literature identified in EPA's 2016 PFOA and PFOS HESDs, which captured literature through 2013 (U.S. EPA, 2016b, c).

A.1.5.1 Literature Search Strategies

The following sections describe literature search strategies used for databases and for additional sources. They also describe methods used to incorporate studies from the 2016 PFOA HESD and other sources into the literature database. The literature search strategy included searches within core literature databases (e.g., PubMed®, Web of Science™) as well as relevant domestic and international non-periodical "gray" literature, such as technical reports, monographs, and conference and symposium proceedings prepared by select committees or bodies (e.g., those convened by the National Academy of Sciences or the World Health Organization (WHO)).

A.1.5.2 Database Searches

The database literature searches for this updated assessment focused only on the chemical name (PFOA and related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, in vitro, in silico) or health outcomes. These searches comprised all literature related to health effects resulting from acute, subchronic, and chronic exposure durations, and from inhalation, oral, dermal, and injection exposure studies. Epidemiological, animal toxicological, and in vitro studies that provide MOA information were included, and data specifically useful for addressing risks to children and other susceptible populations (e.g., the elderly, pregnant or lactating women, genetically susceptible populations) were identified. The searches likewise included ADME studies and models useful for dose-response assessment, such as dosimetry and PBPK models. The initial database search covered from January 2013 through April 11, 2019 (the 2019 literature search). That was subsequently updated by a search covering April 2019 through September 3, 2020 (2020 literature search), another covering September 2020 through February 3, 2022 (2022 literature search), and a final supplemental search covering February 2022 through February 6, 2023 (described in Section A.3 below). The date field tag used for these searches may reflect either the date the article was published in print or e-published, which may result in small amounts of literature being captured in a literature search despite being published prior to the start date. At the recommendation of SAB peer reviewers, the 2022 literature search and supplemental 2023 literature search focused on the five priority health outcomes that have been concluded to have the strongest evidence (developmental, hepatic, immune, and cardiovascular effects, and cancer). EPA considered mechanistic and toxicokinetic data identified through the September 2020 literature search, as well as any more recent studies recommended by the SAB.

The databases listed below were searched for literature containing the search strings identified in Table A-4 and Table A-5:

- Web of Science™ (Thomson Reuters),
- PubMed® (National Library of Medicine),
- ToxLine (incorporated into PubMed post 2019), and
- TSCATS (Toxic Substances Control Act Test Submissions)

Table A-4. Search String for April 2019 Database Searches

Database	Search String	Date Run
WoS	((TS = "perfluorooctanoic acid" OR TS="perfluorooctane sulfonic acid") AND PY=(2013-2019) OR (TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride" OR TS="Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-" OR TS="Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-" OR TS="Pentadecafluoro-1-octanoic acid" OR TS="Pentadecafluoro-n-octanoic acid" OR TS="Pentadecafluorooctanoic acid" OR TS="Perfluorocaprylic acid" OR TS="Perfluorooctanoic acid" OR TS="Perfluoroheptanecarboxylic acid" OR TS="perfluorooctanyl sulfonate" OR TS="Perfluorooctanoic acid" OR TS="Octanoic acid, pentadecafluoro-" OR TS="Perfluorooctanoate" OR TS="perfluorooctane sulfonate" OR TS="A 5717" OR TS="EF 201" OR TS="Eftop EF 201" OR TS="Perfluoro-1-heptanecarboxylic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid" OR TS="1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-" OR TS="1-Perfluorooctanesulfonic acid" OR TS="EF 101" OR TS="Eftop EF 101" OR TS="Heptadecafluoro-1-octanesulfonic acid" OR TS="Heptadecafluorooctane-1-sulphonic acid" OR TS="Perfluorooctane sulfonate" OR TS="perfluorooctane sulfonate" OR TS="Perfluorooctane sulfonic acid" OR TS="Perfluorooctanesulfonic acid" OR TS="Perfluorooctylsulfonic acid" OR TS="perfluorooctane sulphonate" OR TS="perfluorooctane sulfonate" OR TS="1-Octanesulfonic acid, heptadecafluoro-"OR TS="Heptadecafluorooctanesulfonic acid" OR TS="Perfluoro-n-octanesulfonic acid" OR TS="Perfluorooctane Sulphonic Acid" OR TS="Perfluorooctanesulfonate" OR TS="Perfluorooctylsulfonate" OR ((TS="PFOA" OR TS="PFOS") AND (TS="fluorocarbon*" OR TS="fluorotelomer*" OR TS="polyfluoro*" OR TS="perfluoro-*" OR TS="perfluoroa*" OR TS="perfluorob*" OR TS="perfluoroc*" OR TS="perfluorod*" OR TS="perfluoroe*" OR TS="perfluoroh*" OR TS="perfluoron*" OR TS="perfluoroo*" OR TS="perfluorop*" OR TS="perfluoros*" OR TS= "perfluorou*" OR TS="perfluorinated" OR TS="fluorinated" OR TS="PFAS"))) AND PY=(2013-2019))	4/10/2019
PubMed	(335-67-1[rn] OR 1763-23-1[rn] OR 45298-90-6[rn] OR "perfluorooctanoic acid"[nm] OR "perfluorooctane sulfonic acid"[nm]) AND (2013/01/01:3000[pdat] OR 2013/01/01:3000[mhda] OR 2013/01/01:3000[edat] OR 2013/01/01:3000[crdt]) OR (("2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride"[tw] OR "Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-"[tw] OR "Pentadecafluoro-1-	4/10/2019

Database	Search String	Date Run
	octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "perfluorooctanyl sulfonate"[tw] OR "Perfluorooctanoic acid"[tw] OR "Octanoic acid, pentadecafluoro-"[tw] OR "Perfluorooctanoate"[tw] OR "perfluorooctane sulfonate"[tw] OR "A 5717"[tw] OR "EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR "perfluorooctane sulfonate"[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR (("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw])) AND (2013/01/01:3000[pdat] OR 2013/01/01:3000[mhda] OR 2013/01/01:3000[edat] OR 2013/01/01:3000[crdt]))	
Toxline	@AND+@OR+("perfluorooctane sulfonate"+"pfos"+"perfluorooctanesulfonic acid"+"perfluorooctane sulfonic acid"+"perfluorooctane sulphonate"+"perfluorooctane sulfonate"+"perfluorooctanyl sulfonate"+"Heptadecafluorooctane-1-sulphonic"+"Heptadecafluoro-1-octanesulfonic acid"+"1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid"+"perfluorooctanoate"+"perfluorooctanoic acid"+"perfluoroctanoic acid"+"pfoa"+"2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"+"Pentadecafluoro-1-octanoic acid"+"Pentadecafluoro-n-octanoic acid"+"Octanoic acid, pentadecafluoro-"+"Perfluorocaprylic acid"+"Pentadecafluorooctanoic acid"+"perfluoroheptanecarboxylic acid"+@TERM+@rn+335-67-1+@TERM+@rn+1763-23-1+@TERM+@rn+45298-90-6)+@NOT+@org+pubmed+@AND+@RANGE+yr+2013+2019	4/11/2019
TSCATS	@AND+@OR+@rn+"335-67-1"+@AND+@org+TSCATS+@NOT+@org+pubmed @AND+@OR+@rn+"1763-23-1"+@AND+@org+TSCATS+@NOT+@org+pubmed	4/11/2019

Table A-5. Search String for September 2020, February 2022, and February 2023 Database Searches

Database	Search String	Date Run
PubMed Batch IDs: 39678, 46137	(335-67-1[rn] OR 1763-23-1[rn] OR 45298-90-6[rn] OR "perfluorooctanoic acid"[nm] OR "perfluorooctane sulfonic acid"[nm] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid"[tw] OR "3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride"[tw] OR	9/3/2020, 2/2/2022, 2/6/2023

Database	Search String	Date Run
	"3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride"[tw] OR "Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "perfluorooctanyl sulfonate"[tw] OR "Perfluorooctanoic acid"[tw] OR "Octanoic acid, pentadecafluoro-"[tw] OR "Perfluorooctanoate"[tw] OR "perfluorooctane sulfonate"[tw] OR "A 5717"[tw] OR "EF 201"[tw] OR "Eftop EF 201"[tw] OR "Perfluoro-1-heptanecarboxylic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid"[tw] OR "1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-"[tw] OR "1-Perfluorooctanesulfonic acid"[tw] OR "EF 101"[tw] OR "Eftop EF 101"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid"[tw] OR "Perfluorooctanesulfonic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonate" [tw] OR "perfluorooctane sulfonate"[tw] OR "1-Octanesulfonic acid, heptadecafluoro-"[tw] OR "Heptadecafluorooctanesulfonic acid"[tw] OR "Perfluoro-n-octanesulfonic acid"[tw] OR "Perfluorooctane Sulphonic Acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctylsulfonate"[tw] OR (("PFOA"[tw] OR "PFOS"[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw])) AND (2020/09/03:3000[dp])	
Web of Science Batch IDs: 39681, 46144	(TS="perfluorooctanoic acid" OR TS="perfluorooctane sulfonic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-Octanoic acid" OR TS="2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-Hexanoyl fluoride" OR TS="3,3,4,4,5,5,6,6,6-nonafluoro-2-oxohexanoyl fluoride" OR TS="Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-" OR TS="Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-" OR TS="Pentadecafluoro-1-octanoic acid" OR TS="Pentadecafluoro-n-octanoic acid" OR TS="Pentadecafluorooctanoic acid" OR TS="Perfluorocaprylic acid" OR TS="Perfluoroctanoic acid" OR TS="Perfluoroheptanecarboxylic acid" OR TS="perfluorooctanyl sulfonate" OR TS="Perfluorooctanoic acid" OR TS="Octanoic acid, pentadecafluoro-" OR TS="Perfluorooctanoate" OR TS="perfluorooctane sulfonate" OR TS="A 5717" OR TS="EF 201" OR TS="Eftop EF 201" OR TS="Perfluoro-1-heptanecarboxylic acid" OR TS="1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonic acid" OR TS="1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-" OR TS="1-Perfluorooctanesulfonic acid" OR TS="EF 101" OR TS="Eftop EF 101" OR TS="Heptadecafluoro-1-octanesulfonic acid" OR TS="Heptadecafluorooctane-1-sulphonic acid" OR TS="Perfluorooctane sulfonate" OR TS="perfluorooctane sulfonate" OR TS="Perfluorooctane sulfonic acid" OR TS="Perfluorooctanesulfonic acid" OR TS="Perfluorooctylsulfonic acid" OR TS="perfluorooctane sulphonate" OR TS="perfluorooctane sulfonate" OR TS="1-Octanesulfonic acid, heptadecafluoro-"OR TS="Heptadecafluorooctanesulfonic acid" OR TS="Perfluoro-n-octanesulfonic acid" OR TS="Perfluorooctane Sulphonic	9/3/2020, 2/3/2022, 2/6/2023

Database	Search String	Date Run
	Acid" OR TS="Perfluorooctanesulfonate" OR TS="Perfluorooctylsulfonate" OR ((TS="PFOA" OR TS="PFOS") AND (TS="fluorocarbon*" OR TS="fluorotelomer*" OR TS="polyfluoro*" OR TS="perfluoro-*" OR TS="perfluoroa*" OR TS="perfluorob*" OR TS="perfluoroc*" OR TS="perfluorod*" OR TS="perfluoroe*" OR TS="perfluoroh*" OR TS="perfluoron*" OR TS="perfluoroo*" OR TS="perfluorop*" OR TS="perfluoros*" OR TS= "perfluorou*" OR TS="perfluorinated" OR TS="fluorinated" OR TS="PFAS"))) AND PY=(2020-2022)	
TOXLINE	TOXLINE taken down, cannot search.	–
TSCATS	Incorporated into PubMed post 2019.	–

The database searches were conducted by EPA and/or contractor information specialists and librarians on April 11, 2019, September 3, 2020, February 2 and 3, 2022, and February 6, 2023 and all search results were stored in the Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608). After deduplication (i.e., removal of duplicate results) in HERO, the database search results were imported into SWIFT Review software for filtering/prioritization. SWIFT Review identifies those references most likely to be applicable to human health risk assessment (https://www.sciome.com/swift-review/; see also (Howard et al., 2016)). In brief, SWIFT Review has preset literature search strategies ("filters") developed and applied by information specialists to identify and prioritize studies that are most likely to be useful for identifying human health content from those that likely are not (e.g., studies on analytical methods). The filters function like a typical search strategy in which studies are tagged as belonging to a certain category if the terms in the filter literature search strategy appear in title, abstract, keyword, and/or medical subject headings (MeSH) fields content. The applied SWIFT Review filters focused on the following evidence types: human (epidemiology), animal models for human health, and in vitro studies. The details of the search strategies that underlie the filters are available online (https://hawcprd.epa.gov/media/attachment/SWIFT-Review_Search_Strategies.pdf). The use of SWIFT Review is consistent the IRIS Handbook (U.S. EPA, 2022b) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b)

For all literature searches, the evidence stream filters used were human, animal (all), animal (human health model), [no tag], epidemiological quantitative analysis, and in vitro (with one exception – for the 2022 and 2023 literature searches, the in vitro evidence stream filter was not used because the goal of those literature search was to identify studies relevant to dose response only). Studies not captured using these filters were not considered further. Studies that were captured with these SWIFT Review evidence stream filters were exported as a RIS (Research Information System) file for title and abstract screening using either DistillerSR or SWIFT ActiveScreener software (described in subsequent sections of this appendix).

A.1.5.3 Additional Sources

The literature search strategies used were designed to be broad; however, like any search strategy, studies may be missed (e.g., if the chemical of interest is not mentioned in title, abstract, or keyword content; or if gray literature is not indexed in the databases that were searched).

Thus, additional sources were reviewed to identify studies that could have been missed in the database searches. Reviews of additional sources included the following:

1. Review of studies cited in assessments published by other U.S. federal agencies, as well as international and U.S. state-level agencies (including Agency for Toxic Substances and Disease Registry (ATSDR) and California Environmental Protection Agency (CalEPA) assessments that were ongoing at the time of searching).

    - Manual review of the reference list from ATSDR's Toxicological Profile for Perfluoroalkyls (ATSDR, 2021) (not date limited).

    - Manual review of the reference list from CalEPA's *First Public Review Draft of Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water* (CalEPA, 2021) (not date limited).

    - Manual review of National Toxicology Program (NTP) publications (https://ntp.niehs.nih.gov/data/index.html). In 2020, the NTP website was searched for PFOA toxicity study final reports that could provide relevant health effects information.

    - Manual review of PFAS toxicity studies identified by the New Jersey Department of Environmental Protection (NJDEP).

2. Review of studies identified during mechanistic or toxicokinetic evidence synthesis:

    - Manual review of the reference lists of studies identified as PECO-relevant after full-text review were reviewed at the title level for potential relevance (backward citation search).

    - Manual review of other EPA PFAS assessments or literature searches under development by IRIS.

3. Review of studies identified by the SAB PFAS Panel peer reviewers in their final report (published in August 2022).

4. Review of studies submitted through public comment by May 2023 (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

A.1.5.4 Incorporation of Data From the 2016 PFOA Health Effects Support Document

The 2016 PFOA HESD contains a comprehensive summary of relevant literature based on searches conducted through 2013. The 2016 PFOA HESD underwent a public comment period in February 2014 and an independent external public panel peer review in August 2014. EPA incorporated key studies from the 2016 PFOA HESD that addressed one or more of the five priority health outcomes into this updated PFOA assessment, as described below.

Over 140 epidemiological studies were captured in the 2016 PFOA HESD. The 2016 PFOA HESD did not use the epidemiological data quantitatively. For the current assessment, EPA reviewed the epidemiological studies that were included in the 2016 PFOA HESD summary tables and identified those that were relevant to one or more of the five priority health outcomes

(i.e., developmental, immune, hepatic, cardiovascular, and cancer). A total of 59 epidemiological studies were included and are listed in Table A-6 (studies relevant to more than one health outcome are listed under each applicable category in the table).

Table A-6. Key Epidemiological Studies of Priority Health Outcomes Identified from the 2016 PFOA Health Effects Support Document

HERO ID	Reference	Title
		Cancer
2850946	Barry et al., 2013	Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant
2851186	Bonefeld-Jørgensen et al., 2014	Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort
2150988	Bonefeld-Jørgensen et al., 2011	Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case-control study
2919344	Eriksen et al., 2009	Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population
2968084	Hardell et al., 2014	Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer
2850270	Raleigh et al., 2014	Mortality and cancer incidence in ammonium perfluorooctanoate production workers
2851015	Steenland et al., 2015	A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA)
2919168	Steenland and Woskie, 2012	Cohort mortality study of workers exposed to perfluorooctanoic acid
2919154	Vieira et al., 2013	Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis
		Cardiovascular
1429922	Costa et al., 2009	Thirty years of medical surveillance in perfluorooctanoic acid production workers
1290905	Emmett et al., 2006	Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters
2919150	Eriksen et al., 2013	Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population
2919156	Fisher et al., 2013	Do perfluoroalkyl substances affect metabolic function and plasma lipids? – Analysis of the 2007–2009, Canadian Health Measures Survey (CHMS) Cycle 1
2850962	Fitz-Simon et al., 2013	Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid
1430763	Frisbee et al., 2010	Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project
3749193	Fu et al., 2014	Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population
2850925	Geiger et al., 2014	The association between PFOA, PFOS and serum lipid levels in adolescents
2851286	Geiger et al., 2014	No association between perfluoroalkyl chemicals and hypertension in children

HERO ID	Reference	Title
1290820	Lin et al., 2009	Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults
3981585	Maisonet et al., 2015	Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females
1291110	Nelson et al., 2010	Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population
1290836	Olsen and Zobel, 2007	Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers
1290020	Olsen et al., 2003	Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations
10228462	Olsen et al., 2001	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.
1424954	Olsen et al., 2000	Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers
2850270	Raleigh et al., 2014	Mortality and cancer incidence in ammonium perfluorooctanoate production workers
1291103	Sakr et al., 2007	Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers
1430761	Sakr et al., 2007	Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate
1276141	Savitz et al., 2012	Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community
1424946	Savitz et al., 2012	Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley
2850928	Starling et al., 2014	Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study
2851015	Steenland et al., 2015	A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA)
1291109	Steenland et al., 2009	Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant
2919168	Steenland and Woskie, 2012	Cohort mortality study of workers exposed to perfluorooctanoic acid
1290816	Stein et al., 2009	Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome
2850370	Timmermann et al., 2014	Adiposity and glycemic control in children exposed to perfluorinated compounds
2851142	Winquist and Steenland, 2014	Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts

HERO ID	Reference	Title
		Developmental
1429893	Andersen et al., 2010	Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy
1290833	Apelberg et al., 2007	Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth
1290900	Apelberg et al., 2007	Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland
1332466	Chen et al., 2012	Perfluorinated compounds in umbilical cord blood and adverse birth outcomes
2850274	Darrow et al., 2014	PFOA and PFOS serum levels and miscarriage risk
2850966	Darrow et al., 2013	Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005–2010
1290822	Fei et al., 2008	Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy
2349574	Fei et al., 2008	Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort
1005775	Fei et al., 2007	Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort
1290814	Hamm et al., 2010	Maternal exposure to perfluorinated acids and fetal growth
1332465	Maisonet et al., 2012	Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls
2349575	Monroy et al., 2008	Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples
1290813	Nolan et al., 2010	Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water
2349576	Nolan et al., 2009	The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water
1276141	Savitz et al., 2012	Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community
1424946	Savitz et al., 2012	Relationship of perfluorooctanoic Acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley
1290816	Stein et al., 2009	Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome
1291133	Washino et al., 2009	Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth
		Hepatic
1429922	Costa et al., 2009	Thirty years of medical surveillance in perfluorooctanoic acid production workers
1290905	Emmett et al., 2006	Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters
1276142	Gallo et al., 2012	Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure

HERO ID	Reference	Title
1291111	Lin et al., 2010	Investigation of the Associations Between Low-Dose Serum Perfluorinated Chemicals and Liver Enzymes in U.S. Adults
1290836	Olsen and Zobel, 2007	Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers
1290020	Olsen et al., 2003	Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations
10228462	Olsen et al., 2001	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.
1424954	Olsen et al., 2000	Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers
1291103	Sakr et al., 2007	Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers
1430761	Sakr et al., 2007	Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate
2919168	Steenland and Woskie, 2012	Cohort mortality study of workers exposed to perfluorooctanoic acid
2851015	Steenland et al., 2015	A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA)
		Immune
1429922	Costa et al., 2009	Thirty years of medical surveillance in perfluorooctanoic acid production workers
1937230	Dong et al., 2013	Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children
1290905	Emmett et al., 2006	Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters
1290805	Fei et al., 2010	Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood
1248827	Grandjean et al., 2012	Serum vaccine antibody concentrations in children exposed to perfluorinated compounds
1937228	Granum et al., 2013	Prenatal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood
2851240	Humblet et al., 2014	Perfluoroalkyl chemicals and asthma among children 12–19 yr of age: NHANES (1999–2008)
2850913	Looker et al., 2014	Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate
1332477	Okada et al., 2012	Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants

HERO ID	Reference	Title
2851015	Steenland et al., 2015	A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA)
1424977	Wang et al., 2011	The effect of prenatal perfluorinated chemicals exposures on pediatric atopy

Notes: APFO = ammonium perfluorooctanoate; NHANES = National Health and Examination Survey.

EPA also reviewed the animal toxicological studies in the 2016 PFOA HESD summary tables that were identified as relevant for the five priority health outcomes. A total of 11 "key" animal toxicological studies that were either considered quantitatively in the 2016 PFOA HESD or provided data that may quantitatively impact the assessment conclusions were included and are listed in Table A-7 (studies relevant to more than one health outcome are listed under each applicable category in the table).

Table A-7. Key Animal Toxicological Studies Identified from the 2016 PFOA Health Effects Support Document

HERO ID	Reference	Title
		Cancer
673581	Biegel et al., 2001	Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
		Cardiovascular
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
988599	Loveless et al., 2008	Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate
		Developmental
1335452	Abbott et al., 2007	Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
1276159	Lau et al., 2006	Effects of perfluorooctanoic acid exposure during pregnancy in the mouse
988599	Loveless et al., 2008	Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate
1276151	Macon et al., 2011	Prenatal perfluorooctanoic acid exposure in CD-1 mice: Low-dose developmental effects and internal dosimetry
1332672	Wolf et al., 2007	Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures
		Endocrine
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat

HERO ID	Reference	Title
988599	Loveless et al., 2008	Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate
		Gastrointestinal
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
		Hepatic
1335452	Abbott et al., 2007	Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha
673581	Biegel et al., 2001	Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
1276159	Lau et al., 2006	Effects of perfluorooctanoic acid exposure during pregnancy in the mouse
988599	Loveless et al., 2008	Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate
1276151	Macon et al., 2011	Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry
1291118	Perkins et al., 2004	13-wk dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats
1332672	Wolf et al., 2007	Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures
		Immune
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
1290826	Dewitt et al., 2008	Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice
988599	Loveless et al., 2008	Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate
		Metabolic
1335452	Abbott et al., 2007	Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
		Nervous
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat

HERO ID	Reference	Title
1276151	Macon et al., 2011	Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry
		Renal
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
		Reproductive
1335452	Abbott et al., 2007	Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha
673581	Biegel et al., 2001	Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
1291118	Perkins et al., 2004	13-wk dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats
		Respiratory
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats`
1291118	Perkins et al., 2004	13-wk dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats
		Systemic
1335452	Abbott et al., 2007	Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha
2919192	Butenhoff et al., 2012	Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats
1291063	Butenhoff et al., 2004	The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat
1290826	Dewitt et al., 2008	Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice
1276159	Lau et al., 2006	Effects of perfluorooctanoic acid exposure during pregnancy in the mouse
1291118	Perkins et al., 2004	13-wk dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats
1332672	Wolf et al., 2007	Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures
3981487	Yu et al., 2016	Effects of perfluorooctanoic acid on metabolic profiles in brain and liver of mouse revealed by a high-throughput targeted metabolomics approach

A.1.6 Literature Screening Process to Target Dose-Response Studies and PK Models

This section summarizes the methods used to screen the literature search results against the PECO criteria to identify relevant studies potentially suitable for use in dose-response analyses and studies featuring PK models. Literature search results were screened at both title/abstract and full-text levels. These screening steps are described further below.

The PECO criteria used to screen the literature search results are the same as those used to frame the initial literature search (Table A-1) and are outlined again in Table A-8 below.

Table A-8. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria for a Systematic Review on the Health Effects from Exposure to PFOA and PFOS

PECO Element	Inclusion Criteria
Population	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). In vitro/cell studies or in silico/modeling toxicity studies should be tagged as supplemental.
Exposure	**Any exposure to PFOA, PFOS, and/or the salts of PFOA/PFOS, including but not limited to**: PFOA (*Chemical Abstracts Service (CAS) number 335-67-1*). Other names: perfluorooctanoate; perfluorooctanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic-acid, pentadecafluoro- Relevant Salts of PFOA: ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, potassium perfluorooctanoate PFOS (*CAS number 1763-23-1*). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, Heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid Relevant Salts of PFOS: lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, sodium perfluorooctanesulfonate **Human:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, or unknown/multiple routes will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." **Animal:** Any exposure to PFOA or PFOS via oral routes. Other exposure routes, including inhalation, dermal, injection or unknown/multiple routes, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." Studies involving exposures to mixtures will be included only if they include exposure to PFOA or PFOS alone. Studies with less than 28 d of dosing, with the exception of reproductive, developmental, immune and neurological health outcome studies, should be tagged as supplemental.
Comparator	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcome	All health outcomes (both cancer and noncancer).
PBPK Models	Studies describing PBPK models will be included.

Notes: PBPK = physiologically based pharmacokinetic.

Following SWIFT Review filtering (see Section A.1.5.2), literature search results were imported into either DistillerSR (Evidence Partners; https://www.evidencepartners.com/products/distillersr-systematic-review-software) or SWIFT ActiveScreener (Sciome; https://www.sciome.com/swift-activescreener/) software and were screened against the PECO criteria at the title and abstract level to identify PECO-relevant studies that could influence the derivation of an oral RfD and/or CSF. Studies that met the PECO criteria were tagged as having relevant human data, relevant animal data (in a mammalian model), or a PBPK model. Studies that did not meet the PECO criteria as determined by title/abstract screening but did appear to include potentially important supplemental information were categorized according to the type of supplemental information they contained (e.g., mechanistic, ADME). Following completion of title/abstract screening (described further in Sections A.1.6.3 and A.1.6.4), the literature search results were re-screened at the full-text level (described further in Section A.1.6.5).

The title/abstract and full-text level screenings were performed by two independent reviewers using structured forms in DistillerSR, with a process for conflict resolution that included discussion of conflicts with the screening team. During full-text screening, literature inventories identifying evidence types and health effect systems were created for PECO-relevant studies and studies tagged as containing potentially relevant supplemental material to facilitate review of studies by topic-specific experts. These procedures are consistent with those outlined in the IRIS Handbook (U.S. EPA, 2022c).

Studies that did not meet the PECO criteria but contained potentially relevant supplemental information were inventoried during the literature screening process. Potentially relevant supplemental material included the following (see Table A-11 for full list):

- Mechanistic data (including in vitro/ex vivo/in silico studies),
- Studies in nonmammalian or transgenic mammalian model systems,
- Nonoral routes of administration (for animal toxicological studies),
- ADME and toxicokinetic studies (including the application of existing PBPK models),
- Exposure assessment or characterization studies (no health outcome assessment),
- Mixture studies (animal toxicological studies on mixtures of PFOA and other substances or epidemiological studies that only report associations based on sum or total PFAS),
- Human case reports (n = 1–3 cases per report),
- Records or other assessments with no original data (e.g., reviews, editorials, commentaries),
- Conference abstracts, and
- Non-English language studies.

Following title/abstract and full-text level screening, studies tagged as containing potentially relevant mechanistic, ADME, or toxicokinetic data underwent additional screening and data extraction steps that were separate from steps followed for PECO-relevant studies. Additionally, studies that were tagged as containing relevant PBPK models were sent to the modeling technical experts for scientific and technical review. Details on the screening and data extraction methods for ADME and mechanistic studies are described below.

A.1.6.1 Screening ADME Studies

Studies identified as containing potentially relevant supplemental ADME data during title/abstract and/or full-text screening underwent further screening against the ADME-specific PECO criteria outlined in Table A-2. For studies that met the ADME-specific PECO criteria (see Table A-2), key study information was extracted using litstream™ software. Methods for this ADME screening and extraction of some key study information into litstream is described further in Section A.1.6.7.

A.1.6.2 Screening Mechanistic Studies

Studies identified as containing potentially relevant supplemental mechanistic data during title/abstract and/or full-text screening underwent further screening against the mechanistic-specific PECO criteria outlined in Table A-3. Studies that met the mechanistic-specific PECO criteria were extracted into litstream™. Methods for this mechanistic information screening and extraction of some key study information into litstream is described further in Section A.1.6.8.

A.1.6.3 Title/Abstract Screening Questions – DistillerSR

Studies identified from the 2016 PFOA HESD and recent systematic literature search and review efforts (searches through 2020) were imported into DistillerSR software for title/abstract screening. For each study, the screeners reviewed the title and abstract and responded to a series of prompts within structured DistillerSR forms to assess PECO relevance and identify evidence stream(s). Table A-9 below lists the prompts within the DistillerSR forms used for title/abstract screening and the response options for each prompt.

Table A-9. DistillerSR Form for Title/Abstract Screening

	Question/Prompt	Response Options
1	**Does the article meet PECO criteria?** *[Select one]*	• Yes[a] • No • Tag as potentially relevant supplemental material • Unclear
If "Yes" to Question #1:		
2a	**What type of evidence?** *[Select all that apply]*	• Human • Animal (mammalian models) • PBPK model
If "Tag as potentially relevant supplemental material" to Question #1:		
2b	**What kind of supplemental material?[b]** *[Select all that apply]*	• Mechanistic[c] • Nonmammalian model • ADME/toxicokinetic • Acute/short-term duration exposures • Non-oral route of administration • Exposure characteristics (no health outcome) • Susceptible population (no health outcome) • Environmental fate or occurrence (including food) • Mixture study • Case study or case series • Other assessments or records with no original data (e.g., reviews, editorials, commentaries)

Question/Prompt	Response Options
	• Conference abstract • Bioaccumulation data in fish

Notes: PBPK = physiologically based pharmacokinetic.
[a] Errata, corrections, and corrigenda were tagged to the original study and not considered a separate relevant record.
[b] Refer to list of supplemental tags in Appendix A.1.6.4.1.
[c] Refer to list of mechanistic information in Appendix A.1.6.4.2.

A.1.6.4 Title/Abstract Screening Questions – SWIFT-Active

Studies identified from the most recent literature search (2020–2022) were imported into SWIFT-Active Screener software for title/abstract screening. For each study, screeners reviewed the title and abstract and responded to a set of prompts designed to ascertain PECO relevance and identify evidence stream(s). Table A-10 below lists the prompts within SWIFT-Active that were used for title/abstract screening and the response options for each prompt.

Table A-10. SWIFT-Active Form for Title/Abstract Screening

	Question/Prompt	Response Options
1	**Include this reference?** Select "Yes, include the reference" if unsure. *[Select one]*	• Yes, include the reference[a] • No, exclude the reference
If "Yes" to Question #1:		
2a	**Identify the Type of Evidence** *[Select all that apply]*	• Human/Epidemiological • Animal • Unsure
If "No, exclude the reference" to Question #1:		
2b	**Not Relevant or Supplemental?[b]** Select whether the reference is not relevant to PECO and should be excluded or if the reference contains supplemental information. *[Select all that apply]*	• Exclude/Not Relevant • Supplemental

Notes:
[a] Errata, corrections, and corrigenda were tagged to the original study and not considered a separate relevant record.
[b] Refer to the list of supplemental tags in Section A.1.6.4.2.

A.1.6.4.1 Supplemental Tags

The categories shown in Table A-11 were considered supplemental throughout the title/abstract and full-text screening processes. With the exception of studies tagged as containing ADME/TK or mechanistic information, which were further considered as described in Section A.1.6.7 and Section A.1.6.8 of this appendix, studies identified as not PECO-relevant but containing potentially useful supplemental material were not considered for the subsequent steps of the systematic review process.

Table A-11. Supplemental Tags for Title/Abstract and Full-Text Screening

Category	Evidence
Mechanistic Studies	Studies reporting measurements related to a health outcome that inform the biological or chemical events associated with phenotypic effects, in both mammalian and nonmammalian model systems, including in vitro, in vivo (by various routes of exposure), ex vivo, and in silico studies. When possible, mechanistic studies will be sub-tagged as pertinent to cancer, noncancer, or unclear/unknown.
Non-Mammalian Model Systems	Studies in nonmammalian model systems, e.g., fish, birds, *C. elegans*
ADME and Toxicokinetic	Studies designed to capture information regarding absorption, distribution, metabolism, and excretion, including toxicokinetic studies. Such information may be helpful in updating or revising the parameters used in existing PBPK models.

Category	Evidence
Acute/Short-Term Duration Exposures	Animal studies of less than 28 d (unless the study is a developmental/reproductive, neurological, or immune study)
Only One Exposure Group	Animal studies with only one exposure group, e.g., control and 1 mg/kg/day PFOA.
Non-Oral Routes of Exposure	Studies not addressing routes of exposure that fall outside the PECO scope, include inhalation and dermal exposure routes
Exposure Characteristics (No Health Outcome)	Exposure characteristic studies include data that are unrelated to toxicological endpoints, but which provide information on exposure sources or measurement properties of the environmental agent (e.g., demonstrate a biomarker of exposure).
Susceptible Populations (No Health Outcome)	Studies that identify potentially susceptible subgroups; for example, studies that focus on a specific demographic, lifestage, or genotype.
Environmental Fate or Occurrence (Including Food)	Studies that focus on describing where the chemical will end up after it is used and released into the environment.
Mixture Studies	Mixture studies that are not considered PECO-relevant because they do not contain an exposure or treatment group assessing only the chemical of interest.
Case Studies or Case Series	Case reports and case series will be tracked as potentially relevant supplemental information.
Records With No Original Data	Records that do not contain original data, such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Other Assessments or Records With No Original Data (e.g., Reviews, Editorials, Commentaries)	Secondary studies (e.g., reviews, editorials, commentaries, assessments) that do not provide any primary research/results.
Conference Abstracts	Records that do not contain sufficient documentation to support study evaluation and data extraction.
Bioaccumulation in Fish	Retained records relevant to other EPA projects mentioned in the PFAS Action Plan.
Non-English Reports	Studies not reported in English.

Notes: C. elegans = Caenorhabditis elegans.

A.1.6.4.2 Mechanistic Study Categories and Keywords

The following categories were considered mechanistic throughout the title/abstract and full-text screening (Table A-12). Studies tagged as containing potentially relevant supplemental mechanistic information were further considered as described in Section A.1.6.8 of this appendix.

Table A-12. Mechanistic Study Categories Considered as Supplemental

Category	Examples of Keywords
Chromosome or DNA structure, function, repair, or integrity	genotoxicity, micronuclei, DNA strand break, sister chromatid exchange, aneuploidy, genomic instability, gene amplification, epigenomics, DNA methylation, DNA methyltransferase, histone, DNA repair, base excision repair, nucleotide excision repair, DNA mismatch repair
Gene expression and transcription	individual genes, pathway-related genes, transcriptomics, epigenetics, transcription factors, microRNAs, noncoding RNAs
Protein synthesis, folding, function, transport, localization, or degradation	proteomics, translation, ribosomes, chaperones, heat shock proteins, ubiquitin, proteasome, ER stress, UPR, PERK
Metabolism	anabolic or catabolic pathways for lipids, carbohydrates, amino acids, nucleotides; energy metabolism; biochemical pathways; metabolomics; lipidomics; enzyme or coenzyme activity or function.

Category	Examples of Keywords
Cell signaling or signal transduction pathway	ligand interactions with membrane, cytoplasmic and nuclear receptors (e.g., AHR, ER, AR, CAR, RAR, neurotransmitter receptors, insulin receptor, G-protein coupled receptors), tyrosine kinases, phosphatase, phospholipases, GTPase, second messengers (calcium, diacylglycerol, ceramide, NO), signaling pathways (NF-ƙB, MAPK/ERK, AKT, mTOR, IP3/DAG, cAMP-dependent, Wnt, β-catenin, TGFβ, etc.)
Cell or organelle structure, motility, integrity	membrane integrity, cell scaffolding, cytoskeleton, actin, microtubules, ER, Golgi, mitochondria, lysosome, endosome, phagosome, nucleus, chemotaxis, atrophy, hypertrophy
Extracellular matrix or molecules	ECM proteins (collagens, elastins, fibronectins and laminins), proteoglycans, matrix metalloproteinases (MMPs)
Cell growth, differentiation, proliferation, or viability	cell cycle (G1, S, G2, M), cyclins, CDKs, p53, p27, Rb, E2F stem cell, progenitor, apoptosis, Annexin V, TUNEL, necrosis, blebbing, pyknosis, Bax, Bcl-2, hyperplasia, dysplasia
Activation of intrinsic cell defense molecules or systems	cytokines, chemokines, caspases, MHC/HLA molecules, pattern recognition receptors (PRRs), NLR, proteasomes, autophagy
Oxidative stress	reactive oxygen species (ROS), oxidative stress, hydroxyl radical, hydrogen peroxide, reactive nitrogen species, superoxide anion, peroxyl radicals, antioxidant response, catalase, superoxide dismutase, EROD, glutathione (GSH), GSH peroxidase, glutathione-S-transferase, 8-OHdG
Hormone function	GnRH, CRF, ADH/vasopressin, FSH, LH, ACTH, GH, TH, TSH, PTH, cortisol, epinephrine/norepinephrine, melatonin, oxytocin, estrogen, testosterone, adiponectin, leptin, insulin, glucagon
Biomarkers of cerebral function	Apoptotic neurodegeneration protein markers, cerebral glucose metabolism, brain glucose levels
Other (provide details)	Please provide specific details regarding reason for supplemental tag in the notes section.

Notes: 8-OHdG = 8-hydroxy-2'-deoxyguanosine; ACTH = adrenocorticotropic hormone; ADH = antidiuretic hormone; AHR = aryl hydrocarbon receptor; Bcl-2 = B-cell lymphoma 2; CAR = constitutive androstane receptor; CDK = cyclin-dependent kinase; CRF = corticotropin-releasing factor; DAG = diacylglycerol; DNA = deoxyribonucleic acid; ECM = extracellular matrix; ER = estrogen receptor; EROD = ethoxyresorufin-O-dealkylase; FSH = follicle stimulating hormone; GH = growth hormone; GTPase = guanosine triphosphate; GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; MHC/NHLA = major histocompatibility complex/human leukocyte antigen; microRNA = micro ribonucleic acid; mTOR = rapamycin; NF-ƙB = nuclear factor kappa B; NLR = nucleotide-binding oligomerization domain-like receptors; NO = nitric oxide; PERK = protein kinase R-like endoplasmic reticulum kinase; PTH = parathyroid hormone; RAR = retinoic acid receptor; RNA = ribonucleic acid; TH = thyroid hormone; TGFβ = transforming growth factor beta; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; UPR = unfolded protein response.

A.1.6.5 Full-Text Screening Questions

All studies identified as PECO-relevant from title/abstract screening advanced to full-text screening, which was performed in DistillerSR. Screeners reviewed each full study report and any supplemental study materials to respond to prompts pertaining to PECO relevance, evidence stream, health outcome(s), and whether PFOA and/or PFOS was evaluated (some screening efforts for PFOA and PFOS were performed concurrently). Table A-13 below lists the prompts and response options that were used for full-text screening.

Table A-13. DistillerSR Form for Full-Text Screening

	Question/Prompt	Response Options
1	**Source of study if not identified from database search.** *[Select one]*	• Source other than HERO database search
2	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No • Tag as potentially relevant supplemental material • Unclear
If "Yes" to Question #1:		
3a	**If meets PECO, what type of evidence?** *[Select all that apply]*	• Human • Animal (mammalian models) • PBPK model
4a	**If meets PECO, which health outcome(s) apply?**[a] *[Select all that apply]*	• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and developmental • Hematological, including nonimmune/hepatic/renal clinical chemistry measures • Hepatic, including liver measures and serum markers (e.g., ALT, AST) • Immune/inflammation • Musculoskeletal • Nervous system, including behavior and sensory function • Nutrition and metabolic • Ocular

	Question/Prompt	Response Options
		• PBPK or PK model • Renal, including urinary measures (e.g., protein) • Reproductive • Respiratory • Skin and connective tissue effects • Dermal • Unsure • Other
		If meets PECO and endocrine outcome, which endocrine tags apply? *[Select all that apply]* • Adrenal • Sex hormones (e.g., androgen, estrogen, progesterone) • Neuroendocrine • Pituitary • Steroidogenesis • Thyroid
		If "Unsure" or "Other" is selected for health outcome, write reasoning in the respective free-text box. *[Free-text]*
If "Tag as potentially relevant supplemental material" to Question #1:		
3b	**If supplemental, what type of information?[b,c]** *[Select all that apply]*	• Mechanistic • Nonmammalian model • ADME/toxicokinetic • Acute/short-term duration exposures[d] • Non-oral route of administration • Exposure characteristics (no health outcome) • Susceptible population (no health outcome) • Environmental fate or occurrence (including food) • Mixture study • Case study or case series • Other assessments or records with no original data (e.g., reviews, editorials, commentaries) • Conference abstract • Bioaccumulation data in fish
4b		**If "Acute," which health outcome(s) apply?** *[Select all that apply]*

	Question/Prompt	Response Options
		• General toxicity, including body weight, mortality, and survival • Cancer • Cardiovascular, including serum lipids • Endocrine (hormone) • Gastrointestinal • Genotoxicity • Growth (early life) and developmental • Hematological, including nonimmune/hepatic/renal clinical chemistry measures • Hepatic, including liver measures and serum markers (e.g., ALT, AST) • Immune/inflammation • Musculoskeletal • Nervous system, including behavior and sensory function • Nutrition and metabolic • Ocular • PBPK or PK model • Renal, including urinary measures (e.g., protein) • Reproductive • Respiratory • Skin and connective tissue effects • Dermal • Unsure
If "Yes," "Tag as potentially relevant supplemental material," or "Unclear" to Question #1:		
5	**Which PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS • Other PFAS

Notes: ALT = alanine transaminase; AST = aspartate aminotransferase; PBPK = physiologically based pharmacokinetic; PK = pharmacokinetic.
[a] Refer to list of health outcomes and examples in Appendix A.1.6.5.1.
[b] Refer to list of supplemental tags in Appendix A.1.6.4.1.
[c] Refer to list of mechanistic information in Appendix A.1.6.4.2.
[d] Refer to definition of acute/short-term duration exposures in Appendix A.1.6.6.

A.1.6.5.1 Health Effect Categories and Example Outcomes for Epidemiological Studies

The following health effects categories were considered throughout the full-text screening and subsequent steps of the systematic review process for epidemiological studies (Table A-14).

Table A-14. Health Effect Categories Considered for Epidemiological Studies

Health Effect Category	Example Health Outcomes	Notes
Cancer	• Tumors • Precancerous lesions (e.g., dysplasia)	–
Cardiovascular	• Serum lipids (e.g., cholesterol, LDL, HDL, triglycerides) • Blood pressure • Hypertension • Atherosclerosis • Coronary heart disease • Other cardiovascular disease	–
Dermal	• Skin sensitivity	–
Developmental	• Birth size (birth weight; birth length; small for gestational age) • Preterm birth • Sex ratio • Postnatal growth	• Markers of development specific to other systems are organ/system-specific (e.g., tests of sensory maturation are under **Nervous System**). • Pubertal development is under **Reproductive**.
Endocrine	• Thyroid hormones (e.g., T3, T4, TSH) • Thyroid weight and histopathology • Hormonal measures in any tissue or blood (non-reproductive)	• Reproductive hormones (e.g., estrogen, progesterone, testosterone) are under **Reproductive**.
Gastrointestinal	• Symptoms of the stomach and intestines (e.g., diarrhea, nausea, vomiting, abdominal pain and cramps)	–
Hematologic	• Blood count • Red blood cells • Blood Hematocrit or hemoglobin • Corpuscular volume • Blood Platelets or reticulocytes • Blood biochemical measures (e.g., sodium, calcium, phosphorus)	• White blood cell counts and globulin are under **Immune**. • Serum lipids are under **Cardiovascular**. • Serum liver markers are under **Hepatic**.
Hepatic	• Liver enzymes (e.g., ALT; AST; ALP) • Liver disease • Liver-specific serum biochemistry (e.g., albumin)	• Serum lipids are under **Cardiovascular**. • Biochemical markers, such as albumin, are under **Hepatic**. Liver tissue cytokines are under **Immune**. • Globulin is under **Immune**. • Serum glucose is under **Metabolic.**
Immune	• Asthma • Allergy • Atopic dermatitis/eczema • Vaccine response • IgE	• Red blood cells are under **Hematological**. • Non-immune measures of pulmonary function are under **Respiratory**.

Health Effect Category	Example Health Outcomes	Notes
	• Autoimmune or infectious disease • Hypersensitivity • General immune assays (e.g., white blood cell counts) • Immune responses in the respiratory system • Stress-related factors in blood (e.g., glucocorticoids or other adrenal markers)	• Interleukin 6 (IL-6) is considered a **Mechanistic** outcome.
Metabolic/Systemic	• Obesity • BMI • Adiposity • Diabetes (including gestational diabetes) • Insulin resistance • Blood glucose • Allostatic load • Metabolic syndrome	• Waist circumference, ponderal index, BMI SDS, BMI z-scores, are all included here. • Gestational weight gain, adult weight change also included here.
Musculoskeletal/Connective Tissue	• Bone health • Osteoporosis • Bone density	–
Nervous	• Cognition • Behavior • Autism • Attention (ADHD) • Depression • Communication • Motor	–
Ocular	• Vison changes • Eye irritation	–
Reproductive, female	• Reproductive hormones • Breastfeeding • Fecundity • PCOS • Spontaneous abortion • Menopause • Endometriosis • Pubertal development • Menstrual cycle characteristics • Anogenital distance (females)	• If data indicate altered birth parameters are likely attributable to female fertility, these data may be discussed under **Female Reproductive**.
Reproductive, male	• Reproductive hormones • Semen parameters • Sperm DNA damage • Pubertal development • Anogenital distance (males)	–
Respiratory	• Non-immune measures of pulmonary (lung) function (e.g., FEV1, FVC, lung capacity)	• Asthma, wheeze, lower/upper respiratory trat infections are **Immune**.
Renal	• GFR • Uric acid • Creatinine • Renal function • Urinary measures (e.g., protein; volume; pH; specific gravity)	–

Health Effect Category	Example Health Outcomes	Notes
Other	• Select this category if the outcome does not fit in any of the above categories	–

Notes: ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; GFR = glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PBPK = physiologically based pharmacokinetic; PCOS = polycystic ovary syndrome; PK = pharmacokinetic; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone.

A.1.6.6 Animal Toxicological Study Design Definitions

The following definitions were used throughout full-text screening and data extraction for animal toxicological studies:

- Acute/short-term: Exposure duration between 1–28 days.
- Sub-chronic: Exposure duration between 28–90 days.
- Chronic: Exposure duration greater than 90 days.
- Developmental: Exposure occurs during gestation and dams are sacrificed prior to birth. These studies are typically focused on the pups and evaluate viability, developmental milestones, and other growth and developmental effects in pups.
- Reproductive: Exposure begins prior to mating and may continue through birth and, in some cases, through a second generation. These studies will typically evaluate reproductive outcomes in the dams (e.g., copulation and fertility indices, numbers of corpora lutea and implantation sites, pre- and post-implantation loss).

A.1.6.7 ADME Screening and Light Data Extraction

All studies identified as containing ADME data during title/abstract or full-text screening were imported into litstream and underwent additional screening. Studies that met certain criteria (e.g., PECO relevant and evaluated multiple timepoints, tissues, and/or dose levels) underwent light data extraction. For each study, at least two reviewers (one primary screener/extractor and one quality assurance (QA) reviewer) reviewed the full study and any supplemental study materials to respond to prompts pertaining to key study elements (e.g., tested species or population, tissues evaluated, dose levels tested, ADME endpoints measured). Table A-15 below describes the prompts and response options that were used for ADME screening of epidemiological or animal toxicological studies.

Table A-15. Litstream Forms for ADME Screening and Light Data Extraction

	Question/Prompt	Response Options	Suggested Considerations
1	**General Questions**		
1.1	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No	• Use ADME-specific PECO statement (see Toxicity Assessment, (U.S. EPA, 2024b)) and "Draft EPA IRIS Handbook: Principles and Procedures for Integrated Risk Information System (IRIS) Toxicological Reviews" to inform the answer. • Examples of exclusions may include abstract-only, foreign language, secondary data sources, exposure studies, physical-chemical properties, and species that are not relevant. • If "No" is selected, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly explaining why the study does not meet PECO.
1.2	**What PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS	–
1.3	**Does this study contain multiple time points, multiple tissues, and/or multiple doses?** *[Select one]*	• Yes • No	• If "No" is selected, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly explaining why the study meets PECO but does not contain multiple time points, multiple tissues, and/or multiple doses.

	Question/Prompt	Response Options	Suggested Considerations
1.4	**Does this study contain supporting epidemiological information?** *[Select one]*	• Yes • No	• Supporting epidemiological information includes studies that compare PFAS levels in women of different parity status or week of breastfeeding as well as studies that compare PFAS levels across multiple age groups or multiple time points even if it is not the same individuals who are being followed over time (e.g., a cross-sectional study that enrolls people of various ages and compares PFOS/PFOA levels in a specific tissue in children vs. older adults).
1.5	**Indicate if there is supplemental data for this study.** *[Select all that apply; Free-text]*	• MOA/Mechanistic • Exposure Study	• Use the free-text field below to provide a brief description of the type of MOA/mechanistic (refer to Appendix A.1.6.4.2 for examples) and/or exposure information that is available. • Examples of exposure information include studies of PFAS levels in environmental media not directly linked to human exposure (e.g., soil, sediment, microbes, water [except drinking water], birds, or fish [except those typically consumed by humans]).
1.6	**Identify the species, system, or model.** *[Select all that apply]*	• Human • Non-human primate • Rat • Mouse • Mammalian cells (in vitro studies) • PBPK/TK models (or in silico studies)	• If a study only contains PBPK/TK models, do not move forward with the light extraction. Finish filling out Section 1 – General Questions (if applicable) and add a note in Section 5 – Notes under "Notes from Initial Extractor to QA/QC team" briefly describing the model.
2	**Human Studies Sub-Form** If the study does **not** contain a human study, **skip** this section and move on to Section 3 – Animal Studies Sub-Form.		
2.1	**Population Name** *[Free-Text]*	–	• Name a population (e.g., Females – pregnant, PFOS). • Separate populations should be made for each chemical, population sex, lifestage where ADME data were collected, exposure route, etc. combination.
2.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• Note: PFOA and PFOS are not metabolized so "metabolism" is an unlikely selection.
2.3	**List the specific ADME endpoints addressed.**	–	• List all the ADME endpoints analyzed for this population.

	Question/Prompt	Response Options	Suggested Considerations
	[Free-text]		
2.4	**Exposure Category** Use the free-text field if additional information is needed (e.g., it is a unique exposure, occupational setting). *[Select one; Free-text]*	• General environmental • Poisoning • Occupational • Developmental	–
2.5	**Identify the Exposure Route** *[Select one; Free-text]*	• Inhalation • Oral • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intranasal)	• If "other" option is selected, use the free-text field to describe exposure route. • If the study population is exposed through more than one route (e.g., oral and dermal), select one route from the list and use the free-text field to describe the other exposure routes listed in the paper. • If the study population is offspring that were exposed "in utero/placental" AND by "lactational transfer," select "in utero/placental" and use the free-text field to note that lactational transfer also occurred. • If exposure route is unknown, select "other" option and write in "Unknown" in the free-text field. • If the route is unspecified or multiple routes were suspected based on the exposure vehicle, select "other" and write in suspected exposure route in the free-text field.
2.6	**What is the exposure vehicle?** *[Select one]*	• Drinking water • Diet • Breast milk • In utero/placental transfer • Occupational • Unknown • Other	• If "other" option is selected, use the free-text field to describe exposure vehicle. • If the study population is offspring that were exposed "in utero/placental" AND by "breast milk," select "in utero/placental" and use the free-text field to note that lactational transfer also occurred via breast milk. • If "occupational" option is selected, use the free-text field to describe exposure vehicle.
2.7	**What is the sex of the population?** *[Select one]*	• Male • Female • Unspecified	• If results are given separately for each sex, separate sub-forms should be used for each population.
2.8	**Number of Subjects**	–	• Example: Total number of subjects = 428.

	Question/Prompt	Response Options	Suggested Considerations
	Use the free-text field to add additional details on number of subjects if they are broken up by groups or quartiles. *[Free-text]*		
2.9	**What is the lifestage when the ADME data were collected?** Use the free-text field to provide additional lifestage notes. *[Select one; Free-text]*	• Prenatal: conception to birth • Infancy: 0–12 mo • Childhood: 13 mo to 11 yr • Adolescence: 12 to 20 yr • Adult: 21 to 65 yr • Elderly: >65 yr	• If there is more than one lifestage when ADME data were collected, add an additional population in another form.
2.10	**Exposure Levels** Use the free-text field to enter the numeric exposure levels (if known/estimated in an environmental medium such as air, water, dust, food, breast milk, etc.). *[Free-text]*	–	• Do not report levels in serum or urine for this question.
2.11	**Exposure Units** Use the free-text field to report the exposure units as presented in the paper. *[Free-text]*	–	• Examples: mg/kg-d; mg/m^3; ppm • Use "Not Reported" if appropriate.
2.12	**Exposure Duration** Use the free-text field to enter the details of the exposure duration if known. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y). ○ Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.
2.13	**Time Points Analyzed** Use the free-text field to enter the time points data were analyzed. *[Free-text]*	–	• Use abbreviations (h, d, wk, mon, y). ○ Examples: 28 d; 13 wk; 2 y • Use "Not Reported" if appropriate.
2.14	**Measured Tissues** Use the free-text field to enter the tissues measured in the study (e.g., plasma, breast milk, cord blood).	–	–

	Question/Prompt	Response Options	Suggested Considerations
	[Free-text]		
3	**Animal Studies** If the study does not contain an animal study, skip this section and move on to Section 4 – Mammalian Cells/In Vitro.		
3.1	**Population Name** *[Free-text]*	–	• Name a population (e.g., Females dams, PFOS). • Separate populations should be made for each chemical, species, population sex, lifestage where ADME data were collected, exposure route, etc. combination.
3.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• PFOA and PFOS are not metabolized, so "metabolism" is an unlikely selection.
3.3	**List the specific ADME Endpoints addressed.** Use the free-text field below to list all the ADME endpoints analyzed for this population. *[Free-text]*	–	–
3.4	**Identify the Exposure Route** *[Select one]*	• Inhalation (nose only) • Inhalation (whole head exposure) • Inhalation (whole body exposure) • Oral (diet) • Oral (drinking water) • Oral (gavage) • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intravenous, intranasal)	• If "other" option is selected, use the free-text field below to describe exposure route. • If the study population is offspring that were exposed "in utero/placental" AND by "lactational transfer," select "in utero/placental" and use the free-text field to note that lactational transfer also occurred. • If there is more than one exposure route identified, add an additional population in another form.
3.5	**What is the exposure vehicle?** *[Select one]*	• Diet • Water • Breast milk • In utero/placental transfer • Corn oil • Filtered air	• If "other" option is selected, use the free-text field below to describe exposure vehicle • If the study population is offspring that were exposed "in utero/placental" AND by "breast milk," select "in utero/placental" and use the free-text field to note that lactational transfer also occurred via breast milk.

	Question/Prompt	Response Options	Suggested Considerations
		• Olive oil • Ethanol • DMSO • Mineral oil • Corn oil:acetone • Other	
3.6	**What is the strain?** Use the free-text field to list the strain (e.g., Sprague-Dawley). *[Free-text]*	–	• If there is more than one species studied, add an additional population in another form.
3.7	**What is the sex?** *[Select one]*	• Male • Female • Male and Female	• If results are given separately for each sex, add an additional population in another form.
3.8	**What is the lifestage when the animal was dosed?** *[Select all that apply]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Prenatal o Non-human primates: conception to birth o Rodents: GD 0 to birth • Weaning o Non-human primates: 1–130 d (0.35 yr) o Rodents: PND 1−21 • Adolescent o Non-human primates: 130–1,825 d (0.35–5 yr) o Rodents: 21–50 d (3–7 wk) • Adult o Non-human primates: 5–35 yr o Rodents: >50 d (>7 wk) • Elderly o Non-human primates: >35 yr
3.9	**What is the reported average age of the animals when dosing began?** *[Free-text]*	–	• Use “Not Reported” if appropriate.
3.10	**What is the average initial body weight of the animals when dosing began?** *[Free-text]*	–	• Use “Not Reported” if appropriate.
3.11	**What is the lifestage when the ADME data were collected?**	• Prenatal • Weaning	• Prenatal o Non-human primates: conception to birth

	Question/Prompt	Response Options	Suggested Considerations
	[Select all that apply; Free-text]	• Adolescent • Adult • Elderly	○ Rodents: GD 0 to birth • Weaning ○ Non-human primates: 1–130 d (0.35 yr) ○ Rodents: PND 1−21 • Adolescent ○ Non-human primates: 130–1,825 d (0.35–5 yr) ○ Rodents: 21–50 d (3–7 wk) • Adult ○ Non-human primates: 5–35 yr ○ Rodents: >50 d (>7 wk) • Elderly ○ Non-human primates: >35 yr • Use the free-text field to provide additional lifestage notes. • If there is more than one lifestage when ADME data were collected, add an additional population in another form.
3.12	**What is the number of animals per dosing group?** Use the free-text field to report the number of animals per dosing group. *[Free-text]*	–	• Example: Control = 10, low dose = 20, high dose = 20; All groups = 20. • Use "Not Reported" if appropriate.
3.13	**Dose Levels** Use the free-text field to enter the numeric dose levels. *[Free-text]*	–	• Example: 0, 450, 900.
3.14	**Dose Units** Use the free-text field to report the dosage units as presented in the paper. *[Free-text]*	–	• Examples: mg/kg-d; mg/m^3; ppm • Use "Not Reported" if appropriate.
3.15	**Dose Duration** Use the free-text field to enter the details of the dose duration if known. *[Free-text]*	–	• Use abbreviations (h, d, wk, mo, y). • For reproductive and developmental studies, where possible instead include abbreviated age descriptions such as "GD 1–10" or "GD 2–PND10." ○ Examples: 14 d, 13 w (6 h/d x 5 d/wk); GD 2–PND 10.

	Question/Prompt	Response Options	Suggested Considerations
			• Use "Not Reported" if appropriate.
3.16	**Time Points Analyzed** Use the free-text field to enter the time points data were analyzed. *[Free-text]*	–	• Use abbreviations (h, d, wk, mo, y). ○ Examples: 14 or 28 d; 13 wk; 2 y. • Use "Not Reported" if appropriate.
3.17	**Measured Tissues** Use the free-text field to enter the tissues measured in the study (e.g., plasma, liver, adipose). *[Free-text]*	–	–
4	**Mammalian Cells/In Vitro** If the study does not contain an in vitro component, skip this section and move on to Section 5 – Notes.		
4.1	**Population Name** *[Free-text]*	–	• Name a population (e.g., Primary Human Hepatic, PFOA; A549, PFOS). • Separate populations should be made for each chemical, population sex, lifestage where ADME data were collected, exposure route, etc. combination. Use the "Clone" button to copy forms/information for easier extraction if the study populations are similar.
4.2	**Select whether the study looks at absorption, distribution, metabolism, and/or excretion.** *[Select all that apply]*	• Absorption • Distribution • Metabolism • Excretion	• PFOA and PFOS are not metabolized so "metabolism" is an unlikely selection.
4.3	**List the specific ADME Endpoints addressed.** Use the free-text field below to list all the ADME endpoints analyzed for this population. *[Free-text]*	–	–
4.4	**Does the study present data on protein binding?** *[Select one; Free-text]*	• Yes • No	• If "Yes" option is selected, use the free-text field to list the binding proteins.
4.5	**Does the study present data on active transport?** *[Select one; Free-text]*	• Yes • No	• If "Yes" option is selected, use the free-text field to list the transporters.

	Question/Prompt	Response Options	Suggested Considerations
4.6	**Cell Line Name or Tissue Source** Use the free-text field to list the cell line name or tissue source the cells were derived from. *[Free-text]*	–	• Examples: A549; liver tissue from adult Sprague-Dawley female rats. • If there is more than one cell line name or tissue source studied, add an additional population in another form.
4.7	**In vitro System** *[Select one; Free-text]*	• Mammalian cells • Cell-free system • In silico system • Other	• If "other" option is selected, use the free-text field below to describe the in vitro system. • If there is more than one in vitro source studied, add an additional population in another form.
4.8	**Select all study design elements that apply.** *[Select all that apply]*	• Multiple time points • Multiple cell/tissue types • Multiple dose levels	–
4.9	**Exposure Design** Use the free-text field to describe the exposure design, be as succinct as possible. *[Free-text]*	–	–
4.10	**What is the exposure vehicle?** Use the free-text field to describe the exposure vehicle, be as succinct as possible *[Free-text]*	–	–
4.11	**Dose Levels** Use the free-text field to enter the numeric dose levels. *[Free-text]*		• Example: 0, 450, 900.
4.12	**Dose Units** Use the free-text field to report the dosage units as presented in the paper. *[Free-text]*		• Examples: ppm; mg/mL • Use "Not Reported" if appropriate.
4.13	**Dose Duration** Use the free-text field to enter the details of the exposure duration. *[Free-text]*		• Use abbreviations (h, d, wk, mon, y). o Examples: 28 d; 13 wk; 2 y. • Use "Not Reported" if appropriate.
4.14	**Time Points Analyzed**		• Use abbreviations (h, d, wk, mon, y).

	Question/Prompt	Response Options	Suggested Considerations
	Use the free-text field to enter the time points data were analyzed. *[Free-text]*		○ Examples: 28 d; 13 wk; 2 y. • Use "Not Reported" if appropriate.
5	**Notes**		
5.1	**General Study Notes** *[Free-text]* Use the free-text field to add any general study notes not captured above that may be of interest to the QC reviewer or PBPK modelers	–	• Please indicate whether the study contains information on PFOA/PFOS that is broken up by linear/branched isomers. Use the following phrase: "Contains linear/branched isomer information."
5.2	**Notes from Initial Extractor to QA/QC Team** Use the free-text field to add any general study notes not captured above that may be of interest to the QC reviewer. *[Free-text]*	–	–
5.3	**Notes from QA/QC Team** Use the free-text field to add any general study notes not captured above that may be of interest to the PBPK modelers. *[Free-text]*	–	–

Notes: GD = gestational day; MOA = mode of action; PBPK = physiologically based pharmacokinetic; PND = postnatal day; ppm = parts per million; QA/QC = quality assurance/quality control; TK = toxicokinetic.

A.1.6.8 Mechanistic Screening and Light Data Extraction

All studies identified as mechanistic in title/abstract or full-text screening were imported into litstream and underwent additional screening. Studies that were confirmed to be PECO relevant underwent light data extraction. For each study, at least two reviewers (one primary screener/extractor and one QA reviewer) reviewed the full study and any study materials to respond to prompts pertaining to key study elements (e.g., tested species or population, mechanistic endpoint(s) evaluated, lifestage(s) at which evaluations were performed). Table A-16 below describes the prompts and response options that were used for screening studies with mechanistic evidence.

Table A-16. litstream Forms for Mechanistic Screening and Light Data Extraction

	Question/Prompt	Response Options	Suggested Considerations
1	**General Questions**		
1.1	**Does the article meet PECO criteria?** *[Select one]*	• Yes • No	–
1.2	**What PFAS did the study report?** *[Select all that apply]*	• PFOA • PFOS	–
1.3	**Publication Type** *[Select one]*	• Primary research • Review article	–
1.4	**Indicate if there is hazard ID or supplemental data for this study.** *[Select all that apply; Free-text]*	• Animal tox • Epi • ADME	• Use free-text field to provide an explanation.
2	**Human Studies Sub-Form** If the study does not contain a human study, skip this section and move on to Section 3 – Animal Studies Sub-Form.		
2.1	**Population/Study Group Name** *[Free-text]*	–	–
2.2	**Exposure Category** *[Select one; Free-text]*	• General environmental • Poisoning • Occupational • Developmental • Controlled experimental	• Free-text field if additional information is needed.
2.3	**Identify the Exposure Route** *[Select all that apply]*	• Inhalation • Oral • Dermal • Lactational transfer	• Free-text field to elaborate on “other” and “unknown” options.

	Question/Prompt	Response Options	Suggested Considerations
		• In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intranasal) • Unknown	
2.4	**What is the exposure vehicle?** *[Select one]*	• Drinking water • Diet • Breast milk • In utero/placental transfer • Occupational • Unknown • Other	• Free-text field to elaborate on "other" and "unknown" options.
2.5	**What is the lifestage when the mechanistic data were collected?** *[Select one; Free-text]*	• Prenatal • Infancy • Childhood • Adolescence • Adult • Elderly	• Free-text for lifestage notes.
2.6	**What is the corresponding health outcome system?** *[Select one]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.

	Question/Prompt	Response Options	Suggested Considerations
2.7	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics • Chromosome/DNA structure, function, repair or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, Morphology, or Morphometry • Other	• Free-text field for "other" option.
2.8	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, nontargeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction • Extracellular matrix or molecules; Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and Immune Response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	• Free-text field for "other" option.
2.9	**Mechanistic Endpoints** *[Free-text]*	–	• Free-text field to list mechanistic endpoints.
3	**Animal Studies Sub-Form** If the study does not contain an animal study, skip this section and move on to Section 4 – In Vitro Sub-Form.		
3.1	**Population/Study Group Name** *[Free-text]*	–	–
3.2	**What is the species?** *[Select one; Free-text]*	• Non-human primate • Zebrafish • Rat • Mouse • Rabbit • Guinea pig	• Free-text field to list species for "other rodent model" option.

	Question/Prompt	Response Options	Suggested Considerations
		• Other rodent model	
3.3	**What is the strain?** *[Free-text]*	–	–
3.4	**Identify the Exposure Route** *[Select one]*	• Inhalation (nose only) • Inhalation (whole head exposure) • Inhalation (whole body exposure) • Oral (diet) • Oral (drinking water) • Oral (gavage) • Dermal • Lactational transfer • In utero/placental transfer • Other (e.g., intraperitoneal, intramuscular, intravenous, intranasal)	• Free-text field for "other" option.
3.5	**What is the exposure vehicle?** *[Select one]*	• Diet • Water • Breast milk • In utero/placental transfer • Corn oil • Filtered air • Olive oil • Ethanol • DMSO • Mineral oil • Corn oil:acetone • Other	• Free-text field for other "other" option.
3.6	**What is the lifestage when the animal was dosed?** *[Select one; Free-text]*	• Prenatal • Weaning • Adolescent • Adult • Elderly	• Free-text field for lifestage notes.
3.7	**What is the lifestage when the mechanistic data were collected?** *[Select one; Free-text]*	• Prenatal • Weaning • Adolescent • Adult	• Free-text field for lifestage notes.

	Question/Prompt	Response Options	Suggested Considerations
		• Elderly	
3.8	**What is the corresponding health outcome system?** *[Select all that apply; Free-text]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free-text field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.
3.9	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics chromosome/DNA structure, function, repair, or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, Morphology, or Morphometry • Other	• Free-text field for "other" option.
3.10	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, nontargeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction	• Free-text field for "other" option.

	Question/Prompt	Response Options	Suggested Considerations
		• Extracellular matrix or molecules • Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and Immune Response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	
3.11	**Mechanistic Endpoints** *[Free-text]*		• Free-text field to list mechanistic endpoints.
4	**In Vitro Sub-Form** If the study does not contain an in vitro component, skip this section and move on to Section 5 – Notes.		
4.1	**Population/Study Group Name** *[Free-text]*	–	–
4.2	**Does the study present data on protein binding?** *[Select one; Free-text]*	• Yes • No	• Free-text field if "Yes" to list binding proteins.
4.3	**Does the study present data on active transport?** *[Select one; Free-text]*	• Yes • No	• Free-text field if "Yes" to list transporters.
4.4	**In Vitro System** *[Select one; Free-text]*	• Mammalian cells • Cell-free system • In silico system • Other	• Free-text field for "other" option.
4.5	**If a cellular model is used, is it a cell line or primary cells?** *[Select one]*	• Cell line • Primary cell	–
4.6	**Cell Or Tissue Source for In Vitro/Ex Vivo Studies** *[Select one; Free-text]*	• Human • Zebrafish • Non-human primate • Rat • Mouse • Rabbit • Guinea pig	• Free-text field to list "other rodent model" option.

	Question/Prompt	Response Options	Suggested Considerations
		• Other rodent model	
4.7	**What is the corresponding health outcome system?** *[Select all that apply; Free-text]*	• Cancer • Cardiovascular • Dental • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Lymphatic • Metabolic • Musculoskeletal/connective tissue • Nervous • Ocular • Renal • Reproductive • Respiratory • Systemic/whole body • Other	• Free-text field for "other" option, includes endpoints that do not fit neatly into any one health outcome system.
4.8	**Mechanistic Category** *[Select all that apply; Free-text]*	• Epigenetics chromosome/DNA structure, function, repair, or integrity • Gene expression and transcription • Protein expression, synthesis, folding, function, transport, localization, or degradation • Metabolomics • Cell or organelle structure, motility, or integrity • Structure, morphology, or morphometry • Other	• Free-text field for "other" option.
4.9	**Mechanistic Pathway** *[Select all that apply; Free-text]*	• Angiogenic, antiangiogenic, vascular tissue remodeling • Atherogenesis and clot formation • Big data, nontargeted analysis • Cell growth, differentiation, proliferation, or viability • Cell signaling or signal transduction	• Free-text field for "other" option.

	Question/Prompt	Response Options	Suggested Considerations
		• Extracellular matrix or molecules • Fatty acid synthesis, metabolism, storage, transport, binding, β-oxidation • Hormone function • Inflammation and immune response • Oxidative stress • Renal dysfunction • Vasoconstriction/vasodilation • Xenobiotic metabolism • Other	
4.10	**Mechanistic Endpoints** *[Free-text]*	–	–
5	**Notes**		
5.1	**General Study Notes** Use the free-text field to add any general study notes not captured above that may be of interest to the QC reviewer or PBPK modelers. *[Free-text]*	–	• Please indicate whether the study contains information on PFOA/PFOS that is broken up by linear/branched isomers. Use the following phrase: "Contains linear/branched isomer information."
5.2	**Notes from Initial Extractor to QA/QC Team** Use the free-text field to add any general study notes not captured above that may be of interest to the QC reviewer. *[Free-text]*	–	–
5.3	**Notes from QA/QC Team** Use the free-text field to add any general study notes not captured above that may be of interest to the PBPK modelers. *[Free-text]*	–	–

Notes: DMSO = dimethyl sulfoxide; DNA = deoxyribonucleic acid; QA/QA = quality assurance/quality control.

A.1.7 Study Quality Evaluation Overview

After literature search results were screened and inventoried, epidemiological and animal toxicological studies that met PECO criteria underwent study quality evaluation to assess each study's validity and utility. As outlined in the IRIS Handbook (U.S. EPA, 2022c), the key concerns during the review of epidemiological and animal toxicological studies are potential bias (factors that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect; low sensitivity is a bias toward the null when an effect exists). Study quality evaluations produce overall judgments about confidence in the reliability of study results. The general approach for study quality evaluation is outlined in Figure A-1, which has been adapted from Figure 4-1 in the IRIS Handbook (U.S. EPA, 2022c) (previously Figure 6-1 in the draft IRIS Handbook (U.S. EPA, 2020a)). Study quality evaluations were performed using the structured platform for study evaluation housed within EPA's Health Assessment Workplace Collaborative (HAWC).

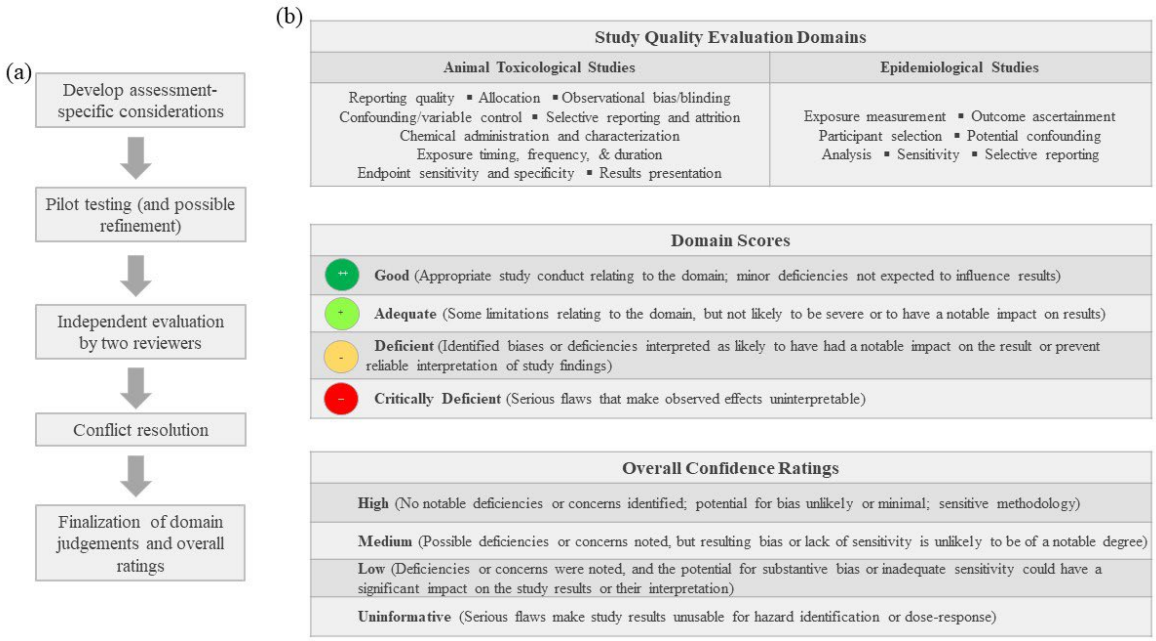


Figure A-1. Overview of Study Quality Evaluation Approach

(a) An overview of the study quality evaluation process; (b) Evaluation domains and ratings definitions (i.e., domain scores and overall confidence ratings, performed on an outcome-specific basis as applicable).

The overall aims of study quality evaluation are the same for both epidemiological and animal toxicological studies, but some aspects of the approaches are different. Therefore, study quality evaluation procedures for epidemiological and animal toxicological studies are described separately in the following sections. In brief, at least two primary reviewers independently judged the reliability of the study results according to multiple study quality evaluation domains presented in the IRIS Handbook. Domain-specific core and prompting questions are provided to guide the reviewer in assessing different aspects of study design and conduct related to reporting, risk of bias, and study sensitivity. For each domain, each reviewer assigned a rating of good, adequate, deficient (or "not reported," which carried the same functional interpretation as deficient), or critically deficient (see Figure A-1 and Table A-17). A QA reviewer (in accordance

with protocols outlined in the IRIS Handbook) engaged in conflict resolution with the two independent reviewers as needed and made a final determination (reflected as study confidence ratings; see Figure A-1 and Table A-18) regarding each health outcome or outcome grouping of interest; thus, different judgments were possible for different health outcomes within the same study. The overall confidence rating should, to the extent possible, reflect interpretations of the potential influence on the results across all domains. The rationale supporting the overall confidence rating is documented clearly and consistently and includes a brief description of any important study strengths and/or limitations and their potential impact(s) on the overall confidence.

The specific study limitations identified during study quality evaluation were carried forward to inform the synthesis of findings within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform judgments in isolation).

Studies containing PBPK, mechanistic or ADME data did not undergo study quality evaluation, as study quality domains for these types of studies are not currently available (U.S. EPA, 2022b).

Table A-17. Possible Domain Scores for Study Quality Evaluation

Good	Intended to represent a judgment that there was appropriate study conduct relating to the domain (as defined by consideration of the criteria listed below), and any minor deficiencies that were noted would not be expected to influence interpretation of the study findings.
Adequate	Indicates a judgment that there were study design limitations relating to the domain (as defined by consideration of the criteria listed below), but that those limitations are not likely to be severe and are expected to have minimal impact on interpretation of the study findings.
Deficient	Denotes identified biases or limitations that are interpreted as likely to have had a substantial impact on the results or that prevent reliable interpretation of the study findings. **Note: Not reported** indicates that the information necessary to evaluate the domain was not available in the study. Generally, this term carries the same functional interpretation as **Deficient** for the purposes of the study confidence classification.
Critically Deficient	Reflects a judgment that the study design limitations relating to the domain introduced a flaw so serious that the study should not be used without exceptional justification (e.g., it is the only study of its kind and may highlight possible research gaps). This judgment should only be used if there is an interpretation that the limitation(s) would be the primary driver of any observed effect(s), or if it makes the study findings uninterpretable.

Table A-18. Overall Study Confidence Classifications

***High* Confidence**	No notable concerns were identified (e.g., most or all domains rated **Good**).
***Medium* Confidence**	Some concerns are identified but expected to have minimal impact on the interpretation of the results (e.g., most domains rated **Adequate** or **Good**; may include studies with **Deficient** ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.

***Low* Confidence**	Identified concerns are expected to significantly impact the study results or their interpretation (e.g., generally, **Deficient** ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis.
Uninformative	Serious flaw(s) make the study results unusable for informing hazard identification (e.g., generally, **Critically Deficient** rating in any domain; many **Deficient** ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.

A.1.7.1 Study Quality Evaluation for Epidemiological Studies

Study quality evaluation domains for assessing risk of bias and sensitivity in epidemiology studies of health effects are: participant selection, exposure measurement, outcome ascertainment, potential confounding, analysis, study sensitivity, and selective reporting. As noted in the IRIS Handbook, this framework is adapted from the Risk Of Bias in Nonrandomized Studies of Interventions (ROBINS-I) tool (https://methods.cochrane.org/methods-cochrane/robins-i-tool), modified by IRIS for use with the types of studies more typically encountered in EPA's work. As outlined in Section A.1.7 of this appendix, study quality evaluations are performed for a set of established domains, and core and prompting questions are provided for each domain to guide the reviewer. Each domain is assigned a score of **Good**, **Adequate**, **Deficient**, **Not Reported**, or **Critically Deficient**, and rationales to support the scores are developed. Once all domains are evaluated, a confidence rating of ***High***, ***Medium***, or ***Low*** confidence or ***Uninformative*** is assigned.

The tables presented in the following sections describe the epidemiological study quality evaluation domains and the prompting questions and considerations for assessing study quality in relation to each domain.

A.1.7.1.1 Participant Selection

The aim of study quality evaluation for this domain is to ascertain whether the reported information indicates that selection in or out of the study (or analysis sample) and participation was not likely to be biased (i.e., the exposure-outcome distribution of the participants is likely representative of the exposure-outcome distribution in the overall population of eligible persons) (Table A-19).

Table A-19. Study Quality Evaluation Considerations for Participant Selection

Core Question: Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
For longitudinal cohort: Did participants volunteer for the cohort based on knowledge of exposure and/or preclinical disease symptoms? Was entry into the cohort or continuation in the cohort related to exposure and outcome? ***For occupational cohort:*** Did entry into the cohort begin with the start of the exposure? Was follow-up or outcome assessment incomplete, and if so, was follow-up related to both exposure and outcome status? Could exposure produce symptoms that would result in a change in work assignment/work status ("healthy worker survivor effect")? ***For case-control study:*** Were controls representative of population and time periods from which cases were drawn?	Were differences in participant enrollment and follow-up evaluated to assess the potential for bias? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)? Were appropriate analyses performed to address changing exposures over time in relation to symptoms? Is there a comparison of participants and	**Good**	• Minimal concern for selection bias based on description of recruitment process (e.g., selection of comparison population, population-based random sample selection, recruitment from sampling frame including current and previous employees) such that study participants were unlikely to differ from a larger cohort based on recruitment or enrollment methods (or data provided to confirm a lack of difference). • Exclusion and inclusion criteria specified and would not be likely to induce bias. • Participation rate is reported at all steps of study (e.g., initial enrollment, follow-up, selection into analysis sample). If rate is not high, there is appropriate rationale for why it is unlikely to be related to exposure (e.g., comparison between participants and nonparticipants or other available information indicates differential selection is not likely). • Comparison groups are similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers).

Core Question: Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?			
Are hospital controls selected from a group whose reason for admission is independent of exposure? Could recruitment strategies, eligibility criteria, or participation rates result in differential participation relating to both disease and exposure? ***For population-based survey:*** Was recruitment based on advertisement to people with knowledge of exposure, outcome, and hypothesis?	nonparticipants to address whether differential selection is likely?	**Adequate**	• Enough of a description of the recruitment process (i.e., recruitment strategy, participant selection or case ascertainment) to be comfortable that there is no serious risk of bias. • Inclusion and exclusion criteria specified and would not induce bias. • Participation rate is incompletely reported for some steps of the study, but available information indicates participation is unlikely to be related to exposure. • Comparison groups are largely similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers) or these are mostly accounted for in the study analysis.
		Deficient	• Little information on recruitment process, selection strategy, sampling framework and/or participation OR aspects of these processes raises the likelihood of bias (e.g., healthy worker effect, survivor bias). *Example: Enrollment of "cases" from a specific clinic setting (e.g., diagnosed autism), which could be biased by referral practices and services availability, without consideration of similar selection forces affecting recruitment of controls.*
		Critically Deficient	• Aspects of the processes for recruitment, selection strategy, sampling framework, or participation result in concern that the likelihood of selection bias is high (e.g., convenience sample with no information about recruitment and selection, cases and controls are recruited from different sources with different likelihood of exposure, recruitment materials stated outcome of interest and potential participants are aware of or are concerned about specific exposures). • Convenience sample, and recruitment and selection not described. • Case report, case series, or other study designs lacking a comparison group (these should be excluded if they do not meet assessment PECO criteria).

A.1.7.1.2 Exposure Measurement

This domain may need to be evaluated multiple times for a single study if more than one measurement of exposure is assessed. Therefore, different sets of criteria may be applied for different exposure assessments in the same study. Table A-20 outlines criteria that apply across exposure assessments (first row), and specific *additional* criteria for specific types of exposure assessments (e.g., biomarkers, occupational) in subsequent rows.

Table A-20. Study Quality Evaluation Considerations for Exposure Measurement

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?			
Prompting Questions	**Follow-Up Questions**	**Suggested Considerations**	
Does the exposure measure capture the variability in exposure among the participants, considering intensity, frequency, and duration of exposure? Does the exposure measure reflect a relevant time window? If not, can the relationship between measures in this time and the relevant time window be estimated reliably? Was the exposure measurement likely to be affected by a knowledge of the outcome? Was the exposure measurement likely to be affected by the presence of the outcome (i.e., reverse causality)?	Is the degree of exposure misclassification likely to vary by exposure level? If the correlation between exposure measurements is of concern, is there an adequate statistical approach to ameliorate variability in measurements? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Valid exposure assessment methods used, which represent the etiologically relevant time period for reported effects (e.g., exposure during a critical developmental window or exposure preceding the evaluation of the outcome). • Exposure misclassification is expected to be minimal.
		Adequate	• Valid exposure assessment methods used, which represent the etiologically relevant time period of interest. • Exposure misclassification may exist but is not expected to greatly impact the effect estimate.
		Deficient	• Specific knowledge about the exposure and outcome raise concerns about reverse causality, but there is uncertainty whether it is influencing the effect estimate. • Exposed groups are expected to contain a notable proportion of unexposed or minimally exposed individuals, the method did not capture important temporal or spatial variation, or there is other evidence of exposure misclassification that would be expected to notably change the effect estimate.
		Critically Deficient	• Exposure measurement does not characterize the etiologically relevant time period of exposure or is not valid. • There is evidence that reverse causality is very likely to account for the observed association. • Exposure measurement was not independent of outcome status.

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?

Additional prompting questions for biomarkers of exposure: Is a standard assay used? What are the intra- and inter-assay coefficients of variation? Is the assay likely to be affected by contamination? Are values less than the limit of detection dealt with adequately? What exposure time period is reflected by the biomarker? If the half-life is short, what is the correlation between serial measurements of exposure?	***Additional suggested considerations for biomarkers of exposure (should be evaluated in addition to the general considerations above):***	
	Good	• Use of appropriate analytic method such as [specific gold standard exposure assessment method for the exposure of interest].
	Adequate	• Use of appropriate (but not gold standard) analytic method.
	Deficient	• Did not identify analytical methods used to measure exposure. • Failure to report LOD, percentage less than LOD, and methods used to account for values below the LOD. • Failure to report QA/QC measures and results.
	Critically Deficient	• Use of inappropriate analytical method or use of an appropriate method with measurement issues that are likely to impact the interpretation of results.
Additional prompting questions for case-control studies of occupational exposures: Is exposure based on a comprehensive job history describing tasks, setting, time period, and use of specific materials?	***Additional suggested considerations for occupational exposures (should be evaluated in addition to the general considerations above):***	
	Good	• Describes the use of personal protective equipment. • Confirmed contrast in exposure between groups using biomarker measurements. • Expert assessment method based on a detailed lifetime occupational history and using a high-quality, validated job exposure matrix (JEM) or a JEM that incorporates industry, time period, population/country, tasks, and material used.
	Adequate	• Describes the use of personal protective equipment. • Confirmed contrast in exposure between groups using biomarker measurements.
	Deficient	• Expert assessment method based on incomplete occupational history information (lacking job titles, employers, industries, start and finish years, number of hours worked per day, number of days worked per week, tasks performed, or materials used) – may be Critically Deficient, depending on severity of this limitation.

Core Question: Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?		
	Critically Deficient	• JEM with data indicating it cannot differentiate between exposure levels over time, area, or between individuals.

Notes: JEM = job exposure matrix; LOD = limit of detection; QA/QC = quality assurance/quality control.

PFAS-Specific Exposure Measurement Study Quality Evaluation Criteria

Standard analytical methods of individual PFAS in serum or whole blood using quantitative techniques, such as liquid chromatography triple quadrupole mass spectrometry, are considered well-established methods (Table A-21).

Table A-21. Study Quality Evaluation Considerations for PFAS-Specific Exposure Measurement

Rating	Criteria
Good	• Evidence that exposure was consistently assessed using well-established analytical methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma). **OR** • Exposure was assessed using less established methods (e.g., measurement of PFAS in breast milk) or methods that indirectly measure exposure (e.g., drinking water concentrations and residential location/history, questionnaire or occupational exposure assessment by a certified industrial hygienist) that are supported by well-established methods (i.e., inter-methods validation: one method vs. another) in the target population of interest. **And all the following:** • Exposure was assessed in a relevant time-window (i.e., temporality is established, and sufficient latency occurred prior to disease onset) for development of the outcome based on current biological understanding. • There is evidence that sufficient exposure data measurements are above the limit of quantification for the assay. • The laboratory analysis included data on standard quality control measures with demonstrated precision and accuracy.
Adequate	• Exposure was assessed using less established methods or indirect measures that are validated but not in the target population of interest. **OR** • Evidence that exposure was consistently assessed using methods described in Good, but there were some concerns about quality control measures or other potential for non-differential misclassification. **And all the following:** • Exposure was assessed in a relevant time-window for development of the outcome. • There is evidence that sufficient exposure data measurements are above the limit of quantification for the assay. • The laboratory analysis included some data on standard quality control measures with demonstrated precision and accuracy.
Deficient	**Any of the following:** • Some concern, but no direct evidence, that the exposure was assessed using methods that have not been validated or empirically shown to be consistent with methods that directly measure exposure. • Exposure was assessed in a relevant time window(s) for development of the outcome, but there could be some concern about the potential for bias due to reverse causality[a] between exposure and outcome, yet no direct evidence that it is present; or has somehow been mitigated by the design, etc.
Critically Deficient	**Any of the following:** • Exposure was assessed in a time window that is unknown or not relevant for development of the outcome. This could be due to clear evidence of bias from reverse causality between exposure and outcome, or other concerns such as the lack of temporal ordering of exposure and disease onset, insufficient latency, or having exposure measurements that are not reliable measures of exposure during the etiologic window(s). • Direct evidence that bias was likely because the exposure was assessed using methods with poor validity.

Rating	Criteria
	• Evidence of differential exposure misclassification (e.g., differential recall of self-reported exposure). • There is evidence that an insufficient number of the exposure data measurements were above the limit of quantification for the assay.

Notes:

[a] Reverse causality refers to a situation where an observed association between exposure and outcome is not due to causality from exposure to outcome, but rather due to the outcome of interest causing a change in the measured exposure.

A.1.7.1.3 Outcome Ascertainment

This domain may need to be evaluated multiple times for a single study if more than one PECO-relevant outcome is reported. Therefore, outcome-specific criteria (Radke et al., 2019) may be applied for each outcome measured in a study. Table A-22 presents criteria that apply across outcomes.

Table A-22. Study Quality Evaluation Considerations for Outcome Ascertainment

Core Question: Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?

Prompting Questions	Follow-Up Questions		Suggested Considerations
Is outcome ascertainment likely to be affected by knowledge of, or presence of, exposure (e.g., consider access to health care, if based on self-reported history of diagnosis)? ***For case-control studies:*** Is the comparison group without the outcome (e.g., controls in a case-control study) based on objective criteria with little or no likelihood of inclusion of people with the disease? ***For mortality measures:*** How well does cause of death data reflect occurrence of the disease in an individual? How well do mortality data reflect incidence of the disease? ***For diagnosis of disease measures:*** Is the diagnosis based on standard clinical criteria? If it is based on self-report of the diagnosis, what is the validity of this measure? ***For laboratory-based measures (e.g., hormone levels):*** Is a standard assay used? Does the assay have an acceptable level of inter-assay variability? Is the sensitivity of the assay appropriate for the	Is there a concern that any outcome misclassification is nondifferential, differential, or both? What is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• High certainty in the outcome definition (i.e., specificity and sensitivity), minimal concerns with respect to misclassification. • Assessment instrument was validated in a population comparable to the one from which the study group was selected.

Core Question: Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?

outcome measure in this study population? Were QA/QC measures and results reported?		
	Adequate	• Moderate confidence that outcome definition was specific and sensitive, some uncertainty with respect to misclassification but not expected to greatly change the effect estimate. • Assessment instrument was validated but not necessarily in a population comparable to the study group.
	Deficient	• Outcome definition was not specific or sensitive. • Uncertainty regarding validity of assessment instrument.
	Critically Deficient	• Invalid/insensitive marker of outcome. • Outcome ascertainment is very likely to be affected by knowledge of, or presence of, exposure. **Note:** Lack of blinding should not be automatically construed to be ***Critically Deficient***.

A.1.7.1.4 Potential Confounding

The aim of evaluating this domain is to ascertain whether confounding of the relationship between the exposure and health outcome of interest is likely to exist, and if so, whether it was considered in the design and/or analysis of the study (Table A-23). Co-exposures to other PFAS were considered in this domain.

Table A-23. Study Quality Evaluation Considerations for Confounding

Core Question: Is confounding of the effect of the exposure likely?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
Is confounding adequately addressed by considerations in: • Participant selection (matching or restriction)? • Accurate information on potential confounders and statistical adjustment procedures? • Lack of association between confounder and outcome, or confounder and exposure in the study? • Information from other sources? Is the assessment of confounders based on a thoughtful review of published literature, potential relationships (e.g., as can be gained through directed acyclic graphing), and minimizing potential overcontrol (e.g., inclusion of a variable on the pathway between exposure and outcome)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Conveys strategy for identifying key confounders. This may include: a priori biological considerations, published literature, causal diagrams, or statistical analyses; with recognition that not all "risk factors" are confounders. • Inclusion of potential confounders in statistical models not based solely on statistical significance criteria (e.g.,$p < 0.05$ from stepwise regression). • Does not include variables in the models that are likely to be influential colliders or intermediates on the causal pathway. • Key confounders are evaluated appropriately and considered to be unlikely sources of substantial confounding. This often will include: o Presenting the distribution of potential confounders by levels of the exposure of interest and/or the outcomes of interest (with amount of missing data noted); o Consideration that potential confounders were rare among the study population, or were expected to be poorly correlated with exposure of interest; o Consideration of the most relevant functional forms of potential confounders; o Examination of the potential impact of measurement error or missing data on confounder adjustment; o Presenting a progression of model results with adjustments for different potential confounders, if warranted.
		Adequate	• Similar to **Good** but may not have considered all potential confounders (though all key confounders were considered), or less detail may be available on the evaluation of confounders (e.g., sub-bullets in **Good**). It is possible that residual confounding could explain part of the observed effect, but concern is minimal.

Core Question: Is confounding of the effect of the exposure likely?		
	Deficient	• All key confounders were not considered by design or in the statistical analysis. • Assessed an outcome based on report of medical diagnosis that would have required access to a health professional (e.g., autism, ADHD, depression) and failed to consider some marker of socioeconomic status (e.g., maternal education, household income, marital status, crowding, poverty, job status) as a potential confounder. • Does not include variables in the models that are likely to be influential colliders or intermediates on the causal pathway. **And any of the following:** • The potential for bias to explain some of the results is high based on an inability to rule out residual confounding, such as a lack of demonstration that key confounders of the exposure-outcome relationships were considered; • Descriptive information on key confounders (e.g., their relationship relative to the outcomes and exposure levels) is not presented; or • Strategy of evaluating confounding is unclear or is not recommended (e.g., only based on statistical significance criteria or stepwise regression (forward or backward elimination)).
	Critically Deficient	• Includes variables in the models that are colliders and/or intermediates in the causal pathway, indicating that substantial bias is likely from this adjustment; or • Substantial confounding is likely present and not accounted for, such that all of the results were most likely due to bias. • If confounders not considered by design or in the analysis (e.g., only simple correlations presented).

Notes: ADHD = attention deficit hyperactivity disorder.

A.1.7.1.5 Analysis

Information relevant to evaluation of analysis includes, but is not limited to, the extent (and if applicable, treatment) of missing data for exposure, outcome, and confounders, approach to modeling, classification of exposure and outcome variables (continuous vs. categorical), testing of assumptions, sample size for specific analyses, and relevant sensitivity analyses (Table A-24).

Table A-24. Study Quality Evaluation Considerations for Analysis

Core Question: Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?			
Prompting Questions	**Follow-Up Questions**	**Suggested Considerations**	
Are missing outcome, exposure, and covariate data recognized, and if necessary, accounted for in the analysis? Does the analysis appropriately consider variable distributions and modeling assumptions? Does the analysis appropriately consider subgroups of interest (e.g., based on variability in exposure level or duration or susceptibility)? Is an appropriate analysis used for the study design? Is effect modification considered, based on considerations developed a priori? Does the study include additional analyses addressing potential biases or limitations (i.e., sensitivity analyses)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Good**	• Use of an optimal characterization of the outcome variable. • Quantitative results presented (effect estimates and confidence limits or variability in estimates (e.g., standard error, standard deviation); i.e., not presented only as a p-value or "significant"/"not significant"). • Descriptive information about outcome and exposure provided (where applicable). • Amount of missing data noted and addressed appropriately (discussion of selection issues—missing at random vs. differential). • Where applicable, for exposure, includes LOD (and percentage below the LOD), and decision to use log transformation. • Includes analyses that address robustness of findings, e.g., examination of exposure-response (explicit consideration of nonlinear possibilities, quadratic, spline, or threshold/ceiling effects included, when feasible); relevant sensitivity analyses; effect modification examined based only on a priori rationale with sufficient numbers. • No deficiencies in analysis evident. Discussion of some details may be absent (e.g., examination of outliers).
		Adequate	Same as Good, except: • Descriptive information about exposure provided (where applicable) but may be incomplete; might not have discussed missing data, cut points, or shape of distribution. • Includes analyses that address robustness of findings (examples in Good), but some important analyses are not performed.

Core Question: Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?		
	Deficient	• Descriptive information about exposure levels not provided (where applicable). • Effect estimate and p-value presented, without standard error or confidence interval (where applicable). • Results presented as statistically "significant"/"not significant."
	Critically Deficient	• Results of analyses of effect modification examined without clear a priori rationale and without providing main/principal effects (e.g., presentation only of statistically significant interactions that were not hypothesis driven). • Analysis methods are not appropriate for design or data of the study.

Notes: LOD = limit of detection.

A.1.7.1.6 Selective Reporting

This domain concerns the potential for misleading results that can arise from selective reporting (e.g., of only a subset of the measures or analyses that were conducted). The concept of selective reporting involves the selection of results from among multiple outcome measures, multiple analyses, or different subgroups, based on the direction or magnitude of these results (e.g., presenting "positive" results) (Table A-25).

Table A-25. Study Quality Evaluation Considerations for Selective Reporting

Core Question: Is there reason to be concerned about selective reporting?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
Were results provided for all the primary analyses described in the methods section? Is there appropriate justification for restricting the amount and type of results that are shown? Are only statistically significant results presented?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?	**Adequate**	• The results reported by study authors are consistent with the primary and secondary analyses described in a registered protocol or methods paper. **OR** • The authors described their primary (and secondary) analyses in the methods section and results were reported for all primary analyses.
	–	**Deficient**	• Concerns were raised based on previous publications, a methods paper, or a registered protocol indicating that analyses were planned or conducted that were not reported, or that hypotheses originally considered to be secondary were represented as primary in the reviewed paper. • Only subgroup analyses were reported; results for the entire group were omitted without any justification (e.g., to address effect measure modification). • Of the PECO-relevant outcomes examined, only statistically significant results were reported.

A.1.7.1.7 Study Sensitivity

The aim of evaluation of this domain is to determine if there are features of the study that affect its ability to detect a true association (Table A-26). Some of the study features that can affect study sensitivity may have already been included in the outcome, exposure, or other categories, such as the validity of a method used to ascertain an outcome, the ability to characterize exposure in a relevant time period for the outcome under consideration, selection of affected individuals out of the study population, or inappropriate inclusion of intermediaries in a model.

Other features may not have been addressed, and so should be included here. Examples include the exposure range (e.g., the contrast between the "low" and "high" exposure groups within a study), the level or duration of exposure, and the length of follow-up. In some cases (for very rare outcomes), sample size or number of observed cases may also be considered within this "sensitivity" category.

Table A-26. Study Quality Evaluation Considerations for Study Sensitivity

Core Question: Is there a concern that sensitivity of the study is not adequate to detect an effect?			
Prompting Questions	**Follow-Up Questions**		**Suggested Considerations**
Is the exposure range/contrast adequate to detect associations that are present? Was the appropriate (at risk) population included? Was the length of follow-up adequate? Is the time/age of outcome ascertainment optimal given the interval of exposure and the health outcome? Are there other aspects related to risk of bias or otherwise that raise concerns about sensitivity?	–	**Adequate**	• The range of exposure levels provides adequate variability to evaluate primary hypotheses in study. • The population was exposed to levels expected to have an impact on response. • The study population was sensitive to the development of the outcomes of interest (e.g., ages, lifestage, sex). • The timing of outcome ascertainment was appropriate given expected latency for outcome development (i.e., adequate follow-up interval). • The main effects and stratified analyses were fairly precise (relatively small confidence bounds). • The study was adequately powered to observe an effect. Consider sample size, precision (e.g., width of confidence intervals), anticipated power, exposure ranges and contrasts. • No other concerns raised regarding study sensitivity.
	–	**Deficient**	• Concerns were raised about the issues described for ***Adequate*** that are expected to notably decrease the sensitivity of the study to detect associations for the outcome.

A.1.7.1.8 Overall Confidence

Table A-27. Study Quality Evaluation Considerations for Overall Study Confidence – Epidemiological Studies

Provide judgment and rationale for each endpoint or groups of endpoints. The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results. Evaluation Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?

Prompting Questions		Suggested Considerations
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects? *NOTE: Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias toward the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	**High confidence**	• No notable concerns are identified (e.g., most or all domains rated Good).
	Medium confidence	• Some concerns are identified but expected to have minimal impact on the interpretation of the results. (e.g., most domains rated Adequate or Good; may include studies with Deficient ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.
	Low confidence	• Identified concerns are expected to significantly impact on the study results or their interpretation (e.g., generally, Deficient ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note).
	Uninformative	• Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, Critically Deficient rating in any domain; many Deficient ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.

A.1.7.2 Study Quality Evaluation for Animal Toxicological Studies

As noted in the IRIS Handbook, the approach to evaluating study quality for animal toxicological studies considers study design and experimental conduct in the context of reporting quality, risk of bias, and study sensitivity. As outlined in Section A.1.7 of this appendix, study quality evaluations are performed for a set of established domains, and core and prompting questions are provided for each domain to guide the reviewer. Each domain is assigned a score of **Good**, **Adequate**, **Deficient**, **Not Reported**, or **Critically Deficient,** and rationales to support the scores are developed. Once all domains are evaluated, a confidence rating of ***High***, ***Medium***, or ***Low*** confidence or ***Uninformative*** is assigned for each endpoint/outcome from the study.

The tables in the following sections describe the core and prompting questions and considerations for assessing each domain during animal toxicological study quality evaluation. Tables within each section also provide example evaluations for each domain.

A.1.7.2.1 Reporting Quality

Evaluation of this domain is focused on ascertaining whether the study reports enough information to enable evaluation of the study (Table A-28).

Table A-28. Study Evaluation Considerations for Reporting Quality

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?			
Prompting Questions		**Suggested Considerations**	**Example Answers**
Does the study report the following? **<u>Critical information</u> necessary to perform study evaluation:** • Species; test article name; levels and duration of exposure; route (e.g., oral; inhalation); qualitative or quantitative results for at least one endpoint of interest **<u>Important information</u> <u>for evaluating the study methods:</u>** • Test animal: strain, sex, source, and general husbandry procedures • Exposure methods: source, purity, method of administration • Experimental design: frequency of exposure, animal age and lifestage during exposure and at endpoint/outcome evaluation • Endpoint evaluation methods: assays or procedures used to measure the endpoints/outcomes of interest	**Good**	• Minimal concern for selection bias based on description of recruitment process (e.g., selection of comparison population, population-based random sample selection, recruitment from sampling frame including current and previous employees) such that study participants were unlikely to differ from a larger cohort based on recruitment or enrollment methods (or data provided to confirm a lack of difference). • Exclusion and inclusion criteria specified and would not be likely to induce bias. • Participation rate is reported at all steps of study (e.g., initial enrollment, follow-up, selection into analysis sample). If rate is not high, there is appropriate rationale for why it is unlikely to be related to exposure (e.g., comparison between participants and nonparticipants or other available information indicates differential selection is not likely). • Comparison groups are similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers).	Good. Important information is provided for test species, strain, sex, age, exposure methods, experimental design, endpoint evaluations and the presentation of results. The authors report that "the study was conducted in compliance with the OECD guidelines for Good Laboratory Practice [c(81) 30 (Final)]."

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?			
Note: • Reviewers should reach out to authors to obtain missing information when studies are considered key for hazard evaluation and/or dose-response. • This domain is limited to reporting. Other aspects of the exposure methods, experimental design, and endpoint evaluation methods are evaluated using the domains related to risk of bias and study sensitivity.	**Adequate**	• Enough of a description of the recruitment process (i.e., recruitment strategy, participant selection or case ascertainment) to be comfortable that there is no serious risk of bias. • Inclusion and exclusion criteria specified and would not induce bias. • Participation rate is incompletely reported for some steps of the study, but available information indicates participation is unlikely to be related to exposure. • Comparison groups are largely similar with respect to factors expected to influence exposure-outcome relationship (confounders, effect measure modifiers) or these are mostly accounted for in the study analysis.	Adequate. All critical information is reported but some important information is missing. Specifically, it is unclear what strain of rats was used.
	Deficient	• Little information on recruitment process, selection strategy, sampling framework and/or participation OR aspects of these processes raises the likelihood of bias (e.g., healthy worker effect, survivor bias). *Example: Enrollment of "cases" from a specific clinic setting (e.g., diagnosed autism), which could be biased by referral practices and services availability, without consideration of similar selection forces affecting recruitment of controls.*	Deficient. All critical information is reported, but some important information is missing that makes additional study evaluation and interpretation of the results difficult. Specifically, it is not reported (and cannot be inferred) what age/lifestage the animals were at outcome evaluation.
	Critically Deficient	• Aspects of the processes for recruitment, selection strategy, sampling framework, or participation result in concern that the likelihood of selection bias is high (e.g., convenience sample with no information about recruitment and selection, cases and controls are recruited from different sources with different likelihood of exposure, recruitment materials stated outcome of interest and	**Example 1**: Critically Deficient. Critical information is missing. Authors did not report the duration of the exposure or the results (qualitative or quantitative). **Example 2:** Critically Deficient. Critical information is missing. The study reports animals were exposed to per-and polyfluoroalkyl substances (PFAS), but the specific chemicals tested were not provided.

Core Question: Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?		
		potential participants are aware of or are concerned about specific exposures). • Convenience sample, and recruitment and selection not described. • Case report, case series, or other study designs lacking a comparison group (these should be excluded if they do not meet assessment PECO criteria).

Notes: OECD = Organisation for Economic Co-operation and Development.
For the Reporting Quality domain, the **Deficient** rating was used as a flag to potentially reach out to study authors to obtain missing critical information (e.g., blinding, randomization) that may impact the overall confidence rating of the study (e.g., from b confidence to ***Medium*** confidence). A **Deficient** rating does not necessarily relegate the study to ***Low*** confidence, but it is an indicator that obtaining information from the study authors may change the overall confidence rating. EPA could then judge if it was necessary to contact the study authors. If the study received a **Deficient** rating for this domain and correspondence with the study authors could potentially increase the confidence, a statement was added to indicate that obtaining information from the study authors could impact the confidence.
If EPA followed up with authors to obtain missing information, the study details page was updated to note that the authors were contacted and provided the corresponding details.

A.1.7.2.2 Selection and Performance – Allocation

Table A-29. Study Quality Evaluation Considerations for Selection and Performance – Allocation

Core Question: Were animals assigned to experimental groups using a method that minimizes selection bias?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each study: Did each animal or litter have an equal chance of being assigned to any experimental group (i.e., random allocation)? Is the allocation method described? Aside from randomization, were any steps taken to balance variables across experimental groups during allocation?	**Good**	• Experimental groups were randomized and any specific randomization procedure was described or inferable (e.g., computer-generated scheme). (Note that normalization is not the same as randomization (see response for 'Adequate')).	Good. The study authors report that "Fifty males and 50 females were randomly assigned to groups by a computer-generated weight-ordered distribution such that individual body weights did not exceed +20% of the mean weight for each sex."
	Adequate	• Authors report that groups were randomized but do not describe the specific procedure used (e.g., 'animals were randomized'). Alternatively, authors used a non-random method to control for important modifying factors across experimental groups (e.g., body weight normalization).	**Example 1**: Adequate. Randomization was not performed. However, normalization procedures that balance important variables across groups were performed. Specifically, the authors state that animals were "allocated into groups with similar distributions in body weight." **Example 2**: Adequate. The study authors state that "animals were randomly distributed to exposure groups." However, the specific randomization method used was not described. **Example 3**: Adequate. Randomization was not explicitly reported. However, the study was performed according to OECD 416 and EPA OPPT 870.3800 guidelines which both specify randomization, although the specific methods of randomization used in the current study could not be inferred. OECD 416 guidelines state "animals should be

Core Question: Were animals assigned to experimental groups using a method that minimizes selection bias?			
			randomly assigned to the control and treated groups (stratification by body weight is recommended).” EPA OPPT 870.3800 guidelines state “animals should be randomly assigned to the control and treatment groups, in a manner which results in comparable mean body weight values among all groups.” **Example 4:** Adequate. The study authors state that "Animals were randomized by weight into treatment groups," and do not present the specific randomization procedural details.
	Not Reported (Interpreted as Deficient)	• No indication of randomization of groups or other methods (e.g., normalization) to control for important modifying factors across experimental groups.	Not reported (interpreted as Deficient). The authors did not indicate randomization or other normalization procedures for balancing important variables across groups.
	Critically Deficient	• Bias in the animal allocations was reported or inferable.	Critically Deficient. There is direct evidence that animals were allocated to treatment groups in a subjective way, involving the judgment of the investigator. Specifically, the study authors report “the heavier dams were assigned to the higher dose groups to reduce toxicity from [chemical]”; dam weight is an important variable for these developmental outcomes.

Notes: OECD = Organisation for Economic Co-operation and Development; OPPT = Office of Pollution Prevention and Toxics.

A.1.7.2.3 Selection and Performance – Observational Bias/Blinding

Table A-30. Study Quality Evaluation Considerations for Selection and Performance – Observational Bias/Blinding

Core Question: Did the study implement measures to reduce observational bias?

Prompting Questions		Suggested Considerations	Example Answers
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the study report blinding or other methods/procedures for reducing observational bias? If not, did the study use a design or approach for which such procedures can be inferred? What is the expected impact of failure to implement (or report implementation) of these methods/procedures on results?	**Good**	• Measures to reduce observational bias were described (e.g., blinding to conceal treatment groups during endpoint evaluation; consensus-based evaluations of histopathology lesions[a]).	**Example 1:** Good. Histopathology: Although the study did not indicate blinding, blinding during the initial evaluation of tissues for initial or nontargeted evaluations is generally not recommended as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004). The study did include a secondary evaluation by a pathology working group (PWG) review on coded pathology slides, which minimized the potential for observational bias. **Example 2:** Good. Organ weights, FOB, motor activity, swim maze and histopathology: Authors reported that the investigators were blinded to the animal treatment group during evaluation for all outcome measures. Although blinding is not recommended for initial or nontargeted evaluations (Crissman et al., 2004), this study evaluated prespecified outcomes in targeted evaluations for which blinding is appropriate (cell counts in the CA3 region of the hippocampus).
	Adequate	• Methods for reducing observational bias (e.g., blinding) can be inferred or were reported but described incompletely.	Adequate. Histopathology measures: Authors report "lesions were counted by two observers in a blinded fashion" although it should be noted that blinding during the initial evaluation of tissues is generally not

Core Question: Did the study implement measures to reduce observational bias?			
			recommended for initial or nontargeted evaluations as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004).
	Not Reported (Interpreted as Adequate)	• Measures to reduce observational bias were not described. • The potential concern for bias was mitigated based on use of automated/computer driven systems, standard laboratory kits, relatively simple, objective measures (e.g., body or tissue weight), or screening-level evaluations of histopathology.	**Example 1**: Not reported (interpreted as Adequate). Body and organ weights, developmental landmarks, and hormone measures: Authors did not indicate whether investigators were blinded during outcome assessment. Potential concern for bias was mitigated for these endpoints, which were measured using automated/computer driven systems, standard laboratory kits, relatively simple, objective measures (e.g., body or tissue weight). **Example 2**: Not reported (interpreted as Adequate). Histopathology: Blinding during the initial evaluation of tissues is generally not recommended as masked evaluation can make the task of separating treatment-related changes from normal variation more difficult and may result in subtle lesions being overlooked (Crissman et al., 2004). Histopathology was evaluated by an independent laboratory (Toxicology Pathology Associates Little Rock, Arkansas, John Pletcher, D.V.M., DACPV). No subsequent steps to minimize the potential for observational bias were reported (i.e., conducting a secondary targeted blinded review, independent prospective or retrospective peer-review, formation of a pathology working group).

Core Question: Did the study implement measures to reduce observational bias?			
			Example 3: Not reported (interpreted as Adequate). Fetal evaluation for malformations: Blinding during initial evaluation of fetuses is typically not conducted as masked evaluation can make the task of separating treatment-related changes from normal developmental variation more difficult and may result in subtle developmental anomalies being overlooked. Fetal evaluations were conducted in accordance with regulatory test guideline recommendations, using standardized nomenclature. No subsequent steps to minimize the potential for observational bias were reported (e.g., conducting a secondary targeted blinded review, or an independent prospective or retrospective peer-review).
	Not Reported (Interpreted as Deficient)	• Measures to reduce observational bias were not described. • The potential impact on the results is major (e.g., outcome measures are highly subjective).	Not reported (interpreted as Deficient). Neurobehavior (auditory and visual sensory reactivity): Procedural methods addressing observational bias were not described for these endpoints, which were measured using highly subjective methods (i.e., it appears that investigators measured reactivity using manually operated timers).
	Critically Deficient	• Strong evidence for observational bias that could have impacted results.	Critically Deficient. Neurobehavior after restraint stress: There is direct evidence of observational bias in testing methods. Specifically, the study reported that, to minimize stress from changing investigators across trials, one investigator consistently stressed control mice each day for 30 minutes and subsequently tested behaviors, while a separate investigator conducted stress and behavioral testing in treated mice. There was no mention of blinding of investigators.

Notes: FOB = functional observed battery.

[a] For nontargeted or screening-level histopathology outcomes often used in guideline studies, blinding during the initial evaluation of tissues is generally not recommended as masked evaluation can make 'the task of separating treatment-related changes from normal variation more difficult' and 'there is concern that masked review during the initial evaluation may result in missing subtle lesions.' Generally, blinded evaluations are recommended for targeted secondary review of specific tissues or in instances when there is a predefined set of outcomes that is known or predicted to occur (Crissman et al., 2004).

A.1.7.2.4 Confounding/Variable Control

Table A-31. Study Quality Evaluation Considerations for Confounding/Variable Control

Core Question: Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each study: Are there differences across the treatment groups (e.g., co-exposures, vehicle, diet, palatability, husbandry, health status) that could bias the results? If differences are identified, to what extent are they expected to impact the results?	**Good**	• Outside of the exposure of interest, variables that are likely to confound or modify results appear to be controlled for and consistent across experimental groups.	Good. On the basis I study report, vehicle (deionized water with 2% Tween 80) and husbandry practices were inferred to be the same in controls and treatment groups. The experimental conditions described provided no indication of concern for uncontrolled variables or different practices across groups.
	Adequate	• Some concern that variables that were likely to confound or modify results were uncontrolled or inconsistent across groups but are expected to have a minimal impact on the results.	**Example 1 (oral):** Adequate. Hormone measurements: Authors did not use a soy-free diet. Soy-based rodent feeds contain phytoestrogens that may act as a confounder for endocrine-related measures. Since this study includes relatively high doses (100 and 1500 mg/kg/day) the concern is minimal. **Example 2 (inhalation):** Adequate. Behavior, immunological responses, and hormonal changes: control rats did not appear to receive chamber air exposures (they were left in their home cages). As this might introduce a difference in stressors across groups, this difference is interpreted as a possible confounder for measures shown to be sensitive to stress, although the impact of this limitation on the results is expected to be minimal.

Core Question: Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?			
	Deficient	• Notable concern that potentially confounding variables were uncontrolled or inconsistent across groups and are expected to substantially impact the results.	Deficient. Dams in the medium and high-exposure groups (1500 and 15,000 ppm, respectively) showed significantly lower consumption of the treated food throughout the exposure period (gestation) that increased to control levels after the exposure ended. Addition of the test chemical may have affected the palatability of the food and reduced food intake during gestation may have significantly impacted the developmental outcomes in the pups.
	Critically Deficient	• Confounding variables were presumed to be uncontrolled or inconsistent across groups, and are expected to be a primary driver of the results.	Critically Deficient. The study did not include a vehicle-only control group, and, given the high concentration of DMSO required to solubilize the test article in other experiments using a similar exposure design, this is interpreted as likely to be a significant driver of any observed effects.

Notes: ppm = parts per million; DMSO = dimethyl sulfoxide.

A.1.7.2.5 Reporting and Attrition Bias

Table A-32. Study Quality Evaluation Considerations for Selective Reporting and Attrition – Reporting and Attrition Bias

Core Question: Did the study report results for all prespecified outcomes and tested animals?

Prompting Questions	Suggested Considerations		Example Answers
For each study: ***Selective reporting bias:*** Are all results presented for endpoints/outcomes described in the methods (see note)? ***Attrition bias:*** Are all animals accounted for in the results? If there are discrepancies, do authors provide an explanation (e.g., death or unscheduled sacrifice during the study)? If unexplained results omissions and/or attrition are identified, what is the expected impact on the interpretation of the results? *NOTE: This domain does **not** consider the appropriateness of the analysis/results presentation. This aspect of study quality is evaluated in another domain.*	**Good**	• Quantitative or qualitative results were reported for all prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Data not reported in the primary article is available from supplemental material. If results omissions or animal attrition are identified, the authors provide an explanation and these are not expected to impact the interpretation of the results.	Good. Animal loss was reported (the authors treated 10 rats/sex/dose group and noted one death in a high-dose male rat at day 85 of study). All endpoints described in methods were reported qualitatively or quantitatively.
	Adequate	• Quantitative or qualitative results are reported for most prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Omissions and/or attrition are not explained but are not expected to significantly impact the interpretation of the results.	Adequate. Animal loss occurred and was reported (see below), but these are not expected to significantly impact the interpretation of the results. All endpoints described in methods were reported qualitatively or quantitatively. "In the high dose (1000 mg/kg-day) group no male animals were able to complete the entire study; whereas all male rats exposed at other doses completed the 4-wk experiment. In the female group, 1 rat was removed in the 250 mg/kg-day group at day 25, 1 rat in the 500 mg/kg-day was removed at day 21 and 8 rats in the 1000 mg/kg/day group were removed between days 16 and 27 of the experiment." Justification for removals was provided by the study authors.
	Deficient	• Quantitative or qualitative results are missing for many prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints and/or high animal attrition; omissions and/or attrition are not explained and may	**Example 1:** Deficient. Unaccounted for loss of animals was difficult to assess because the study authors do not provide a clear description of the number of animals per exposure group or the selection of animals for outcome analysis. Table 1 states there

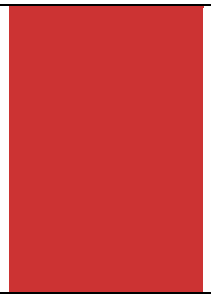
Core Question: Did the study report results for all prespecified outcomes and tested animals?			
		significantly impact the interpretation of the results.	were eight animals used in experiment 1 and 6 animals used in experiments 2 and 3. The figures and tables report data for varying numbers of animals (from 4 to 8), but the authors do not provide a description of the approach used to sample animals for each outcome. **Example 2:** Deficient. Although the authors indicated that "the liver, kidneys, and spleen were weighed and processed for routine histopathology at study termination," qualitative or quantitative findings were not reported for liver or kidney weights, nor for liver, kidney, or spleen histopathology ("spleen weights" were described as unchanged durinA-87ssociatscription of changes in cultured splenic immune cells).
	Critically Deficient	• Extensive results omission and/or animal attrition are identified and prevents comparisons of results across treatment groups.	Critically Deficient. None of the animals in the high and medium dose groups survived and there was high mortality (>75%) in the low-dose group.

A.1.7.2.6 Exposure Methods Sensitivity – Chemical Administration and Characterization

Table A-33. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Chemical Administration and Characterization

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each study: Does the study report the source and purity and/or composition (e.g., identity and percent distribution of different isomers) of the chemical? If not, can the purity and/or composition be obtained from the supplier (e.g., as reported on the website) Was independent analytical verification of the test article purity and composition performed? Did the authors take steps to ensure the reported exposure levels were accurate? For inhalation studies: were target concentrations confirmed using reliable analytical measurements in chamber air? For oral studies: if necessary, based on consideration of chemical-specific knowledge	**Good**	• Chemical administration and characterization are complete (i.e., source, purity, and analytical verification of the test article are provided). There are no concerns about the composition, stability, or purity of the administered chemical, or the specific methods of administration. For inhalation studies, chemical concentrations in the exposure chambers are verified using reliable analytical methods.	**Example 1 (oral):** Good. Source (3M) and purity (98%) are described, and the authors provided verification using analytical methods (GC/MS). Addressing concerns about known instability in solution for this chemical, the authors verified the dosing solutions twice weekly over the course of the experiment. Animals were exposed via gavage with all dose groups receiving the same volume. **Example 2 (inhalation):** Good. Source (3M) and purity (98%) of the test article are described. All animals were transferred to dynamic inhalation exposure chambers for the exposures. The concentration of the test chemical in the air was continuously monitored from the animals' breathing zone throughout the 6-hr exposure periods and mean daily average concentrations and variability were reported.
(e.g., instability in solution; volatility) and/or exposure design (e.g., the frequency and duration of exposure), were chemical concentrations in the dosing solutions or diet analytically confirmed? Are there concerns about the methods used to administer the chemical (e.g., inhalation chamber type, gavage volume)? *NOTE: Consideration of the appropriateness of the route of exposure is not evaluated at the individual study level. Relevance and utility of the*	**Adequate**	• Some uncertainties in the chemical administration and characterization are identified but these are expected to have minimal impact on interpretation of the results (e.g., source and vendor- reported purity are presented, but not independently verified; purity of the test article is sub-optimal but not concerning; For inhalation studies, actual exposure concentrations are missing or verified with less reliable methods).	**Example 1 (oral):** Adequate. Purity (98%) is described, but source is missing. Purity is assumed to be vendor reported because independent analytical verification of the purity is not described. Authors were contacted to try to obtain the vendor information however they did not respond. Stability assessments were not necessary because fresh dosing solutions were prepared daily.

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
routes of exposure are considered in the PECO criteria for study inclusion and during evidence synthesis.			**Example 2 (inhalation):** Adequate. Source (3M) and purity (98%) of the test article are described. All animals were transferred to dynamic inhalation exposure chambers for the exposures. The nominal/target concentrations of the test chemical were not verified by analytical measurements of the chamber air.
	Deficient	• Uncertainties in the exposure characterization are identified and expected to substantially impact the results (e.g., source of the test article is not reported; levels of impurities are substantial or concerning; deficient administration methods, such as use of static inhalation chambers or a gavage volume considered too large for the species and/or lifestage at exposure).	**Example 1 (oral):** Deficient. Test chemical supplied by the chemical manufacturer. Purity and isomeric composition are not described and could not be obtained from manufacturer's website. Analytical verification of the test article's purity and composition was not provided, and the stability of chemical in the diet across the 1-year exposure period does not appear to have been assessed. **Example 2 (inhalation):** Deficient. Source (3M) and vendor-reported purity are described, although these were not independently verified. The animals appear to have been exposed in static (i.e., without dynamic airflow) chambers; this is not interpreted as a critical deficiency due to the relatively short (2-hr) durations of daily exposure.
	Critically Deficient	• Uncertainties in the exposure characterization are identified and there is reasonable certainty that the results are largely attributable to factors other than exposure to the chemical of interest (e.g., identified impurities are expected to be a primary driver of the results).	**Example 1 (oral):** Critically Deficient. The test article contains large amounts of a known impurity [specify] that has previously been shown to cause the outcome(s) of interest. On the bIofthe doses tested (and inferences regarding the administered doses of the impurity), this is likely to be a significant driver of any observed effects.

Core Question: Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?			
			**Example 2 (inhalation):** Critically Deficient. Dams were exposed in static chambers during gestation, and there was evidence of overt toxicity (i.e., gasping) throughout the 12-hr daily exposures at all tested concentrations. This is likely to be a substantial driver of any observed developmental effects.

Notes: GC/MS = gas chromatography mass spectrometry.

A.1.7.2.7 Exposure Methods Sensitivity – Exposure Timing, Frequency, and Duration

Table A-34. Study Quality Evaluation Considerations for Exposure Methods Sensitivity – Exposure Timing, Frequency, and Duration

Core Question: Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the exposure period include the critical window of sensitivity? Was the duration and frequency of exposure sensitive for detecting the endpoint of interest?	**Good**	• The duration and frequency of the exposure was sensitive and the exposure included the critical window of sensitivity (if known).	**Example 1**: Good. Study uses a standard OECD short-term (28-day) study design to examine toxicological effects that are routinely evaluated in this testing guideline. **Example 2:** Good. The experimental design and exposure period were appropriate for evaluation of potential male reproductive and developmental effects. The experiment was designed to evaluate reproductive and developmental outcomes and followed recommendations in (OECD, 2001) and (U.S. EPA, 1998) guidelines.
	Adequate	• The duration and frequency of the exposure was sensitive and the exposure covered most of the critical window of sensitivity (if known).	Adequate. The study does not include the full developmental window of exposure most informative to evaluating potential effects on androgen-dependent development of male reproductive organs. Specifically, the study exposed rats from GD 18−GD 21, whereas the critical window for the development of these endpoints (i.e., cryptorchidism; testes and seminal vesicle weights; and male reproductive organ histopathology) begins on GD 15, and peaks around GD 17 (Scott et al., 2009; NRC, 2008) in rats. The incomplete coverage of this critical window in this study is expected to result in a minor bias toward the null.

Core Question: Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?			
	Deficient	• The duration and/or frequency of the exposure is not sensitive and did not include the majority of the critical window of sensitivity (if known). These limitations are expected to bias the results toward the null.	Deficient. The experimental design is not considered appropriate for evaluation of male fertility. Male rats were exposed for *chemical X* for 1 wk and fertility was assessed on wk 2 of the study. This design is considered deficient because in most rodent species "damage to spermatogonial stem cells will not appear in samples from the cauda epididymis or in ejaculates for 8 to 14 wk" (U.S. EPA, 1996).
	Critically Deficient	• The exposure design was not sensitive and is expected to strongly bias the results toward the null. The rationale should indicate the specific concern(s).	Critically Deficient. The experimental design is not appropriate for evaluation of cancer endpoints. Animals were necropsied and tissues evaluated for the presence of tumors and/or neoplasms 4 wk after only a 28-day exposure period. Notably, because this critical deficiency is due to insensitivity, depending on other identified limitations, the utility of this study will depend on whether effects were observed in the study (i.e., if tumors were observed, this study could be adjusted to a higher rating).

Notes: OECD = Organisation for Economic Co-operation and Development; OPPT = Office of Pollution Prevention and Toxics.

A.1.7.2.8 Outcome Measures and Results Display – Endpoint Sensitivity and Specificity

Table A-35. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Endpoint Sensitivity and Specificity

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?

Prompting Questions	Suggested Considerations		Example Answers
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Are there concerns regarding the specificity and validity of the protocols? Are there serious concerns regarding the sample size (see note)? Are there concerns regarding the timing of the endpoint assessment? *NOTE: Sample size alone is not a reason to conclude an individual study is critically deficient.*	**Good**	–	**Example 1:** Good. Lipid/Lipoproteins: There are no notable concerns about aspects of the procedures, or for the timing of these evaluations. Study authors used standard methodology (i.e., commercial kits) appropriate for use in adult liver tissue samples. **Example 2:** Good. Organ weight, body weights, and hormone measures: no concerns regarding the specificity and validity of the protocols and measures were identified. Study authors used standard methodology for evaluating organ and body weights. Thyroid hormones were measured using commercial electrochemiluminescence-immunoassay methods, and the known diurnal variation in these measures was accounted for during blood collection.
	Adequate	–	**Example 1**: Adequate. Histopathology: Tissues were fixed in 10% neutral buffered formalin, trimmed, sectioned (5 microns) and embedded and stained with H&E. Evaluations included 12 tissues from all animals in the control and highest dose groups. Although not explicitly stated, it is inferred that tissues from animals in the low- and mid-dose groups would have been evaluated if significant increases in lesion incidence were observed at the highest dose. This practice is consistent with NTP pathology guidelines (ref) and is expected to

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			be of minimal concern unless effects are observed at the high dose. Additionally, the report did not provide information on sampling (e.g., # sections evaluated/tissue, sections evaluated at x micron or section intervals). Together, the missing study details introduce some concern for potential insensitivity. **Example 2:** Adequate. Clinical chemistry: Some concern was raised regarding the procedural methods, as no information was provided on the diagnostic kits and, for some of the specific measures (i.e., those without specific data reported), it is unclear whether serum or plasma was analyzed.
	Deficient	–	**Example 1**: Deficient. Histopathology (testis): Concerns regarding the method used to preserve testis for histological analysis: 10% formalin. For evaluation of histopathological effects in the testis, conventional immersion fixation in buffered formalin is not recommended as it gives very poor penetration of fixative and may result in artifacts (Haschek et al., 2009; Foley, 2001). **Example 2:** Deficient. Nipple retention: Concerns for insensitivity were raised due to the timing of endpoint evaluation. Specifically, the authors examined nipple retention in rats at PND 9, whereas this endpoint is more appropriately evaluated around PNDs 12–14. **Example 3:** Deficient. Motor activity: Concerns were raised regarding the small sample sizes used to evaluate these outcomes. Specifically, the authors tested

Core Question: Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest?			
			four animals (sex not specified, but assumed males) per group. Ideally, it is preferable to have more than 10 animals/sex/ group for this type of evaluation, according to OECD guidelines.
	Critically Deficient	–	Critically Deficient. [Endpoint name]: [Assay X] has been shown to be unreliable for evaluating [endpoint of interest]. Currently best practice is to use [Assay Y] for this endpoint.

Notes: NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development.

A.1.7.2.9 Outcome Measures and Results Display – Results Presentation

Table A-36. Study Quality Evaluation Considerations for Outcome Measures and Results Display – Results Presentation

Core Question: Are the results presented in a way that makes the data usable and transparent?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Does the level of detail allow for an informed interpretation of the results? Are the data analyzed, compared, or presented in a way that is inappropriate or misleading?	**Good**	–	Good. There are no notable concerns about the way the results are analyzed or presented.
	Adequate	–	**Example 1:** Adequate. Reproductive organ weights, hormone measures: results are presented graphically; however, the authors do not clarify whether error bars correspond to SD or SE. **Example 2:** Adequate. Developmental effects: the study failed to report information on potential maternal toxicity; however, all tested doses other than the highest dose are not expected to cause overt toxicity in adults, reducing the level of concern. **Example 3:** Adequate. Anogenital distance (AGD): The authors reported AGD without adjusting for body weight, which is preferred (Daston and Kimmel, 1998). However, because the study also provided body weight data, approximation was possible, limiting concern.
	Deficient	–	**Example 1:** Deficient. Histopathology: Incidence and severity of individual effects was unclear, as only scores across multiple, disparate pathological endpoints were reported.

Core Question: Are the results presented in a way that makes the data usable and transparent?			
			Example 2: Deficient. Behavior (neuromuscular function and dexterity): Performance on the rotarod was presented as incidence of falling off the rod within an arbitrary time, rather than as time spent on the rod (the preferred metric). This dichotomization of continuous data without sound justification is expected to strongly bias the results toward observing an effect. **Example 3:** Deficient. Brain weight: Authors presented only relative brain weights, and absolute weights could not be calculated. The adult central nervous system (CNS) is highly protected, and absolute brain weight data are preferred [include reference]. **Example 4:** Deficient. Birth outcomes: Data on pup viability, weights, and malformations were reported as pup averages, without addressing potential litter effects.
	Critically Deficient	–	Critically Deficient. Endpoint name: The study presents the results for this endpoint in both a table and figure; however, the data do not match (e.g., mean ± SE reported for the control group is 2.3 ± 0.5 in the table and 1.9 ± 0.2 in the figure). This reporting discrepancy could not be resolved from the information provided in the study and study authors did not respond to queries for clarification.

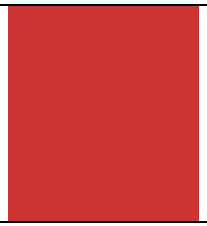
A.1.7.2.10 Overall Confidence

The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias and sensitivity on the results (Table A-37).

Table A-37. Study Quality Evaluation Considerations for Overall Study Confidence – Animal Toxicological Studies

Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?			
Prompting Questions	**Suggested Considerations**		**Example Answers**
For each endpoint/outcome or grouping of endpoints/outcomes in a study: Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects? *NOTE: Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias toward the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	***High* confidence**	• No notable concerns are identified (e.g., most or all domains rated Good).	*High* confidence. Reproductive and developmental effects other than behavior: The study was well-designed for the evaluation of reproductive and developmental toxicity induced by chemical exposure. The study applied established approaches, recommendations, and best practices, and employed an appropriate exposure design for these endpoints. Evidence was presented clearly and transparently.
	***Medium* confidence**	• Some concerns are identified but expected to have minimal impact on the interpretation of the results. (e.g., most domains rated Adequate or Good; may include studies with Deficient ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis.	**Example 1:** *Medium* confidence. Developmental effects: The study was adequately designed for the evaluation of developmental toxicity. Although the authors failed to describe randomized allocation of animals to exposure groups and some concerns were raised regarding the sensitivity (i.e., timing) and sample sizes (i.e., n=6 litters/group) used for the evaluation of potential effects on male reproductive system development with gestational exposure, these limitations are expected to have a minimal impact on the results. **Example 2:** *Medium* confidence. Histopathology: The study authors did not report information on the severity of histological effects for which this is routinely provided. The authors also failed to describe use of methods to reduce potential observational bias.

Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?			
	***Low* confidence**	• Identified concerns are expected to significantly impact on the study results or their interpretation (e.g., generally, Deficient ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note).	**Example 1:** *Low* confidence. Developmental effects: Substantial concerns were raised regarding quantitative analyses without addressing potential litter effects. Other significant limitations included incomplete data presentation (sample sizes for outcome assessment were unclear; no information on maternal toxicity was provided), and methods for selection of animals for outcome assessment. **Example 2:** *Low* confidence. Behavioral measures: The cursory cage-side observations of activity are considered insensitive and nonspecific methods for detecting motor effects, with a strong bias toward the null.
	Uninformative	• Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, Critically Deficient rating in any domain; many Deficient ratings). *Uninformative* studies are not considered further in the synthesis and integration of evidence.	**Example 1:** *Uninformative*. Critical information was not reported. Specifically, the study authors did not report the duration of the exposure or the results (qualitative or quantitative). Given this critical deficiency, the other domains were not evaluated. **Example 2**: *Uninformative.* Concerns were raised over the lack of information on test animal strain and allocation, and chemical source/purity. The lack of information on blinding or other methods to reduce observational blinding is also of significant concern for the endpoints of interest (i.e., follicle counts, ova counts, and evaluation of estrous cyclicity). Finally, concerns were also raised over the apparent self-plagiarism in similar chromium studies published in 1996 by this group of authors. Taken together, this combination of limitations resulted in an interpretation that the results were unreliable. **Example 3:** *Uninformative.* Sperm Measures: Issues were identified with the methods used to prepare samples for analysis, which are likely to introduce artifacts. Concerns were also raised regarding results presentation (i.e., lack of group variability), missing information on sample sizes and loss of animals, and a

Core Question: Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest?		
		lack of information on the timing of these evaluations. Taken together, the evaluation of this endpoint was considered *uninformative.*

A.1.8 Data Extraction for Epidemiological Studies

All epidemiological studies identified as PECO-relevant after full-text screening were considered eligible for data extraction. As noted in the IRIS Handbook (U.S. EPA, 2022c), during data extraction, relevant results from each study are extracted to facilitate organization, visualization, comparison, and analysis of findings and results. Data from PECO-relevant epidemiological were extracted if they received a *medium* or *high* confidence study quality evaluation rating. In cases where data were limited (e.g., thyroid cancer) or when there was a notable effect, results from *low* confidence studies were extracted. Data extracted from *low* confidence studies were considered qualitatively only (e.g., in the evidence synthesis and integration). Studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction. Extraction was targeted toward the five priority health outcomes recommended by the SAB (i.e., cancer, cardiovascular, developmental, hepatic, and immune). Results from main analyses were extracted, and age- and sex-stratified analyses were extracted if available. Results from other stratified and sensitivity analyses were extracted when deemed appropriate for a given outcome (e.g., medication use status for cardiovascular outcomes).

Data extraction of epidemiological studies was carried out using a set of structured forms in DistillerSR. Studies slated for extraction were prescreened by an expert epidemiologist who identified the relevant results to be extracted. Data extraction was performed by one reviewer and then independently verified by at least one other reviewer for quality control. Any conflicts or discrepancies related to data extraction were resolved by discussion and confirmation within the extraction team.

Table A-38 outlines the content of the DistillerSR forms that were populated during data extraction of epidemiological studies, including the extraction questions or prompts and response options.

Table A-38. DistillerSR Form Fields for Data Extraction of Epidemiological Studies

	Question/Prompt	Response Options	Suggested Considerations
1	**Has this study been QC'd?** *[Select one]*	• No (select if doing data extraction) • Yes, no corrections needed • Yes, corrections were needed and completed during QC (please list any major revisions, e.g., incomplete responses, NOEL/LOEL incorrect) • Study is not PECO-relevant (please specify why)	–
2	**Reference (short form)** e.g., Smith et al., 1978 *[Free-text]*	–	• Enter author information; use the format specified in the Distiller form.
3	**Population** *[Select one]*	• General population, adults and children • General population, adults • General population, children and adolescents <18 yr	• Do not select "pregnant women" if pregnant women are only included as part of a general population sample.

	Question/Prompt	Response Options	Suggested Considerations
		• Occupational • Pregnant women • Occupational/general population, adults • Other	• When exposure is measured in cord blood and outcome in children, the study population would be "children."
4	**Population Summary** *[Free-text]*	–	• Briefly describe the study population (e.g., women undergoing fertility treatment, NHANES adults 18+). Try to capture anything outside a typical general population sample. Keep it brief – does not need to be in full sentences. • For studies of mother-child cohorts, when exposure is in maternal blood and outcome is evaluated in children, use "pregnant women and their children." For example, if any of these (non-exhaustive) scenarios apply, capture them in this field: • Known potential for PFAS exposure (e.g., contamination event/lawsuit). • Follow-up timing. • Participants are drawn from a specific population, such as people with a specific health condition, narrow age range within "adults" and "children" (e.g., infants, seniors), specific environments (e.g., assisted living facility, daycare, farmers)
5	**Study Design** *[Select one]*	• Cohort • Case-control • Cross-sectional • Ecological • Controlled trial • Other • Nested caste-control • Cross-sectional and prospective analyses • Cohort and cross-sectional • Case-control and cross-sectional	• See Section A.1.8.1 for different types of study design. • Note: Third trimester samples with outcome measured at birth should be classified as cohort studies. • Cohort studies reporting prospective and cross-sectional analyses should be classified as Cohort and cross-sectional. • Case-control studies reporting cross-sectional analyses among the whole study population or within cases or controls should be classified as Case-control and cross-sectional.
6	**Study Name (if applicable)** *[Free-text]*	–	• Only use the name of an official study or cohort. Leave blank if there is no name.
7	**Country (or Countries)** *[Free-text]*	–	• Use full names such as "United States" (not US).
8	**Year of Data** List which year(s) the data came from. *[Free-text]*	–	• For prospective cohort studies that only state the period the population was recruited (e.g., 2012–2015) and mention the outcomes were assessed at follow-up (e.g., state "5 yr later" but do not provide dates), extract "recruitment 2012–2015, outcome assessed at 5-year follow-up."
9	**Exposure Measurement** *[Select all that apply]*	• Biomonitoring • Air	–

	Question/Prompt	Response Options	Suggested Considerations
		• Food • Drinking water • Occupational (use in cases where exposure is based on factors such as job function, place in building where people worked, job exposure matrices) • Modeled • Questionnaire • Direct administration – oral • Direct administration – inhalation • Other	
10	**If "biomonitoring" was selected, indicate the matrix.** *[Select all that apply]*	• Blood • Serum • Plasma • Maternal blood • Cord blood • Urine • Feces • Breast milk • Hair • Saliva • Nails • Teeth • Semen • Cerebrospinal fluid • Exhaled breath • Other • Glucose • Maternal serum • Amniotic fluid • Maternal Plasma	• For biomonitoring matrix, if PFAS is measured in serum, select serum (and not also blood). Only select blood if something more specific is not specified (e.g., cord blood, maternal blood, plasma, serum).
11	**Quantitative Data Extraction (Sub-Forms)**		
11.1	**Health Effect Category** *[Select one]*	• Cancer • Cardiovascular • Dermal • Developmental • Endocrine • Gastrointestinal • Hematologic • Hepatic • Immune • Metabolic • Musculoskeletal/Connective Tissue • Nervous • Ocular • Reproductive, female	• See Appendix A.1.6.5.1 for what kind of health outcomes are grouped under which health effect category. Please create a separate form for each outcome.

	Question/Prompt	Response Options	Suggested Considerations
		• Reproductive, male • Respiratory • Renal • Other	
11.2	**Measured Outcome/Endpoint** *[Free-text]*	–	• Describe the measured outcome/endpoint and start with most relevant word (e.g., "glucose concentration in serum" preferred to "serum glucose"). • Provide units in parentheses if relevant and readily available. • If the outcome is log transformed, please note it here: o Weight (ln-grams) o Triglyceride (log_{10} mg/dL) • Some outcomes are dichotomous (e.g., high blood pressure, high cholesterol), indicate the outcome definition in parentheses. For example: o High cholesterol (>5.0 mg/dL)
11.3	**If developmental, when was the outcome measured?** *[Select all that apply]*	• ≤2 yr of age • >2–5 yr of age • >5 yr of age	–
11.4	**PFAS** *[Select one]*	• PFOA • PFOS	–
11.5	**For neurodevelopmental outcomes, when was PFAS exposure measured?** *[Select all that apply]*	• Participants were ≤6 mo of age • Participants were >6 mo of age	–
11.6	**Sub-population** *[Free-text]*	–	• If relevant, specify sub-group within the study (e.g., sex, age group, age at outcome and/or exposure measurement). • Leave blank if not applicable.
11.7	**N** *[Free-text]*	–	• N should be for everyone in the analysis, not just one exposure/comparison group. However, if extracting results for specific population subgroups (age category, gender-specific) and if reported, the N should reflect the number of participants in that specific sub-group (e.g., number of boys in the male-specific result extracted).
11.8	**Exposure Levels** *[Free-text]*	–	• Exposure level should be for everyone in the analysis, not just one comparison group. • Ideally extract median and the 25th–75th percentile range for PFAS being extracted. The following format is preferred: median=xx (units) (25th–75th percentile: xx–xx). • Provide labels and units (e.g., median=xx (units) (range: min–max: xx–xx)). o If median is not available, please extract other measures of distribution, such as

	Question/Prompt	Response Options	Suggested Considerations
			mean or geometric mean, range, other percentiles. • Extract levels for the **overall study population**. If only available by subgroups, specify which subgroup. **Example:** • Males: median = 6.4 ng/mL (25th–75th percentile: 3.6–9.2 ng/mL); Females: median=5.8 ng/mL (25th—75th percentile: 3.1–8.3 ng/mL) • Note: sometimes manuscripts will incorrectly use IQR rather than 25th–75th percentile. The IQR is the difference between the 75th and the 25th percentile, so it should be a single number, not a range. If a range is labeled IQR, please use "25th–75th percentile."
11.9	**% with Negligible Exposure (e.g., below the LOD)** *[Free-text]*	–	• Number of samples below LOD/LOQ; do not include the percent sign. • Leave blank if not reported.
11.10	**Description of the Effect Estimate, including Comparison Group if applicable** *[Free-text]*	–	• Describe the effect estimate, including comparison group if applicable. • Brief description of the effect estimate: describe the comparison being made (e.g., beta regression coefficient for IQR increase; OR for Q2 vs. Q1). Make sure to specify unit change for continuous measures (e.g., 1 ln-unit, IQR change, SD increase). • Use ln() over log() for natural log transformations. If not ln, specify log(*base*) (e.g., log10 or log(10)). **Good Examples/Formatting:** • regression coefficient (per 1-log2 ng/mL increase in PFOA). • OR (per 1-ln ng/mL increase in estimated plasma PFOS). • OR (for Q2 vs. Q1). • OR [for Q2 (0.83 ng/mL–1.4 ng/mL) vs. Q1 (0.83 ng/mL)]. • OR [for T2 (0.83 ng/mL–1.4 ng/mL) vs. T1 (<0.83 ng/mL)]. **Bad Examples/Formatting:** • beta coefficient. • linear regression coefficient (standard error) with one unit increase in log-PFC in adults.
11.11	**Rank this Comparison Group by Exposure** *[Free-text]*	–	• For standalone result of unit change, leave blank. • If results are presented for quantiles of exposure, the comparison group for Q2 to Q1

	Question/Prompt	Response Options	Suggested Considerations
			would be ranked as 1, while Q3 to Q1 would be ranked as 2.
11.12	**Effect Estimate Type** *[Select one]*	• Odds Ratio (OR) • Relative Risk Ratio (RR) • Absolute Risk % • Beta Coefficient (b) • Beta Coefficient (standardized) • Standardized Mortality Ratio (SMR) • Standardized Incidence Ratio (SIR) • Incidence Risk Ratio (IRR) • Absolute Risk Reduction/Risk Difference (ARR or RD) • Hazard Ratio (HR) • Comparison of Means • Incidence Rate Ratio • Comparison of Means • Spearman's Correlation Coefficient • Correlation Coefficient • Percent Incidence • Regression Coefficient • Proportionate Mortality Ratio (PMR) • Mean Difference • Percent Difference • Percent Change • Benchmark Dose (BMD) • Mean • Geometric Mean • Least Square Means (LSM) • Geometric Mean Ratio • Fecundability Ratio • Adjusted r^2 • Mean Ratio • Prevalence Ratio (PR)	• If the effect estimate is a regression coefficient (a beta or β), select from the menu "Regression Coefficient" rather than "Beta Coefficient." • If PFOS/PFOA was the outcome of interest (e.g., study looked at the impact of a disease on PFOS/PFOA level), please still extract the data but make a note under the Results Comments (11.19).
11.13	**Effect Estimate** *[Free-text]*	–	• Only report the effect estimate from the adjusted model. If there are multiple adjustment sets, use the final model. • Do not extract the reference group (1) for results comparing exposure levels (i.e., extract OR (for Q2 vs. Q1), but do not extract the OR of 1 for the reference group Q1).
11.14	**CI LCL:** Confidence Interval – Lower Confidence Limit *[Free-text]*	–	–

	Question/Prompt	Response Options	Suggested Considerations
11.15	**CI UCL:** Confidence Internal – Upper Confidence Limit *[Free-text]*	–	–
11.16	**SD or SE** *[Free-text]*	–	• Enter the SD or SE if reported for the effect estimate. • Leave blank if not reported.
11.17	**p-value** *[Free-text]*	–	• Enter the quantitative p-value if available (e.g., "0.0001" or "<0.001") o If the study/table only indicates that p-value is not significant, enter "ns" for not significant. o If the p-value is not reported or does not apply to the estimate being reported, leave blank. o If table footnote mentioned "*p < 0.05" for the results with *, then enter < 0.05. If results do not have a * and no p-value was reported, then leave blank. o If the p-value is not reported and text/methods mention significance level is 0.05, and: ▪ the text mentioned the specific result is statistically significant, then enter <0.05 (and make a note in the Results Comments (11.19) which page is this from). ▪ the text mentioned a result as not statistically significant, then enter "ns" (and make a note in the Results Comments (11.19) which page is this from). • Make sure the p-value reported corresponds to the regression coefficient being extracted. Authors will occasionally report p-values for other things such as the model fit. • Other types of p-values such as interaction p-values or trend p-values are reported, these can be placed in Results Comments (11.19).
11.18	**Covariates in Model** *[Free-text]*	–	• If there are multiple adjustment sets, list covariates in the final model, but make a note in the comment field on the main form (14). that additional adjustment sets were available for sensitivity analyses. • List just the covariates, no need to add "adjusted for…." • Example: age, gender, race, SES.
11.19	**Results Comments** *[Free-text]*	–	• Enter the location of the extracted data (e.g., "Table 3" or "in-text p. 650"). • Enter any relevant p-values, such as interaction p-values or trend p-values. • Enter any additional details on the outcome measurement or definition.

	Question/Prompt	Response Options	Suggested Considerations
12	**Select PFOS or PFOA if it was measured in the study but not analyzed with health effects.**	• PFOS • PFOA	–
13	**Correlations across the included PFAS presented in paper or supplement.** *[Select one]*	• Yes • No	• Note whether the main manuscript or the supplemental material present a table or text describing the (Spearman) correlation coefficients between concentrations of PFAS included in the paper.
14	**Comments** Include brief description of results provided in supplemental materials but not extracted (e.g., stratified analyses, sensitivity analyses). *[Free-text]*	–	• Briefly mention if **effect modification** is analyzed but not extracted (e.g., stratified analyses by race, by BMI categories). Note: **Stratification by sex and age should always be extracted.** • Do not need to specify how values below the LOD were handled. • If data are presented by **sub-group/strata** (e.g., race) in the supplemental material, just note that here. Note: **Stratification by sex and age should always be extracted.** • Briefly, describe any other **supplemental results (e.g., sensitivity analyses)** here; no need to list all confounders other models adjusted for. • Any outcome definitions if study specific (e.g., how was *elevated ALT* defined in a study reporting ORs of elevated ALT).

Notes: QC = quality control; NOEL = no-observed-effect level; LOEL = lowest-observed-effect level; PECO = populations, exposures, comparators, and outcomes; NHANES = National Health and Nutrition Examination Survey; PFAS = per- and polyfluoroalkyl substances; PFOA = perfluorooctanoate aci; PFOS = perfluorooctane sulfonic acid; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; Q2 = quarter 2; Q1 = quarter 1; ln = natural log; SD = standard deviation; T2 = tertile 2S; T1 = tertile 1; PFC = ; Q3 = quarter 3; CI = confidence interval; SE = standard error; ns = not significant; SES = socioeconomic status; BMI = body mass index; ALT = alanine transaminase.

A.1.8.1 Epidemiological Study Design Definitions

Epidemiological studies with cross-sectional, cohort, case-control, ecological, or controlled trial study designs were included. The study design definitions shown in Table A-39 were used throughout full-text screening and data extraction.

Table A-39. Epidemiological Study Design Definitions

Study Design	Description
Cross-sectional	Exposure and outcome are examined at the same point in time in a defined study population. Cannot determine if exposure came before or after outcome.
Cohort	A group of people is examined over time to observe a health outcome. Everyone belongs to the same population (e.g., general U.S. population; an occupational group; cancer survivors). All cohort studies (prospective or retrospective) consider exposure data from before the occurrence of the health outcome.
Case-control	Cases (people with the health outcome) and controls (people without the health outcome) are selected at the start of a study. Exposure is determined and compared between the two groups. A case-control study can be nested within a cohort.

Study Design	Description
Ecological	The unit of observation is at the group level (e.g., zip code; census tract), rather than the individual level. Ecological studies are often used to measure prevalence and incidence of disease. Cannot make inferences about an individual's risk based on an ecological study.
Controlled Trial	Exposure is assigned to subject and then outcome is measured.

A.1.9 Data Extraction for Animal Toxicological Studies

All animal toxicological studies identified as PECO-relevant after full-text screening in DistillerSR were eligible for data extraction. As noted in the IRIS Handbook (U.S. EPA, 2022c), during data extraction, relevant results from each study are extracted to facilitate organization, visualization, comparison, and analysis of findings and results. PECO-relevant animal toxicological studies that received a *medium* or *high* confidence study quality evaluation rating were extracted.

Data extraction was performed using a set of structured forms in HAWC (Table A-40). Studies slated for extraction were prescreened by an expert toxicologist who identified the relevant results. Extraction was performed by one reviewer and then independently verified by at least one other reviewer for quality control. Any conflicts or discrepancies were resolved by discussion and confirmation with a third reviewer.

Table A-40. HAWC Form Fields for Data Extraction of Animal Toxicological Studies

	Questions/Prompts and Options	Suggested Considerations
1	**Experiment**	
1.1	**Name Field** *[Free-text]*	• Name should be short and simple. For example, '28-Day Oral' '2-Year Drinking Water,' '1-Wk Inhalation.' • Reproductive/developmental if appropriate, then route of exposure (oral/inhalation), not number of generations or acute/short-term/sub-chronic/chronic. • If a study includes multiple experiments (e.g., multiple species, varied exposure durations), create separate experiments for each.
1.2	**Type Field** *[Select one]*	• For reproductive and/or developmental studies, select 'reproductive' or 'developmental' as appropriate (recognizing that a study may contain both reproductive and developmental endpoints, but is typically defined as one or the other based on design). • In general, use reproductive when the study begins treatment prior to mating and continues through birth and in some cases through a second generation. These studies will typically evaluate reproductive outcomes in the dams (e.g., copulation and fertility indices, numbers of corpora lutea and implantation sites, pre- and post-implantation loss). Use developmental when the exposure occurs during gestation and dams are sacrificed prior to birth. These studies are typically focused on the pups and evaluate viability, developmental milestones, and other growth and developmental effects in pups and primarily they are looking for abnormalities in the pups. • If reproductive or developmental are selected, indicate if there are data for more than one generation.
1.3	**Chemical Name Field** *[Free-text]*	• Enter the preferred name of the chemical (i.e., PFOA or PFOS). • Refer to the PECO statement in for a list of synonyms for each chemical.
1.4	**Chemical Identifier (CAS) Field** *[Free-text]*	• Be sure to include the dashes in the CAS number. • The CAS number for the chemical can be found in the PECO statement if they are not listed in the paper.

	Questions/Prompts and Options	Suggested Considerations
1.5	**Chemical Source Field** *[Free-text]*	• If the chemical source is not provided by the authors, add in "Not Reported" to this field.
1.6	**Chemical Purity Fields** *[Checkbox]*	• As a default, the 'Chemical purity available?' box will be checked. If the box is checked, entries for 'Purity qualifier' and 'Chemical purity (%)' are required. • Uncheck this box if chemical purity information is not available.
2	**Animal Group**	
2.1	**Name Field** *[Free-text]*	• Name should include sex, common strain name, and species (e.g., Male Sprague-Dawley Rats). • For reproductive or developmental studies, include the generation before sex in title (e.g., F_1 Male Sprague-Dawley Rats or P_0 Female C57 Mice). • If a study combines male and female subjects into one group, use "Male and Female" (e.g., Male and Female Sprague-Dawley Rats). • If gender is unclear, do not mention (e.g., Sprague-Dawley Rats). • Use the plural form for species (e.g., Rats, Mice).
2.2	**Animal Source and Husbandry Field** *[Free-text]*	• Copy and paste details directly from the paper using quotation marks. • If the authors do not provide the animal source, add in "Not Reported" to this field. • For multigenerational reproductive or developmental studies, the animal group dosed might be the parental (or P_0) group. For example, a P_0 female rat may be dosed during pregnancy and/or lactation, and developmental effects are then measured in offspring – or F_1 animals. • For a multigenerational study, specify the 'Generation.'
3	**Add Dosing Regime**	
3.1	**Exposure Duration (Days) Field** *[Free-text]*	• Decimals are allowed, so a 4 h single day study can be represented as 0.17 d. However, decimals are likely not needed for the PFOA/PFOS project since acute studies are not PECO relevant.
3.2	**Exposure Duration (Text) Field** *[Free-text]*	• For all time units, use the following abbreviations: year = yr; month = mo; week = wk; day = d; hour = hr; minute = min; second = sec. • Eliminate unnecessary space between length of time and unit (i.e., "2wk" instead of "2 wk").
3.3	**Description Field** *[Free-text]*	• Include dosing description from materials and methods. Be sure to use quotation marks around all text directly copied/pasted from the paper. • Include any information on how dosing solutions were prepared. • Summarize any results the authors present on analytical work conducted to confirm dose, stability, and purity.
3.4	**Dose Groups Field** *[Free-text]*	• Dose groups should be listed lowest to highest (dose group 1 = 0 mg/kg-d). • For visualization purposes dose units need to be in mg/kg-d. For studies that provide the units, please use those for extraction purposes. • For dietary or drinking water studies, if they provide BOTH concentration of the dose formulation (e.g., ppm) AND doses as mg/kg-d, please extract both.

	Questions/Prompts and Options	Suggested Considerations
		• For dietary or drinking water studies that ONLY provide the dose concentration, enter the dose concentrations as reported in the study and then utilize the conversions spreadsheet to convert the dosage into mg/kg-day (note that mg/kg body weight/day is the same as mg/kg-d so you just need to use the mg/kg-d). • If PFOA/PFOS are administered as salts and the doses are presented as salts of PFOA/PFOS, please contact senior-level extractors before using the conversion spreadsheet. • If converting doses, add in "Data extractor calculated [PFOS/PFOA] equivalent doses for mg/kg-day" into the "Description" box. • When defining the dosing regime for a multigenerational experiment, creating a new dosing regime may not be needed; instead specify the existing dosing regime of the P_0 (dosed during gestation and/or lactation). • A new dosing regime may be needed if offspring were exposed after weaning and, if applicable, acknowledge parental exposure in the 'Description' field on the 'Dosing regime' page. • If the authors provide internal measurements of PFOS/PFOA in any tissue, add this information in as an additional dose group using the mean tissue levels as the value and the tissue as part of the dose units (e.g., mg/kg bone, ppm brain).
4	**Endpoints (General)**	
4.1	**Endpoint Name Field** *[Free-text]*	• Name should not include descriptive information captured in other fields within HAWC such as sex, strain, species, duration, route, etc. • Include common abbreviation in parenthesis if applicable. • Endpoint detail should be added after main endpoint, ex. "Body Weight, Fetal" NOT "Fetal Body Weight." • In general, specific endpoint names are used except for general categories such as 'Clinical Observations' or histopathology (e.g., 'Kidney Histopathology'), which may comprise a number of observational endpoints. • Examples: Liver Weight, Relative; Triiodothyronine (T3) .
4.2	**System Field** *[Free-text]*	• Represents the appropriate system for the endpoint. • Examples: Hepatic; Endocrine.
4.3	**Organ (and Tissue) Field** *[Free-text]*	• Represents the appropriate organ or tissue for the endpoint. • Examples: Liver; Thyroid.
4.4	**Effect and Effect Subtype Fields** *[Free-text]*	• Represents the appropriate system for the endpoint. • Examples: Hepatic; Endocrine.
4.5	**Observation Time Fields** *[Free-text]*	• The 'Observation time' text field is included in visualizations and should be filled in; the 'Observation time' numeric field and 'Observation time units' can be left blank. • For all time units, use the following abbreviations: year = yr; month = mo; week = wk; day = d; hour = hr • Eliminate unnecessary space between length of time and unit (i.e., "2wk" instead of "2 wk"). • Example: 2yr; 6hr; 45d; 90min. • For developmental and reproductive studies, specify observation time in terms of development (e.g., GD 16, PND 0).

	Questions/Prompts and Options	Suggested Considerations
4.6	**Values Estimated Field** *[Free-text]*	• If data were extracted from a figure into HAWC using a measured ruler, check this box. • For data requiring a digital ruler, use the WebPlotDigitizer tool: https://apps.automeris.io/wpd/. • If there are multiple time points, extract only the latest time point (i.e., end of treatment) or if the last time point is not significant and an earlier time point is, extract the earlier time point (this information should be provided in the data to extract instructions, but this is the general rule in case there are no instructions provided). • Provide additional information in the results comment box to make note of what happened at other timepoints that were not extracted.
4.7	**Litter Effects Field** *[Free-text]*	• If the experiment type has been identified as either 'reproductive' or 'developmental,' the 'Litter effects' will be required, and a choice other than 'not applicable' must be selected.
4.8	**Dataset Type Field** *[Free-text]*	• Select the appropriate dataset type for the endpoint. In general, 'Dataset type' is continuous except for incidence data, which is dichotomous.
4.9	**NOAEL and LOAEL Fields** *[Free-text]*	• Be sure to enter the significance level (e.g., 0.05) for significant results as well as NOAEL/LOAEL. • The **NOAEL** is the highest dose at which there was not an observed toxic or adverse effect. If the LOAEL is the lowest (non-control) dose, then NOAEL should be <None>, not 0. • The **LOAEL** is the lowest dose at which there was an observed toxic or adverse effect. These fields are critical to the visualizations. If there is no LOAEL, leave as <None>. • In cases where the study authors did not conduct statistical tests, use the study authors conclusions to indicate where effects occur. Just make sure to note in the results comments that these were based on author conclusions and no statistical testing was conducted.
4.10	**Statistical Test Field** *[Free-text]*	• If the statistical test is not provided in the study, add "Not Reported" to the text field.
4.11	**Results Notes Field** *[Free-text]*	• If needed, copy and paste details into this field using quotation marks. Although the methods text field can describe all methods used, results comments should be more endpoint specific.
5	**Endpoint (Dummy Variables)** Data to be extracted using dummy variables for the following reasons: • Results that are qualitatively discussed in the text, but actual data are not provided. • For instances where study authors specify that only the significant effects are described – and certain endpoints are then not discussed – assume that no change occurred in these endpoints. Create dummy variables for all	• For endpoints for which no quantitative data are provided, create the endpoint as described above with the exceptions below. • 'Dataset type' is dichotomous or continuous based on the data type if there were data available. • For 'Response units,' use whatever units correspond to the effect for which you are creating the dummy variable (e.g., 'incidence' for histopathology observations, 'grams' for body weight) • Under 'Dose-response data,' fill in with a dummy variable. Use 0 to indicate no change from control, a 1 to indicate an increase from control and a –1 to indicate a decrease from the control. • 'Significance Level' should be populated if the author indicates significance. Otherwise, 'Significance Level' is left blank. • Multiple clinical observations can be grouped together into a single endpoint. • Example: create an endpoint for clinical observations and add dummy variables to indicate no effect.

Questions/Prompts and Options	Suggested Considerations
endpoints stated to be measured with the assumption if they are not discussed they were not significant and make sure to document this in the results comments field. • If an endpoint is discussed in the methods, but there is no mention at all in the results (even to indicate that only significant effects were reported), then create an endpoint only and do not extract any data. In this case, uncheck the 'data reported' and 'data extracted' boxes on the endpoint page. • Organs/tissues that were examined for histopathological changes, but no changes were noted. • Clinical observations in which multiple clinical signs or general observations are grouped together.	• If a single endpoint called "Clinical Observation," create the dummy variables above using all 0 with nothing tagged as significant. • Or if there was an effect, still create a single endpoint called "Clinical Observation" and then put a 1 at the dose where the effects were observed and then in the results comment field indicate the effects that were observed. This would be common in reproductive and developmental studies; indicate if there were "Clinical Observations in Dams" and where they occurred but did not want to have a separate endpoint for each observation. • Example: for any organ listed but not specified any lesions to extract, create a histopathology endpoint and create a dummy variable to indicate no treatment-related effect. • Create an endpoint for each organ (e.g., Liver Histopathology, Kidney Histopathology, Uterus Histopathology), and create the dummy variables described above using all 0 with nothing tagged as significant. • Whenever using dummy variables instead of actual data, make sure to note in the results comment text box that the data are dummy variables using the standard language given in the instructions in HAWC under the 'Results notes' box.

Notes: NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; CAS = Chemical Abstracts Service.

A.1.10 Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes. For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review, considering the conclusions from the individual study quality evaluations. Syntheses of the evidence for human and animal health effects are based primarily on studies of *high* and *medium* confidence; *low* confidence results were given less weight compared with *high* or *medium* confidence results during evidence synthesis and integration. However, in certain instances (i.e., for health outcomes for which few or no studies with higher confidence are available), *low* confidence studies might be used to help evaluate consistency, or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect.

The available human and animal evidence pertaining to the potential health effects of PFOA were synthesized separately, and a summary discussion of the available evidence was developed for each evidence stream. For the five priority health outcomes, mechanistic evidence was also considered in the development of each synthesis. Strength-of-evidence judgments were made for each health outcome within each evidence stream (i.e., human or animal) using standard terminology (i.e., *robust, moderate, slight, indeterminate*) and definitions according to the framework described in the IRIS Handbook and outlined in Table A-41 and Table A-42.

Following evidence synthesis, the evidence for humans and animals was integrated for each health outcome. The evidence integration was conducted following the guidance outlined in the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). Integrated judgments were drawn across all lines of evidence for each assessed health outcome as to whether, and to what extent, the evidence supports that exposure to PFOA has the potential to be hazardous to humans. The evidence integration provided a summary of the causal interpretations from the available studies, as well as mechanistic evidence for the five priority health outcomes. Mechanistic evidence was organized by pathway or other categories (e.g., key characteristics of carcinogens) as relevant to each outcome. The integrated judgments are developed through structured review of the evidence against an established set of considerations for causality. These considerations include risk of bias, sensitivity, consistency, strength (effect magnitude) and precision, biological gradient/dose-response, coherence, and mechanistic evidence related to biological plausibility. The evidence integration involved an overall judgment on whether there was sufficient evidence or insufficient evidence for each potential human health effect and an evidence basis rationale. During evidence integration, a structured and documented process was used as follows:

- Summarize human and animal health effects studies in parallel but separately, using the set of considerations for causality first introduced by Austin Bradford Hill (Hill, 1965) and relevant mechanistic evidence (or mode of action (MOA) understanding).
- Identify strength of the human and animal health evidence in light of inferences across evidence streams.
- Summarize judgment as to whether the available evidence base for each potential health outcome as a whole indicates that PFOA exposure has the potential to cause adverse health effects in humans (see Table A-43) (“evidence demonstrates,” “evidence indicates

(likely)," "evidence suggests," "evidence is inadequate," or "strong evidence supports no effect").

The decision points within the structured evidence integration process are summarized in an evidence profile table for each assessed health effect.

Table A-41. Framework for Strength-of-Evidence Judgments for Epidemiological Studies[a]

Strength-of-Evidence Judgment	Description
Robust (⊕⊕⊕)	• A set of *high*- or *medium* confidence studies reporting an association between the exposure and the health outcome, with reasonable confidence that alternative explanations, including chance, bias, and confounding, can be ruled out across studies. The set of studies is primarily consistent, with reasonable explanations when results differ; and an exposure-response gradient is demonstrated. Supporting evidence, such as associations with biologically related endpoints in human studies (coherence) or large estimates of risk or severity of the response, may help to rule out alternative explanations. Similarly, mechanistic evidence from exposed humans may serve to address uncertainties relating to exposure-response, temporality, coherence, and biological plausibility (i.e., providing evidence consistent with an explanation for how exposure could cause the health effect based on current biological knowledge) such that the totality of human evidence supports this judgment.
Moderate (⊕⊕⊙)	• Multiple studies showing generally consistent findings, including at least one *high* or *medium* confidence study and supporting evidence, but with some residual uncertainty due to potential chance, bias, or confounding (e.g., effect estimates of low magnitude or small effect sizes given what is known about the endpoint; uninterpretable patterns with respect to exposure levels). Associations with related endpoints, including mechanistic evidence from exposed humans, can address uncertainties relating to exposure response, temporality, coherence, and biological plausibility, and any conflicting evidence is not from a comparable body of higher confidence, sensitive studies • A single *high*- or *medium* confidence study demonstrating an effect with one or more factors that increase evidence strength, such as: a large magnitude or severity of the effect, a dose-response gradient, unique exposure or outcome scenarios (e.g., a natural experiment), or supporting coherent evidence, including mechanistic evidence from exposed humans. There are no comparable studies of similar confidence and sensitivity providing conflicting evidence, or if there are, the differences can be reasonably explained (e.g., by the population or exposure levels studied)
Slight (⊕⊙⊙)	One or more studies reporting an association between exposure and the health outcome, where considerable uncertainty exists: • A body of evidence, including scenarios with one or more *high* or *medium* confidence studies reporting an association between exposure and the health outcome, where either (1) conflicting evidence exists in studies of similar confidence and sensitivity (including mechanistic evidence contradicting the biological plausibility of the reported effects), a (2) a single study without a factor that increases evidence strength (factors described in moderate), OR (3) considerable methodological uncertainties remain across the body of evidence (typically related to exposure or outcome ascertainment, including temporality), AND there is no supporting coherent evidence that increases the overall evidence strength. • A set of only *low* confidence studies that are largely consistent. • Strong mechanistic evidence in well-conducted studies of exposed humans (*medium* or *high* confidence) or human cells, in the absence of other substantive data, where an informed evaluation has determined that the data are reliable for assessing the health effect of interest and the mechanistic events have been reasonably linked to the development of that health effect.

Strength-of-Evidence Judgment	Description
Indeterminate (⊙⊙⊙)	• No studies in humans or well-conducted studies of human cells. • Situations when the evidence is highly inconsistent and primarily of *low* confidence. • May include situations with *medium* or *high* confidence studies, but unexplained heterogeneity exists (in studies of similar confidence and sensitivity), and there are additional outstanding concerns such as effect estimates of low magnitude, uninterpretable patterns with respect to exposure levels, or uncertainties or methodological limitations that result in an inability to discern effects from exposure. • A set of largely null studies that does not meet the criteria for compelling evidence of no effect, including evidence bases with inadequate testing of susceptible populations and lifestages.
Compelling evidence of no effect (– – –)	• Several *high* confidence studies showing null results (for example, an odds ratio of 1.0), ruling out alternative explanations including chance, bias, and confounding with reasonable confidence. Each of the studies should have used an optimal outcome and exposure assessment and adequate sample size (specifically for higher exposure groups and for susceptible populations). The set as a whole should include the full range of levels of exposures that human beings are known to encounter, an evaluation of an exposure-response gradient, and an examination of at-risk populations and lifestages.

Notes:
[a] Table adapted from Table 11-3 in the IRIS Handbook.

Table A-42. Framework for Strength-of-Evidence Judgments for Animal Toxicological Studies[a]

Strength-of-Evidence Judgment	Description
Robust (⊕⊕⊕)	• A set of *high*- or *medium* confidence studies with consistent findings of adverse or toxicologically significant effects across multiple laboratories, exposure routes, experimental designs (e.g., a subchronic study and a two-generation study), or species; and the experiments reasonably rule out the potential for nonspecific effects to have caused the effects of interest. Any inconsistent evidence (evidence that cannot be reasonably explained based on study design or differences in animal model) is from a set of experiments of lower confidence or sensitivity. To reasonably rule out alternative explanations, multiple additional factors in the set of experiments exist, such as: coherent effects across biologically related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; or consistent observations across animal lifestages, sexes, or strains. Similarly, mechanistic evidence (e.g., precursor events linked to adverse outcomes) in animal models may exist to address uncertainties in the evidence base such that the totality of animal evidence supports this judgment.
Moderate (⊕⊕⊙)	• At least one *high*- or *medium* confidence study with supporting information increasing the strength of the evidence. Although the results are largely consistent, notable uncertainties remain. However, in scenarios when inconsistent evidence or evidence indicating nonspecific effects exist, it is not judged to reduce or discount the level of concern regarding the positive findings, or it is not from a comparable body of higher confidence, sensitive studies. The additional support provided includes either consistent effects across laboratories or species; coherent effects across multiple related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; or consistent observations across exposure scenarios (e.g., route, timing, duration), sexes, or animal strains. Mechanistic evidence in animals may serve to provide this support or otherwise address residual uncertainties.

Strength-of-Evidence Judgment	Description
	• A single *high* or *medium* confidence experiment demonstrating an effect in the absence of comparable experiment(s) of similar confidence and sensitivity providing conflicting evidence, namely evidence that cannot be reasonably explained (e.g., by respective study designs or differences in animal model).
Slight (⊕⊙⊙)	Scenarios in which there is a signal of a possible effect, but the evidence is conflicting or weak: • A body of evidence, including scenarios with one or more *high* or *medium* confidence experiments reporting effects but without supporting or coherent evidence (see description in moderate) that increases the overall evidence strength, where conflicting evidence exists from a set of sensitive experiments of similar or higher confidence (including mechanistic evidence contradicting the biological plausibility of the reported effects). • A set of only *low* confidence experiments that are largely consistent. • Strong mechanistic evidence in well-conducted studies of animals or animal cells, in the absence of other substantive data, where an informed evaluation has determined the assays are reliable for assessing the health effect of interest and the mechanistic events have been reasonably linked to the development of that health effect.
Indeterminate (⊙⊙⊙)	• No animal studies or well-conducted studies of animal cells. • The available models (not considering human relevance) or endpoints are not informative to the hazard question under evaluation. • The evidence is inconsistent and primarily of *low* confidence. • May include situations with *medium* or *high* confidence studies, but there is unexplained heterogeneity and additional concerns such as small effect sizes (given what is known about the endpoint) or a lack of dose-dependence. • A set of largely null studies that does not meet the criteria for compelling evidence of no effect.
Compelling evidence of no effect (– – –)	• A set of *high* confidence experiments examining a reasonable spectrum of endpoints relevant to a type of toxicity that demonstrate a lack of biologically significant effects across multiple species, both sexes, and a broad range of exposure levels. The data are compelling in that the experiments have examined the range of scenarios across which health effects in animals could be observed, and an alternative explanation (e.g., inadequately controlled features of the studies' experimental designs; inadequate sample sizes) for the observed lack of effects is not available. The experiments were designed to specifically test for effects of interest, including suitable exposure timing and duration, post exposure latency, and endpoint evaluation procedures, and to address potentially susceptible populations and lifestages. Mechanistic data in animals (in vivo or in vitro) that address the above considerations or that provide information supporting the A-118ssociaten association between exposure and effect with reasonable confidence may provide additional support such that the totality of evidence supports this judgment.

Notes:
[a] Table adapted from Table 11-4 in the IRIS Handbook.

Table A-43. Evidence Integration Judgments for Characterizing Potential Human Health Effects in the Evidence Integration[a]

Evidence integration judgment level	Explanation and example scenarios
Evidence demonstrates	• A strong evidence base demonstrating that [chemical] exposure causes [health effect] in humans • For when there is robust human evidence supporting an effect

Evidence integration judgment level	Explanation and example scenarios
	• Could also be used when there is moderate human evidence and robust animal evidence if there is strong mechanistic evidence that MOA(s) or key precursors identified in animals are expected to occur and progress in humans
Evidence indicates (likely)	• An evidence base that indicates that [chemical] exposure likely causes [health effect] in humans, although there may be outstanding questions or limitations. • Used if there is robust animal evidence supporting an effect and slight or indeterminate human evidence, or with moderate human evidence when strong mechanistic evidence is lacking • Could also be used with moderate human evidence supporting an effect and slight or indeterminate animal evidence, or with moderate animal evidence supporting an effect and slight or indeterminate human evidence. In these scenarios, any uncertainties in the moderate evidence are not sufficient to substantially reduce confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., precursors) exists to increase confidence in the reliability of the moderate evidence • A decision between "evidence indicates" and "evidence suggests" considers the extent to which findings are coherent or biologically consistent across lines of evidence streams, and may incorporate other supplemental evidence (e.g., structure-activity data; chemical class information)
Evidence suggests	• An evidence base that suggests that [chemical] exposure may cause [health effect] in humans, but there are very few studies that contributed to the evaluation, the evidence is weak or conflicting, and/or the methodological conduct of the studies is poor. • Used if there is slight human evidence and indeterminate or slight animal evidence • Used with slight animal evidence and indeterminate or slight human evidence • Could also be used with moderate human evidence and slight or indeterminate animal evidence, or with moderate animal evidence and slight or indeterminate human evidence. In these scenarios, there are outstanding issues regarding the moderate evidence that substantially reduced confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., null results in well-conducted evaluations of precursors) exists to decrease confidence in the reliability of the moderate evidence • When there is general scientific understanding of mechanistic events that result in a health effect, this judgment level could also be used if there is strong mechanistic evidence that is sufficient to highlight potential human toxicity in the absence of informative conventional studies in humans or in animals
Evidence inadequate[b]	• This conveys either a lack of information or an inability to interpret the available evidence for [health effect]. On an assessment-specific basis, a single use of this "evidence inadequate" judgment might be used to characterize the evidence for multiple health effect categories. • Used if there is indeterminate human and animal evidence • Used if there is slight animal evidence and compelling evidence of no effect human evidence • Could also be used with slight or robust animal evidence and indeterminate human evidence if strong mechanistic information indicated that the animal evidence is unlikely to be relevant to humans
Strong evidence supports no effect	• Extensive evidence across a range of populations and exposure levels has identified no effects/associations. This scenario requires a high degree of confidence in the conduct of individual studies, including consideration of study sensitivity, and comprehensive assessments of the endpoints and lifestages of exposure potentially relevant to the health effect of interest. • Used if there is compelling evidence of no effect in human studies and compelling evidence of no effect or indeterminate animal evidence • Also used if there is indeterminate human evidence and compelling evidence of no effect animal evidence in models judged as relevant to humans

Evidence integration judgment level	Explanation and example scenarios
	• Could also be used with compelling evidence of no effect in human studies and moderate or robust animal evidence if strong mechanistic information indicated that the animal evidence is unlikely to be relevant to humans

Notes: MOA = mode of action.
[a] Table adapted from Table 11-5 in the IRIS Handbook.
[b] An "evidence inadequate" judgment is not a determination that the chemical does not cause the indicated human health effect(s), but rather an indication that the available evidence is insufficient to reach a judgment.

A.1.10.1 Epidemiological Studies Included from 2016 PFOA HESD

For the five priority health outcomes (i.e., developmental, immune, hepatic, cardiovascular, and cancer), epidemiological studies identified and reviewed in the 2016 PFOA HESD were included in the evidence synthesis, including discussion of study quality considerations, according to the recommendations from the SAB. Inferences drawn from studies included from the 2016 PFOA HESD were considered in drawing health effects conclusions

For all nonpriority health outcomes, epidemiological studies identified and reviewed in the 2016 PFOA HESD were included in the evidence syntheses in summary paragraphs describing previously reached conclusions for each health outcome. Study quality was considered, but domain-based, structured study quality evaluations were not performed for 2016 PFOA HESD studies. Inferences drawn from evidence in the current literature search were compared with the results described from 2016 studies.

A.1.10.2 Epidemiological Studies Excluded from Synthesis

Some epidemiological studies were not included in the evidence synthesis narrative if they included overlapping results (e.g., overlapping NHANES studies). Studies reporting results from the same cohort with the same health outcome were considered overlapping evidence, and these studies were not discussed in the synthesis narrative to avoid duplication or overrepresentation of results from the same group of participants. When participants from the same cohort were included in more than one eligible study, the study with the largest number of participants was included in the evidence synthesis narrative. In general, to best gauge consistency and magnitude of reported associations, EPA focused on the most accurate and most prevalent measures. In some cases, such as developmental outcomes, studies on the same population providing more accurate outcome measures (e.g., birthweight and birth length for fetal growth restriction) were given preference over studies providing less accurate outcome measures (e.g., ponderal index for fetal growth restriction). Overlapping studies were included in study quality figures.

Meta-analyses were considered during evidence integration as support of consistent effects across studies. Details of the identified meta-analyses and assessment implications are summarized in Section A.2.

A.1.11 Dose-Response Assessment: Selecting Studies and Quantitative Analysis

As noted in the IRIS Handbook, selection of studies and endpoints for dose-response assessment involves considerations of the data that build from judgments and decisions made during earlier steps of the systematic review and assessment process. EPA guidance and support documents that describe data requirements and other considerations for dose-response modeling include EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

Dose-response assessments are performed for both noncancer and cancer oral health hazards, if supported by existing data. For noncancer hazards, an oral RfD will be derived when possible. 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, 2002). Reference values are not predictive risk values; that is, they provide no information about risks at higher or lower exposure levels.

For cancer hazards, a CSF will be derived to estimate human cancer risk when low-dose linear extrapolation for cancer effects is supported. 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). In contrast to RfDs, CSFs can be used in conjunction with exposure information to predict cancer risk at a given dose.

The derivation of reference values will depend on the conclusions drawn during previous steps of this protocol. Specifically, EPA will attempt dose-response assessments for noncancer outcomes when the evidence integration judgments indicate stronger evidence of hazard (i.e., *evidence demonstrates* and *evidence indicates* integration judgments). Quantitative analyses are generally not attempted for other evidence integration conclusions. Similarly, EPA will attempt dose-response assessments for cancer outcomes for chemicals that are classified as *Carcinogenic* or *Likely to be Carcinogenic to Humans*. When there is *Suggestive Evidence of Carcinogenic Potential to Humans*, EPA generally does not conduct dose-response assessment unless a well-conducted study is available and a quantitative analysis is deemed useful.

A.1.11.1 Study Selection

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*. 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. When multiple endpoints for a health outcome are available, endpoints are selected for dose-response analysis based on rationale describing how the endpoint is representative of the broader health outcome (U.S. EPA, 2022c). Studies were evaluated for use in POD derivation following considerations described in Table 7-2 (Table A-44) of the IRIS Handbook (U.S. EPA, 2022c). These attributes support a more complete characterization of the shape of the exposure-response curve and decrease the uncertainty in the associated exposure-response metric (e.g., RfD) by reducing statistical uncertainty in the POD and minimizing the need for low-dose extrapolation. Some important considerations include:

- Human data are preferred over animal data to eliminate interspecies extrapolation uncertainties,
- Animal species known to respond similarly to humans are preferred over studies of other species,
- *High* or *medium* confidence studies are preferred over *low* confidence studies,
- Chronic or subchronic studies, or studies encompassing a sensitive lifestage (i.e., gestational) are preferred for the derivation of chronic toxicity values over acute studies,
- Studies with a design or analysis that addresses relevant confounding for a given outcome are preferred,
- human studies providing the most updated data on a population are preferred over prior publications,
- and studies reporting all necessary data (e.g., total population or quartile exposure concentrations) for dose-response analysis are preferred.

The number of studies considered for toxicity value derivation was reduced based on these considerations and others described in EPA (U.S. EPA, 2022c, 2012).

Table A-44. Attributes used to evaluate studies for derivation of toxicity values (adapted from ORD Staff Handbook for Developing IRIS Assessments Table 7-2).

Study attributes		Considerations	
		Human studies	**Animal studies**
Study confidence		*High* or *medium* confidence studies are highly preferred over *low* confidence studies. The selection of *low* confidence studies should include an additional explanatory justification (e.g., only *low* confidence studies had adequate data for toxicity value derivation). The available high and medium confidence studies are further differentiated on the basis of the study attributes below, as well as a reconsideration of the specific limitations identified and their potential impact on dose-response analyses.	
Rationale for choice of species		Human data are preferred over animal data to eliminate interspecies extrapolation uncertainties (e.g., in pharmacodynamics, dose-response pattern in relevant dose range, relevance of specific health outcomes to humans).	Animal studies provide supporting evidence when adequate human studies are available, and they are considered the studies of primary interest when adequate human studies are not available. For some hazards, studies of particular animal species known to respond similarly to humans would be preferred over studies of other species.
Relevance of exposure paradigm	Exposure route	Studies involving human environmental exposures (oral, inhalation).	Studies by a route of administration relevant to human environmental exposure are preferred. A validated pharmacokinetic model can also be used to extrapolate across exposure routes.
	Exposure durations	When developing a chronic toxicity value, chronic or subchronic studies are preferred over studies of acute exposure durations. Exceptions exist, such as when a susceptible population or lifestage is more sensitive in a particular time window (e.g., developmental exposure).	
	Exposure levels	Exposures near the range of typical environmental human exposures are preferred. Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship (see the EPA *Benchmark Dose Technical Guidance*, §2.1.1) and facilitate extrapolation to more relevant (generally lower) exposures.	
Subject selection		Studies that provide risk estimates in the most susceptible groups are preferred.	
Controls for possible confounding		Studies with a design (e.g., matching procedures, blocking) or analysis (e.g., covariates or other procedures for statistical adjustment) that adequately address the relevant sources of potential critical confounding for a given outcome are preferred.	
Measurement of exposure		Studies that can reliably distinguish between levels of exposure in a time window considered most relevant for development of a causal effect are preferred. Exposure assessment methods that provide measurements at the level of the individual and that reduce measurement error are preferred. Measurements of exposure should not be influenced by knowledge of health outcome status.	Studies providing actual measurements of exposure (e.g., analytical inhalation concentrations vs. target concentrations) are preferred. Relevant internal dose measures might facilitate extrapolation to humans, as would availability of a suitable animal PBPK model in conjunction with an animal study reported in terms of administered exposure.
Health outcome(s)		Studies that can reliably distinguish the presence or absence (or degree of severity) of the outcome are preferred. Outcome ascertainment methods using generally accepted or standardized approaches are preferred.	
		Studies with individual data are preferred in general. For example, individual data allow you to characterize experimental variability more realistically and to characterize overall incidence of individuals affected by related outcomes (e.g., phthalate syndrome).	

Study attributes	Considerations	
	Human studies	**Animal studies**
	Among several relevant health outcomes, preference is generally given to those outcomes with less concerns for indirectness or with greater biological significance.	
Study size and design	Preference is given to studies using designs reasonably expected to have power to detect responses of suitable magnitude This does not mean that studies with substantial responses, but low power would be ignored, but that they should be interpreted in light of a confidence interval or variance for the response. Studies that address changes in the number at risk (through decreased survival, loss to follow-up) are preferred.	

Notes: PBPK = physiologically based pharmacokinetic.

A.1.11.2 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, 2002). 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, 2002). For PFOA, multiple candidate RfDs were derived within a health outcome as described in Section 4 of the Toxicity Assessment (U.S. EPA, 2024a).

Considerations for BMR selection are discussed in detail in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). For the derivation of RfDs, the BMR selected should correspond to a low or minimal level of response in a population for the outcome of interest and is generally the same across assessments, though the BMR could change over time based on new data or developments. The following general recommendations for BMR selection were considered for this assessment:

- For dichotomous data (e.g., presence or absence), a BMR of 10% extra risk is generally used for minimally adverse effects. Lower BMRs (5% or lower) can be selected for severe or frank effects. For example, developmental effects are relatively serious effects, and BMDs derived for these effects could use a 5% extra risk BMR. Developmental malformations considered severe enough to lead to early mortality could use an even lower BMR (U.S. EPA, 2022c, 2012).
- For continuous data, a BMR is ideally based on an established definition of biologic significance in the effect of interest. In the absence of such a definition, a difference of one standard deviation (SD) from the mean response of the control mean is often used and one-half the standard deviation is used for more severe effects. Note that the standard deviation used should reflect underlying variability in the outcome to the extent possible separate from variability attributable to laboratory procedures, etc. (U.S. EPA, 2022c, 2012).

Deviations of these recommendations, if any, are described in Section 4 of the Toxicity Assessment.

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. 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, 2012). 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, 2012). 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, 2022b, 2012). 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, 2012). 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, 2012). See Appendix E 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, 2012) because the EPA was able to define a level considered clinically adverse for these outcomes (see Appendix E). 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, 2012). See Appendix E 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. 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 For each noncancer dataset analyzed for dose-response, 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: extrapolation from animals to

humans, human variation, the type of POD being used for reference value derivation, extrapolation to chronic exposure duration, and extrapolation to a minimal level of risk (if not observed in the dataset). The particular value for these adjustments is usually 10, 3, or 1, but different values may be applied based on chemical-specific information if sufficient information exists in the chemical database. The assessment discusses the scientific bases for estimating these data-based adjustments and uncertainty factors (UFs). 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, 2002).

- Animal-to-human extrapolation: If animal results are used to make inferences about humans, the toxicity value incorporates cross-species differences, which may arise from differences in toxicokinetics or toxicodynamics. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. Otherwise, if the POD is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences.
- Human variation: The assessment accounts for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. If population-based data for the effect or for characterizing the internal dose are available, the potential for data-based adjustments for toxicodynamics or toxicokinetics is considered. Further, "when sufficient data are available, an intraspecies UF either less than or greater than 10× may be justified (U.S. EPA, 2002). However, a reduction from the default (10) is only considered in cases when there are dose-response data for the most susceptible population" (U.S. EPA, 2002). This factor is reduced only if the POD is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, 1991). Otherwise, a factor of 10 is generally used to account for this variation.
- LOAEL to NOAEL: If a POD is based on a LOAEL or a BMDL associated with an adverse effect level, the assessment must infer an exposure level where such effects are not expected. This can be a matter of great uncertainty if there is no evidence available at lower exposures. A factor of up to 10 is generally applied to extrapolate to a lower exposure expected to be without appreciable effects. A factor other than 10 may be used depending on the magnitude and nature of the response and the shape of the dose-response curve.
- Subchronic-to-chronic exposure: If a chronic reference value is being developed and a POD is based on subchronic evidence, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of up to 10 is applied when using subchronic studies to make inferences about lifetime exposure. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response. This factor may also be applied, albeit rarely, for developmental or reproductive effects if exposure covered less than the full critical period.
- In addition to the adjustments above, if database deficiencies raise concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database UF (U.S. EPA, 2002, 1991). The size of the factor depends on the nature of the database deficiency. For example, EPA typically follows the suggestion that a factor of 10 be applied if a prenatal toxicity study and a two-generation reproduction

study are both missing, and a factor of $10^{1/2}$ (rounded to 3) if either one or the other is missing. A database UF would still be applied if this type of study were available but considered to be a *low* confidence study.

The POD for a particular RfD is divided by the product of these factors. The RfD review recommends that any composite factor that exceeds 3,000 represents excessive uncertainty and recommends against relying on the associated RfD.

A.1.11.3 Cancer Assessment

A.1.11.3.1 Cancer Assessment

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 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).

A.1.11.3.2 Approach for Deriving Candidate CSFs

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).

[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.

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 Toxicity Assessment, (U.S. EPA, 2024b)). Then, BMDS fits multistage models, the preferred model type (U.S. EPA, 2012), 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, 2012). 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 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 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.

A.1.11.4 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, 2022c) 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 basing a more robust result on multiple data sets.

[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.

A.2 Meta-Analysis Table

Studies identified in title/abstract and full-text screening as assessments or records with no original data were considered supplemental material. Meta-analysis studies were included among those secondary studies. Consideration of meta-analyses alongside original epidemiology studies could lead to duplication of results and give greater weight to studies included in meta-analyses; therefore, meta-analysis studies were summarized separately. For PFOA, 25 epidemiological and 1 animal toxicity meta-analysis studies were identified and summarized below (Table A-45, Table A-46).

Table A-45. Epidemiologic Meta-Analysis Studies Identified from Literature Review

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Meta-Analysis Studies Identified before February 2022				
Johnson et al. (Johnson et al., 2014)	9	Canada, Denmark, Germany, Japan, South Korea, Taiwan, United Kingdom, United States	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in serum or plasma PFOA (9 studies) = –18.9 g (–29.8, –7.9), I^2 = 38% **Length:** • Pooled β per 1 ng/mL increase in serum or plasma PFOA (5 studies) = –0.06 cm (–0.09, –0.02), I^2 = 0% **Ponderal Index:** • Pooled β per 1 ng/mL increase in serum or plasma PFOA (5 studies) = –0.01 g/cm^3 (–0.03, 0.01), I^2 = 63% **Head Circumference:** • Pooled β per 1 ng/mL increase in serum or plasma PFOA (4 studies) = –0.03 cm (–0.08, 0.01), I^2 = 26%
Verner et al. (Verner et al., 2015)	7	Canada, Denmark, Japan, Norway, Taiwan, United Kingdom, United States	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in PFOA in maternal or cord blood (7 studies) = –14.72 g (–8.92, –1.09) • PBPK model simulations suggest that the association between PFAS levels and birthweight may be confounded by changes in glomerular filtration rate and due to blood draw timing
Negri et al. (Negri et al., 2017)	16	Canada, China, Denmark, Germany, Greenland, Japan, Norway, Poland, South Korea, Taiwan, Ukraine, United	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in PFOA (12 studies) = –12.8 g (–23.2, 2.4), I^2 = 53% • Pooled β per 1-ln ng/mL increase in PFOA (9 studies) = –27.1 g (–50.6, –3.6), I^2 = 28%

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
		Kingdom, United States		
Steenland et al. (Steenland et al., 2018a)	24	NR	Developmental	**Birthweight:** • Pooled β per 1 ng/mL increase in PFOA in maternal or cord blood (24 studies) = −10.5 g (−16.7, −4.4), I^2 = 63% • After inclusion of one additional large study (Savitz et al., 2012a) (25 studies) = −1.0 g (−2.4, 0.4) • Cord blood studies only (9 studies) = −13.3 g (−24.7, −1.8), I2 = 47% • Maternal blood studies only (15 studies) = −9.2 g (−15.6, −2.8), I^2 = 66% • Difference between pooled effect estimates from studies with early- and late-pregnancy blood sampling had a p-value of 0.02 • Early-pregnancy blood PFOA (9 studies) = –3.3 g (–9.6, 3.0), I^2 = 68% • Late-pregnancy blood PFOA (17 studies) = –17.8 g (–25.0, –10.6), I^2 = 29%
Cao et al. (Cao et al., 2021)	6	South Korea, Spain, Taiwan, United States	Developmental	**LBW:** • Pooled OR for maternal PFOA (6 studies) = OR: 0.90 (0.80–1.01), I^2 = 18.4%
Deji et al. (Deji et al., 2021)	21	Brazil, Canada, China, Denmark, Norway, Spain, United States	Developmental, Female Reproductive	**Miscarriage:** • Pooled OR (6 studies) = 0.98 (0.92, 1.05), I^2 = 0%, heterogeneity p = 0.502 **PTB:** • Pooled OR (16 studies) = 0.98 (0.89, 1.08), I^2 = 54.6%, heterogeneity p = 0.005
Gao et al. (Gao et al., 2021)	29	Brazil, Canada, China, Denmark, Norway, Spain, Sweden, United States	Developmental, Female Reproductive	**PTB**[c]: • (8 studies) inverted U-shaped association, increased risk in middle exposure range (p-value for nonlinear trend = 0.030) • **GDM** (7 studies), **miscarriage** (2 studies), **preeclampsia** (4 studies), **pregnancy-induced hypertension** (2 studies), **SGA** (6 studies), **LBW** (2 studies): Associations not statistically significant
Yang et al. (Yang et al., 2022b)	23	Belgium, Canada, China, Denmark, Netherlands, Norway, Slovakia, Spain,	Developmental	**PTB:** • Pooled OR (14 studies) = 1.22 (0.95, 1.57), I^2 = 58.8% ○ Significant associations for PFOA in maternal blood sampled in 3rd trimester to delivery (2 studies, pooled OR = 2.25 [1.07,

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
		Sweden, United States		4.74], I^2 = 0%), and for maternal blood sample type overall (13 studies, pooled OR = 1.29 [1.01, 1.66], I^2 = 52.6%) **Miscarriage:** • Pooled OR for PFOA in maternal blood (5 studies) = 1.40 (1.15, 1.70), I^2 = 0% o Pooled OR for PFOA in maternal blood sampled in 1st–2nd trimester (3 studies) = 1.50 (1.16, 1.95), I^2 = 0% **SGA:** • Pooled OR (11 studies) = 1.08 (0.93, 1.27), I^2 = 0% **LBW:** • Pooled OR (7 studies) = 1.02 (0.80, 1.29), I^2 = 0%
Costello et al. (Costello et al., 2022)	25	Belgium, China, Finland, France, Greece, Japan, Lithuania, Norway, Spain, Sweden, United Kingdom, United States	Hepatic	**ALT:** • PFOA was associated with higher ALT levels in adults and adolescents o Cross-sectional (8 studies) weighted z-score = 6.20, $p < 0.001$ o Longitudinal (3 studies) weighted z-score = 5.12, $p < 0.001$ o In children less than 12 yr of age, associations were not statistically significant (2 studies) **GGT:** • PFOA was associated with higher GGT levels in adults o Cross-sectional (8 studies) weighted z-score = 4.13, $p < 0.001$ o Longitudinal (1 study) reported a positive association **AST**, **other liver enzymes**: • Associations for PFOA not statistically significant
Abdullah Soheimi et al. (Abdullah Soheimi et al., 2021)	29	Canada, China, Denmark, Italy, Norway, Spain, Sweden, Taiwan, United States	Cardiovascular (18 studies)	**CVD Risk:** • Small overall effect between serum PFOA and CVD risk (16 studies); z = 1.56, p = 0.12, I^2 = 72.1% • Inconsistent associations between serum PFOA and coronary heart disease and stroke
			Serum Lipids (11 studies)	• Consistent associations between serum PFOA and increased serum TC, LDL, TG levels, and uric acid
			Metabolic (3 studies)	• Inconsistent associations between serum PFOA and increased GDM in pregnant mothers compared with non-pregnant mothers
Kim et al. (Kim et al., 2018)	11	China, South Korea, Japan, Norway, Taiwan, United States	Endocrine	**Total T3:** • Pooled z-value (7 studies) = 0.03 (0.00, 0.06), I^2 = 43%

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
				• **Free T4** (8 studies), **Total T4** (8 studies), **TSH** (11 studies): Associations not statistically significant ○ Sensitivity analyses removed one outlier for **total T4**; z-value = −0.06 (−0.08, −0.03), I^2 = 47% • Subgroup analyses stratified by PFOA levels or pregnancy status: Associations not statistically significant
Liu et al. (Liu et al., 2018c)	10	Denmark, Faroe Islands, Greenland, Norway, Spain, Sweden, Taiwan, Ukraine, United States	Metabolic	**Overweight risk:** • Overall effect size for maternal plasma/serum PFOA and childhood overweight risk (8 studies) = 1.25 (1.04, 1.50), I^2 = 40.5% **BMI z-score:** • Pooled β for maternal plasma/serum (9 studies) = 0.10 (0.03, 0.17), I^2 = 27.9% • Significant association between early-life exposure to PFOA and childhood BMI z-score from studies in Europe (7 studies), but not studies in North America (3 studies) or Asia (1 study) • Subgroup of analyses adjusted by maternal parity (7 studies) = β = 0.13 (0.02, 0.24), I^2 = 47.4% • Subgroup of analyses stratified by sex (4 studies): Associations not statistically significant for either sex
Zare Jeddi et al. (Zare Jeddi et al., 2021b)	7	Canada, China, Croatia, Italy, United States	Metabolic	**Metabolic syndrome:** • Pooled OR: 1.06 (0.9, 2.34), I^2 = 67.6%
Stratakis et al. (Stratakis et al., 2022)	21	China, Demark, Faroe Islands, Greenland, Netherlands, Norway, Spain, Sweden, Taiwan, Ukraine, United Kingdom, United States	Metabolic	**BMI z-score:** • Inverse association reported between prenatal PFOA exposure and BMI-z in infancy (3 studies): β = −0.02 (−0.08, 0.05), I^2 = 70.9% • BMI-z in childhood (2–9 yr) (10 studies): β = 0.03 (−0.02, 0.08), I^2 = 55.5% • Waist circumference in childhood (4 studies): β = 0.30 (−0.50, 1.09), I^2 = 85.7% • Inconsistent associations between PFOA exposure and fat mass, overweight risk
Qu et al. (Qu et al., 2021)	8	Denmark, Greenland, Norway, Poland, Sweden, Ukraine, United States	Neurodevelopmental	**ADHD:** • Pooled OR = 1.00 (0.75, 1.25), I^2 = 76.6% • Subgroup analyses for differences by region or exposure type not significant

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
Bartell and Vieira (Bartell and Vieira, 2021)	7	NR	Cancer	**Kidney cancer:** • Concluded that PFOA is a likely cause of kidney cancer and testicular cancer in humans • Rate ratio for cancer incidence per 10 ng/mL increase in serum PFOA: o Kidney cancer (7 studies) = 1.16 (1.03, 1.30) o Testicular cancer (3 studies) = 1.03 (1.02, 1.04)
Meta-Analysis and Pooled Studies Identified after February 2022				
Jiang et al. (Jiang et al., 2022)	8	China, Denmark, France, Japan, The Philippines, United States	Cancer	**Breast cancer:** • PFOA was positively correlated with breast cancer risk: pooled OR = 1.32 (1.19, 1.46), I^2 = 98.5% • Results influenced by the largest study (Omoike et al., 2021). • Serious methodological limitations warrant cautious interpretations of results from this publication.
Gui et al. (Gui et al., 2022a)	46	Australia, Brazil, Canada, China, Denmark, England, Faroe Islands, Germany, Greenland, Japan, Norway, Poland, South Korea, Spain, Sweden, Taiwan, Ukraine, United States	Developmental	Meta-analysis of 24 studies, pooled change in birthweight per 1 ng/mL increase in PFOA (unadjusted for gestational age/unstandardized birth weight). Significant effects observed for birth weight, birth length, and ponderal index. No significant associations observed for head circumference, preterm birth, low birth weight, or small-for-gestational age. Subgroup analyses were included, by fetal gender, time of blood sample collection, blood sample type and whether adjusted for GA/parity, study design, and geographic region. Described assessment of risk of bias for studies included in the meta-analyses. **Birth weight:** • Pooled β per 1 ln(ng/mL) increase in PFOA (24 studies) = –37.02 g (–54.37, –19.66), I^2 = 56.5% **Birth length:** • Pooled β for the highest vs. lowest PFOA exposure (4 studies) = –0.301 cm (–0.529, –0.073), I^2 = 36.6% **Ponderal index:** • Pooled β per 1 ng/mL increase in PFOA (3 studies) = –0.412 g/cm^2/100 (–0.787, –0.037), I^2 = 0.0% **Head circumference:** • Pooled β per 1 ln(ng/mL) increase in PFOA (11 studies) = –0.20 cm (–0.47, 0.07), I^2 = 0.0%

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
				PTB • Associations not statistically significant. **LBW** • Associations not statistically significant. **SGA:** • Associations not statistically significant.
Zhang et al. (Zhang et al., 2022b)	9	Faroe Islands, Germany, Greenland, Guinea-Bissau, Norway, United States	Immune	**Vaccine antibody production in children:** **Tetanus antibodies:** • Pooled effect estimate (3 studies, 5 results) = –20.11 (–29.45, –10.77), p-value for heterogeneity = 0.26 • Unclear what the effect estimate measures reported are and what units were used for PFOA exposure. **Diphtheria antibodies:** • No association for PFOA exposure.
Gui et al. (Gui et al., 2022b)	22	China, Norway, Sweden, South Korea, Taiwan, United States	Metabolic	**Diabetes:** • Case-control studies (number of studies not reported): pooled OR of T2DM incidence for high vs. low PFOA exposure = 0.53 (0.28, 1.00), I^2 = 88%; OR per ln-ng/mL increase in PFOA = 0.11 (0.04, 0.34), I^2 = 75% • Cohort studies (6 studies): pooled HR per ln-ng/mL increase in PFOA = 1.51 (1.09, 2.10), I^2 = 96% • No significant association with PFOA in case-control and cross-sectional studies combined.
Steenland et al. (Steenland et al., 2022)	2	United States	Cancer	**RCC:** • 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. Pooled odds ratios of RCC were evaluated using a linear spline model with a knot at 12.5 ng/mL. • Pooled OR per 1 ng/mL increase in PFOA from the median up to 12.5 ng/mL= 2.02, 95% CI: 1.45, 2.80 • The estimated lifetime excess risk associated with an exposure to 1 ng/mL PFOA (i.e., cancer slope factor) was 0.0018.
Wang et al. (Wang et al., 2022a)	7	China, Denmark, Faroe Islands, Greenland, Poland,	Male Reproductive	**Semen quality:** • PFOA inversely associated with sperm progressive motility • Pooled β (3 studies), β = −1.38 (−2.44, −0.32), I^2 = 2.6%

Reference	Number of Studies	Countries	Health Outcome	Results/Conclusions[a]
		Ukraine, United States		• Change in PFOA associated with pooled β not reported. • No association with other semen quality parameters (i.e., sperm concentration, sperm motility, semen volume, sperm count, and sperm morphology).
Pan et al. (Pan et al., 2023)	12	China, Italy, Norway, Sweden, United States	Cardiovascular	**Hypertension:** • Pooled OR (12 studies, 13 results) = 1.12 (1.02–1.23), I^2 = 75.4% • Unit change in PFOA associated with pooled OR not reported. • Serious methodological limitations warrant cautious interpretations of results from this publication. These include missing studies, inclusion of studies with overlapping populations, lack of effect estimate with common unit change in exposure.
Yu et al. (Yu et al., 2023)	9	NR	Renal	**Hyperuricemia:** • Pooled OR (6 studies) = 1.44 (1.15, 1.79), I^2 = 60% • Change in PFOA associated with pooled OR not reported.
Zhang et al. (Zhang et al., 2023a)	13	Canada, China, Denmark, Norway, Spain, South Korea, Taiwan, United States,	Endocrine	**TSH during pregnancy:** • Pooled β per ng/mL increase in PFOA (13 studies) = 0.010 (0.009, 0.011), I^2 = 0.0% • No significant associations with other thyroid hormones (e.g., total T3, total T4, free T3, free T4)

Notes: PFOA = perfluorooctanoate; PBPK = Physiologically based pharmacokinetic; NR = not reported; OR = odds ratio; GDM = gestational diabetes mellitus; SGA = small for gestational age; LBW = low birth weight; PTB = preterm birth; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; AST = aspartate aminotransferase; CVD = cardiovascular disease; TC = total cholesterol; LDL = low-density lipoproteins; TG = triglyceride; T4 = thyroxine; TSH = thyroid stimulating hormone; BMI = body mass index; ADHD = attention deficit hyperactivity disorder; GA = gestational age; PTB = preterm birth; LBW = low birth weight; T2DM = type 2 diabetes mellitus; HR = hazard ratio; NCI = National Cancer Institute; RCC = renal cell carcinoma; T3 = triiodothyronine.

[a] Results reported as effect estimate and 95% confidence interval (CI) unless otherwise stated.

[b] Toxicological study data included in these publications were not subject to meta-analysis.

[c] Preterm birth was defined as birth ≤37 wk of gestation.

Table A-46. Animal Toxicity Meta-Analysis Studies Identified From Literature Review

Reference	Number of Studies	Animal Sex and Model/Species	Health Outcome(s)	Results/Conclusions[a]
Wang et al. (Wang et al., 2021)	16	Male Rats, Male Mice	Male Reproductive (including Cancer)	• SMD for reproductive toxicity (14 studies) = −0.39 (−0.71, −0.07), p = 0.02, I^2 = 80% • SMD for serum testosterone levels (6 studies) = −0.54 (−0.95, −0.13), p = 0.01, I^2 = 71% • Mean difference for serum estradiol levels (3 studies) = 4.75 (2.29, 7.21), p = 0.0002, I^2 = 91% • SMD for absolute testicular weight (7 studies) =: −0.20 (−0.33, −0.06), p = 0.005, I^2 = 44% • SMD for absolute epididymis weight (2 studies) = −0.01 (−0.02, −0.01), p < 0.0001, I^2 = 39% • OR for incidence of Leydig cell adenoma (2 studies) = 8.47 (2.74, 26.18), p = 0.0002, I^2 = 0% • Mean difference for percentage of abnormal sperm (2 studies) = 1.48 (0.65, 2.30), p = 0.0004, I^2 = 87% • Day of preputial separation, risk of testis atrophy, risk of epididymis tubular atrophy, sperm motility: Associations not statistically significant

Notes: SMD = standard mean difference; OR = odds ratio.
[a] Results reported as effect estimate and 95% confidence interval (CI) unless otherwise stated.

A.3 Studies Identified In Supplemental Literature Search Assessment Literature Cut-Off Date

The EPA conducted a supplemental literature search in 2023. Consistent with the final IRIS handbook (U.S. EPA, 2022c), 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, 2022b). 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) for PFOA or alter the cancer classification.

The EPA has defined a systematic process for assessing the potential for a quantitative impact that is consistent with the final IRIS Handbook. First, the EPA reviewed studies against two broad inclusion criteria for new relevant health effects studies: 1) the study met the predefined PECO criteria and 2) following the SAB PFAS Review Panel's recommendation, the health effect/endpoint described in the study was within the one of the five health outcomes determined to have the strongest weight of evidence (i.e., developmental, hepatic, immune, cardiovascular, and cancer) (U.S. EPA, 2022c). Second, for studies that met these two inclusion criteria, two or more subject matter experts (e.g., epidemiologists and/or toxicologists) independently reviewed the studies to determine whether the study conclusions potentially impacted assessment conclusions. Subject matter experts considered a variety of factors to determine this, including, but not limited to, whether the publication provided 1) information on a health effect (within the five priority health outcomes) that was not previously quantitatively considered for dose response; 2) information on health effects that were previously considered quantitatively and potentially indicated effects at lower doses than the critical studies selected for the draft points of departure (PODs), RfD, or CSF; or 3) information on health effects that were previously considered quantitively and may have improved study design or data analyses compared with those that were selected for POD, RfD, or CSF derivation. If the subject matter experts disagreed about a study's potential quantitative impact, an additional expert independently reviewed the rationale and made a final decision. The EPA provides the rationales for study inclusion decisions in Table A-47 and Table A-48. For PFOA, 52 epidemiological and 3 animal toxicity studies were identified after the updated literature search in 2022 and underwent title/abstract and full-text screening according to Section A.1.6. These studies are summarized below (Table A-47 and Table A-48). Studies that were selected for inclusion proceeded to study quality evaluation and were incorporated into the relevant evidence synthesis and dose-response analysis when the study was determined to be *medium* or *high* confidence.

Numerous studies identified in the supplemental literature search examined associations between elevated exposure to PFOA and the priority health outcomes described in the Toxicity Assessment (U.S. EPA, 2024b) (i.e., cancer, hepatic, immune, cardiovascular, and developmental). Specifically, there were eight studies examining the effects of exposure to PFOA on cancer, 11 studies examining the effects of exposure to PFOA on serum lipids, seven studies examining the effects of exposure to PFOA on birth weight, one study examining the effect of exposure to PFOA on antibody response in children, and four studies examined the effect of exposure to PFOA on ALT concentrations. Summaries of these studies and their

potential impact to the evidence base, as well as additional studies examining outcomes belonging to the five priority health outcomes, are provided in Table A-47.

No studies identified in the supplemental literature search provided new evidence for kidney or testicular cancer. One study (Zhang et al., 2023c) examining immune effects was determined to impact assessment conclusions and proceeded through systematic review steps, including study quality evaluation, extraction, incorporation into the evidence synthesis, and considered for dose-response analysis. The study reported a decreased antibody response to rubella in adolescents associated with elevated exposure to PFOA. This effect was consistent with other studies reporting decreased antibody response to other pathogens (i.e., tetanus and diphtheria), but provided additional evidence for a different pathogen.

Table A-47. Epidemiological Studies Identified After 2022 Updated Literature Search (Published or Identified Between February 2022 and February 2023)

Reference	Major Findings	Assessment Implications
	Cancer	
Cao et al. (2022)	Case-control study conducted in Hangzhou, China of 203 liver cancer cases and 203 healthy controls (2019–2021). The odds of liver cancer incidence were elevated with increasing PFOA exposure (OR = 1.036, 95% CI: 1.002, 1.070), but the study authors noted the result did not reach significance (p for trend = 0.07).	*Liver Cancer:* Exposure to PFOA may be associated with increased risk of liver cancer in adults. In the updated evidence base, there was one study (1/6) that reported significantly increased risk of liver cancer. Considering both studies post-dating the 2022 literature search, there was only one study reporting significantly increased risk of liver cancer (1/8). However, one newly identified study reported a marginally significant increased risk (Cao et al., 2022) and the other study reported a suggestive positive association (2022 10369722). Both studies were considered for deriving PODs for PFOA and were moved forward and integrated into the MCLG synthesis for cancer (see Toxicity Assessment, (U.S. EPA, 2024b)). Cao et al. (2022) was determined to be *low* confidence during study quality evaluation and was not modeled. For Goodrich et al. (2022), the study had a limited number of cases (n = 11) and controls (n = 4) in the highest exposure group and was not modeled due to low sensitivity.
Goodrich et al. (2022)	Nested case-control study within the MEC Study, including incident, non-viral HCC cases (n = 50) and healthy controls (n = 50). Nonsignificant increase in risk in in those with high PFOA exposure (>85th percentile; >8.6 µg/L) vs. low exposure (<85th percentile; <8.6 µg/L) (OR = 1.20, 95% CI: 0.49, 2.80).	
Feng et al. (2022b)	Case-cohort study within the Dongfeng-Tongji cohort, including incident breast cancer cases (n = 226) and a random sub-cohort (n = 990). Significant increase in risk per each 1-ln ng/mL increase in PFOA (HR = 1.35, 95% CI: 1.03, 1.78), in the highest (≥1.80 ng/mL) vs. lowest quartile (<0.84 ng/mL) (HR = 1.69, 95% CI: 1.05, 2.70)), and among postmenopausal women (HR = 1.34, 95% CI: 1.01, 1.77). Quantile g-computation analysis observed a 19% increased incident risk of breast cancer along with each simultaneous quartile increase in all ln-transformed PFOA concentrations (HR = 1.19, 95% CI: 1.01, 1.41), with PFOA accounting for 56% of the positive effect.	*Breast Cancer:* Exposure to PFOA may be associated with increased risk of breast cancer in postmenopausal women. Evidence for breast cancer was mixed in the updated evidence base with two *medium* confidence studies reporting an increased risk (2/8). Significant increases in risk were only observed in some subpopulations (e.g., stratified by genotype) and for some specific types of breast cancer (e.g., ER– and PR–

Reference	Major Findings	Assessment Implications
Li et al. (2022)	Case-control study (2012–2016) of incident Chinese breast cancer cases (n = 373) and healthy controls (n = 657). The risk of breast cancer was significantly elevated with increasing PFOA exposure (OR = 3.32, 95% CI: 2.32, 4.75). Significant associations for PFOA were also observed in analyses of breast cancer subtypes, including increased risk of ER+, PR+, Luminal A, and HER2+ breast cancers.	breast cancers). A meta-analysis also reported an increased risk of breast cancer, although there were methodological imitations that warrant cautions interpretations of results (Jiang et al., 2022). Considering the studies post-dating the 2022 updated literature review, two additional studies (2/3) reported significantly increased risk of breast cancer. Altogether, four studies (4/11) reported an increased risk of breast cancer, which provides an indication of a potentially increased risk of breast cancer with increasing PFOA exposure, but the overall evidence remains mixed. The studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Velarde et al. (2022)	Case-control study of 150 Filipino women (75 breast cancer cases and 75 controls). PFOA was not statistically significantly associated with breast cancer risk.	
Wen et al. (2022)	Population-based cohort study of 11,747 participants from 1999–2014 NHANES followed up to December 2015. PFOA was not statistically significantly associated with cancer mortality.	*All-cause Cancer:* There was concern for the self-reported outcome in Girardi et al. (Girardi et al., 2022) and the lack of specificity of all-cause cancer in this study. One study (1/2) from the updated evidence base reported a significantly increased risk of all-cause cancer but was considered *low* confidence. Considering both studies post-dating the 2022 updated literature did not observe associations (0/2), there was altogether mixed evidence for all-cause cancer (1/3). The studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Girardi et al. (2022)	Cohort study (TEDDY-Child Study) evaluating perceived health risks and self-reported health outcomes in mothers located in a high-exposure community (Veneto, Italy). No associations were observed for cancer.	
Messmer et al. (2022)	Ecological study of 24 types of cancer incidence (2005–2014) in Merrimack, New Hampshire compared with local and national incidence rates. Geometric mean levels of PFOA in blood were elevated among Merrimack, New Hampshire residents (GM = 3.9 μg/L). Significantly increased risks of incident thyroid cancer (RR = 1.47, 95% CI: 1.12, 1.93), bladder cancer (RR = 1.45, 95% CI: 1.17, 1.81), esophageal cancer (RR = 1.71, 95% CI: 1.1, 2.65), and mesothelioma (RR = 2.41, 95% CI: 1.09, 5.34) were observed compared with national rates.	*Other cancer types:* Exposure to PFOA may be associated with thyroid cancer, bladder cancer, esophageal cancer, and mesothelioma. In the updated evidence base, increases in risk were observed for one study (1/7) on bladder cancer and two studies (2/3) on thyroid cancer, although one was considered *low* confidence. Increases in risk for esophageal cancer and mesothelioma were not identified from any

Reference	Major Findings	Assessment Implications
		prior study. The evidence for these cancer types remains mixed, and interpretations of individual-level risk from this study may be limited by the study design.
	Cardiovascular	
Batzella et al. (2022b)	Cross-sectional study of residents (n = 36,517; aged 20-64) of the Veneto Region, Italy, a high-exposure community. In single-pollutant models, PFOA was significantly associated with increased TC (β per 1-ln-ng/mL increase in PFOA: 1.83, 95% CI: 1.51, 2.15), HDL-C (β: 0.32, 95% CI: 0.20, 0.44), and LDL-C (β: 1.10, 95% CI: 0.81, 1.38). Significant positive associations were observed for all three lipid measurements in PFAS mixture analyses (WQS), with PFOA identified as a primary contributor to the association between increased PFAS exposure and elevated HDL-C in females (weight: 0.29).	*Total Cholesterol:* Eleven studies identified after the 2022 updated literature search evaluated changes in TC, and six (6/11) reported significant increases in TC with elevated exposure to PFOA (Batzella et al., 2022b; Cakmak et al., 2022; Cheng et al., 2022; Dunder et al., 2022; Maranhao Neto et al., 2022; Nilsson et al., 2022). In the updated evidence base, there was evidence of increases in TC (19/23) associated with elevated PFOA exposure in studies of adults (see Toxicity Assessment, (U.S. EPA, 2024b)). Considering the updated evidence base and studies post-dating the 2022 literature search together, there were 24 of 33 general population adult studies reporting positive associations for TC. Overall, these studies support EPA's conclusion of *evidence indicates* that elevated exposures to PFOA are associated with adverse cardiovascular effects, specifically serum lipids, as well as EPA's selection of increased TC in adults for dose-response modeling.
Batzella et al. (2022a)	Cross-sectional occupational study of 232 retired and former male workers at a PFAS production plant located in Veneto, Italy (2018–2020). SBP was significantly elevated in the highest quartile of PFOA exposure compared with the lowest (β = 7.26, 95% CI: 2.66, 11.86), and in analyses of continuous exposure (β per 1-ln-ng/mL increase on PFOA = 1.04, 95% CI: 0.32, 1.77; β per IQR increase = 3.60, 95% CI: 0.86, 5.86). SBP was also significantly elevated in WQS regression analyses of PFAS mixture, with PFOA identified as a main contributor (weight: 0.31). No significant associations observed for TC, HDL-C, LDL-C, and DBP.	
Cheng et al. (2022)	Cross-sectional study of 98 patients recruited from Shiyan Renmin Hospital (Hubei, China; 2018–2019). Plasma PFOA concentrations were significantly associated with increased TC (β per 1-ln-ng/mL increase in PFOA = 5.812, 95% CI: 0.171, 11.801) and LDL-C (β: 8.325, 95% CI: 2.588, 14.383). No significant associations were observed for HDL-C or TG. Associations were partly mediated by methylation of genes related to lipid metabolism.	
Cakmak et al. (2022)	Population-based cross-sectional study (CHMS) of 6,768 participants aged 3–79 yr old. Increases in PFOA were significantly associated with elevated TC (2.5, 95% CI: 0.6, 4.4) and an increased TC/HDL-C ratio (4.5, 95% CI: 1.0, 8.2). No significant associations observed for TG, LDL-C, or HDL-C.	
Maranhao Neto et al. (2022)	Cross-sectional study of 479 adult participants (aged 25–89) from the Kardiovize study, Czech Republic. Serum PFOA was significantly	

Reference	Major Findings	Assessment Implications
	associated with increased SBP (β per 1-ln-ng/mL increase in PFOA = 0.76, SE = 0.32), DBP (β = 0.65, SE = 0.18), TC (β = 0.07, SE = 0.02), and LDL-C (β = 0.04, SE = 0.02). No significant associations were observed for HDL-C or TG.	*LDL-C, HDL-C, and TG:* Ten studies identified after the 2022 updated literature search evaluated changes in LDL-C, and three studies (3/10) reported significant increases in LDL-C with elevated exposure to PFOA (Batzella et al., 2022b; Cheng et al., 2022; Maranhao Neto et al., 2022). In the updated evidence base, 12 general population adult studies (12/17) reported positive associations for LDL-C. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were 15 general population adult studies (15/27) reporting positive associations for LDL-C. The findings for HDL-C and TG in these 10 studies were mixed, similar to results provided in the updated evidence base. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward; however, these studies support EPA's conclusion of *evidence indicates* that elevated exposures to PFOA are associated with adverse cardiovascular effects, specifically serum lipids.
Rosen et al. (2022)	Cross-sectional study of 326 participants in the GenX Exposure Study (2017–2018) in Wilmington, North Carolina. No associations were observed between serum PFOA measurements and TC, LDL-C, HDL-C, TG, or total non-HDL-C.	
Schillemans et al. (2022)	Population-based nested case-control study of Swedish adults (n = 1,528) in the SMC-C and the 60YO Cohort, including first incident myocardial infarction (n = 345) and stroke (n = 354) cases. In cross-sectional analyses among 128 controls from the 60YO cohort, baseline plasma PFOA was significantly associated with HDL-C in T2 and T3 compared with T1, and per 1-SD increase (T2 β = 0.11, 95% CI: 0.03, 0.20; T3 β = 0.15, 95% CI: 0.06, 0.24; per 1-SD-ln- ng/mL PFOA β = 0.05, 95% CI: 0.01, 0.09) and apoA1 in the highest tertile (T3 β = 0.09, 95% CI: 0.03, 0.16). No significant associations were observed among controls from the 60YO cohort between baseline PFOA plasma concentrations and serum TC, LDL-C, TG, or apoB. In prospective analyses of the pooled cohorts, there was no association between baseline plasma PFOA and risk of subsequent CVD, MI, or stroke.	
Papadopoulou et al. (2022)	Study of 127 Norwegian adults ages 24–72 yr old from the EuroMix study. Serum PFOA was significantly associated with a decrease in day 1 and day 2 triglycerides (percent change per IQR change in PFOA: −23%, 95% CI: −38, −4%). No significant associations were observed for TC, HDL-C, LDL-C, and VLDL.	
Nilsson et al. (2022)	Prospective occupational study of Australian firefighters who had used AFFF reporting cross-sectional (n = 783) and longitudinal (n = 130) analyses. PFOA was significantly associated with increased TC (β per doubling PFOA = 0.111, 95% CI: 0.026, 0.195) and LDL-C (β = 0.104, 95% CI: 0.03, 0.178) in cross-sectional analyses. No significant associations were observed for serum lipids in longitudinal analyses.	
Linakis et al. (2022)	Cross-sectional study of 7,242 NHANES participants (cycles 2003–2016). Serum PFOA was positively associated with increased ln-TC, and the magnitude of the association was not substantially altered by adjustment for energy intake-adjusted fiber.	

Reference	Major Findings	Assessment Implications
Dunder et al. (2022)	Prospective cohort study (PIVUS) of seniors at age 70 (n = 864) and followed up at age 75 (n = 614) and age 80 (n = 404) in Sweden. Increases in PFOA over the 10-year follow-up period were significantly associated with increases in TC (β = 0.14, 95% CI: 0.06, 0.22, p = 0.001), TG (β = 0.06, 95% CI: 0.03, 0.10, p < 0.0001), and HDL-C (β = 0.07, 95% CI: 0.04, 0.09, p < 0.0001). No significant association between changes in PFOA and LDL-C.	*Blood pressure and hypertension:* Measures of blood pressure and hypertension were examined in seven studies identified after the updated 2022 literature search, and five studies (5/7) reported significant increases in blood pressure or increased risk of hypertension (Tian et al., 2023; Batzella et al., 2022a; Ding et al., 2022; Maranhao Neto et al., 2022; Ward-Caviness et al., 2022). One meta-analysis post-dating the 2022 literature search reported a significantly increased risk of hypertension in adults, but there were some methodological limitations, which warrant cautious interpretations of results (Pan et al., 2023). In the updated evidence base, there was positive evidence in adult studies for increases in systolic (6/8) and diastolic blood pressure (7/8), as well as an increased risk of hypertension (9/10). Considering the updated evidence base and studies post-dating the 2022 literature search together, there were nine general population adult studies (9/12) reporting increases in systolic blood pressure and diastolic blood pressure (9/12); and 11 (11/13) general population adult studies reporting increases in risk for hypertension. Evidence for changes in blood pressure and increases in risk for hypertension were supportive of a conclusion of *moderate* evidence for cardiovascular effects, specifically serum lipids, although blood pressure and hypertension were not selected as outcomes for modeling.
Ding et al. (2022)	Cohort study of 1,058 women (ages 42–52 yr) with no hypertension from the multiethnic and multiracial SWAN. There was significantly increased risk of hypertension per doubling of n-PFOA (HR = 1.17, 95% CI: 1.07, 1.27, p < 0.0001), and across tertiles of baseline serum n-PFOA compared with the lowest tertile (p-trend < 0.0001). In the mixture analysis, women in the highest tertile of PFAS concentrations had a significantly higher risk of hypertension compared with those in the lowest tertile (HR = 1.71 ,95% CI: 1.15, 2.54; p-trend = 0.008).	
Yang et al. (2022a)	Prospective study of 826 pregnant women from the Jiashan Birth Cohort (enrollment 2016–2018), Jiashan, Zhejiang, China. No significant associations observed between plasma PFOA measured within 16 wk of gestation and gestational hypertension, or blood pressure measurements in any trimester of pregnancy.	
Tian et al. (2023)	Case-control study of pregnant women from Hangzhou, China, with (n = 82) and without (n = 169) preeclampsia. PFOA exposure measured 1-2 d before delivery was significantly associated with increased SBP (β per 1-log10-ng/mL increase in PFOA = 0.262, 95% CI: 0.147, 0.377) and DBP (β = 0.224, 95% CI: 0.133, 0.314).	
Zhang et al. (2022d)	Prospective study of 1,080 participants in the Dongfeng-Tongji cohort of retired workers in China established in 2008 and followed for approximately 5 yr. Baseline serum PFOA concentrations were significantly inversely associated with changes in SBP (β per log10-ng/mL increase in PFOA: −1.53, 95% CI: −2.93, −0.12). PFOA was not significantly associated with changes in DBP or with risk of incident hypertension.	
Feng et al. (2022a)	Population-based cross-sectional study (NHANES, cycles 2003–2012) of 7,904 adults. No significant associations were observed between PFOA exposure and heart failure, coronary heart disease, angina, heart attack, stroke, or total CVD.	

Reference	Major Findings	Assessment Implications
Li et al. (2023)	Hospital-based case-control study of adults with and without ACS (355 cases, 355 age- and sex-matched controls) recruited in 2022 in Shijiazhuang, Hebei, China. In single PFAS models, plasma PFOA was significantly associated with ACS (OR per 1-ln-ng/mL increase in PFOA = 2.43, 95% CI: 1.34, 4.39). The association between PFOA and ACS remained significant in multiple-PFAS models. No significant associations were observed with PFAS mixtures.	*Cardiovascular disease:* A variety of cardiovascular diseases, including heart arrythmia, myocardial infarction, stroke, angina, heart disease, and acute coronary syndrome were examined in six studies identified after the 2022 updated literature search, and two studies (2/6) reported significant increases in risk for cardiovascular disease (Li et al., 2023; Ward-Caviness et al., 2022). In the updated evidence base, evidence was limited for cardiovascular diseases with one study reporting increased risk of myocardial infarction (1/1) and one study reporting increased risk of stroke (1/2). Other cardiovascular diseases were examined in single studies, and no associations were observed. Considering the updated evidence base and studies post-dating the 2022 literature search together, evidence was mixed for myocardial infarction (1/3) and stroke (1/4). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward
Wen et al. (2022)	Population-based cohort study of 11,747 participants from 1999–2014 NHANES followed up to December 2015. PFOA was not statistically significantly associated with heart disease mortality.	
Ward-Caviness et al. (2022)	Cross-sectional study of electronic health records of 10,168 patients from the University all of North Carolina Healthcare System as a part of the EPA CARES resource (2004–2016). Presence of PFOA in the water system was significantly associated with higher prevalence of arrythmia (OR = 1.30, 95% CI: 1.05, 1.62) and hypertension (OR = 1.25, 95% CI: 1.08, 1.45). Marginally significant (p = 0.05) positive association between presence of PFOA in the water system and ischemic heart disease (OR = 1.26, 95% CI: 1.00, 1.60).	
Girardi et al. (2022)	Cohort study (TEDDY-Child Study) evaluating perceived health risks and self-reported health outcomes in mothers located in a high-exposure community (Veneto, Italy). No associations were observed for cardiovascular diseases.	
	Developmental	
Sevelsted et al. (2022)	Prospective study of 738 maternal-child pairs enrolled in a population-based birth cohort study (COPSAC-2010) in Zealand, Denmark (2008–2010). Maternal plasma PFOA (measured at 24 wk GA and 1 wk postpartum) was significantly associated with lower birth BMI z-score (β per 1-ng/mL increase = −0.19, 95% CI: −0.33, −0.05), decreased birth weight percentile for sex and GA (β = −4.28, 95% CI: −8.17, −0.39).	*Birth weight:* Seven studies identified after the updated literature search evaluated changes in birth weight (i.e., birth weight and birth weight for sex and GA), and three studies reported significant decreases. Of the studies reporting significant results, one study examined changes in birth weight in relation to PFOA concentrations measured in early pregnancy (Wang et al., 2023b), and two studies examined changes in relation to PFOA concentrations measured in later pregnancy (Tian et al., 2023; Sevelsted et al., 2022). Other studies not observing decreases in birth
Tian et al. (2023)	Case-control study of pregnant women from Hangzhou, China, with (n = 82) and without (n = 169) preeclampsia. PFOA exposure measured 1-2 d before delivery was significantly associated with decrease in birth weight (β per 1-log10-ng/mL increase in PFOA = −8.9, 95% CI: −13.9, −3.89) and with increased risk of LBW (OR per 1-log10-ng/mL increase in PFOA = 2.01, 95% CI: 1.98, 2.43, p = 0.002) and SGA (OR = 1.03, 95% CI: 1.00, 1.05, p-0.049).	

Reference	Major Findings	Assessment Implications
Jia et al. (2023)	Cross-sectional study of 66 infants born to women at a maternity hospital in Shijiazhuang, Hebei, China in 2022. No significant association between umbilical cord serum PFOA and birth weight.	weight were generally smaller (i.e., <200 participants) (Jia et al., 2023; Wang et al., 2023a; Hall et al., 2022), and frequently measured PFOA in samples collected in later pregnancy, at birth (i.e., umbilical cord or placenta PFOA), or were not reported (Jia et al., 2023; Hall et al., 2022; Shen et al., 2022). In the updated evidence base, there were 30 studies reporting deficits in birth weight (30/42). Considering the updated evidence base and studies post-dating the 2022 literature search together, deficits in birth weight were observed in 33 studies (33/49). Overall, these studies support EPA's conclusion of evidence indicates that elevated exposures to PFOA are associated with adverse developmental effects, as well as EPA's selection of decreased birth weight for dose-response modeling. *Other FGR:* Regarding other fetal growth restriction outcomes, one study identified after the updated literature search evaluated changes in other measures of fetal growth restriction (e.g., birth length, head circumference, and ponderal index) and observed a significant decrease in head circumference (Wang et al., 2023a). No associations were observed for birth length or ponderal index. In the updated evidence base, there was some evidence of adverse effects for birth length (13/31) and head circumference (14/25), but the evidence was generally mixed. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Gestational duration and PTB:* Preterm birth was examined in three studies, and all three
Hall et al. (2022)	Prospective birth cohort study of 120 mother-child pairs enrolled in the HPHB cohort in Durham, North Carolina (enrollment 2010–2011). No associations were observed between placental PFOA exposure and birth weight percentile, GA, or birth weight for GA.	
Shen et al. (2022)	Prospective study of 506 maternal-child pairs enrolled in a birth cohort study in Hangzhou, China (2020–2021). Maternal serum PFOA (GA at assessment not specified) was associated with significantly increased odds of PTB (OR per log10-ng/mL increase: 2.17, 95% CI: 1.27, 3.71). No significant associations were observed between maternal serum PFOA and birth weight or Apgar scores after adjustment for confounders.	
Wang et al. (2023a)	Prospective study of 180 maternal-child pairs enrolled in a birth cohort study in Tangshan City, Hebei province, China (2013–2014). Placental PFOA was inversely associated with head circumference (β per 1-ln-ng/g increase: −0.214, 95% CI: −0.381, −0.046). No associations were observed between PFOA and other birth outcomes (birth weight, birth length, and ponderal index).	
Wang et al. (2023b)	Prospective study of 1,405 maternal-child pairs enrolled in the Shanghai Birth Cohort in Shanghai, China (2013–2016). Maternal first trimester plasma PFOA was associated with lower birth weight z-score (β per doubling of PFOA = −0.34, 95% CI: −0.66, −0.03) in children of women in the high fasting plasma third trimester glucose group (>5.0 mmol/L). Stronger associations were observed at higher cut-off levels used to define the high glucose group. No significant associations were observed in the low fasting plasma glucose group.	
Peterson et al. (2022)	Prospective study of pregnant women and their fetuses (n = 335 mother-fetus pairs) from the MADRES pregnancy cohort. Exposure to maternal serum PFOA measured during pregnancy (median = 19 wk, range = 5.7–38.3 wk GA) was significantly associated with smaller average fetal head circumference (β: −2.5 mm, 95% CI: −4.2, −0.8) and fetal biparietal diameter (β: −0.7 mm, 95% CI: −1.3, −0.2) when comparing pregnant women with detectable PFOA levels and those without detectable PFOA at a fixed gestational age. Associations were stronger in women reporting higher levels of perceived stress: head circumference, β: −3.5 mm, 95% CI: −5.8, −1.3; biparietal diameter, β: −0.8 mm, 95% CI: −1.6, −0.03. No	

Reference	Major Findings	Assessment Implications
	significant associations were observed between PFOA exposure and estimated fetal weight, femur bone length or abdominal circumference.	studies reported significantly increased risks (Liao et al., 2022; Shen et al., 2022; Yu et al., 2022). Exposure sample timing differed between the three studies, with one cohort study colleting maternal samples in the first trimester (Liao et al., 2022), one study colleting maternal samples in the third trimester (Yu et al., 2022), and one cohort study that did not report sample collection (Shen et al., 2022). In the updated evidence base, there are 11 studies (11/20) reporting increased risk of preterm birth. Considering the updated evidence base and studies post-dating the 2022 literature search together, there are 14 studies reporting increased risk of preterm birth (14/23). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Pregnancy loss:* Pregnancy loss was examined in two studies, and neither study reported significantly increased risks (Mi et al., 2022; Nian et al., 2022). Timing of exposure sample collection was reported in one case-control study analyzing prepregnancy plasma samples (Nian et al., 2022) and one nested case-control study did not report exposure sample timing (Nian et al., 2022). In the updated evidence base, there are three studies (3/9) reporting increased risk of pregnancy loss. Considering the updated evidence base and studies post-dating the 2022 literature search together, there are three studies reporting increased risk of pregnancy loss (3/11). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Liao et al. (2022)	Prospective study of 1,341 maternal-child pairs enrolled in the Guangxi Zhuang Birth Cohort study in Guangxi, China {2015–2019}. PFOA exposure (measured at 12 wk GA) was associated with significantly lower risk of preterm birth (RR = 0.599, 95% CI: 0.370, 0.967 for the 2nd versus the 1st quartile). There was no significant trend across quartiles and no association was observed between continuous PFOA exposure and risk of PTB.	
Yu et al. (2022)	Case-control study of 836 maternal-child pairs enrolled in the Maoming Cohort Study in Maoming, China between 2015–2018. Maternal third trimester serum PFOA was associated with preterm birth (OR per ln-ng/mL increase = 1.51, 95% CI: 1.27, 1.80). No associations were observed with paternal or neonatal PFOA.	
Nian et al. (2022)	Case-control study (464 cases, 440 controls) of women with and without URSA in Shandong and Zhejiang provinces, China (2014–2016). No association was observed between prepregnancy plasma PFOA and URSA.	
Mi et al. (2022)	Nested case-control study of women with and without early pregnancy loss (41 cases, 47 controls) in Beijing, China (2018–2020). No association observed between prenatal PFOA exposure (GA not specified) and early pregnancy loss.	
Romano et al. (2022)	Prospective study of 481 maternal-child pairs enrolled in the NHBCS with at least four child anthropometric measurements in the first year of life, (2009–2018). Among boys, maternal second trimester PFOA was associated with a decreased risk of following a growth trajectory in which BMI steeply increases in months 1–3 of life and then stabilizes compared with a growth trajectory in which BMI increases gradually and plateaus around 3 mo (relative risk ratio per twofold increase: 0.4, 95% CI: 0.1, 0.9). No associations observed for risk of following other growth patterns or with changes in BMI at each timepoint (2 wk, 6 mo, and 12 mo).	
Zeng et al. (2023)	Prospective study of mother-child pairs (n = 1,671) from the Shanghai Birth Cohort in China (2013–2016). Child anthropometric measures were taken at 6, 12, 24, and 48 mo. No significant associations were observed between plasma PFOA measured in early pregnancy and BMI-for-age z-scores trajectories.	
Cai et al. (2023)	Prospective study of mother-child pairs (n = 207) from two birth cohorts from the FLEHS: FLEHS I (2002–2004) and FLEHS II (2008–2009). No	

Reference	Major Findings	Assessment Implications
	statistically significant associations were observed between cord blood PFOA and infant growth in single- or multipollutant models.	*Post-natal growth:* Four studies examined post-natal growth in early childhood, and one study reported a decreased risk of following a growth trajectory in which BMI steeply increases in months 1–3 of life and then stabilizes compared with a growth trajectory in which BMI increases gradually and plateaus around 3 mo (Romano et al., 2022). No significant associations were reported from other studies examining post-natal growth from studies on birth cohorts such as the Shanghai Birth Cohort (Zeng et al., 2023), the Flemish Environmental Health Study (Cai et al., 2023), or the Danish National Birth Cohort (Luo et al., 2022). In the updated evidence base, increased risk for adverse changes in post-natal weight changes in infancy were observed in nine (9/11) studies. Considering the updated evidence base and studies post-dating the 2022 literature search together, there are 10 studies reporting increased risk adverse effects on post-natal growth (10/15). Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Luo et al. (2022)	Prospective study in the Danish National Birth Cohort, 656 children. Prenatal exposure to PFOA was not associated with facial features (measures of palpebral fissure length, philtrum groove, and upper-lip thickness) in children at age 5.	
	Immune	
Zhang et al. (2023c)	Population-based cross-sectional study of adolescents aged 12–19 (n = 819) from the NHANES 2009–2010 and 2013–2014 cycles. The study population was stratified in two groups of lower (n = 552) and upper (n = 267) folate levels based on the <66th percentile. Significant inverse associations were observed for rubella antibodies in adolescents in the lower folate group (% change per 2.7-fold increase in PFOA = −10.87, 95% CI: −19.27, −1.61). Significant inverse associations were observed for mumps antibodies in the total population (% change per 2.7-fold increase in PFOA = −11.05, 95% CI: −18.56, −2.85) and the lower folate group (% change = −14.79, 95% CI: −24.46, −3.89). No significant associations were observed for measles antibodies in total or folate groups, and no significant	*Vaccine response:* Three studies identified after the updated literature search evaluated antibody responses to multiple pathogens in different populations, and all three observed an effect (Kaur et al., 2023; Zhang et al., 2023c; Porter et al., 2022). The only study examining rubella antibody response observed a significant decrease (Zhang et al., 2023c). In the updated evidence base, there was one study (1/2) that reported significant decreases in rubella antibody response in children and

Reference	Major Findings	Assessment Implications
	associations were observed for any measured antibodies (rubella, mumps, and measles) in the upper folate group.	adolescents. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were two studies (2/3) in children and adolescents reporting significantly decreased rubella antibody response. Zhang et al. (Zhang et al., 2023c) was considered for deriving PODs for PFOA and was moved forward and integrated into the MCLG synthesis for immune effects (see Toxicity Assessment, (U.S. EPA, 2024b)). Both studies (2/2) examining SARS-CoV-2 antibody response reported significant inverse associations (Kaur et al., 2023; Porter et al., 2022). There were a limited number of studies examining SARS-CoV-2 in the studies captured in updated 2022 evidence base, but these studies post-dating the 2022 updated literature search suggest there may be an association between exposure to PFOA and decreased SARS-CoV-2 antibody response, coherent with decreases in the antibody response for other pathogens. Overall, these studies provide additional evidence for decreased antibody response for multiple pathogens, including in populations located in the United States, and support EPA's conclusion of *evidence indicates* that elevated exposures to PFOA are associated with immunological effects in humans, as well as EPA's selection of decreased vaccine response in children for dose-response modeling. *Infectious disease:* One study identified after the updated 2022 literature search examined infectious disease in children and no association was observed (Zhang et al.,
Kaur et al. (2023)	Cross-sectional study of pregnant participants with past SARS-CoV-2 infection (n = 72) from the Generation C Study. A significant inverse association was observed between maternal plasma n-PFOA and SARS-CoV-2 anti-spike IgG titers (β: −0.62, 95% CI: −1.11, −0.12, p-value = 0.017). Maternal SARS-CoV-2 anti-spike IgG titers were also significantly decreased in WQS regression analysis of a PFAS mixture index (β: −0.35, 95% CI: −0.52, −0.17, p-value = 0.0003), with PFOA accounting for approximately 10% of the effect.	
Porter et al. (2022)	Longitudinal study of current and retired workers (n = 415; 757 observations) of 3M facilities in Decatur, Alabama and Menomonie, Wisconsin (Spring 2021). Serum PFOA was associated with decreases in SARS-CoV-2 anti-spike IgG antibody and SARS-CoV-2 neutralizing antibody response after adjustment for age, gender, race, BMI, location, smoking, immunocompromising conditions or recent corticosteroid use, and time since antigenic stimulus. Associations were not significant after further adjustment for the antigenic stimulus group.	
Jones et al. (2022)	Cross-sectional analysis of infants (n = 3,448) from the Upstate KIDS Study Birth Cohort (2008–2010). PFOA and immunoglobulins were both quantified in infant heel stick blood spots. Significant inverse associations were observed between PFOA and IgE (β per 1 ln ng/mL increase in PFOA: −0.12, 95% CI: −0.065, −0.17). Significant positive associations were observed for IgG_2 (β: 0.22, 95% CI: 0.15, 0.27), IgM (β: 0.11, 95% CI: 0.08, 0.14), and IgA (β: 0.15, 95% CI: 0.07, 0.13). No significant associations were observed in IgG_3, IgG_4, and IgG_1.	
Zhang et al. (2022c)	Population-based cross-sectional study of children aged 3–11 (n = 517) and adolescents aged 12–19 (n = 2,732) from the NHANES 2013–2014 cycle and 2003–2016 cycles, respectively. No significant associations were observed between PFOA and recent incidence (i.e., past 30 d) of the common cold in children.	
Qu et al. (2022)	Case-control study from the Second Affiliated Hospital of Zhejiang University School of Medicine (2019–2020), including RA patients (n = 156) and healthy controls (n = 156). The adjusted odds of RA were significantly increased per 1-log ng/mL increase in serum PFOA concentration (OR = 1.998, 95% CI: 1.623, 2.361).	

Reference	Major Findings	Assessment Implications
Zhao et al. (2022b)	Case-control study of RA patients (n = 294) and volunteer controls (n = 280) in Hangzhou, China from January 2018–December 2020. Significant positive associations were observed for several immune parameters related to RA, including IgG (β per each 1-ln ng/mL increase in PFOA= 0.25, 95% CI: 0.21, 0.29), ACPA (β = 0.59, 95% CI: 0.37, 0.81), C-RP (β = 0.52, 95% CI: 0.40, 0.65), and RF (β = 0.57, 95% CI: 0.34, 0.80). No significant associations were observed for IgA, IgM, C4, C3, KAP, and LAM.	2022c). In the updated evidence base, results were mixed for infectious disease in children, with five studies (5/12) reporting positive associations or increased risk. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were five studies (5/13) reporting positive associations or increased risk of infectious disease in children. Overall, the study was judged to not quantitatively impact assessment conclusions and were not moved forward. *Immunoglobulins:* Two studies identified after the updated 2022 literature search examined immunoglobulins, and one study (1/2) observed an effect (Jones et al., 2022). In the updated evidence base, five studies examined immunoglobulins in a variety of populations, with mixed evidence. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Autoimmune disease:* Three studies examining RA were identified after the updated 2022 literature search, and all three studies (3/3) observed significantly increased RA biomarkers (Zhao et al., 2022b), increased RA severity scores (Zhao et al., 2022a), or increased risk of RA (Qu et al., 2022). In the updated evidence base, two studies examined RA, and both studies (2/2) reported positive associations, including one significant positive trend. While all studies observed increases in risk or evidence of increased biomarkers related to RA, the methods of examination differed between the studies, limiting comparability of the results. Evidence for other autoimmune diseases in the updated evidence base was mixed and limited
Zhao et al. (2022a)	Case-control study from the Second Affiliated Hospital of Zhejiang University School of Medicine (2019– 2020), including RA patients (n = 155) and healthy controls (n = 145). Participants were categorized by their DAS28 (inactivity, low activity, moderate activity, and high activity). Significant differences (p = 0.0001) in median serum PFOA concentrations were observed between the four groups of DAS28, with the highest median PFOA concentrations observed among those with the highest DAS28 (≥ 5.1). Comparing subjects categorized as with leukopenia (WBC < 4.0 x 10^9/L) to those without leukopenia (WBC ≥ 4.0 x 10^9/L), serum PFOA levels were higher in the non-leukopenia group. No significant associations were observed between PFOA exposure and interstitial lung disease.	
Girardi et al. (2022)	Cohort study (TEDDY-Child Study) evaluating perceived health risks and self-reported health outcomes in mothers located in a high-exposure community (Veneto, Italy). No associations were observed for autoimmune disorders.	

Reference	Major Findings	Assessment Implications
		to a small number of studies. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
	Hepatic	
Liu et al. (2022)	Community-based cross-sectional study of adults (n = 1,303) living in Guangzhou, China. Positive dose-response relationships between PFOA and liver enzymes, except for ALP. Significant associations were observed for the 50th compared with the 25th percentile of PFOA for liver function biomarkers (percentage differences): ALB (3.04, 95% CI: 2.72, 3.37) ALT (6.08, 95% CI: 4.21, 8.00), AST (2.68, 95% CI: 1.48, 3.90), GGT (6.56, 95% CI: 4.33, 8.83), and DBIL (2.46, 95% CI: 1.11, 3.82). Associations remained significant for other comparisons (75th percentile vs. 25th percentile and 95th percentile vs. 25th percentile). Significant positive associations were observed for abnormal liver function at the 50th percentile (OR = 1.34, 95% CI: 1.21, 1.48). No significant associations were observed for ALP.	*ALT:* Four studies identified after the updated 2022 literature search examined ALT, and two (2/4) reported significant increases (Cakmak et al., 2022; Liu et al., 2022). In the updated evidence base, there were nine (9/11) studies reporting increased ALT in adults. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were 11 (11/15) studies reporting increases in ALT in adults. Overall, the studies support EPA's conclusion that *evidence indicates* that PFOA exposure is likely to cause hepatotoxicity in humans, specifically increased ALT in adults; however, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Other liver enzymes:* Three studies identified after the updated 2022 literature search examined liver enzymes besides ALT, and all three studies (3/3) observed effects (Borghese et al., 2022; Cakmak et al., 2022; Liu et al., 2022). In the updated evidence base, there were five studies (5/7) reporting increases in AST and seven studies (7/10) reporting increases in GGT in adults. Results for other liver enzymes in adults were generally mixed. Considering the updated evidence base and studies post-dating the 2022 literature search together, there were five studies (8/10) reporting increases in AST and seven studies
Borghese et al. (2022)	Population-based cross-sectional study of adults (n = 4,657) from three cycles of the CHMS. A twofold increase in serum PFOA was significantly associated with elevated AST (% change = 7.9, 95% CI: 5.4, 10.4) and ALP (% change = 3.9, 95% CI: 1.7, 6.1). GGT was significantly elevated for not physically active individuals only. No significant associations were observed for ALT and total bilirubin.	
Cakmak et al. (2022)	Population-based cross-sectional study of 6,768 participants aged 3–79 yr old from the CHMS. Increases in PFOA were significantly associated with elevated AST (percent change per GM [1.90 µg/L] increase in PFOA = 3.7, 95% CI: 1.1, 6.4), GGT (11.8, 95% CI: 2.5, 21.8), ALT (3.2, 95% CI: 0.5, 5.9), and bilirubin (3.6, 95% CI: 2.7, 4.5). No significant associations observed for ALP.	
Nilsson et al. (2022)	Cross-sectional occupational study of Australian firefighters who had used AFFF (n = 783). No significant associations were observed for ALT and self-reported liver problems.	
E et al. (2023)	Population-based cross-sectional study of adults (n = 3,464) from NHANES (2005–2018). A significant increase in risk for NAFLD was observed in women (RR per 1-log ng/mL increase in PFOA = 1.354, 95% CI: 1.110, 1.651). Associations in continuous analyses remained significant after stratification by menopausal status. Significant monotonic trend in	

Reference	Major Findings	Assessment Implications
	women overall, in premenopausal women, and in postmenopausal women (p for trend = 0.002, 0.002, and 0.004, respectively) across quartiles of PFOA. No significant associations were observed in all participants or in men only.	(10/13) reporting increases in GGT in adults. Overall, the studies support EPA's conclusion that *evidence indicates* that PFOA exposure is likely to cause hepatotoxicity in humans, specifically increased ALT in adults; however, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward. *Liver disease:* Four studies identified after the updated 2022 literature search examined liver disease, and one study reported a significant increase in risk of NAFLD (E et al., 2023). The other three studies did not report associations for any liver disease (1/4). In the updated evidence base, there were three studies examining all liver disease and none reported significant associations (0/3). NAFLD was not specifically examined in the updated evidence base. Overall, the studies were judged to not quantitatively impact assessment conclusions and were not moved forward.
Ward-Caviness et al. (2022)	Cross-sectional study of electronic health records of 10,168 patients from the University all of North Carolina Healthcare System as a part of the EPA (CARES) resource (2004–2016). Presence of PFOA in the water system was not significantly associated with higher prevalence of liver disease.	
Girardi et al. (2022)	Cohort study (TEDDY-Child Study) evaluating perceived health risks and self-reported health outcomes in mothers located in a high-exposure community (Veneto, Italy). No associations were observed for liver disease.	

Notes: OR = odds ratio; CI = confidence interval; PFOA = perfluorooctanoate; POD = point of departure; MEC = Multiethnic Cohort Study; HCC = hepatocellular carcinoma; HR = hazard ratio; ln = natural log; ER+ = estrogen receptor positive; PR+ = progesterone receptor positive; HER2+ = human epidermal growth factor receptor positive; NHANES = National Health and Nutrition Examination Survey; TEDDY = The Environmental Determinants of Diabetes in the Young; GM = geometric mean; RR = relative risk; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PFAS = per- and polyfluoroalkyl substances; WQS = weighted quantile sum; SBP = systolic blood pressure; IQR = interquartile range; DBP = diastolic blood pressure; CHMS = Canadian Health Measures Survey; TG = triglycerides; SMC-C = Swedish Mammography Cohort-Clinical; 60YO = 60-year-olds; MI = myocardial infarction; T2 = tertile 2; T3 = tertile 3; T1 = tertile 1; SD = standard deviation; apoA1 = apolipoprotein A1; apoB = apolipoprotein B; CVD = cardiovascular disease; VLDL = very low-density lipoprotein; AFFF = aqueous film forming foams; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors Study; SWAN = Study of Women's Health Across the Nation; ACS = acute coronary syndrome; EPA = Environmental Protection Agency; CARES = Clinical and Archived records Research for the Environmental Studies; COPSAC-2010 = Copenhagen Prospective Studies of Asthma in Childhood 2010; GA = gestational age; BMI = body mass index; LBW = low birth weight; SGA = small for gestational age; HPHB = Healthy Pregnancy, Healthy Baby; PTB = preterm birth; MADRES = Maternal and Developmental Risks from Environmental and Social Stressors; URSA = unexplained recurrent spontaneous abortion; NHBCS = New Hampshire Birth Cohort Study; FLEHS = Flemish Environment and Health Studies; RBC = red blood cell; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; IgG = immunoglobulin G; IgE = immunoglobulin E; IgG_2 = immunoglobulin G subclass 2; IgM = immunoglobulin M; C-RP = c-reactive protein; IgA = immunoglobulin A; IgG_3 = immunoglobulin G subclass 3; IgG_4 = immunoglobulin G subclass 4; IgG_1 = immunoglobulin G subclass 1; RA = rheumatoid arthritis; ACPA = anti-citrullinated protein antibodies; RF = rheumatoid factor; C4 = complement 4; C3 = complement 3; KAP = light chain kappa isotype; LAM = light chain lambda isotype; DAS28 = Disease Activity Score28; WBC = white blood cell; ALP = alkaline phosphatase; ALB = albumin; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase; DBIL = direct bilirubin; NAFLD = nonalcoholic fatty liver disease

Table A-48. Animal Toxicity Studies Identified After Updated Literature Review (Published or Identified After February 2022)

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Attema et al. (Attema et al., 2022	Cardiovascular, Hepatic	PFOA exposure (0.05 or 0.3 mg/kg/day) in male mice via drinking water for 20 wk led to disruption of hepatic lipid metabolism, increased liver weight, reduced plasma triglycerides and cholesterol, and increased hepatic triglycerides. Effects of PFOA were mediated by multiple nuclear receptors, including PPARα, PXR, and CAR. Experiments were conducted in wild-type and in PPARα-knockout mice, and mice were fed a high-fat diet during exposure.	General Notes: All animals fed only a high-fat diet (45% fat) with no groups being fed a normal diet. Cardiovascular: Subchronic exposure design but the direction of effect for cardiovascular endpoints are in the same direction as other studies within the PFOA animal database with short-term, chronic, and developmental exposure design. These effects (e.g., decreased serum cholesterol levels) are not concordant with the changes seen in humans. Dose levels are similar to the lowest dose previously tested (0.08 mg/kg/day). Hepatic: One of three subchronic studies investigating hepatic endpoints. Relative liver weight increase is concordant with most other studies. No histopathology aside from lipid accumulation and no serum enzyme levels. Overall Assessment Conclusion: Effects are generally consistent with current database, using generally similar dose levels, and endpoints measured for hepatic and cardiovascular were not endpoints that were brought forward previously for dose response. All data are present only in a high-fat diet group without a normal diet comparator. Generally, data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.
Zhang et al. (Zhang et al., 2022a)	Developmental	PFOA exposure (1 or 5 mg/kg/day) via drinking water for 28 d in female mice prior to mating and gestation led to impaired oocyte maturation and disrupted mitochondrial metabolism. Serum estradiol and progesterone were reduced at both doses. Ovary weight (relative and absolute) was reduced at both doses. Time to mating was increased while litter size was decreased.	General Notes: Only two dose groups, both of which are relatively high compared with other studies in the PFOA database. Developmental: Exposure in female mice ends prior to mating and gestation, which is not as sensitive to measuring developmental endpoints (e.g., maternal gestational body weight, fetal and pup body weight, offspring mortality) compared with other exposure paradigms. Decreased litter size is consistent with PFOA database.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
			Overall Assessment Conclusion: Effects are generally consistent with current database, although other studies investigating developmental endpoints tended to use lower dose levels. Data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.
Conley et al. (Conley et al., 2022)	Developmental, Hepatic	Pregnant rats were exposed to PFOA (10, 30, 62.5, 125, or 250 mg/kg/day) via oral gavage from GD 8 to PND 2. Maternal gestational and postnatal body weights were decreased, and relative liver weight was increased. Offspring effects were also observed and included decreased litter size, pup body weight, survival, liver glycogen, and altered relative and absolute liver weight.	General Notes: A large number of dose groups at levels that are relatively high compared with other studies in the PFOA database. Developmental: Decreased maternal body weight in the two highest dose groups are consistent with PFOA database; however, other studies found this effect at lower dose levels. Decreased pup weight at most dose groups is consistent with PFOA database; however, other studies found this effect at lower dose levels. Pup survival decrease in the high-dose group is consistent with PFOA database; however, other studies found this effect at lower dose levels. Hepatic: Maternal liver weight was increased in the high-dose group while pup absolute liver weight displayed a non-monotonic decrease. Serum enzyme levels were all nonsignificant. Overall Assessment Conclusion: Effects are generally consistent with current database, although other studies investigating developmental endpoints tended to use lower dose levels. Endpoints measured for hepatic (e.g., liver weight, serum enzymes, bilirubin) were not endpoints that were brought forward previously for dose response (e.g., necrosis in the liver). Generally, data are supportive of endpoints currently modeled but would not impact conclusions of assessment. Study will not move forward for further evaluation.

Notes: PFOA = perfluorooctanoate; PPARα = peroxisome proliferator-activated receptor-alpha; PXR = pregnane X receptor; CAR = constitutive androstane receptor; GD = gestational day; PND = postnatal day.

A.4 Studies Identified After Assessment Literature Searches

Studies identified after the updated literature review (February 2023) did not undergo the systematic review protocol. Studies were reviewed for major findings and how those findings may affect the assessment. For PFOA, 16 epidemiological studies were identified after the updated literature search in 2023 and are summarized below (Table A-49).

Table A-49. Epidemiological Studies Identified After 2023 Updated Literature Search (Published or Identified After February 2023)

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		Primary Epidemiologic Studies	
Purdue et al. (2023)	Cancer	Nested case-control study of 530 matched pairs of U.S. Air Force Servicemen conducted using serum samples from the DoD Serum Repository and the DoD Cancer Registry (1990–2018). No association was observed between prediagnosis serum PFOA concentrations and testicular germ cell tumors among active-duty U.S. Air Force Servicemen, before or after adjustment for other PFAS.	No change.
Kang et al. (2023)	Cardiovascular	Prospective study of 1,130 women from the Study of Women's Health Across the Nation 45–56 yr old at baseline (1999–2000) followed through 2016. Serum lipids were collected at multiple timepoints over the course of 17 yr, and high, medium, and low trajectories for serum lipids were identified using a latent class growth model. In PFAS mixture analyses, significant increases in risk were observed for belonging to the medium or high trajectory class for TC and LDL-C. No association was observed between PFOA and the risk of being in the medium or high trajectory class compared with the low trajectory class for TC, LDL-C, HDL-C, and TG. However, PFOA was associated with decreased TG in cross-sectional analyses of PFAS and lipids at baseline. No associations were observed for TC, LDL-C, or HDL-C in cross-sectional analyses of baseline data.	Exposure to PFOA may be associated with changes to TG. No change.
Tan et al. (2023)	Immune	Prospective study of 425 pregnant women from the Atlanta African American Maternal-Child Cohort. The association between serum PFAS mixture, collected at 8–14 wk gestation, and serum inflammatory biomarkers was analyzed using	Exposure to PFAS mixture may be associated with increased cytokine and inflammatory markers. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		mixture modeling approaches, including quantile g-computation, BKMR, BWS, and WQS. Serum PFAS mixture was associated with significantly increased serum concentrations of multiple cytokines and inflammatory markers (i.e., IFN-γ IL-10, and TNF-α) in both cross-sectional analyses (i.e., 8–14 wk gestation) and at a later follow-up visit at 24–30 wk gestation. The effect was reported to be stronger for inflammatory biomarkers measured at the 24–30-wk visit.	
Andersson et al. (2023)	Immune	Prospective study of adults (20–60 yr old) from Ronneby, Sweden comparing a group of 309 adults with high-exposure (median PFOA concentration = 2 ng/mL) and 47 adults with background exposure (median PFOA concentration = 1 ng/mL). In quartile analyses, serum PFOA measured at baseline was associated with a significant increase in SARS-CoV-2 anti-spike antibody levels 6 mo post-vaccination for those in the fourth quartile of exposure. No significant association was observed between baseline serum PFOA concentrations and SARS-CoV-2 anti-spike antibody levels at 5 wk post-vaccination or 6 mo post-vaccination. Similarly, no association was observed at 5 wk or 6 mo post-vaccination for PFAS mixture (summed PFOA, PFOS, PFHxS, and PFNA).	Exposure to PFOA may be associated with changes to SARS-CoV-2 antibody response. No change.
Zheng et al. (2023)	Developmental	Cohort study of 97 pregnant women enrolled in the Collaborative Perinatal Project (CPP) Study (1960–1966). Sample collection timing was not reported. Birth weight was significantly reduced for mothers above the median PFOA exposure level compared with mothers below the median PFOA exposure level ($\beta = -0.233$, $p = 0.03$). No significant association for birth height or ponderal index.	Supports an association between exposure to PFOA and decreased birthweight. No change.
Ma et al. (2023)	Hepatic	Cross-sectional study of 11,794 participants from NHANES (2003–2016). Serum PFOA was significantly associated with increased ALT (β per doubling in PFOA = 0.87, 95% CI: 0.49, 1.25). Elevated exposure to PFOA was associated with a significantly increased risk for having high (>95th percentile) ALT (OR per doubling in PFOA = 1.17, 95% CI: 1.03, 1.32). A similar increase in risk for high AST was observed in continuous analyses of PFOA exposure (OR = 1.15, 95% CI:	Supports an association between exposure to PFOA and increased ALT. Exposure to PFOA may be associated with increased AST. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		1.02, 1.30, p for trend = 0.03). No associations observed for total bilirubin, ALP, or GGT.	
Gump et al. (2023)	Cardiovascular	Cross-sectional study of 291 children (9–11 yr old) from the EECHO study located in upstate New York (2013–2017). Blood pressure reactivity to acute stress was examined by measuring blood pressure after three acute stress computer tasks. Elevated exposure to PFOA was associated with significantly increased PP reactivity (β per ln-ng/mL = 0.25, 95% CI: 0.11, 0.38) and significantly increased CO reactivity (β = 0.17, 95% CI: 0.02, 0.30). Elevated exposure to PFOA was also associated with significantly decreased DBP reactivity (β = −0.19, 95% CI: −0.32, −0.05) and significantly decreased TPR reactivity (β = −0.20, 95% CI: −0.34, −0.06). Significant interactions were observed between PFOA and blood Pb for DBP reactivity and TPR reactivity. Marginally significant ($p < 0.1$) associations were observed between elevated PFOA exposure and increased resting DBP and PEP at baseline. In BKMR analyses, PFOA was associated with significantly higher PP reactivity. No association between elevated exposure to PFOA and CIMT, cfPVW, LV mass index, resting SBP, HR, or PP; or SBP, HR, and PEP reactivity.	Elevated exposure to PFOA may be associated with changes to PP, CO, DBP, and TPR in response to acute stress. No change.
Xu et al. (2023)	Cardiovascular	Prospective study of 129 mother-child pairs from the Shanghai Birth Cohort (SBC) (recruitment: 2013–2016). Exposure to PFOS was measured in cord blood at birth, and blood pressure was measured at a follow-up visit at 4 yr of age (2018–2021). Exposure to PFAS mixture was significantly associated with decreased SBP, DBP, and mean artery pressure in BKMR and WQS regression analyses. No significant associations observed for SBP, DBP, mean artery pressure, and pulse pressure in single-pollutant models.	No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
Pumarega et al. (2023)	Immune	Prospective study of 240 adults from Barcelona, Spain (2016–2021). Exposure to PFOA was measured in blood collected in 2016–2017, and SARS-CoV-2 infection was detected in nasopharyngeal swabs or blood samples collected in 2020–2021. No association was observed for PFOA or PFAS mixture and SARS-CoV-2 seropositivity or COVID-19 disease.	No change.
Rhee et al. (2023)	Cancer	Nested case-control study of 428 matched pairs of renal cell carcinoma (RCC) cases and healthy controls from the Multiethnic Cohort (MEC) Study. Suggestive associations were observed for elevated exposure to PFOA and increased risk of RCC in White participants (OR per doubling in PFOA = 2.12, 95% CI: 0.87, 5.18) and those providing their serum sample prior to 2002 (OR = 1.49, 95% CI: 0.77, 2.87). No significant association was observed between elevated exposure to PFOA and increased risk of RCC.	No change.
Zhang et al. (2023b)	Cancer	Two individual nested case-control studies conducted on 251 matched pairs from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) and 360 matched pairs from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). In 50–69-year-old Finnish men from ATBC (1985–1988), elevated exposure to PFOA was significantly associated with an increased risk of pancreatic ductal adenocarcinoma (PDAC) (OR per SD increase in PFOA = 1.27, 95% CI: 1.04, 1.56). Significantly elevated odds ratios were also reported for participants in the fourth and fifth quintile of PFOA exposure compared with participants in the first PFOA exposure quintile, including a significant trend (p for trend = 0.01). No significant association was observed in 50–74-year-old American men and women (1993–2001) from PLCO for PDAC.	No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
van Gerwen et al. (2023)	Cancer	Nested case-control study of 88 matched pairs of thyroid cancer patients and healthy controls from the Bio*Me* Biobank, medical record-linked biobank of participants from New York City (2008–2021). No significant association was observed for the association between elevated exposure to FPOA and thyroid cancer.	No change.
Kim et al. (2023)	Hepatic	Cross-sectional study of 1,404 adults from the Korean National Environmental Health Survey (KoNEHS), Cycle 3 (2015–2017). Significant positive associations were observed between serum PFOA concentrations and levels of ALT, AST, and GGT in single-pollutant models. In sex-stratified analyses, associations remained significant for men and women for all three liver enzymes. In analyses stratified by BMI status, significant positive associations were observed for all three liver enzymes in individuals with a BMI <25 but were not significant for those with a BMI of 25 or greater. PFAS mixture was analyzed using quantile g-computation, and significant positive associations were observed for ALT, AST, and GGT. Partial effects (weights) from quantile g-computation were reported and demonstrated PFOA contributing to the positive effects for ALT (PFOA weight: 0.21), AST (0.22), and GGT (0.35).	No change.
Zell-Baran et al. (2023)	Immune	Prospective cohort study of 145 mother-child pairs from the Healthy Start cohort study (enrollment: 2009–2014) with antibody levels measured at a follow-up visit at a mean age of 5 yr old (2015–2019). An increased risk of having a low antibody titer for measles and mumps was observed, including a significantly increased risk for low antibody titer for mumps (OR per 1 ln ng/mL increase in PFOA = 2.46, 95% CI: 1.28, 4.75). In quantile g-computation analyses, an increased risk of having a low antibody was observed for both measles and mumps, and positive weights were observed for PFOA for measles (weight: 0.68) and mumps (1.00). In linear regression analyses, an inverse association was observed for rubella antibody titer, but no association was observed for varicella antibody titer. In quantile g-computation analyses, a positive	Supports an association between elevated exposure to PFOA and increased risk of decreased antibody response in children. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		association was observed for PFAS mixture and rubella antibody titer, however, the weight for PFOA was inverse (weight: –0.86). PFAS mixture was not associated with changes in varicella antibody titers.	
Winquist et al. (2023)	Cancer	Case-cohort study of 999 participants without cancer at enrollment and 3,762 incident cancer cases within the American Cancer Society's prospective Cancer Prevention Study II (CPS-II) (1998–2001). An increased risk of kidney cancer was observed in the overall population, which was driven by a positive association in females in sex-stratified analyses. In analyses of histological subtypes, a significantly increased risk of renal cell carcinoma (RCC) was observed for females (HR per doubling in PFOA concentration = 1.54, 95% CI: 1.05, 2.26). A decreased risk of multiple myeloma was observed in analyses of combined sexes and in males. A decreased risk was also observed for myeloma in females. No associations were observed for hematological malignancies, bladder, kidney, pancreatic cancer in site-level analyses.	Supports an association between elevated exposure to PFOA and increased risk of RCC. No change.
		Meta-analysis and Pooled Analysis Studies	
Padula et al. (2023)	Developmental	Pooled analysis of 3,339 mother-child pairs from 11 prospective birth cohorts in the ECHO program across the U.S. Prenatal PFOA concentrations were significantly associated with decreases in birthweight-for-gestational-age z-score (β per ln-ng/mL increase in PFOA = −0.15, 95% CI: −0.27, −0.03) and gestational age at birth (β = −0.22, 95% CI: −0.43, −0.01). Results were similar in sex-stratified analyses. Nonsignificant increases in risk of term low birth weight (OR per ln-ng/mL increase in PFOA = 1.43, 95% CI: 0.54, 3.80) and preterm birth (OR: 1.41, 95% CI: 0.93, 2.14). Associations were stronger between increased PFOA in the first trimester and lower birthweight-for-gestational-age z-score and increased risk of term low birth weight and SGA. PFAS mixture was inversely associated with birthweight-for-	Supports an association between exposure to PFOA and decreased birthweight. Exposure to PFOA may be associated with decreased gestational age. No change.

Reference	Health Outcome(s)	Major Findings	Assessment Implications
		gestational-age z-score (PFOA weight: 0.15) and gestational age at birth (PFOA weight: 0.26), and the association was not significant for gestational age at birth. No associations were observed for SGA or LGA.	

Notes: DoD = Department of Defense; PFOA = perfluorooctanoic acid; PFAS = Per- and polyfluoroalkyl substances; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; BKMR = Bayesian Kernel Machine Regression; BWS = Bayesian Weighted Sums; WQS = weighted quantile sum regression; IL-γ = interleukin gamma; IL-10 = interleukin 10; TNF-α = tumor necrosis factor alpha; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; PFOS = perfluorooctane sulfonic acid; PFHxS = perfluorohexanesulfonic acid; PFNA = perfluorononanoic acid; CPP = Collaborative Perinatal Project; NHANES = National Health and Nutrition Examination Survey; ALT = alanine transaminase; AST = aspartate transaminase; OR = odds ratio; ALP = alkaline phosphatase; GGT = gamma-glutamyltransferase; EECHO = Environmental Exposures and Child Health Outcomes; PP = pulse pressure; CO = cardiac output; DBP = diastolic blood pressure; TPR = total peripheral resistance; Pb = lead; PEP = pre-ejection period; CIMT = carotid intima-media thickness; cfPWV = carotid-femoral pulse wave velocity; LV = left ventricular; SBP = systolic blood pressure; HR = heart rate; SBC = Shanghai Birth Cohort; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; COVID-19 = coronavirus disease 2019; MEC = Multiethnic Cohort Study; RCC = renal cell carcinoma; ATBC = Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; PDAC = pancreatic ductal adenocarcinoma; KoNEHS = Korean National Environmental Health Survey; ECHO = Environmental influences on Child Health Outcomes; ln = natural log; CI = confidence interval; OR = odds ratio; SGA = small for gestational age; LGA = large-for-gestational-age.

Appendix B. Detailed Toxicokinetics

B.1 Absorption

A summary of studies that provide information on perfluorooctanoic acid (PFOA) absorption from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA Health Effects Support Document (HESD) is shown in Figure B-1.

Evidence Stream	Grand Total
Animal	11
Human	2
In Vitro	7
Grand Total	19

Figure B-1. Summary of PFOA Absorption Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOA HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.1.1 Cellular Uptake

Several studies using cell lines transfected with specific transporters or vector controls support cellular accumulation of PFOA through facilitated transport. Several transporters classically considered specific to renal or enterohepatic resorption have also been found to be expressed in tissues relevant to absorption. Specifically, organic anion transporter 2 (OAT2) transcripts have been identified in several tissues in addition to kidney including the small intestine (Cropp et al., 2008). OATP1A2 expression has also been identified in intestine (Kullak-Ublick et al., 1995).

A single study in immortalized intestinal Caco-2 cells found that uptake was fast and saturable, supporting a carrier-mediated uptake process. The K_m for PFOA uptake was calculated to be 8.3 ± 1.2 µM and uptake clearance (V_{max}/K_m) was 55.0 µL/mg protein/min. Uptake was found to be independent of sodium ions, while concentration, temperature, and pH all influenced uptake. Substrates or inhibitors of organic anion transporting polypeptides (OATPs) significantly decreased the uptake of PFOA, suggesting that the uptake of PFOA from the apical membranes of Caco-2 cells was at least partially mediated by OATPs (Kimura et al., 2017).

Lipid binding may influence PFOA accumulation in various cell types relevant to absorption as well as distribution. Sanchez Garcia et al. (2018) compared PFOA and PFOS in their ability to accumulate and be retained in cells including lung epithelial cells (NCI-H292). Cellular accumulation and retention of PFOS was observed in lung cells at higher levels compared with azithromycin-dihydrate, a lysosomotropic cationic amphiphilic drug used as a reference compound. In contrast, PFOA only accumulated to very low levels (Table B-1). Phospholipid binding was assessed by measuring the relative affinity for a phosphatidylcholine (PC)-coated column at pH7.4 to calculate a chromatographic index (CHIIAM7.4). Lipid binding (LogD7.4) was determined by measuring the relative affinity of compounds for a C18-coated liquid chromatography column at pH7.4. LogP values obtained from the PubChem database were used

as a comparative lipophilicity measure. Phospholipophilicity correlated ($r^2 = 0.75$) 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.

Table B-1. Cellular Accumulation and Retention Relative to Lipophilicity and Phospholipophilicity as Reported by Sanchez Garcia et al. (2018)

Chemical	Cellular Accumulation and Retention		Lipophilicity		
	Accumulation in Lung Epithelium (% AZI)	Retention in Lung Epithelium	Phospholipid Binding (CHIIAM7.4)	Lipid Binding (LogD7.4)	LogP
PFOS	313 ± 101*	26 ± 4	39 ± 3*	2.33 ± 0.11*	5
PFOA	15 ± 3	ND	29 ± 1	1.29 ± 0.02	4.9

Notes: AZI = azithromycin-dihydrate; ND = not determined.
*Statistically significant at $p \leq 0.05$ from PFOA.

The study by Sanchez Garcia et al. (2018) raises the possibility of passive uptake of PFOA into cells. This is consistent with observations that cells transfected with vector only could take up PFOA, albeit at lower levels than cells transfected with PFOA-specific transporters (discussed further in Section B.4.2.1). Ebert et al. (2020) determined membrane/water partition coefficients ($K_{mem/w}$) for PFOA and examined passable permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. Membrane permeability and partition coefficients were predicted using an approach developed to model molecules in micellar systems and biomembranes (COSMOmic and related tools, (Klamt et al., 2008). The predicted log ($K_{mem/w}$/[LW/kg_{mem}]) for PFOA was 3.93, similar to the experimentally determined value of 3.52 ± 0.08. $K_{mem/w}$ values increase with increasing chain length, reflecting increased surface area for van der Waals interactions. The authors observed that perfluoroalkanesulfonic acids (PFSAs) adsorb about 1.2 log units more strongly to the membrane than perfluorocarboxylates (PFCAs) with the same number of perfluorinated carbons. Permeability showed the same chain-length dependence as $K_{mem/w}$ values. The predicted anionic permeability ($logP_{ion}$/[cm/s]) for PFOA ranged from −6.89 to –7.45, considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes. The extent to which passive uptake influences absorption in vivo remains to be determined.

B.1.2 Oral Exposure

According to animal data, PFOA is well absorbed following oral exposure. Studies on male rats administered PFOA by gavage using a single dose (11.4 mg/kg, CD rats) or daily doses over 28 days (5 or 20 mg/kg/day, Sprague-Dawley rats) all estimated dose absorption of at least 92.3% (Cui et al., 2010; Gibson and Johnson, 1979).

Toxicokinetic parameters informing absorption were derived by comparing oral to intravenous (IV) dosing in two studies conducted in rats (Dzierlenga et al., 2019; Kim et al., 2016b). In the study by Kim and colleagues, rapid differences in absorption based on sex were observed for PFOA but not PFOS (Kim et al., 2016b). Male and female Sprague-Dawley rats were administered 1 mg/kg by either the IV or oral route. Urine and feces were collected daily for both males and females, and blood was collected at 11 time points on the first day (females) or 3 time

points on the first day and then up to 12 days after exposure (males). The time to reach the maximum PFOA plasma concentration (T_{max}) following oral exposure in females was 1.44 hours versus 2.07 days in males. Dzierlenga et al. (2019) administered a single bolus IV (6 mg/kg) or gavage dose (6, 12, or 48 mg/kg) to adult male Sprague-Dawley rats and a single bolus IV (40 mg/kg) or gavage dose (40, 80, or 320 mg/kg) to adult female Sprague-Dawley rats. Blood and urine were collected for up to 8 time points during the first 24 hours and then up to 12 (females) or 50 (males) days post-dosing. T_{max} in rats administered these doses via gavage ranged from 2.33 to 3.22 hours in females and 4.86 to 8.33 hours in males for PFOA. In females, maximum blood concentration (C_{max}) per dose (mM/mmol/kg) decreased with increasing dose suggesting saturation of absorption kinetics at higher doses. Similar to the Kim et al. (2016b) study, shorter T_{max} values were observed in females compared with males at all doses.

The data from studies of adverse effects on monkeys, rats, and mice receiving PFOA in capsules, food, or drinking water demonstrate gastrointestinal absorption. In cynomolgus monkeys, steady-state serum and urine PFOA levels were reached 4–6 weeks after dosing with capsules containing 3, 10, or 20 mg/kg PFOA for 6 months (Butenhoff et al., 2004b). Serum PFOA concentrations in male Crl:CD BR rats fed diets containing 0.06, 0.64, 1.94, or 6.5 mg PFOA/kg for 90 days were 7.1, 41, 70, and 138 µg/mL, respectively (Perkins et al., 2004). Peak blood levels of PFOA were attained 1–2 hours following a 25 mg/kg dose to male and female rats (Kennedy et al., 2004). Studies on same-sex rats found no differentiation in blood or plasma levels of PFOA when comparing single and repeated daily PFOA dose administrations (Elcombe et al., 2010; Kennedy et al., 2004).

In rats, marked sex differences in serum and tissue PFOA levels exist following PFOA administration. Males consistently have much higher levels than females with differences maintained and becoming more pronounced over time. Female rats show much greater urinary excretion of PFOA than do male rats with serum half-life values in hours for females compared with days for males. These differences account for variability in postexposure serum PFOA concentrations between males and females.

B.1.3 Inhalation Exposure

Data on exposure to PFOA by inhalation remains unchanged since Hinderliter et al. (2006a) measured the serum concentrations of PFOA following single and repeated nose-only aerosol inhalation exposures of 0, 1, 10, or 25 mg/m^3 PFOA in Sprague-Dawley rats, which found that PFOA plasma concentrations increased proportional to aerosol exposure concentrations. The male plasma C_{max} values were approximately 2–3 times higher than the female plasma C_{max} values. The female C_{max} occurred approximately 1 hour after the exposure period with plasma concentrations then declining. In males, C_{max} was observed immediately after the exposure period ended and persisted for up to 6 hours. These data demonstrate absorption of PFOA via inhalation and provide evidence of the sex differences consistent with rate of excretion.

B.1.4 Dermal Exposure

Evidence that PFOA is absorbed following dermal exposure remains unchanged since 2005, with in vitro percutaneous absorption studies of PFOA through rat and human skin allowing calculation of permeability coefficients for PFOA in rat skin to be 3.25×10^{-5} cm/hr, and that of human skin to be 9.49×10^{-7} cm/hr (Fasano et al., 2005b). Previously, O'Malley and Ebbins

(1981) utilized mortality as an indicator of dermal uptake across groups of two male and two female New Zealand white rabbits receiving 0, 100, 1,000, or 2,000 mg/kg PFOA; after 14 daily dermal doses, all of the animals died at the highest dose, three of four animals died in the mid-dose group, and no animals died in the low-dose group. Kennedy (1985) detected elevated blood organofluorine levels in male New Zealand white albino rabbits and male and female Crl:CD rats that were dermally treated with a total of 10 applications of PFOA at doses of 0, 20, 200, or 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner.

B.1.5 Developmental Exposure

The literature contains no studies on PFOA absorption following developmental exposure. Additional information on PFOA distribution during reproduction and development is found in Section B.2.4.

B.1.6 Bioavailability

One study measured PFOA after a controlled exposure in humans. Dourson et al. (2019) used serum measurements in cancer patients administered PFOA for therapeutic ends (Elcombe, 2013). The original study administered PFOA in capsules up to 1,200 mg once per week for 6 weeks to 43 males and females with different cancers as part of a Phase 1 trial. Dourson and colleagues (2019) reported that C_{max} values in these patients increased after weekly exposure. The average human C_{max} value was 732 μM/mg/kg-day (303 mg/L). In the nine patients dosed beyond 6 weeks, the patients appeared to reach a steady state with C_{max} values 1.6-fold higher than their individual 6-week averages (as measured in weeks 12–36). C_{max} values from humans and mice were used to derive a data derived extrapolation factor (DDEF) for humans (estimated at 1.3 for single dose and 14 for 6+ weeks of dosing).

The Kim and Dzierlenga studies discussed above also observed very high bioavailability in rats (Table B-2) (Dzierlenga et al., 2019; Kim et al., 2016b). At a lower dose of 1 mg/kg, Kim et al. (2016b) found that C_{max} values after oral administration were 85% and 92% of values obtained after IV administration (bioavailability values were not reported in this study). In the Dzierlenga et al. (2019) study, bioavailability (calculated by dividing the dose-adjusted gavage area under the curve (AUC) by the IV AUC) was 140% in males administered 6 mg/kg and 182% in females administered 40 mg/kg. The authors suggested that the high bioavailability of PFOA may be attributed to increased reabsorption by intestinal transporters by the oral route.

Table B-2. PFOA Parameters From Toxicokinetic Studies Informing Bioavailability in Sprague-Dawley Rats

Study	Dose (mg/kg)	Route	Sex	C_{max} (μg/mL)	T_{max} (hours)[a]
Kim et al. (2016b)	1	Oral	Male	7.55 ± 0.51	49.68 ± 5.04
		IV	Male	8.92 ± 2.34	NA
	1	Oral	Female	5.41 ± 0.38	1.44 ± 0.096
		IV	Female	5.84 ± 0.38	NA
Dzierlenga et al. (2019)	6	Oral	Male	36.85 ± 2.90	4.86 ± 0.81
		IV	Male	52.59 ± 2.5	NA
	40	Oral	Female	240.16 ± 24.84	3.22 ± 0.32

Study	Dose (mg/kg)	Route	Sex	C_{max} (µg/mL)	T_{max} (hours)[a]
		IV	Female	369.76 ± 81.16	NA

Notes: C_{max} = maximum serum concentration; IV = intravenous; NA = not applicable; T_{max} = time to C_{max}.
[a] Converted published T_{max} (days) to T_{max} (hours) for Kim et al. (2016b).

Li et al. (2015) examined bioavailability from food sources in female BALB/c mice and using in vitro methods. In mice, PFOA was mixed with foods of different nutritional compositions (e.g., meat, seafood, milk, and fruits/vegetables) and fed to mice over a 7-day period. By comparing PFOA administration via food mixtures to administration in water, relative bioavailability was assessed by measuring accumulation in liver. PFOA bioavailability relative to water ranged from 4.30 ± 0.80 to 69.0 ± 11.9% and was negatively correlated with lipid content (r = 0.76). The authors suggest that sorption by free fatty acids in foods could limit PFOA access to intestinal transporters. Another possibility is cations in the gastrointestinal tract, such as Ca^{2+} and mg^{2+}, can complex PFOA promoting partitioning to the lipid phase. Three different in vitro methods were used to measure bioavailability using these food mixtures including the in vitro digestion method (IVD) (James et al., 2011), the unified BARGE method (UBM) (Smith et al., 2012), and the physiologically based extraction test (PBET) (Tilston et al., 2011). Instead of soil, 0.3 g of food was used at sample/solution ratios of 1:97.5 for UBM, 1:100 for PBET, and 1:150 for IVD. PFOA bioaccessibility varied by the method (8.7%−73% for UBM, 9.8%−99% for PBET, and 21%−114% for IVD). As observed in the in vivo study, bioaccessibility was negatively correlated with lipid content for the UBM method (r = 0.82) but not for other in vitro methods (r = 0.11−0.22). The authors suggest that the UBM method can be used to model bioaccessibility, possibly because this method is associated with higher lipolysis and better mimics cations in gastrointestinal fluid of UBM. This may lower the potential to form stable micelles using this method compared with PBET and IVD methods. Together, these findings suggest PFOA bioavailability is strongly influenced by diet, with high fat diets associated with reduced absorption, and that an important factor influencing PFOA bioaccessibility is colloidal stability in intestinal solutions.

B.2 Distribution

A summary of studies that provide information on PFOA distribution from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD is shown in Figure B-2.

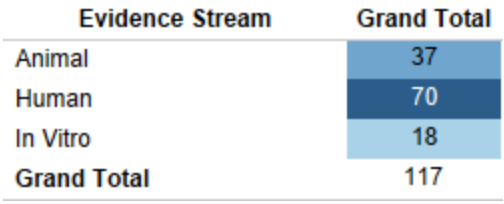


Figure B-2. Summary of PFOA Distribution Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOA HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.2.1 Protein Binding

Kerstner-Wood et al. (2003) used in vitro methods to evaluate PFOA binding to protein in plasma from humans, cynomolgus monkeys, and rats. In all species, plasma proteins were able to bind 97%–100% of the PFOA added at concentrations ranging from 1 to 500 ppm. In humans, serum albumin carried the largest portion of PFOA among the protein components of human plasma. Serum albumin is a common carrier of hydrophobic materials in the blood (Fasano et al., 2005a) and approximately 60% of the serum protein in humans and rats is albumin (Saladin, 2004; Harkness and Wagner, 1983).

Han et al. (2003) investigated the binding of PFOA to rat and human plasma proteins in vitro and determined that the primary PFOA binding protein in plasma was serum albumin. No significant differences in binding to the serum albumin were found between humans and rats. Calculation of disassociation constants (K_d) for PFOA, conducted using purified rodent and human serum albumin binding using labeled ^{19}F nuclear magnetic resonance (NMR) and micro-size-exclusion chromatography and the estimated number of binding sites from this study are presented in Table B-3.

Table B-3. Dissociation Constants of Binding Between PFOA and Albumin as Reported by Han et al. (2003)

Parameter	Method	Rat Serum Albumin	Human Serum Albumin
K_d (mM)	NMR[a]	0.29 ± 0.10[b]	ND
K_d (mM)	micro-SEC[c]	0.36 ± 0.08[b]	0.38 ± 0.04
Number of Binding Sites	micro-SEC[c]	7.8 ± 1.5	7.2 ± 1.3

Notes: K_d = dissociation constant; micro-SEC = micro-size-exclusion chromatography; ND = not determined; NMR = nuclear magnetic resonance.
[a] Average of the two K_d values (0.31 ± 0.15 and 0.27 ± 0.05 mM) obtained by NMR.
[b] On the basis of the result of unpaired t-test at 95% confidence interval, the difference of K_d values determined by NMR and micro-SEC is statistically insignificant.
[c] Values were obtained from three independent experiments and their standard deviations are shown.

Several studies have examined the interactions between PFOA and human serum albumin (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; Kerstner-Wood et al., 2003; Luebker et al., 2002). Wu et al. (2009) examined whether PFOA, after absorption, was transported bound to albumin by dialyzing PFOA solutions in the presence and absence of human serum albumin. The authors found that, in the absence of albumin, 98% of the dissolved PFOA crossed the dialysis membrane into the dialysate within 4 hours. In the presence of albumin, the amount of PFOA found in the dialysate decreased in direct proportion to the albumin concentration, demonstrating binding to the protein. No albumin was identified in the dialysate. Circular dichroism measurements of the albumin/PFOA complex suggested a conformational change in the protein as a result of the PFOA binding. These conformational changes could interfere with the functional properties of serum albumin or other serum proteins impacted by surface monolayers of PFOA. For example, albumin's ability to transport its natural ligands could be decreased by the presence of PFOA on the protein surface (Wu et al., 2009).

MacManus-Spencer et al. (2010) used a variety of approaches to quantify the binding of PFOA to serum albumin (e.g., surface tension measurements, ^{19}F NMR spectroscopy, fluorescence

spectroscopy) using bovine serum albumin. Taken together, the results from these analyses suggested the presence of primary and secondary binding sites on albumin. The results of the fluorescence spectroscopy suggested a conformational change in albumin following binding of PFOA that moved tryptophan residue 214 from a slightly polar region of the protein to a less polar region. Qin et al. (2010) also used fluorescence spectroscopy quenching analysis to study PFOA binding to bovine serum albumin and reported that albumin underwent a conformational change following the binding of PFOA. They also suggested that van der Waals forces and hydrogen bonds were the dominant intermolecular binding forces. Similar findings were observed more recently (Chen et al., 2020) for human serum albumin. This study used infrared spectroscopy to examine PFOA-mediated effects on albumin secondary structure and found that PFOA binding led to a decrease in the β-sheet and α-helix conformations.

Salvalaglio et al. (2010) conducted a modeling study to determine the binding sites of PFOA on human serum albumin and classify them by their interaction energy using molecular modeling. They estimated a maximum number of nine PFOA binding sites on human serum albumin and determined that these site locations were common to the natural binding sites for fatty acids, thyroxine (T4), Warfarin, indole, and benzodiazepine. The binding site closest to tryptophan residue 214 had the highest binding affinity.

Beesoon and Martin (2015) examined differences in the binding of the linear and branched-chain PFOA isomers to calf serum albumin and human serum proteins. The linear PFOA isomer bound more strongly to calf serum albumin than the branched-chain isomers. When arranged in order of increasing binding, the order was 4m < 3m < 5m < 6m (iso) < linear. In the isomer-specific binding to spiked total human serum protein, the linear molecule clearly had the strongest binding potential with about 7%–10% free. The relationship for the other isomers was 5m > 6m > 4m > 3m (15%–30% free).

Weiss et al. (2009) screened PFOA and 29 other perfluorinated compounds – differing by carbon chain length (C4–18), fluorination degree, and functional groups – for potential binding to the serum thyroid hormone transport protein, transthyretin (TTR), using a radioligand-binding assay. The natural ligand of TTR is T4. Human TTR was incubated overnight with ^{125}I-labeled T4, unlabeled T4 (reference), and 10–10,000 nmol PFOA as a competitor for the T4 binding sites. The authors concluded that the binding affinity for TTR was highest for the fully fluorinated compounds tested and those having at least a carbon chain length of 8, characteristics that apply to PFOA. PFOA demonstrated a high binding affinity for TTR with 949 nmol, causing a 50% inhibition of T4 binding to TTR.

Binding to albumin and other serum proteins may affect transfer of PFOA from maternal blood to the fetus. Gao et al. (2019) correlated placental transfer with experimentally measured K_d to human serum binding proteins, serum albumin, and L-FABP. For PFOA, K_ds were calculated to be 115 ± 16 μM for albumin, 166 ± 10 μM for serum binding proteins, and 197 ± 13 μM for L-FABP. These K_ds significantly correlated with placental transfer efficiencies measured in 132 maternal blood–cord blood pairs from subjects in Beijing, China, suggesting serum and binding proteins, especially albumin, play an important role in placental transfer efficiency. 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 the concentration of cord serum albumin was associated with higher transfer

efficiencies (increase of 4.1% per 1 g/L albumin). However, maternal serum albumin concentration was associated with reduced transfer efficiency (decrease of 2.5% per 1 g/L albumin). Because albumin cannot cross the placental barrier, the authors speculate that binding of PFOA to maternal serum albumin can reduce the free PFOA available to move across the barrier through passive diffusion. Similarly, higher fetal albumin levels will lead to less free PFOA in cord blood, which may facilitate the rate of placental transfer via passive diffusion.

In contrast to serum proteins, little is known regarding PFOA binding to proteins in the gut. Yue et al. (2016) examined whether PFOA that enters the digestive tract binds to gastric enzymes, specifically pepsin. Binding to pepsin was examined using fluorescence quenching of pepsin's intrinsic fluorescent properties. Scatchard analysis was used to estimate a binding constant of 0.717×10^4 at 298 K. Spectroscopy including ultraviolet-visible absorption, Fourier transform infrared fluorescence, and circular dichroism indicated that PFOA induces a conformation change in pepsin associated with decreased α-helical and β-sheet content. Molecular docking analysis suggested that PFOA interacts with 16 amino acid residues of pepsin. It is unclear whether PFOA-pepsin interactions impact absorption or distribution from the gut to other compartments in the body.

PFAS also binds intracellular proteins. Luebker et al. (2002), Zhang et al. (2013a), and Yang et al. (2020a) conducted in vitro studies that examined the binding of PFOA and other PFAS to the liver fatty acid binding protein (L-FABP). 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. Luebker et al. (2002) evaluated the ability of perfluorinated chemicals to displace a fluorescent substrate from L-FABP and reported that PFOA exhibited some binding to L-FABP, but its binding potential was about 50% less than that of PFOS and far less than that of oleic acid. Zhang et al. (2013a) cloned the human L-FABP gene and used it to produce purified protein for evaluation of the binding of PFOA and PFOS. The median inhibiting concentrations (IC_{50}s) for PFOA and PFOS were 9.0 ± 0.7 and 3.3 ± 0.1 μmol, respectively, suggesting that PFOA has a lower binding affinity than PFOS. PFOA was bound to the carrier protein in a 1:1 ratio, and the interaction was mediated by electrostatic interactions and hydrogen binding with the fatty acid binding site. Using size-exclusion column coelution and nontarget analysis to identify additional PFAS ligands from contaminated environmental sources, Yang et al. (2020a) also found that that both polar and hydrophobic interactions are crucial for binding affinities to L-FABP for PFOA and PFOS.

B.2.2 Subcellular Distribution

Han et al. (2005) examined the subcellular distribution of PFOA in the liver and kidney of male and female rats. Male and female Sprague-Dawley Crl:CD (SD)IGS BR rats were gavage-dosed with 25 mg/kg [^{14}C]PFOA and necropsied 2 hours after dosing. Blood was collected and the liver and kidneys were removed. Five subcellular fractions (nuclei and cell debris, lysosome and mitochondria, microsome, light microsome and ribosome, and membrane-free cytosol) were obtained by differential centrifugation. In the male liver, the highest proportion of total reactive residues (TRR) of PFOA was located in the nuclei and cell debris (40%), followed by membrane-free cytosol (26% TRR), lysosome and mitochondria (~14% TRR), microsome (~16% TRR), and light microsome and ribosome (~1% TRR). In the female liver, the highest

proportion of TRR of PFOA was found in the membrane-free cytosol (48%), followed by nuclei and cell debris (~31% TRR), lysosome and mitochondria (~12% TRR), microsome (~8% TRR), and light microsome and ribosome (~1% TRR). Given the results, the authors concluded that subcellular distribution of PFOA in the rat liver was sex-dependent because 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. They also hypothesized that the unknown protein or protein complex might normally aid in transport of fatty acids from the liver. In the kidney, the subcellular distribution did not show the sex difference seen with the liver; however, the protein-bound fraction for the males (42%) was about twice that for the females (17%).

Zhang et al. (2020a) examined the subcellular distribution of PFOA in human colorectal cancer cells (DLD-1), human lung epithelial cells (A549), and human normal liver cells (L-02). Cells were incubated with 100 or 300 μM PFOA for 48 hours and mitochondria, nucleus, and cytosol were isolated and examined for PFOA levels. Accumulation in these subcellular compartments corresponded to exposure levels with the highest amounts accumulating in cytosol followed by nuclei and mitochondria. Cytosolic accumulation was more than 100 times greater than accumulation in the other analyzed subcellular compartments. The PFOA concentration in cytosol was highest for liver cells and was comparable between colorectal cancer and lung epithelial cells. The patterns of accumulation (cytosol > nuclei > mitochondria) were also comparable.

B.2.3 Tissue Distribution

B.2.3.1 Human Studies

B.2.3.1.1 Distribution in Blood Fractions

Human blood is a major site of PFOA accumulation. A recent example measured PFAS in blood samples from 344 Wilmington, NC residents (289 adults and 55 children) exposed to contaminated drinking water from release of PFAS chemicals into the Cape Fear River between 1980 and 2017. The mean serum PFOA concentration was 4.8 ng/mL in adults and 3.0 ng/mL in children (Kotlarz et al., 2020). This value was similar to the estimate of 3.8 ng/mL predicted using a pharmacokinetic model based on drinking water containing 15 ng/L PFOA and using the average length of residence of 20 years for the participants. A slightly lower mean serum PFOA concentration of 2 ng/mL was measured in 41 Norwegian women (Haug et al., 2011). Using adjusted multiple linear regression models, PFOA serum concentrations were significantly correlated with age, weight, and the number of months since breastfeeding ended, but not consumption of fish. Household dust concentrations were significantly correlated with serum levels, indicating indoor environments are an important exposure pathway in these women.

PFOA accumulation in blood impacts distribution to various tissues and organs, but few studies have examined PFOA partitioning to human blood fractions. Forsthuber et al. (2020) measured the distribution of PFOA in blood fractions including plasma, albumin, and lipoprotein fractions (e.g., very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)). Blood from four young healthy volunteers (two women, two men, 23–31 years old) were separated into fractions using size fractionation (for proteins) and serial

ultracentrifugation. Results found that albumin was the most important carrier for PFOA and that there was no affinity for lipoproteins. The concentration of PFOA in these fractions was below the limit of detection (LOD).

Jin et al. (2016) analyzed 60 blood samples from a Chinese population, and three whole blood samples from an exposed Canadian family to investigate the partitioning of PFAS of different chain lengths and their major isomers between human blood and plasma. Increasing chain length for PFAS correlated with an increased mass fraction in human plasma from C6 (mean 0.24) to C11 (0.87). The PFOA plasma:whole blood ratio in the Jin et al. (2016) study was lower (1.2 ± 0.43) compared with the mean plasma:whole blood (2.0–2.1) (Ehresman et al., 2007) and serum:whole blood (1.4–2.2) (Hanssen et al., 2013; Kärrman et al., 2006) ratios previously reported. In blood samples obtained from three highly exposed Canadian subjects, the highest levels of PFOA were measured in plasma (0.27 ng/mL) compared with red blood cells (RBCs, 0.13 ng/mL) and washed RBCs (0.12 ng/mL). The authors suggest that these values could be used as more accurate conversion factors to convert concentrations between whole blood and plasma.

In an analysis of Faroese children (ages 5–14) from three birth cohorts, PFOA made up 11%–24% of the PFAS in serum with PFOS accounting for the largest fraction (54%–74%) of the PFAS in serum (Dassuncao et al., 2018). Fractionation to blood fractions was also examined in 61 male and female participants from Oslo, Norway in 2013−2014 (Poothong et al., 2017). The median relative PFAS compositions in serum, plasma, and whole blood were dominated by PFOS, followed by PFOA (representing 60%–70% of blood PFAS), relative to the other 23 PFAS chemicals analyzed. Median PFOA concentrations in plasma, serum, and whole blood were 1.90, 1.60 and 0.93 ng/mL, respectively. Similar to other studies, PFOA preferentially accumulated in plasma relative to other blood fractions and also suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum will not provide accurate estimates for PFOA.

In another study (De Toni et al., 2020) in which blood from healthy low-exposed donors was exposed to PFOA, platelets were identified as the preferential site of PFOA accumulation. The concentrations observed among blood cell components were below the limit of quantification (LOQ) in erythrocytes, 6.2 ± 0.4 pg/10^6 cells in leukocytes, and 243.9 ± 122.6 pg/10^6 cells in platelets. The authors also incubated platelets with Merocyanine 540, a fluorescent dye that has been used as a marker of membrane fluidity. Fluorescence intensity increased in a dose-dependent manner up to, but not beyond, 400 ng/mL. The authors suggest these findings support an association between PFOA accumulation and increased membrane fluidity.

B.2.3.1.2 Distribution in Tissues

No clinical studies are available that examined tissue distribution in humans following administration of a controlled dose of PFOA. However, samples collected in biomonitoring and epidemiological studies provide data showing distribution of PFOA.

Pirali et al. (2009) measured intrathyroidal PFOA levels (0.4–6.0 ng/g) in thyroid surgical patients and found no correlation between serum and thyroid PFOA concentrations. PFOA has been detected in breast milk samples (Tao et al., 2008; Völkel et al., 2008), cord blood samples (Monroy et al., 2008; Apelberg et al., 2007), and follicular fluid samples (Kang et al., 2020) at concentrations above the LOQ. Ex vivo studies also demonstrated PFOA accumulation in human

sperm membranes by a process that can be reversed by water-soluble lipid-sequestrants, such as β-cyclodextrin (Šabović et al., 2020). These studies indicate that PFOA is widely distributed within the body, including reproductive tissues.

PFOA concentrations above the LOQ were detected in 5 of 6 postmortem liver samples from males in Catalonia, Spain. In females, only 1 of 6 liver samples was above LOQ of 0.77 ng/g (Kärrman et al., 2010). Pérez et al. (2013) collected tissue samples (liver, kidney, brain, lung, and bone) in the first 24 hours after death from 20 adult subjects (aged 28–83 years) who had been living in Catalonia, Spain. PFOA was present in 45% of the samples but could be quantified in only 20% (median 1.9 ng/g). PFOA accumulated primarily in the bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below LOD (2.4 ng/g) in the brain. Maestri et al. (Maestri et al., 2006) examined pooled postmortem tissues from five males and two females from northern Italy ranging in age from 12 to 83 years. Of the 12 tissues analyzed, the highest levels were detected in lung, kidney, and liver (3.8, 3.5, and 3.1 ng/g, respectively) and the lowest levels were detected in skeletal muscle, brain, and basal ganglia (0.6, 0.5, and 0.3 ng/g, respectively). PFOA was also detected in the cranium, humerus, and rib bone samples from a cadaver of a 46-year-old year-old and from biopsies from live subjects in a bone bank in Finland (Koskela et al., 2017). However, PFOA was below the detection limit in femur, tibia, and fibula samples but was detected in other soft tissues including brain, liver, lung, and subcutaneous fat.

Two studies examined accumulation of PFOA in cerebrospinal fluid and serum (Wang et al., 2018b; Fujii et al., 2015). In both studies, PFOA levels in cerebrospinal fluid were two orders of magnitude lower than in the serum. These results indicate that PFOA does not easily cross the adult blood-brain barrier (BBB). Consistent with limited passage across the BBB, another study detected PFOA in brain tissue (0.5 ng/g) and basal ganglia (0.3 ng/g) at relatively low levels. Higher levels were observed in the pituitary gland (2.0 ng/g), gonads (1.9 ng/g), adipose tissue (1.4 ng/g), and other tissues (Maestri et al., 2006).

Balk et al. (2019) developed a one-compartment PBPK model to analyze intake in children from 1 to 10.5 years of age. Measured serum concentrations were derived from a subgroup of a longitudinal child study (LUKAS 2) (Koponen et al., 2018). Estimated daily intakes ranged between 0.16 and 0.55 ng/kg body weight (BW)/day for low and high exposure scenarios. Measured PFOA serum concentrations (5th–95th percentile) ranged from 1.9–4.1 ng/mL (age 6) to 1.0–2.1 ng/mL (age 10.5). The model reconstructed median PFOA serum concentrations compared with corresponding measured median serum concentrations and predicted that growth dilution contributed from 63%–77% of total PFOA loss, with elimination pathways accounting for the remaining PFOA loss in children.

B.2.3.2 Animal Studies

Studies of tissue distributions are available for several species including nonhuman primates, rats, and mice. Experiments in nonhuman primates provide evidence of serum and liver accumulation of PFOA. While only a few studies exist, they document distribution with repeated measurements over long periods of time and include recovery time after exposure termination. Mouse studies demonstrate that PFOA primarily distributes to serum, liver, lungs, and kidney; however, several of these studies detect PFOA in additional organs and tissues. These tissues include the central nervous system, cardiovascular, gastrointestinal, renal, reproductive,

endocrine, and musculoskeletal systems. Recent studies have also indicated that a moderate amount of PFOA enters bone and even crosses the barriers into the central nervous system. Adipose tissue was observed as a site that contained very little amounts of PFOA accumulation.

These data are characterized based on dosing (low, medium, and high), time exposed (acute vs. chronic), and any sex differences between males and females. Ranges of dose regimens indicate changes in deposition patterns as animals are exposed to increased concentrations of PFOA, indicating possible changes in excretion through bile and urine. Several studies corroborate to show that there are sex-specific deposition patterns, primarily that male animals accumulate more PFOA in serum and some tissues including liver. Overall, these studies provide a wide range of deposition data that can illustrate short- and long-term accumulation of PFOA in animal tissues.

B.2.3.2.1 Nonhuman Primates

One of the few studies in cynomolgus monkey that measured distribution of PFOA was performed by Butenhoff et al. (2004b; 2002). The study followed four to six male monkeys that received PFOA (0, 3, 10, or 20 mg/kg) daily via oral capsule. Serum, urine, and fecal samples were collected at 2-week intervals and liver samples were collected at necropsy. Steady-state concentrations of PFOA in serum were 77 ± 39, 86 ± 33, and 158 ± 100 µg/mL after 6 weeks and 81 ± 40, 99 ± 50, and 156 ± 103 µg/mL after 6 months for the 3 , 10 , and 20 mg/kg dose groups, respectively (Butenhoff et al., 2004b; Butenhoff et al., 2002). The mean serum concentration of PFOA in control monkeys was 0.134–0.203 µg/mL. Urine PFOA concentrations reached steady state after 4 weeks and were 53 ± 25, 166 ± 83, and 181 ± 100 µg/mL in the 3, 10, and 20 mg/kg dose groups, respectively, for the duration of the study. Liver PFOA concentrations at necropsy in the 3 mg/kg and 10 mg/kg dose groups were similar and ranged from 6.29 to 21.9 µg/g, while concentrations in two monkeys exposed to 20 mg/kg were 16.0 and 83.3 µg/g. Liver PFOA concentrations in two monkeys dosed with 10 mg/kg/day at the end of a 13-week recovery period were 0.08 and 0.15 µg/g (Butenhoff et al., 2004b).

An earlier study (Griffith and Long, 1980) administered 0, 3, 10, 30 and 100 mg/kg/day by stomach tubes to groups of 4–5 male and female Rhesus monkeys. All monkeys at the highest dose died between weeks 2 and 5, and one male and two females died at the 30 mg/kg/day dose. Monkeys at the 30 mg/kg/day dose all exhibited overt toxicity. Serum and livers were analyzed for organic fluorine using a chromatographic method. The authors noted the data suggested dose-related increases in serum and liver values. For example, at the 3 mg/kg/day dose, serum levels ranged from 48 to 65 ppm in serum and 3 to 7 ppm in liver. At the 10 mg/kg/day dose, levels ranged from 45 to 79 ppm in serum and 910 ppm in liver.

B.2.3.2.2 Rats

Numerous studies have been performed on PFOA distribution in rats. These studies range from acute (hours) to chronic (2 years) and include various levels of dosing. Previous studies have indicated that humans and rats have similar serum albumin binding, suggesting circulation of PFOA in the body would be similar (Saladin, 2004; Harkness and Wagner, 1983).

In adult male Sprague-Dawley rats, animals were exposed by gavage to PFOA (20 mg/kg/day) for 1, 3, or 5 days (Martin et al., 2007). While serum data was only presented for 3-day exposure animals, it is clear that serum levels had a moderate accumulation of 245 ± 41 µg/mL.

Additionally, liver concentrations were 92 ± 6, 250 ± 32, and 243 ± 23 μg/g after 1, 3, and five daily doses, respectively. Liver accumulation appeared to reach its peak by day 3 and remained steady at this level through day 5. While limited serum levels were presented, data indicates that at day 3, serum and liver levels were in a 1:1 ratio.

Several studies indicate that the major target organs of PFOA accumulation are liver, kidneys, and lungs with a large amount of PFOA remaining in blood serum. In an earlier study of PFOA, Ylinen et al. (1990) administered male and female Wistar rat doses of 3, 10, and 30 mg/kg/day PFOA via gavage for 28 days. At necropsy, serum, brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected (Table B-4).

Kudo et al. (2007) intravenously administered [^{14}C]PFOA to male Wistar rats via the jugular vein at the doses of 0.041, 0.12, 0.41, 1.23, 4.14 and 16.56 mg/kg BW. Tissue distribution was examined 2 hours after injection, a time point identified as the distribution phase in a 2-compartment model. Distribution patterns varied by dose. Distribution of PFOA to liver decreased with increasing dose. Liver represented 52% of the dose administered for 0.041 mg/kg BW dose but fell to 27% at the highest dose of 16.56 mg/kg BW. At the highest dose, more PFOA was distributed to extrahepatic tissues including blood, carcass, intestine, lung, stomach, epididymal fat and heart. The ratio of hepatic to serum of PFOA concentration (2.22) was 2.7 times larger at the lowest dose compared with the highest dose. Subcellular fractionation analyses of liver cells showed that PFOA was enriched in the cytosolic fraction, where 43% of PFOA was distributed in animals exposed to the highest dose.

Interestingly, measurements of PFOA from adipose tissue resulted in no detectable levels at any dose or timepoint. For the 3 mg/kg/day dose group, male rats exhibited the highest concentration of PFOA in their serum followed by, liver, kidneys and then lungs with notable accumulation in testis. In higher doses of 10 and 30 mg/kg/day, male rats had a significant increase in kidney PFOA concentration. The levels of PFOA in male rat serum were generally lower in the 30 mg/kg/day dose group than in the 10 mg/kg/day dose group, presumably due to increased urinary elimination in the 30 mg/kg/day group as a result of saturation of PFOA binding sites in serum. The PFOA tissue levels were otherwise similar for the 10 and 30 mg/kg/day dose groups of male rats. In comparison, female rats exhibited much lower serum concentrations that the males; the female serum PFOA concentrations were approximately 5%–27% of the male concentrations.

Lower PFOA concentrations were also seen in the female rats' solid tissues as liver and kidney measurements were ~10% and 30% of the concentrations detected in males, respectively. In females, there was a dose-related increase in tissue and serum PFOA concentrations. Concentrations of PFOA for female rats at the low dose were highest in serum, followed by liver, lungs, and spleen. At the higher doses of 10 and 30 mg/kg/day, the highest PFOA concentrations were found in the serum and kidney, a pattern also observed in male rats.

Table B-4. Tissue Distribution of PFOA in Wistar Rats After Exposure via Gavage for 28 Days as Reported by Ylinen et al. (1990)

Tissue[a]	Males			Females		
	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Serum (μg/mL)	48.60 ± 10.30	87.27 ± 20.09	51.65 ± 11.47	2.40[b]	12.47 ± 4.07	13.92 ± 6.06

Tissue[a]	Males			Females		
	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Liver (µg/g)	39.90 ± 7.25	51.71 ± 11.18	49.77 ± 10.76	1.81 ± 0.49	3.45 ± 1.36	6.64 ± 2.64
Kidney (µg/g)	1.55 ± 0.71	40.56 ± 14.94	39.81 ± 17.67	0.06 ± 0.02	7.36 ± 3.19	12.54 ± 8.24
Spleen (µg/g)	4.75 ± 1.66	7.59 ± 3.50	4.10 ± 1.57	0.15 ± 0.04	0.38 ± 0.17	1.59 ± 0.49
Lung (µg/g)	2.95 ± 0.54	22.58 ± 4.59	23.71 ± 5.42	0.24[b]	0.22 ± 0.15	0.75 ± 0.26
Brain (µg/g)	0.398 ± 0.144	1.464 ± 0.211	0.710 ± 0.320	<LOQ[c]	0.029 ± 0.019	0.044 ± 0.018
Ovary (µg/g)	–	–	–	<LOQ	0.41 ± 0.27	1.16 ± 0.58
Testis (µg/g)	6.24 ± 2.04	9.35 ± 4.02	7.22 ± 3.17	–	–	–

Notes: LOQ = limit of quantification.
[a] Data are presented as mean ± standard deviation (n = 6).
[b] Data are presented as the mean (n = 3).
[c] LOQ = 1 µg/mL.

Kawabata et al. (2017) measured PFOA in the tissues of male Wistar rats (including brains) 9 days after administration of a single dose of 50 mg/kg. Serum PFOA concentrations were 33.3 µg/mL and liver concentrations were 58.7 µg/g. However, PFOA levels in brain were below the LOD (i.e., <0.8 µg/g). Although levels are low and detection is variable, these studies do support PFOA accumulation to low levels in brains of adult rats.

PFOA distribution followed a similar pattern in Sprague-Dawley rats administered a single [^{14}C]PFOA dose via oral gavage to male (Table B-5) and female (Table B-6) rats (Kemper, 2003). Tissues from male rats were collected at 10.5 hours (T_{max}) and 171 hours ($T_{max/2}$) (time to return to 50% maximum plasma concentration) after dosing. Tissues from female rats were collected at 1.25 hours (T_{max}) and 4 hours ($T_{max/2}$) after dosing. Liver, blood, skin, muscle, bone, gastrointestinal tract, and adipose were the primary tissues for distribution of [^{14}C]PFOA. In males, the fraction of dose found in the liver increased between T_{max} and $T_{max/2}$ but remained constant or decreased in other tissues. In females, the fraction of the dose present in all tissues remained constant or decreased between T_{max} and $T_{max/2}$. Liver:blood ratios for [^{14}C]PFOA at T_{max} in males were approximately 1:1 but increased between T_{max} and $T_{max/2}$. In females, the liver:blood ratio was ~1.2:1 at the low dose but increased to ~1.5 at higher doses. In males, the PFOA blood concentration was tenfold or higher than the kidney concentration at T_{max} and declined slightly at $T_{max/2}$. In the female tissues at $T_{max/2}$, ~30% of the dosed PFOA retained was present in the liver, blood, kidney, muscle, and skin tissues in decreasing amounts. This study confirmed sex-specific differences in PFOA distribution and identified accumulation in reproductive tissues including testes and ovaries.

Table B-5. Distribution of PFOA in Male Sprague-Dawley Rats After a Single Oral Gavage Dose[a] as Reported by Kemper et al. (2003)

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
Prostate	0.083 ± 0.039	0.030 ± 0.002	0.071 ± 0.045	0.057 ± 0.020	0.067 ± 0.018	0.028 ± 0.012
Skin[b]	14.772 ± 2.135	6.061 ± 0.274	15.565 ± 0.899	7.233 ± 0.430	13.836 ± 0.969	5.419 ± 0.237
Blood[b]	22.148 ± 0.692	8.232 ± 1.218	24.919 ± 1.942	11.140 ± 0.624	22.905 ± 1.177	7.904 ± 1.032
Brain	0.071 ± 0.018	0.022 ± 0.002	0.051 ± 0.021	0.023 ± 0.008	0.063 ± 0.007	0.019 ± 0.002
Fat[b]	2.281 ± 0.467	0.593 ± 0.136	2.815 ± 0.225	0.916 ± 0.205	2.153 ± 0.430	0.628 ± 0.110
Heart	0.451 ± 0.119	0.195 ± 0.024	0.443 ± 0.037	0.252 ± 0.030	0.461 ± 0.053	0.164 ± 0.032
Lungs	0.740 ± 0.147	0.341 ± 0.043	0.593 ± 0.376	0.344 ± 0.194	0.863 ± 0.103	0.303 ± 0.057
Spleen	0.086 ± 0.011	0.045 ± 0.006	0.096 ± 0.017	0.060 ± 0.007	0.106 ± 0.015	0.042 ± 0.005
Liver	21.708 ± 5.627	32.627 ± 3.601	18.750 ± 2.434	25.231 ± 1.289	17.528 ± 0.900	20.145 ± 3.098
Kidney	1.949 ± 0.402	1.140 ± 0.215	2.170 ± 0.354	1.212 ± 0.115	2.293 ± 0.286	1.003 ± 0.122
G.I. tract	2.930 ± 0.929	0.980 ± 0.300	2.508 ± 0.713	1.052 ± 0.202	2.784 ± 0.608	0.808 ± 0.189
G.I. contents	2.083 ± 0.625	0.239 ± 0.025	2.632 ± 0.934	0.270 ± 0.028	4.186 ± 1.349	0.210 ± 0.084
Thyroid	0.008 ± 0.005	0.004 ± 0.003	0.011 ± 0.006	0.004 ± 0.002	0.009 ± 0.002	0.005 ± 0.001
Thymus	0.085 ± 0.008	0.051 ± 0.018	0.085 ± 0.012	0.053 ± 0.003	0.120 ± 0.025	0.045 ± 0.010
Testes	0.755 ± 0.079	0.356 ± 0.037	0.693 ± 0.180	0.372 ± 0.062	0.623 ± 0.098	0.224 ± 0.031
Adrenals	0.019 ± 0.004	0.010 ± 0.001	0.022 ± 0.004	0.009 ± 0.001	0.026 ± 0.004	0.009 ± 0.003
Muscle[b]	12.025 ± 0.648	4.984 ± 0.745	13.565 ± 0.576	6.429 ± 0.648	12.855 ± 0.841	4.253 ± 0.358
Bone[b]	3.273 ± 0.538	1.120 ± 0.094	3.047 ± 0.544	1.375 ± 0.169	3.062 ± 0.438	0.906 ± 0.100
Total[c]	85.465 ± 6.426	57.026 ± 3.379	88.033 ± 1.420	56.031 ± 1.025	83.937 ± 3.680	42.112 ± 4.740

Notes: G.I. = gastrointestinal; T_{max} = time to reach maximum plasma concentration; $T_{max/2}$ = time to return to 50% maximum plasma concentration.

[a] Data are presented as mean percent of dose ± standard deviation recovered at T_{max} and $T_{max/2}$ in tissues.

[b] Percent recovery scaled to whole animal assuming the following: skin = 19%, whole blood = 7.4%, fat = 7%, muscle = 40.4%, bone = 7.3% of body weight.

[c] Totals are calculated from individual animal data.

Table B-6. Distribution of PFOA in Female Sprague-Dawley Rats After a Single Oral Gavage Dose[a] as Reported by Kemper et al. (2003)

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
Skin[b]	0.434 ± 0.162	0.403 ± 0.096	0.624 ± 0.142	0.307 ± 0.121	0.380 ± 0.166	0.415 ± 0.175
Blood[b]	5.740 ± 1.507	4.438 ± 1.625	8.089 ± 2.080	5.411 ± 1.466	7.158 ± 2.232	6.407 ± 1.406
Brain	0.037 ± 0.009	0.047 ± 0.008	0.066 ± 0.019	0.045 ± 0.010	0.058 ± 0.008	0.058 ± 0.018
Fat[b]	0.134 ± 0.032	0.164 ± 0.079	0.220 ± 0.111	0.110 ± 0.069	0.147 ± 0.053	0.148 ± 0.065
Heart	0.198 ± 0.079	0.253 ± 0.055	0.388 ± 0.057	0.236 ± 0.051	0.317 ± 0.035	0.287 ± 0.069
Lungs	0.454 ± 0.148	0.546 ± 0.082	0.827 ± 0.102	0.570 ± 0.179	0.678 ± 0.067	0.775 ± 0.204
Spleen	0.063 ± 0.027	0.058 ± 0.006	0.101 ± 0.021	0.060 ± 0.012	0.091 ± 0.007	0.070 ± 0.002
Liver	7.060 ± 1.266	6.817 ± 1.537	11.190 ± 2.192	7.176 ± 0.982	10.538 ± 1.723	9.080 ± 0.895
Kidney	3.288 ± 0.948	2.769 ± 0.784	4.293 ± 0.771	2.685 ± 0.736	5.867 ± 0.946	4.749 ± 0.393
G.I. tract	10.699 ± 9.066	8.462 ± 6.519	7.142 ± 2.594	8.255 ± 8.967	6.923 ± 1.846	3.547 ± 1.306
G.I. contents	21.956 ± 13.48	3.891 ± 2.395	2.896 ± 2.305	5.601 ± 6.165	2.491 ± 1.548	1.121 ± 1.010
Thyroid	0.010 ± 0.003	0.016 ± 0.021	0.008 ± 0.002	0.006 ± 0.002	0.009 ± 0.003	0.007 ± 0.002
Thymus	0.052 ± 0.017	0.058 ± 0.024	0.105 ± 0.030	0.068 ± 0.021	0.091 ± 0.032	0.077 ± 0.020
Ovaries	0.047 ± 0.019	0.048 ± 0.006	0.071 ± 0.012	0.041 ± 0.012	0.071 ± 0.012	0.070 ± 0.012
Adrenals	0.014 ± 0.005	0.018 ± 0.004	0.026 ± 0.005	0.015 ± 0.004	0.031 ± 0.005	0.021 ± 0.001
Muscle[b]	0.170 ± 0.051	0.258 ± 0.089	0.325 ± 0.010	0.229 ± 0.031	0.441 ± 0.116	0.304 ± 0.099
Uterus	0.243 ± 0.091	0.374 ± 0.247	0.354 ± 0.046	0.247 ± 0.068	0.358 ± 0.124	0.365 ± 0.029
Bone[b]	0.101 ± 0.017	0.153 ± 0.052	0.174 ± 0.057	0.142 ± 0.078	0.157 ± 0.072	0.181 ± 0.090
Total[c]	50.698 ± 16.485	28.772 ± 10.976	36.897 ± 3.187	31.201 ± 12.63	35.803 ± 2.554	27.680 ± 2.569

Notes: G.I. = gastrointestinal; T_{max} = time to reach maximum plasma concentration; $T_{max/2}$ = time to return to 50% maximum plasma concentration.

[a] Data are presented as mean percent of dose ± standard deviation recovered at T_{max} and $T_{max/2}$ in tissues.

[b] Percent recovery scaled to whole animal assuming the following: skin = 19%, whole blood = 7.4%, fat = 7%, muscle = 40.4%, bone = 7.3% of body weight.

[c] Totals are calculated from individual animal data.

Sex-dependent dose distribution similar to results found in Ylinen et al. (1990) have also been found in several other reports (Lau et al., 2006; Kemper, 2003). According to Kemper (2003), plasma concentration occurred 10 times faster and at much lower levels in females when compared with males. Lau et al. (2006) dosed male and female Sprague-Dawley rats with 10 mg/kg for 20 days and necropsied them 24 hours after the last dose. Male rats had serum PFOA levels of 111 μg/mL compared with 0.69 μg/mL in female rats, a sex ratio that was in line with the Kemper et al. results.

Kemper (2003) observed levels of PFOA accumulation in the kidneys of females that were consistently elevated compared with males, indicating that excretion of PFOA may play a role in the sex differences of PFOA distribution. The results suggest females absorb and excrete PFOA more rapidly than males. This study also confirmed PFOA can accumulate in reproductive organs (testes) and observed PFOA accumulation in endocrine (thyroid, adrenals) and immune (thymus) tissues.

Furthermore, at $T_{max/2}$ there was only ~1% of the dosed [^{14}C]PFOA in the gastrointestinal tissues and contents in males, compared with ~14% in females. However, samples were collected at 1.25 and 4 hours in females and 10.5 and 171 hours in males (the timing was based on previous toxicokinetic experiments determining the T_{max} and $T_{max/2}$), thus providing more time for absorption in the males (Kemper, 2003).

Two NTP studies exemplify sex-specific patterns of PFOA accumulation in blood and liver. PFOA levels were measured in the context of both a 28-day toxicity study (NTP, 2019a) and a 2-year chronic toxicity study (NTP, 2020). In the 28-day study (NTP, 2019a), male and female Sprague-Dawley rats were administered 0 to 10 mg/kg/day (males) or 0 to 100 mg/kg/day (females) of PFOA by oral gavage. Although females were administered a 10-fold higher dose of PFOA, males exhibited higher plasma concentrations than females across all dose groups. The plasma concentrations in males were 50.7 ± 2.2 and 148.6 ± 15.4 μg/mL at the lowest and highest dose groups, respectively. In females, plasma concentrations were 0.4905 ± 0.072.1 and 23.444 ± 3.247 μg/mL at the lowest highest dose groups, respectively. When normalized to dose administered (μM/mmol/kg), males had a 1,000-fold higher level than females at the lowest dose and a 63-fold higher level at the highest dose. Males exhibited a decreasing normalized plasma concentration with dose, whereas females exhibited an increasing normalized plasma concentration with dose. PFOA in liver was only measured in males, and the liver:plasma ratios were fairly consistent across dose groups, ranging from 0.87 to 1.17.

In the 2-year study (NTP, 2020), Sprague-Dawley rats were exposed to 0, 150, or 300 ppm PFOA during the perinatal periods. During the postweaning period, first generation (F_1) male rats were provided 0, 150, or 300 ppm and F_1 female rats were provided 0, 300, or 1,000 ppm PFOA via feed. Plasma and liver PFOA levels were measured at the 16-week interval. Plasma and liver PFOA concentrations in males were within 10% of each other regardless of whether animals were also dosed during the perinatal period. Plasma concentrations in females showed a similar pattern to the males (e.g., minor differences between perinatal exposures and liver patterns). Although exposures in females were 2–3 times higher than in males, PFOA plasma concentrations were much lower compared with males. For example, at the highest dose in rats exposed during both perinatal and postweaning periods, plasma concentrations were 223.4 ± 8.4 μg/mL in males compared with 70.2 ± 6.9 μg/mL in females. The liver:plasma ratios

were again fairly consistent across dose groups, ranging from 0.73 to 0.88 in males and from 0.81 to 0.99 in females.

In a repeated inhalation exposure study, Hinderliter et al. (2006a) exposed male and female rats to 0, 1, 10, or 25 mg/m^3 aerosol concentrations of PFOA for 6 hours per day, 5 days per week for 3 weeks. Blood was collected immediately before and after the daily exposure period 3 days per week. The aerosols had mass median aerodynamic diameters of 1.3–1.9 μm with geometric standard deviations (GSDs) of 1.5–2.1. PFOA plasma concentrations were proportional to the inhalation exposure concentrations, and repeated exposures produced little plasma carryover in females, but significant day-to-day carryover in males. By 3 weeks, males reached steady-state plasma levels of 8, 21, and 36 μg/mL for the 1, 10, and 25 mg/m^3 groups, respectively. In females, the postexposure plasma levels were 1, 2, and 4 μg/mL for the 1, 10, and 25 mg/m^3 groups, respectively. When measured immediately before the next daily exposure, plasma levels had returned to baseline in females, demonstrating clearance within 24 hours of each daily dose.

Vanden Heuvel et al. (1991) measured distribution between 2 hours and 28 days in male and female rats exposed to a single i.p. dose of 4 mg/kg. In males, liver and plasma exhibited higher PFOA levels compared with other tissues. Female rats showed a distinct distribution with roughly equal levels distributing to plasma, kidney, and liver.

B.2.3.2.3 Mice

Serum PFOA concentrations exhibited a strong dose-dependent correlation in prepubertal CD-1 female mice subjected to short-term gavage dosing (PND days 18–20) at 0.005, 0.01, 0.02, 0.05, 0.1, and 1.0 mg/kg/day. PFOA serum levels ranged from 0.005 ± 0.4 μg/mL in mice at the low dose to 1.17 ± 0.097 μg/mL in mice at the high dose (r^2 = 0.99 by linear regression) (Yao et al., 2014). A similar dose-dependency was observed in male BALB/c mice (6–8 weeks of age) administered between 0.08 and 20 mg/kg/day daily for 28 days (Yan et al., 2014) and either 0.5 or 2.5 mg/kg/day for 28 days (Yu et al., 2016). Dose-dependent PFOA serum levels were also observed in APOE*3-Leiden.CETP mice that exhibit more human-like lipoprotein metabolism (Pouwer et al., 2019). Serum levels of 0.049 ± 0.004, 1.350 ± 0.088, and 90.7 ± 8.87 μg/mL were observed in animals dosed with 10, 300, and 30,000 ng/g/day, respectively 4 weeks into exposure. In C57BL/6 mice (6–8 weeks old) administered PFOA in drinking water for 5 weeks (0.1, 1, and 5 mg/kg BW), dose-related increases in serum levels were observed (Crebelli et al., 2019), but serum levels were not directly proportional to dose. The fold-increase of internal exposure (serum levels) relative to drinking water concentration ranged from 5.6 (low dose) to 3.0 (high dose).

Measurements of serum PFOA concentrations in mice have differed from results in rat studies. Lau and colleagues (2006) dosed male and female CD-1 mice with 20 mg/kg/day of PFOA for 7 or 17 days and analyzed serum levels. After 7 days, male mice had serum PFOA levels of 181 μg/mL and females had levels of 178 μg/mL. After 17 days of treatment, male mice had serum PFOA levels of 199 μg/mL and females had levels of 171 μg/mL (Lau et al., 2006). Additionally, in a separate experiment performed by Lou et al. (2009) female CD-1 mice were dosed with 20 mg/kg/day for 17 days (Lou et al., 2009). Serum samples were collected 24 hours after the final dose and analyzed for PFOA. The mean serum concentration was 130 ± 23 mg/L, which is comparable to the reported value of 171 μg/mL reported above by Lau et al. (2006).

These data suggest that the sex difference observed by Lau et al. (2006) in rats was not seen in the mice under the conditions of this study.

Lou et al. (2009) measured pharmacokinetics of PFOA in mice administered single doses of 1 and 10 mg/kg to groups of male and female CD-1 mice. Plasma, liver, and kidney tissues were collected at multiple early time points (4, 8, 12, and 24 hours) as well as a dozen time points between 3 and 80 days. In female mice, peak serum concentrations were measured at 10 and 100 mg/L and declined to 2 mg/L and <0.2 mg/L after 80 days for the 1 and 10 mg/kg/day doses, respectively. Peak serum concentrations were slightly lower in the males at ~8 and 80 mg/L, but final serum concentrations were higher in the males at ~0.5 and 8 mg/L, respectively. Liver and kidney concentrations also were higher in males than in females for each of the two doses. These data suggest a longer half-life in males than in females. Additionally, this group dosed 60 mg/kg to female mice and measured serum levels over the course of 28 days. The authors' findings suggest that these mice were able to clear a higher dose of PFOA much more quickly than animals who had received a 1 or 10 mg/kg dose (Lou et al., 2009). The 60 mg/kg dose animals were able to return to a 0.4 mg/L serum concentration in about 28 days while the 10 mg/kg and 1 mg/kg groups took 61 days and 70 days to reach 1 mg/L, respectively.

Several studies of short-term distribution of PFOA in mice have been published that vary between 4 hours and 28 days and demonstrate the range of PFOA tissue distribution. One of the earliest of these time points was performed by Burkemper et al. (2017) who used a radioisotope injection [^{18}F]PFOA and measured deposition in 14 different tissues as well as serum 4 hours later. Despite the observation that radiolabel was associated with ~29% of serum protein, the majority of signal was found in the bone (femur), liver, and lungs. The next highest levels of radioisotope detection were in the heart, spleen, large intestines, and then kidneys. These findings were consistent with recent work by Bogdanska et al. (2020). Using a [^{14}C]PFOA radioisotope, authors measured low (0.06 mg/kg/day) and high dose (22 mg/kg/day) PFOA delivered via feed to C57Bl/6 mice and collected measurements at 1, 3 and 5 days postexposure (Table B-7). Like the previous finding in the Burkemper et al. (2017) paper, liver accumulation was consistently 4−5 times greater than what was found in serum at all doses and time points. Yan et al. (2015) also demonstrated a dose-dependent accumulation in the livers of male BALB/c mice exposed to 0–20 mg/kg/day PFOA via gavage after 28-day exposure. Lung deposition was also found to be at elevated levels and was measured at nearly half serum concentrations at all doses and time points. In a study by Li et al. (2017a) conducted in BALB/c mice after a 28-day exposure, PFOA concentrations in both liver and serum increased with PFOA dose in mice, with PFOA concentrations being generally higher in the liver than the serum.

Table B-7. Distribution of PFOA in Male C57BL/6 Mice Following Exposure to [^{14}C]PFOA for 1, 3, or 5 days in Feed[a] as Reported by Bogdanska et al. (2020)

Tissue	0.06 mg/kg/day			22 mg/kg/day		
	Dose Duration			Dose Duration		
	1 Day	3 Days	5 Days	1 Day	3 Days	5 Days
Blood	0.328	1.222	1.645	90	183	192
Liver	1.59	5.229	7.507	281	671	756
Lung	0.179	0.606	0.873	40	96	110

Tissue	0.06 mg/kg/day			22 mg/kg/day		
	Dose Duration			Dose Duration		
	1 Day	3 Days	5 Days	1 Day	3 Days	5 Days
Kidney	0.16	0.556	0.783	42	91	104
Pancreas	0.087	0.258	0.344	22	51	61
Thyroid gland	0.082	0.294	0.421	24	48	57
Skin	0.096	0.337	0.501	25	47	52
Stomach	0.125	0.259	0.345	14	45	48
Thymus	0.089	0.197	0.237	16	34	47
Inguinal fat pad	0.064	0.209	0.273	15	37	40
Whole bone	0.105	0.282	0.452	20	30	40
Small intestine	0.057	0.174	0.269	10	37	36
Large intestine	0.05	0.166	0.204	10	32	32
Testis	0.054	0.156	0.235	12	28	29
Epididymal fat	0.053	0.152	0.153	12	23	24
Muscle	0.032	0.116	0.169	9	19	20
Brain	0.008	0.029	0.024	2	3	4
Spleen	0.022	<LOD	<LOD	<LOD	5	1
Heart	<LOD	<LOD	<LOD	14	15	<LOD

Notes: LOD = limit of detection.
[a] Data are presented as mean (nmol/g).

Interestingly, while Burkemper et al. (2017) measured equal levels of kidney and large intestine depositions at very early time points (4 hours), Bogdanska et al. (2020) registered a far greater amount of PFOA in the kidneys at the slightly later time points or 1, 3, and 5 days. This may indicate a change in excretion methods over the course of exposure and/or reflect differential distribution or detection of [^{18}F]PFOA relative to [^{14}C]PFOA. Burkemper et al. also measured a large uptake of [^{18}F]PFOA in mouse femurs at 4 hours, while Bogdanska et al. found moderately low levels at later time points. This difference could be due to rapid fluorine intake of the bone by potential ^{18}F radioisotope artifacts.

Bogdanska et al. (2020) also observed accumulation of 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 (0.31 to 20 mg/kg/day) for 28 days, PFOA levels in the testes increased with increasing dose (Zhang et al., 2014). Further evidence of distribution to reproductive tissues in male mice comes from the finding that PFOA accumulated in the epididymis of BALB/c mice in a dose-dependent manner (Lu et al., 2016b).

Accumulation in both small intestine and the colon was observed in CD-1 mice administered between 1 and 20 mg/kg/day for 10 days (Rashid et al., 2020). Higher levels of PFOA were measured in the small intestine relative to colon. The mean concentration of PFOA in small intestine detected was 1.0, 2.3, 4.4, and 6.5 µg/g in the 1, 5, 10, and 20 mg/kg/day groups, respectively. Dose-dependent accumulation was also seen in the colon, where mean concentrations ranged from 211.12 to 1,834.27 ng/g in colon tissue.

Fujii et al. (2015) performed IV injections of 0.313 μmol/kg of PFOA on male and female animals and collected serum and organ samples after 24 hours. Distribution was calculated as percentage of total recovered dose from serum and organs. The majority of administered PFOA was retained in the serum and liver of mice and less than 2% of administered dose was found in kidney and adipose tissue. While a relatively small amount of PFOA was measured in the brain (0.1%), it is noteworthy that PFOA can cross the BBB in healthy animals. Similar findings were observed in the Burkemper et al. (2017), Bogdanska et al. (2020), and Yu et al. (2016) studies. Levels in female mouse livers were ~30% of the levels measured in male samples. A larger portion of PFOA was not recovered from serum, organ, and excretions of female mice, indicating that there may be further distribution in organs that were not examined in this study. Fujii and colleagues (2015) examined distribution based on chain length. They observed that perfluoroalkyl carboxylic acids (PFCAs) with shorter chain length (C6 and C7) were excreted rapidly through urine, while longer chains (≥C8) accumulated in the liver. Moreover, PFCA with longer chain lengths were found to exhibit increasing affinity for serum and liver fatty acid binding proteins. The authors suggest that lipophilicity driven by chain length may account for the distribution patterns of PFCA, which is consistent with the 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.

Studies that examined PFOA distribution for longer time periods also reveal that the liver is a primary site of PFOA accumulation. Adult male BALB/c mice exposed to PFOA (0.4, 2, and 10 mg/kg/day) via oral gavage for 28 days exhibited dose-dependent increase in both serum and liver (Guo et al., 2019). At every dose tested, liver:serum ratios appeared to stay near 2:1. Additionally, it was found that the liver consistently absorbed 10% of the total PFOA each animal was exposed to. In a study with the same 28-day exposure and similar low dose (1.25 mg/kg/day via oral gavage), Zheng and colleagues found that PFOA distributed in the liver and serum in an ~2.5:1 ratio (Zheng et al., 2017). These findings further corroborate the previous radioisotope studies that PFOA accumulates primarily within the liver and secondarily in serum.

One potential method of removal of PFOA from liver is through activation of 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 the ß-oxidation of fatty acids, induce fatty acid transport across the mitochondrial membrane, decrease hepatic very low-density lipoprotein-triglyceride and apolipoprotein B (apoB) production, and promote lipolysis of triglyceride-rich plasma lipoproteins (Fragki et al., 2021). In an experiment using male wild-type 129S4/SvlmJ mice and PPARα-null 129S4/SvJae-Pparα^{tm1Gonz}/J mice, animals were orally administered 0, 12.5, 25, and 50 μmol/kg/day PFOA (~0, 5.4, 10.8, and 21.6 mg/kg/day PFOA, respectively) for four weeks (Minata et al., 2010). Blood, liver, and bile were collected for determination of PFOA concentration at the end of 4 weeks (Table B-8). The PFOA concentration in whole blood and the liver were similar between wild-type and PPARα-null mice and increased in proportion to dose. In bile, PFOA concentration in wild-type mice increased by a factor of 13.8 from 12.5 to 25 μmol/kg and by a factor of 2.8 from 25 to 50 μmol/kg; however, in bile of PPARα-null mice, PFOA concentration increased by a factor of only 3.2 from 12.5 to 25 μmol/kg and by a factor of 6.1 from 25 to 50 μmol/kg. The liver can transport PFOA from hepatocytes to bile ducts that is

mediated at least partly by PPARα. The lower PFOA levels in bile of PPARα null mice suggest a role for PPARα in PFOA clearance in the liver (Minata et al., 2010).

Table B-8. PFOA Concentrations in Wild-type and PPARα-null Male Mice Exposed to PFOA by Gavage for Four Weeks[a] as Reported by Minata et al. (2010)

Dose (µmol/kg)	Whole Blood		Bile		Liver	
	Wild-type	PPARα-null	Wild-type	PPARα-null	Wild-type	PPARα-null
0	ND	ND	ND	ND	ND	ND
12.5	20.6 ± 2.4[a]	19.3 ± 2.2	56.8 ± 26.9	19.6 ± 2.2	181.2 ± 6.3	172.3 ± 8.9
25	46.9 ± 3.2	36.4 ± 2.7	784 ± 137.6	62.9 ± 16.7	198.8 ± 15.4	218.3 ± 14.5
50	64.2 ± 6.5	71.2 ± 8.0	2,174 ± 322.4	383 ± 109.9	211.6 ± 13.3	239.7 ± 25.0

Notes: ND = not detected; PPARα-null = peroxisome proliferator-activated receptor alpha-null 129S4/SvJae-Pparαtm1Gonz/J mice; Wild-type = 129S4/SvlmJ mice.
[a] Data are presented as mean ± standard deviation (µg/mL).

B.2.3.3 Tissue Transporters

As described earlier, protein transporters from a number of families 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, and 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. Kummu et al. (2015) used placentas donated from healthy mothers to investigate the role of OAT4 and ATP-binding cassette transporter G2 (ABCG2) proteins. Using an ex vivo perfusion system, the authors administered concentrations of PFOA and PFOS (1,000 ng/mL) by perfusing through the maternal circulation. The fetal:maternal ratios of PFOA and PFOS were 0.20 ± 0.04 and 0.26 ± 0.09, which corresponded to transfer index percentages of 12.9 ± 1.5% and 14.4 ± 3.9%, respectively. Immunoblot analysis of OAT4 and AGCG2 in perfused placentas indicated a linear negative correlation between the expression of OAT4 protein (but not ABCG2) and PFOA ($r^2 = 0.92$, $p = 0.043$) and PFOS ($r^2 = 0.99$, $p = 0.007$) transfer at 120 minutes. The authors speculated that OAT4 may play a role in decreasing placental passage of PFAS and intrauterine exposure to these compounds; however, the low number of placentas examined and lack of direct evidence for uptake via OAT4 indicates further studies are needed to understand what, if any, role transporters play in placental transfer of PFOA and PFOS.

To further elucidate the role of placental transporters in facilitating the transfer of maternal PFAS into the fetus, Li et al. (2020a) compared gene expression of selected transporters in preterm and full-term placentas and determined whether the differences in expression could influence the transplacental transfer efficiencies (TTEs). The authors selected nine placental genes with known xenobiotic activity on the maternal side of the placenta: organic cation/carnitine transporter 2, reduced folate carrier 1 (*RFC-1*), equilibrative nucleoside transporter (*ENT1*), folate receptor alpha (*FRα*), heme carrier protein 1, serotonin transporter (*SERT*), p-glycoprotein (*MDR1*),

multi-drug resistance-associated protein 2 (*MRP2*), and breast cancer resistance protein (*BCRP*). *MDR1* expression levels were significantly associated with TTEs of branched PFOS and iso-PFOS, (3 + 4 + 5)m-PFOS, but not linear PFOS or PFOA. *MRP2* expression was associated with total PFOS, linear PFOS, branched PFOS, and iso-PFOS, (3 + 4 + 5)m-PFOS, but not PFOA. *BCRP* expression levels did not significantly change with PFOA or PFOS. Interestingly, the pattern of expression of *MDR1*, *MRP2* and *BCRP* were only observed in full-term placentas. Preterm placentas showed significant expression levels of *ENT1*, *FRα*, and *SERT* and were associated with 1m-PFOS and iso-PFOS. Thus, the expression of transporters and TTEs appear to differ between preterm and full-term placentas. Authors noted that the three transporters that were significantly associated with PFOS (*MDR1*, *MRP2*, and *BCRP*) are also ABC transporters, which play a protective role for the placenta tissue and the fetus by effluxing xenobiotics across the placental barrier thereby reducing exposure to PFOS. It is unclear why there were no correlations with PFOA although this may be related to the fact that gene expression associations with TTE were not confirmed using protein expression data of the candidate genes.

More research is needed to explain how different transporters respond to PFAS and whether physiochemical properties such as chain length and branching may influence the substrate binding capacity of these transplacental transporters.

B.2.4 Distribution During Reproduction and Development

The availability of distribution data from pregnant females plus animal pups and neonates is a strength of the PFOA pharmacokinetic database because it helps to identify those tissues receiving the highest concentration of PFOA during development. For this reason, the information on tissue levels during reproduction and development is presented separately from those that are representative of other life stages.

B.2.4.1 Human Studies

Zhang et al. (2013b) recruited 32 pregnant females (21–39 years) from Tianjin, China, for a study to examine the distribution of PFOA between maternal blood, cord blood, the placenta, and amniotic fluid. Samples were collected at time of delivery (35–37 weeks). The study yielded 31 maternal whole blood samples, 30 cord blood samples, 29 amniotic fluid samples, and 29 placentas. PFOA was found in all fluids/tissues sampled. Maternal blood contained variable levels of 10 PFAS: eight acids and two sulfonates. The mean maternal blood concentration was highest for PFOS (14.6 ng/mL) followed by PFOA (3.35 ng/mL). In both cases, the mean was greater than the median, indicating a distribution skewed toward the higher concentrations. PFOA was transferred to the amniotic fluid to a greater extent than PFOS based on their relative proportions in the maternal blood and cord blood. Compared with mean PFOA blood levels in the pregnant females, the mean levels of PFOA in the cord blood, placenta, and amniotic fluid were 47%, 59%, and 1.3%, respectively, of those in the mother's blood. The correlation coefficients between the maternal PFOA blood levels and placenta, cord blood, and amniotic fluid levels (0.7–0.9) were statistically significant ($p < 0.001$).

B.2.4.1.1 Partitioning to Placenta

The placenta serves as an important link between the mother and the growing fetus throughout gestation. It forms a physiological barrier that facilitates the exchange of nutrients, gases, xenobiotics, and several biological components between maternal and fetal circulation. Several

PFAS compounds including PFOA and PFOS have been identified in amniotic fluid, cord blood, and fetal tissue, indicating that these chemicals cross the transplacental barrier and influence PFAS distribution to the fetus and elimination during pregnancy.

The role of the placenta in facilitating the transport of PFAS compounds to the fetal compartment during gestation is informed by the ratio of placental concentration and matched maternal serum concentration, or R_{PM}. Chen et al. (2017a) examined distribution of PFAS chemicals and their isomers in maternal serum, cord serum, and placentas from 32 pregnant women and their matched infants in Wuhan, China. The mean maternal age for the population was 27.1 years, with average pre-pregnancy body mass index (BMI) of 20.4 and gestational age of 38.9 weeks. PFOA isomers examined included n-PFOA (linear PFOA), iso-PFOA, 5m-PFOA, 4m-PFOA, 3m-PFOA, and tb-PFOA; however, the only isomers detected in maternal serum, cord serum, and/or placenta were linear PFOA, iso-PFOA, and 3m-PFOA. Linear PFOA contributed approximately 89% of cord serum PFOA and 91% of maternal serum PFOA. Branched PFOA, including 3m-PFOA and iso-PFOA, contributed approximately 5% and 6%, respectively, of the total PFOA in cord serum and 5% and 5%, respectively of total PFOA in maternal serum. Notably, the increased proportion of linear isomers was also observed in other PFAS chemicals including PFOS and PFHxS. Similar findings have been reported in Cai et al. (2020) and Li et al. (2020a). The ratio of placental:maternal concentrations (R_{PM}) for 3m-PFOA was greater than that for linear PFOA, suggesting that 3m-PFOA is transferred more efficiently than linear PFOA.

Zhang et al. (2013b) recruited 32 female subjects (mean age of 30.9 years) from a hospital in Tianjin, China, who reported full-term pregnancies (average gestation period of 40.3 weeks). The authors reported an average of 1.58 ng/g of PFOA in the placenta and 3.35 ng/mL in maternal serum (Table B-9). The R_{PM} for total PFOA was approximately 0.47, which is higher than the proportion of total PFOA reported by Chen et al. (2017a). For PFOA levels in maternal serum, Zhang et al. (2013b) reported significantly higher levels which may have contributed to the increased PFOA accumulation. The fact that participants in the Zhang et al. (2013b) study were further along in gestation than participants in the Chen et al. (2017a) study may have contributed to their higher maternal PFOA levels.

Mamsen et al. (2019) demonstrated that factors such as gestational age can affect PFOA concentrations in maternal serum and placentas. Using a linear graph of normalized percentage accumulation as a function of gestational age, the authors found that, for male and female infant placentas, there was a steady increase in PFOA accumulation during gestation days 50 to 300, with male placentas showing higher levels of than female placentas. Authors estimated a placenta PFOA accumulation rate of 0.11% per day during gestation.

In summary, the findings from these studies highlight four important points: 1) Linear PFOA is detected at a higher frequency and at higher concentrations in maternal serum than branched PFOA isomers; 2) branched and linear PFOA cross the placental barrier and are distributed in different proportions within the placenta; 3) branched PFOA is more efficiently transferred into the placenta than linear PFOA; and 4) PFOA concentrations within the placenta increase during gestation from the first to third trimester.

Several studies have investigated distribution from mother to fetus through analysis of detected PFAS chemicals in cord blood. Kato et al. (2014) collected blood samples from 71 mothers and

their infants in a prospective birth cohort in the Cincinnati, Ohio, metropolitan area. They quantified PFAS in maternal blood at 16 weeks of gestation and, at the time of delivery, evaluated the correlation between PFAS levels in maternal serum and matched cord blood. Maternal serum PFOA levels at 16 weeks of gestation and at time of delivery were 4.8 μg/L and 3.3 μg/L, respectively. Authors reported a positive correlation between maternal serum PFOA concentration at 16 weeks of gestation and cord serum (correlation coefficient = 0.94). Similarly, the correlation between maternal serum at the time of delivery and cord serum was also positive (correlation coefficient = 0.88). A strong correlation between PFOA levels in maternal serum (collected within 1 week of delivery) and cord serum (collected at delivery) was also observed in a cohort of 50 mother-infant pairs from the Jiangsu province of China (Pearson correlation coefficient = 0.927, $p < 0.001$) (Yang et al., 2016b). In another study conducted in China, 157 paired maternal and cord serum samples collected in Beijing around delivery were examined (Yang et al., 2016a). PFOS and PFOA were the dominant PFAS contaminants in these samples. Mean PFOA levels were 1.95 ± 1.09 ng/mL and 1.32 ± 0.69 ng/mL in maternal and cord serum, respectively (mean cord:maternal serum ratio was 0.71 ± 0.22:1).

Porpora et al. (2013) quantified PFOA levels in maternal serum and cord blood from 38 mother-infant pairs in Rome, Italy. The women were Italian Caucasian between the ages of 26 and 45 (mean age, 34.5 years). The average gestational age for participants in this study was 39 weeks. Maternal and cord serum PFOA concentrations were 2.9 ng/g and 1.6 ng/g, respectively. A strong positive correlation was observed between maternal and cord serum concentrations ($r = 0.70$, $p < 0.001$). These values suggest a cord to maternal serum ratio of 0.55.

Fromme et al. (2010) measured PFOA in mothers and infants in Munich, Germany. Maternal blood was sampled during pregnancy, at delivery, and 6 months after delivery in mothers aged 21–43 years. PFOA was also measured in cord blood and in infant blood at 6 and 19 months after birth. Maternal PFOA serum concentrations ranged from 0.7 to 7.0 μg/L (38 samples) and cord serum concentrations ranged from 0.5 to 4.2 μg/L (33 samples). The cord to maternal serum mean ratio was 0.7.

Wang et al. (2019d) measured the levels of 10 PFAS chemicals in paired maternal and umbilical cord serum from a prospective birth cohort (n = 369) in Shandong, China. The average maternal and gestational ages of the participants were 28.4 years and 39.4 weeks, respectively. PFOA was detected in all maternal and umbilical cord serum samples at a geometric mean of 39.27 ng/mL (range: 1.16–602.79 ng/mL) in maternal serum and 31.83 ng/mL (range: 1.52–291.56 ng/mL) in cord serum. Of the 10 PFAS chemicals measured, PFOA showed the highest concentration in both maternal and cord serum ($r = 0.908$). The authors did not explain why PFOA levels were high. Comparing the studies in Table B-9, geographic location could be a factor in population exposure to a particular PFAS chemical. In the case of Shandong, China, PFOA production may be a reason for the high PFOA levels in serum samples. According to these studies, cord blood PFOA level is a biomarker for in utero exposure and provides further evidence that PFOA readily accumulates in cord blood during gestation.

Study participants from various geographical locations, whether it be Ohio, USA (Kato et al., 2014), Rome, Italy (Porpora et al., 2013), Spain (Manzano-Salgado et al., 2015), France (Cariou et al., 2015), Faroes Islands, Denmark (Eryasa et al., 2019), Munich, Germany (Fromme et al., 2010), Tianjin Tianjin, China (Zhang et al., 2013b), or Shandong, China (Wang et al., 2019d), mostly show consistently higher levels of PFOA in maternal serum versus cord serum regardless

of gestational age. However, for studies with participants of similar gestational ages, the PFOA concentrations in both maternal and cord serum varied substantially across studies, reflected in RCM ratios that ranged from 0.57 to 1.33 (Table B-9). Factors such as exposure sources, parity, and other maternal demographics can potentially account for the variations in maternal PFOA concentrations. For example, nulliparous mothers generally have significantly higher serum PFOA than parous women (Kato et al., 2014). Conversely, younger women tend to have lower serum PFOA than older women (Kato et al., 2014). Therefore, studies with high percentages of young, multiparous women may report lower levels of PFOA in maternal and cord blood.

To understand the role of the placenta in facilitating the transport of PFAS compounds to the fetal compartment during gestation, it is important to highlight the transplacental transfer efficiency (TTE) and the factors that can potentially modulate in utero transport of PFAS. TTE is a measure of a compound's ability to cross the placenta barrier and is often reported as the ratio of cord blood to maternal blood concentrations (RCM). A summary of recent studies examining RCM is presented in Table B-9. The percentages of maternal PFOA that accumulate in cord blood ranged from 57% to 133% and did not strictly correlate to maternal serum values. This variability suggests that TTE may differ across populations. For example, Manzano-Salgado et al. (2015) demonstrated that the percentage of maternal PFOA that accumulates in cord blood tends to increase with maternal age.

Zhang et al. (2013b) calculated the RCM of 11 PFAS compounds in matched maternal-cord blood from a population of 32 mothers in Tianjin, China, who delivered their infants at full term. Authors noted an interesting trend where the highest RCM was reported for perfluoroheptanoic acid (PFHpA) (C7) and a descending trend of RCM was observed with increasing chain length from PFHpA (C7) to perfluorodecanoic acid (PFDA) (C10). There was then an increasing trend in RCM with increasing chain length from PFDA (C10) to perfluorododecanoic acid (PFDoDA) (C12), creating a "U" shaped curve where the RCM decreases with increasing chain length until a certain threshold is reached and then the RCM increases. The authors suggest that this nonlinear relationship may be due to differential binding affinities to maternal serum proteins and that high-affinity PFAS-serum protein interactions may result in PFAS not being able to cross the placental barrier as efficiently through passive diffusion. In line with most previous reports (Lee et al., 2013; Zhang et al., 2013b; Beesoon et al., 2011; Hanssen et al., 2010), but not all (Gutzkow et al., 2012; Kim et al., 2011), Wang et al. (2019d) reported that short-chain PFAS were transferred to cord serum at higher efficiencies than longer-chain PFAS.

Branching also impacts TTE (Zhao et al., 2017a) with branched isomers transferring more efficiently than their linear isomers. The authors observed a U-shaped trend of TTEs with increasing carbon chain lengths as well as the position of the branching point. TTEs of branched PFOA isomers (iso-, 5m-, and 4m-PFOA) were 0.71, 0.94, and 2.00, respectively compared with a TTE of 0.56 for linear isomer (n-PFOA). Thus, higher efficiencies were observed as the branching point moved closer to the carboxyl moiety of PFOA, which may be due to lower affinities of branched PFOA isomers for HSA allowing for more efficient transfer to the fetus.

The efficiency of the placenta to modulate the transfer of xenobiotic varies during gestation. To determine whether RCMs of PFAS in preterm placentas differed from full-term placentas, Li et al. (2020a) assessed the RCMs of 32 PFAS chemicals in preterm and full-term deliveries in the Maoming Birth Cohort in South China. The concentration of PFOA in maternal blood from preterm subjects (mean = 1.2 ng/mL) did not differ significantly from blood levels in full-term

subjects (mean = 1.34 ng/mL). However, the concentration of PFOA in preterm cord blood (0.70 ng/mL) was significantly lower than full-term cord blood (1.25 ng/mL, $p < 0.001$). Interestingly, the proportion of maternal PFOA in cord blood was 33% higher in full-term pregnancies than in preterm pregnancies. Authors attributed the differences in RCM between preterm and full-term deliveries to several factors, such as the difference in gestational age between the two groups. Full-term deliveries have longer gestation periods which means longer exposure duration. Second, the ability of the placenta to reduce toxin transfer reduces in the later stages of pregnancy, making it easier for PFAS to diffuse into fetal circulation. Third, most preterm pregnancies have impaired uteroplacental circulation, potentially reducing the amount of PFAS entering fetal circulation. Finally, gene expression of RCM transporters varies during the different stages of gestation, consequently affecting placenta barrier efficiency.

Table B-9. PFOA Concentrations in Human Cord Blood, Maternal Blood, and Transplacental Transfer Ratios (RCM)

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOA Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)c	Cord:Maternal Serum Ratios (RCM)[d]
Manzano-Salgado et al. (2015)	Sabadell and Valencia, Spain	53	NR	total PFOA	1.90	2.97	0.746
	Note: Serum concentrations reported as p50. whereas geometric mean concentrations were used by authors to calculate cord:maternal serum ratios. Reported concentrations from 66 maternal plasma samples, and 66 cord blood samples, and 53 maternal serum samples.						
Chen et al. (2017a)	Wuhan, China	32	38.9 ± 1.6	total PFOA	1.237 ± 0.577	1.56 ± 0.611	0.808
				n-PFOA	0.947	1.15	0.842
				Iso-PFOA	0.067	0.053	1.267
				3m-PFOA	0.08	0.06	0.587
Cariou et al. (2015)	Toulouse, France	89	NR	total PFOA	0.919	1.22	0.78
	Note: Concentrations represent mean values from 100 pairs. Semi-quantified values below LOD were taken into account for mean calculation.						
Cai et al. (2020)	Maoming birth cohort, China	424	39.3 ± 1.1	total PFOA	0.85 ± 0.52	1.21 ± 1.01	0.80
	Note: Ratios were calculated from matched maternal and infant pairs for which all cord blood samples were > limit LOD. PFOA was detected 98.28% of samples PFOA.						
Wang et al. (2019d)	Shandong, China	369	39.4 ± 1.3	total PFOA	31.83	39.27	0.83
	Note: PFOA detected in 100% of maternal and cord samples.						
Li et al. (2020a)	Maoming Birth Cohort, China (Pre-term births)	86	33.8 ± 3.0	total PFOA	0.7	1.2	0.57
	Maoming Birth Cohort, China (Full-term births)	187	39.5 ± 1.1	total PFOA	1.25	1.34	0.85
	Note: 273 mother-infant pairs were analyzed, including 86 preterm deliveries and 187 full-term deliveries. Only PFAS quantifiable in >50% of maternal and cord sera were included in generating mean concentration values.						
Li et al. (2020b)	Beijing, China	86	39.0 ± 1.2	total PFOA	4.98	3.63	1.33
	Note: PFOA detection rate was 84.62% in maternal serum and 83.76% in cord serum. For PFOA, 86 of 117 matched cord and maternal serum samples were used to generate RCM.						
Eryasa et al. (2019)	Faroese Birth Cohort, Denmark (cohort 3)	100	39.9 ± 1.3	total PFOA	1.97 (1.42−2.76)	2.33 (1.79−3.29)	0.82

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOA Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)c	Cord:Maternal Serum Ratios (RCM)[d]
	Faroese Birth Cohort, Denmark (cohort 5)	51	39.7 ± 1.1	total PFOA	0.81 (0.56−1.26)	1.03 (0.75−1.41)	0.77
	Note: Cohort 3 included 100 singleton births from 1999 to 2001 and cohort 5 included 51 singleton births from 2008 to 2005. Both cohorts had the same source of exposure and were similar in maternal characteristics. Ratios were reported as median p50. Serum concentrations reported here geometric mean and interquartile ranges(IQR).						
Pan et al. (2017)	Wuhan, China	100	39.4 ± 1.3	total PFOA	1.42	2.19	0.65
	Note: Maternal blood collected in third trimester (38.4 ± 1.6 wk). PFOA was detected in 100% of maternal and cord samples.						
Zhao et al. (2017a)	People's Hospital of Hong'an County, China	63	39.3 ± 0.82	n-PFOA	0.551	0.966	0.59
		49	39.3 ± 0.82	iso-PFOA	0.01	0.014	0.81
		36	39.3 ± 0.82	5m-PFOA	0.003	0.003	1.7
		7	39.3 ± 0.82	4m-PFOA	0.001	0.001	2
		63	39.3 ± 0.82	Total PFOA	0.565	0.984	0.59
	Note: Authors reported that samples <LOD were not included in RCM analysis. Mean ratios reported for matched pairs.						
Beeson et al. (2011)	Chemicals, Health and Pregnancy (CHirP) cohort, Vancouver, Canada	20	NR	Total PFOA	1.1	1.8	0.61
		20	NR	n-PFOA	NR	NR	0.62
		20	NR	Iso-PFOA	NR	NR	0.84
		4	NR	5m-PFOA	NR	NR	0.86
		19	NR	4m-PFOA	NR	NR	0.64
		18	NR	3m-PFOA	NR	NR	0.76
	Note: First trimester samples collected between gestation weeks 4 and 14. Timing of second trimester blood collection was not reported. Ratios and concentrations were generated from blood samples collected from 50 randomly selected matched maternal-cord pairs that met study criteria (from a total of = 80,678 maternal participants in the cohort).						
Fei et al. (2007)	Danish National Birth Cohort, maternal blood obtained in first trimester	50	40.06 ± 1.57	total PFOA	3.7 ± 4.7	5.6 ± 2.5	0.55
	Danish National Birth Cohort, maternal blood obtained in second trimester	50	40.06 ± 1.57	total PFOA	3.7 ± 4.7	4.5 ± 1.9	0.68
	Note: Authors did not specify if matched maternal and cord blood samples were used to derive ratios.						

Study	Country, Cohort	Number of Maternal-Infant Pairs[a]	Mean Gestational Age (weeks)[b]	PFOA Measurement	Cord Serum (ng/mL)[c]	Maternal Serum (ng/mL)c	Cord:Maternal Serum Ratios (RCM)[d]
Hanssen et al. (2010)	Johannesburg, South Africa	71 maternal serum, 58 cord blood	NR	total PFOA	1.3	1.3	0.71
	Note: Maternal and cord blood samples taken at time of delivery.						
Fromme et al. (2010)	Munich, Germany	38 maternal and 33 cord serum	NR	total PFOA	1.4	1.9	1.02
	Note: Maternal and cord blood samples taken at time of delivery.						
Kim et al. (2011)	Seoul and Gumi, South Korea	44 mothers, 43 infants	39 ± 1.6	total PFOA	1.15 (0.95−1.86)	1.46 (1.15−1.91)	0.98
	Note: Median serum concentrations reported. Values in parentheses are 25%–75% IQRs.						
Needham et al. (2011)	Faroe Islands	12	NR	total PFOA	3.1	4.2	0.72
	Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.						
Liu et al. (2011)	Jinhu, China	50 (all)	NR	total PFOA	1.5	1.655	0.91
		26 (male infants)	NR	total PFOA	NR	NR	0.87
		24 (female infants)	NR	total PFOA	NR	NR	0.95
	Note: Maternal samples collected in the first weeks after delivery.						
Midasch et al. (2007)	NR	11	NR	total PFOA	3.4	2.6	1.26
	Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.						
Verner et al. (2016)	NA	NA	NA	NA	NA	NA	0.78
	Note: Authors developed a two-compartment, two-generation pharmacokinetic model of prenatal and postnatal exposure to PFOA and PFOS. R_{CMs} applied in model were derived from average of studies reported in Aylward et al. (2014).						

Notes: IQR = interquartile range; LOD = level of detection; NA = not applicable; NR = not reported; RCM = ratio between cord and maternal blood.

[a] Number represents number of matched pairs used for RCM calculation unless otherwise noted in comments.

[b] Gestational age reported as mean ± SD, represents gestational age at the time of cord blood sampling (delivery) and may not be the same as age at the time of maternal blood sampling.

[c] Concentrations in cord or maternal samples are reported as means with or without SD or IQR unless otherwise noted in comments. Note that several studies, the mean serum concentrations may be derived from more subjects than values used for RCM calculation, which typically included only matched pairs for which both cord and maternal serum concentrations were above the limit of detection.

[d] Data are presented as a ratio of cord serum to maternal serum concentrations unless otherwise noted in comments.

B.2.4.1.2 Partitioning to Amniotic Fluid

Zhang et al. (2013b) measured the levels of 11 PFAS chemicals in maternal blood, cord blood, and placenta. All 11 PFAS were detected in their respective biological tissues at different concentrations. The mean concentration ratio between amniotic fluid and maternal blood (AF:MB) was higher in PFOA (0.13) than in PFOS (0.0014). Similarly, the mean concentration ratio between amniotic fluid and cord blood (AF:CB) was higher in PFOA (0.023) than in PFOS (0.0065). Authors attributed the differences in ratios between the two compartments to the solubility of PFOS and PFOA and their respective protein binding capacities in the two matrices. The authors reported a positive correlation between PFOA in amniotic fluid and maternal blood ($r = 0.621$, $p < 0.01$) and cord blood ($r = 0.664$, $p < 0.01$), adding to the evidence that PFOA levels in amniotic fluid is a potential biomarker for fetal exposure during pregnancy.

Table B-10 presents means or medians and ranges of measured and estimated PFOA concentrations in maternal blood from recent studies (2013 to present) that also measured fetal indicators of exposure (cord blood, placenta, and amniotic fluid). These studies demonstrate the variability of PFOA accumulation in these tissues across geographic regions. Maternal serum values ranged from 0.02 ng/mL in Rome, Italy (Porpora et al., 2013) to 602.79 ng/mL in Shandong, China (Wang et al., 2019d). These same studies also showed the greatest range of PFOA in cord blood (0.17–291.56 ng/mL). Fewer studies measured PFOA in placentas and amniotic fluid. Placenta values ranged from <LOQ at hospitals in Skelby ad Randers, Denmark (Mamsen et al., 2017) to 3.57 ng/g in Tianjin, China (Zhang et al., 2013b). The same two studies provided ranges detected in amniotic fluid (<LOQ to 0.145 ng/mL), which were lower than those observed in placentas. The very wide concentration ranges observed across these geographic locations and matrices highlight the challenges of comparing the partitioning of PFOA from mother to fetus across studies.

In addition to geographic variation, inter-individual variability likely plays an important role in the range of concentrations observed in maternal and fetal tissues and matrices. Variability was examined by Brochot et al. (2019) using a PBPK model calibrated in a population framework to provide quantitative estimates for the PFOA and PFOS placental transfers in humans. The measured values of maternal plasma:cord serum input in their model were, on average, close to 1 but showed a variability close to tenfold. The measured transfer rates of PFOA and PFOS used were also quite variable, indicating that PFOA crosses the placental barrier at a rate 3 times higher than PFOS. The coefficients of variation of the maximal transfer rate across subjects were estimated at 75% for PFOA and 55% for PFOS. Variation was also observed in the ranking of PFOA and PFOS when comparing exposure levels to fetal indicators of exposure. Maternal daily intake estimates were then used as inputs to the PBPK model to simulate the fetal exposure in several target organs over the whole pregnancy. The PFOA and PFOS fetal plasma concentrations are quite similar at the end of pregnancy for the whole cohort. This similarity was also predicted for the brain, but not in the kidneys and liver. When examined at the individual level, the ranking of PFOA and PFOS exposure exhibited a wide range of variability. Interestingly, the model estimated that approximately one-third of the population has levels of one compound always higher than levels of the other compound, whereas the remaining two-thirds exhibited differing patterns of accumulation for PFOA and PFOS. The majority, however, were predicted to accumulate PFOA at higher levels than PFOS levels for most of the fetal indicators of exposure. The authors concluded that differences in fetal exposure are not predicted by the measurement of the maternal concentration during pregnancy.

Table B-10. PFOA Concentrations in Human Maternal Blood, Cord Blood, Placenta and Amniotic Fluid Across Studies

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
Porpora et al. (2013) (Italy)	Maternal serum Mean: 2.9 ng/g Median: 2.4 ng/g Range: 0.20–9.1 ng/g	Cord serum Mean: 1.6 ng/g Median: 1.6 ng/g Range: 0.17–5.0 ng/g	NR	NR
Zhang et al. (2014) (Tianjin, China)	NR	NR	Mean: 1.58 ng/g Median: 1.41 ng/g	Mean: 0.044 ng/mL Median: 0.043 ng/mL
Yang et al. (2016a) (Jiangsu, China)	Maternal serum Mean: 1.64 ng/mL SD: 1.11 ng/mL Median: 1.24 ng/mL Range: 0.34–5.30 ng/mL	Cord serum Mean: 1.45 ng/mL SD: 1.14 ng/mL Median: 1.03 ng/mL Range: 0.16–6.77 ng/mL	NR	NR
Manzano-Salgado et al. (2015) (Sabadell and Valencia, Spain)	Maternal plasma Median: 2.85 ng/mL Range: 0.78–11.93 ng/mL IQR: 1.87–6.00 ng/mL Maternal serum Median: 2.97 ng/mL Range: 0.86–14.54 ng/mL IQR: 2.26–4.85 ng/mL	Cord serum Median: 1.90 ng/mL Range: 0.60–10.56 ng/mL IQR: 1.45–4.70 ng/mL	NR	NR
Zhang et al. (2013b) (Tianjin, China)	Mean: 3.35 ng/mL RSD: 1.03 Range: 1.17–8.94 ng/mL	1.95 ng/mL RSD: 0.71 Range: 0.70–4.31 ng/mL	Mean: 1.58 ng/g RSD: 0.54 Range: 0.45–3.57 ng/g	Mean: 0.044 ng/mL RSD: 0.021 Range: <LOQ–0.145 ng/mL
Cariou et al. (2015) (Toulouse, France)	Maternal serum Mean: 1.22 ng/mL Median: 1.045 ng/mL Range: 0.309–7.31 ng/mL	Cord serum Mean: 0.919 ng/mL Median: 0.860 ng/mL Range: 0.311–7.06 ng/mL	NR	NR
Pan et al. (2017) (Wuhan, China)[a,c]	Maternal Serum T1 Mean: 3.15 ng/mL Median: 3.24 ng/mL IQR: 2.44–3.88 ng/mL	Cord serum Mean: 1.42 ng/mL Median: 1.41 ng/mL IQR: 1.14–1.84 ng/mL	NR	NR
	T2 serum Mean: 2.52 ng/mL Median: 2.50 ng/mL IQR: 2.05–3.13 ng/mL		NR	NR
	T3 serum Mean: 2.19 ng/mL Median: 2.16 ng/mL IQR: 1.81–2.73 ng/mL		NR	NR
Caserta et al. (2018) (Rome, Italy)	Mean: 1.05 ng/mL SD: 0.35 ng/mL	Mean: 0.98 ng/mL SD: 0.54 ng/mL	NR	NR

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
	Range: 0.45–1.9 ng/mL	Range: 0.30–2.50 ng/mL		
Wang et al. (2019d) (Shandong, China)	Maternal serum GM: 39.27 ng/mL Median: 42.83 ng/mL Range: 1.16– 602.79 ng/mL	Cord serum GM: 31.83 ng/mL Median: 34.67 ng/mL Range: 1.52– 291.56 ng/mL	NR	NR
Zhao et al. (2017a) (Hubei, China)	Maternal blood Mean: 0.984 ng/mL Median: 0.907 ng/mL Range: 0.274–2.72 ng/mL	Cord blood Mean: 0.565 ng/mL Median: 0.535 ng/mL Range:0.126 – 1.44 ng/mL	NR	NR
Brochot et al. (2019) (INMA prospective birth cohort, Spain)[a,d]	Group 1 mean (plasma): 3.26 ± 1.87 (0.39– 11.93) ng/mL Group 2 mean (plasma): 2.78 ± 2.18 (0.20– 31.64) ng/mL	Mean: 2.54 ± 1.64 (0.86– 10.56) ng/mL	NR	NR
Gao et al. (2019) (Beijing, China)	Mean: 2.85 ng/mL Median: 2.21 ng/mL Range: <LOD– 25.4 ng/mL	Mean: 2.29 ng/mL Median: 1.88 ng/mL Range: 0.03–10.2 ng/mL	NR	NR
Eryasa et al. (2019) (Faroese Birth Cohorts, Denmark)[b] (Cohort 3)	GM serum: 2.33 ng/mL SD: 0.12 ng/mL IQR: 1.79–3.29 ng/mL	Cord serum Mean: 1.97 ng/mL SD: 0.10 ng/mL IQR: 1.42–2.76 ng/mL Whole cord blood Mean: 1.08 ng/mL SD: 0.05 ng/mL IQR: 0.8–1.45 ng/mL	NR	NR
Eryasa et al. (2019) (Faroese Birth Cohorts, Denmark)[b] (Cohort 5)	Mean: 1.03 ng/mL SD: 0.08 ng/mL IQR: 0.75–1.41 ng/mL	Cord serum Mean: 0.81 ng/mL SD: 0.07 ng/mL IQR: 0.56–1.26 ng/mL Whole cord blood Mean: 0.41 ng/mL SD: 0.03 ng/mL IQR: 0.29–0.59 ng/mL	NR	NR
Cai et al. (2020) (Maoming Birth Cohort, China)	Maternal serum Mean: 1.21 ng/mL SD: 1.01 ng/mL Median: 0.99 ng/mL IQR: 0.74–1.37/mL	Cord serum Mean: 0.85 ng/mL SD: 0.52 ng/mL Median: 0.75 ng/mL IQR: 0.52–1.09 ng/mL	NR	NR

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
Li et al. (2020a) (Maoming Birth Cohort, China)	Preterm delivery Mean serum: 1.20 ng/mL Median: 1.00 ng/mL IQR: 0.69–1.47 Full-term delivery Mean: 1.34 Median: 1.13 ng/mL IQR 0.72–1.74	Preterm delivery Mean: 0.70 ng/mL Median: 0.57 ng/mL IQR: 0.43–0.91 Full-term delivery Mean: 1.25 ng/mL Median: 0.99 ng/mL IQR 0.64–1.49	NR	NR
Li et al. (2020b) (Beijing, China)	Mean serum: 3.63 ng/mL (95% CI: 3.26, 4.49) Median: 3.20 ng/mL	Mean: 4.98 ng/mL (95% CI: 4.41, 7.38) Median: 3.80 ng/mL	NR	NR
Mamsen et al. (2017) (Hospitals in Skelby and Randers, Denmark)	Mean: 2.1 ng/g, Range: 0.6–8.0 ng/g	NR	Mean: 0.23 ng/g, Range: 0.04–0.45 ng/g	NR
Mamsen et al. (2019) (Denmark)[a]	T1 serum Mean: 2.04 ng/mL SD: 1.63 ng/mL Median: 1.51 ng/mL Range: 0.55–7.95 ng/mL	NR	Mean: 0.28 ng/g SD: 0.09 ng/g Median: 0.27 ng/g Range: 0.15–0.45 ng/g	NR
	T2 serum Mean: 1.62 ng/mL SD: 0.71 ng/mL Median: 1.58 ng/mL Range: 0.72–3.78 ng/mL	NR	Mean: 0.39 ng/g SD: 0.26 ng/g Median: 0.26 ng/g Range: 0.19–0.99 ng/g	NR
	T3 serum Mean: 1.62 ng/mL SD: 0.85 ng/mL Median: 1.36 ng/mL Range: 0.62–4.62 ng/mL	NR	Mean: 0.43 ng/g SD: 0.16 ng/g Median: 0.36 ng/g Range: 0.21–0.82 ng/g	NR
Hanssen et al. (2013) (Norilsk, Russia)[e]	Plasma Median: 1.61 ng/mL Mean: 1.50 ng/mL Range: 0.63–2.48 ng/mL	Cord plasma Median: 1.00 ng/mL Mean: 1.26 ng/mL Range: 0.36–2.32 ng/mL	NR	NR
	Whole blood Median: 0.89 ng/mL Mean: 0.89 ng/mL Range: 0.33–1.40 ng/mL	Cord whole blood Median: 0.49 ng/mL Mean: 0.58 ng/mL Range: 0.15–1.12 ng/mL	NR	NR
Kato et al. (2014) (Ohio, USA)[f]	Maternal Serum at 16 wk Median: 4.80 µg/L Maternal serum at delivery Median:3.30 µg/L	Cord serum at delivery Median:3.10 µg/L		

Notes: CI = confidence interval; GM = geometric mean; LOD = limit of detection; LOQ = limit of quantification; IQR = interquartile range; NR = not reported; SD = standard deviations; T1 = first trimester; T2 = second trimester; T3 = third trimester; wk = week(s).

[a] For studies that quantified PFOA at different trimesters, first trimester (T1), second trimester (T2) and third trimester (T3).

[b] Eryasa et al. (2019) sampled participants from two birth cohorts: Cohort 3 (100 Singleton births from 1999 to 2001), and cohort 5 (50 singleton birth from 2008 to 2005). Both cohorts had the same source of exposure and were similar in maternal characteristics.
[c] Pan et al. (2017) measured PFOA in maternal serum at first, second and third trimester and measured cord blood only at the time of full-term delivery.
[d] Brochot et al. (2019) collected samples from women in 2 cohorts: Group 1 consist of 52 mother-child pairs that had available samples of maternal blood and cord serum-PFAS during pregnancy. Group 2 consists of 355 mothers who provided maternal blood during pregnancy. Cord blood was not collected for the Group 2 cohort.
[e] Hanssen et al. (2013) measured PFOA in whole blood and plasma from mothers and their infants at the time of delivery.
[f] Kato et al. (2014) measured PFOA in 71 matched maternal and cord serum pairs. Maternal serum samples were collected at 16 weeks of gestation and at the time of delivery.

B.2.4.1.3 Distribution in Fetal Tissues

Mamsen et al. (2017) measured the concentrations of 5 PFAS chemicals in human fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark, who legally terminated their pregnancies before gestational week 12 for reasons other than fetal abnormality. The samples collected included 24 maternal blood, 34 placenta, and 108 fetal organs. The participants were healthy women ages 18–46 years with an average BMI of 22.7. About 51% of the mothers smoked during pregnancy at an average of 10 cigarettes per day or were exposed to secondhand cigarette smoke for an average of 1.8 hours per day. PFOA was detected in placenta, fetal liver, extremities, heart, intestines, lungs, connective tissues, spinal cord, and ribs at different concentrations. Notably, PFOA levels were highest in the placenta and lung. Mean concentrations of PFOA in maternal serum, placenta, and fetal organs were reported as 1.9 (0.6–4.1), 0.2 (0.0–0.4), and 0.1 (0−0.3) ng/g, respectively. Mean concentrations of PFOS in maternal serum, placenta, and fetal organs were reported as 8.2 (2.5–16.7), 1.0 (0.3–2.6), and 0.3 (0–0.7) ng/g, respectively. The concentrations of PFOS in all three matrices were significantly higher than PFOA. For 21 of the samples where all three specimens (maternal plasma, placenta, and fetal tissues) were collected from the same women, the concentration of PFOA decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. The relative concentration of PFOA in the placenta was 11% of the concentrations found in maternal plasma and was further reduced to 7% in fetal tissues. In general, a positive trend was observed between fetal tissue-maternal serum ratio and gestational age. Although the gestational age reported in this study is short (37–68 days post conception), the results suggest that PFOA is retained in several fetal organs and may potentially continue to accumulate across gestation.

To determine whether PFOA accumulation in fetal organs changes across trimesters during gestation, Mamsen et al. (2019) quantified PFAS levels in embryos and fetuses at gestational weeks 7–42 and serum from their matched maternal pairs. Like Mamsen et al. (2017), participants were similar in age (18–46 years) and BMI (22.8 (first trimester)). However, the smoking status of the women in this study was not reported and the majority of the pregnancies were terminated due to intrauterine fetal death (IUFD) caused by placental insufficiency and intrauterine growth restriction (58%), and infection (13%). A total of 78 pregnant women were enrolled in the study. Fetal tissues (placenta, liver, lung, heart, central nervous system (CNS), and adipose) were collected from 38 first-trimester pregnancies, 18 second-trimester pregnancies, and 22 third-trimester pregnancies. 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 ratios of PFOA in fetal tissue increased from first trimester to third trimester except for the liver and heart which showed the highest tissue:maternal serum ratios in the second trimester compared with the third trimester. The fetal tissue:maternal serum ratio of

PFOA was highest in adipose tissue during the second trimester than in any other tissue across gestation.

Interestingly, PFOA concentration in the liver was also highest in the second trimester compared with the first and third trimesters. Authors attributed this phenomenon to the unique architecture of the fetal liver during early gestation when oxygenated cord venous blood bypasses the liver into the heart through the ductus venosus and is then delivered throughout the fetus. This pattern of blood distribution changes between week 20 and 26 of gestation (late second trimester). The amount of blood shunted from the liver is reduced from 60% to 30% in the second trimester Pennati et al. (2003). This reduction results in increased flow of cord blood through the liver, thus increasing levels of PFOA and PFOS during the second trimester. Furthermore, Mamsen et al. (2019) observed that PFOA and PFOS levels were lowest in the CNS than any of the tissues examined, suggesting that the CNS has less PFAS exposure and may be protected by the BBB. When interpreting these results, it is important to note that second and third trimester fetal tissues were obtained from patients with IUFD and may not be comparable to normal pregnancies as the fetus died in utero of placental insufficiency and intrauterine growth restriction. Placental insufficiency can potentially reduce the amount of PFAS crossing the placenta. In addition, the PFAS exposure level in this cohort may vary due to different geographical locations of the participants. The first trimester participants were from Denmark and the second and third trimester participants came from Sweden.

B.2.4.1.4 Partitioning to Infants

Four studies shown in Table B-11 analyzed PFOA levels in maternal serum and levels in breast milk and/or infant blood. Maternal and infant serum PFOA levels were an order of magnitude higher in subjects in the United States exposed to contaminated drinking water (Mondal et al., 2014) compared with subjects analyzed in France, Denmark (Faroe Islands), or Sweden (Gyllenhammar et al., 2018a; Cariou et al., 2015; Mogensen et al., 2015b). In the Mondal study, geometric mean maternal serum PFOA concentrations were lower in breastfeeding mothers (18.32 ng/mL) versus non-breastfeeding mothers (19.26 ng/mL). Conversely, breastfed infants had higher geometric mean serum PFOA (48.55 g/mL) than infants who were never breastfed (21.74 ng/mL).

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 (n = 10). The authors noted that the transfer rates from serum to breastmilk of PFAAs were lower compared with other lipophilic persistent organic pollutants such as polychlorinated biphenyls. In this study, four PFAS compounds were analyzed (PFOA, PFOS, PFNA, and PFHxS), and the individual patterns for these compounds exhibited important inter-individual variability. While PFOS was the main contributor in serum, PFOA and PFOS were found to be the main contributors in breastmilk. Interestingly, while the number of pregnancies was inversely correlated with maternal serum levels, after adjustment, the correlation with parity did not reach significance for PFOA, although it did reach significance for PFHxS. Only PFOA exhibited a significant correlation between the total duration of breastfeeding and serum PFOA levels after adjustment (0.87 (0.80–0.94), p = 0.0007).

Mogensen et al. (2015b) relied on maternal PFOA serum concentrations measured at 32 weeks of pregnancy to assess prenatal exposure and measured concentrations in the serum of children at

11 and 18 months of age. They applied linear mixed models to estimate age-dependent serum concentrations for up to 5 years after birth. The only other exposure source adjusted for in this study was the eating whale meat by the infants. As shown in Table B-11, the increases in infant blood PFOA concentrations over time, with the greatest increases found at the end of the breastfeeding period, suggest that breastfeeding is the primary exposure source during infancy.

Gyllenhammar et al. (2018a) used multiple linear regression and general linear model analysis to investigate associations between serum PFOA concentrations in 2−4-month-old infants and maternal PFOA concentrations close to delivery, duration of in utero exposure (gestational age at delivery), duration of breastfeeding, and other parameters. The authors examined PFAAs of various chain lengths and observed decreased strength of association between maternal and infant concentrations with increased PFAA carbon chain length among breastfed infants. Of note, the authors observed that variation in maternal PFOA concentrations explained 53% of the infant concentration variation, whereas only 13% of the variation in infant PFUnDA was explained by maternal variation. Also, the PFOA infant:maternal serum ratio was higher than ratios for other PFAAs (2.8 (0.43−5.7)).

Table B-11. Summary of Studies Evaluating PFOA Concentrations in Maternal Serum, Breast Milk, and Infant Serum

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Mondal et al. (2014)	A subcohort of the C8 Science Panel Study (exposed to contaminated drinking water in six water districts near Parkersburg, West Virginia) who had a child <3.5 yr of age and who provided blood samples and reported detailed information on breastfeeding at the time of survey (633 mothers and 49 infants included). PFAA serum concentrations were available for all mothers and 8% (n = 49) of the infants. Maternal and infant serum concentrations were regressed on duration of breastfeeding.	Maternal serum Breastfed and not breastfed Mean: 18.69 ng/mL 95% CI: 17.13, 20.28 Breastfed GM: 18.32 ng/mL 95% CI: 16.36, 20.50 Not breastfed GM: 19.26 ng/mL 95% CI: 16.80, 22.08	NR	Infant serum Breastfed and not breastfed Mean: 36.14 ng/mL 95% CI: 24.87, 52.52 Breastfed GM: 48.55 g/mL 95% CI: 31.17, 75.61 Not breastfed GM: 21.74 ng/mL 95% CI: 11.21, 42.17
Cariou et al. (2015)	Female volunteers hospitalized between June 2010 and January 2013 for planned cesarean delivery in France. Maternal blood samples (n = 100) were collected during cesarean delivery and breast milk samples (61) were collected between the 4th and 5th day after delivery.	Mean: 1.22 ng/mL Median: 1.045 ng/mL Range: 0.309–7.31 ng/mL	Mean: 0.041 ng/mL Median: <LOQ LOQ = 0.050 ng/mL Range: <LOD–0.308 ng/mL	NR

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Mogensen et al. (2015b)[a]	80 singleton children in Faroese birth cohort born between 1997 and 2000. The children were breastfed exclusively for a median of 4.5 mo, followed by partial breastfeeding with supplementary baby food for a median of 4 mo. A piece-wise linear model was used to estimate the age dependence of the PFOA concentration.	NR	NR	Median at birth: 2.0 ng/mL (IQR 1.7,2.7) Median at 11 mo: 8.2 ng/mL (IQR 6.1, 10.9) Median at 18 mo: 6.1 ng/mL (IQR 5.1, 10) Median at 60 mo: 3.8 ng/mL (IQR 3.1, 4.9)
Gyllenhammar et al. (2018a)	Primaparae mother/child pairs in 1996–1999 recruited in Sweden. 101 maternal and 107 infant samples were available for PFAA analyses. Serum concentrations were determined in mothers 3 wk after delivery and in 2–4-mo-old infants.	Maternal serum Mean: 2.8 ng/g SD: 0.96 ng/g Median: 2.7 ng/g Range: 1.2–6.7 ng/g	NR	Infant serum Mean: 7.7 ng/g SD: 3.7 ng/g Median: 7.2 ng/g Range: 1.3–20 ng/g
Haug et al. (2011)	41 female volunteers from Oslo, Norway, of which 19 submitted breast milk samples. The timing of serum or milk samples obtained from breastfeeding women was not reported.	Maternal serum Mean: 2 ng/mL Range: 0.28–22 ng/mL	Mean: 0.76 ng/mL Range: <0.018 (below LOQ) – 0.83 ng/mL	NR

Notes: CI = confidence interval; GM = geometric mean; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; mo = months; NR = not reported; SD = standard deviation.

[a] Neonatal serum-PFAS concentrations was calculated based on PFAS ratios between cord and maternal pregnancy serum concentrations previously estimated for the same cohort (0.34 for PFOA) from Needham et al. (2011).

Mondal et al. (2014) also examined the change in maternal and infant PFOA levels with duration of breastfeeding (Table B-12). Maternal serum concentrations decreased with each month of breastfeeding (−3%; 95% CI: −5%, −2%) with the greatest decrease observed after 12 months of breastfeeding (−41%). Correspondingly, the infant PFOA serum concentrations increased by 6% (95% CI: 1%, 10%) with each month of breastfeeding, lower than the estimate of 30% per month in Swedish infants found by Gyllenhammar et al. (2018a). Increases were modest in the first 6 months (13%) but increased to 141% after 12 months of breastfeeding. Using mixed linear model regression (Table B-13), Mogensen et al. (2015b) calculated that, during months with exclusive breastfeeding, significant increases in the PFOA concentrations in infant serum were estimated (27.8% and 31.2% per month at 18 and 60 months, respectively). These levels were higher than the continuous (per month) 6% estimated increases in the Mondal study, respectively. Increases were less striking for months with partial breastfeeding and small or none for months without breastfeeding. Haug et al. (2011) reported a significant positive correlation between maternal serum and breast milk for PFOA ($r = 0.97$, $n = 10$) with the average breast milk concentration representing 3.8% of the corresponding serum concentration. Altogether,

these findings support breastfeeding as the primary source of infant PFOA accumulation and that distribution to the infant correlates with the length of breastfeeding.

Table B-12. Percent Change in PFOA Ratios in Maternal Serum to Breast Milk and Breast Milk to Infant Serum by Infant Age in Humans as Reported by Mondal et al. (2014)

PFOA (Mondal et al., 2014)	Maternal Serum: Breast Milk		Breastmilk: Infant Serum	
Infant Age	**Percent Change**	**95% CI**	**Percent Change**	**95% CI**
≤6 mo	−5%	(−18, 8)	13%	(−46, 139)
7−12 mo	−29%	(−41, −13)	82%	(−23, −334)
>12 mo	−41%	(−57, −17)	141%	(4, 460)
Continuous (per month)	−3%	(−5, −2)	6%	(1, 10)

Notes: CI = confidence interval; mo = months.

Table B-13. Percent Change in PFOA Serum Concentration by Exclusive, Mixed or No Breastfeeding per Month in Humans as Reported by Mogensen et al. (2015b)

Variable	Mixed Model up to 18 Months			Mixed Model up to 60 Months		
	Percent Change	**95% CI**	**p-value**	**Percent Change**	**95% CI**	**p-value**
Exclusive	27.8	(23.6, 32.1)	<0.0001	31.2	(28.0, 34.5)	<0.0001
Partial	3.9	(0.5, 7.3)	0.0252	0.1	(−1.6, 1.9)	0.8951
None	0.7	(−1.1, 2.5)	0.4528	−1.3	(−1.5, −1.0)	<0.0001

Notes: CI = confidence interval.

The contributions of placental transfer, breastfeeding, and ingestion of PFAA-contaminated drinking water to early life PFOA levels in children were analyzed (Gyllenhammar et al., 2019). This study measured PFOA concentrations in children aged 4, 8, and 12 years (n = 57, 55, and 119, respectively) between 2008 and 2015 as part of the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study in Sweden. Mixed linear regression (MLR) models were used to ascertain associations with PFOA for these exposure modes. PFOA concentrations increased 10% per unit (ng/g serum) of increase in the maternal serum level at delivery. The association was strongest in 4-year-old children. Duration of breastfeeding only correlated with 4-year-old children but not older children in the MLR model (partial $R^2 = 0.05$ for children in this age group). PFOA increased 1.2% per month of cumulative drinking water exposure. The authors suggested that, in addition to exposure in utero and through lactation, drinking water with low-to-moderate PFOA contamination is an important source of exposure for children.

B.2.4.2 Animal Studies

B.2.4.2.1 Rats

PFOA levels during gestation and lactation were studied by Hinderliter et al. (2005) (publication of data reported by Mylchreest (2003)). Time-mated female Sprague-Dawley rats were dosed with 0, 3, 10, or 30 mg/kg/day of PFOA during days 4–10, 4–15, and 4–21 of gestation, or from GD 4 to LD 21. Maternal blood samples were collected at 2 hours ± 30 minutes post-dose on a daily basis. Plasma, milk, amniotic fluid, and tissue homogenate (placenta, embryo, and fetus)

supernatants were analyzed for PFOA concentrations by high-performance liquid chromatography mass spectrometry (HPLC/MS). Maternal PFOA plasma levels during gestation and lactation are presented in Table B-14. Maternal plasma levels at 2 hours post-dosing (approximately the time of peak blood levels following a gavage dose) were fairly similar during the course of the study with mean levels of 11.2, 26.8, and 66.6 µg/mL in the 3, 10, and 30 mg/kg/day groups, respectively; PFOA levels in the control group were below the LOQ (0.05 µg/mL). The stability of the maternal plasma PFOA concentrations day-to-day indicates that PFOA was not accumulating in plasma, despite repeated exposures. This is possibly because the female rats were able to completely excrete the PFOA dose (3, 10, or 30 mg/kg/day) within 24 hours, before the next dose was administered via gavage.

Table B-14. Maternal Plasma PFOA Levels in Sprague-Dawley Rats During Gestation and Lactation[a] as Reported by Hinderliter et al. (2005)

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD4–GD 10	GD 10 plasma	8.53 ± 1.06	23.32 ± 2.15	70.49 ± 8.94
GD4–GD 15	GD 15 plasma	15.92 ± 12.96	29.40 ± 14.19	79.55 ± 3.11
GD4–GD 21	GD 21 plasma	14.04 ± 2.27	34.20 ± 6.68	76.36 ± 14.76
GD4–LD 3	LD 3 plasma	11.01 ± 2.11	22.47 ± 2.74	54.39 ± 17.86
GD4–LD 7	LD 7 plasma	10.09 ± 2.90	25.83 ± 2.07	66.91 ± 11.82
GD4–LD 14	LD 14 plasma	9.69 ± 0.92	23.79 ± 2.81	54.65 ± 11.63
GD4–LD 21	LD 21 plasma	9.04 ± 1.01	28.84 ± 5.15	64.13 ± 1.45
NA	Average plasma	11.19 ± 2.76	26.84 ± 4.21	66.64 ± 9.80

Notes: GD = gestation day; LD = lactation day; NA = not applicable.
[a] Data are presented as mean ± standard deviation (µg/mL).

PFOA levels in the placenta, amniotic fluid, and embryo/fetus are presented in Table B-15. The levels of PFOA in the placenta on GD 21 were approximately twice the levels observed on GD 15, and the levels of PFOA in the amniotic fluid were approximately 4 times higher on GD 21 than on GD 15. The concentration of PFOA in the embryo/fetus was highest in the GD 10 embryo and lowest in the GD 15 embryo; PFOA levels in the GD 21 fetus were intermediate.

Fetal and pup PFOA plasma levels during gestation and lactation are presented in Table B-16, and PFOA levels in maternal milk during lactation are provided in Table B-17. The concentrations of PFOA in the plasma of the GD21 fetus (5.88, 14.48, and 33.11 µg/mL, respectively, in the 3, 10, and 30 mg/kg/day groups) were approximately half the levels observed in the maternal plasma (Table B-14). Pup plasma levels decreased between birth and LD 7 (Table B-16) and were, thereafter, similar to the levels observed in the milk (Table B-17). The pups were not separated by sex. The concentrations of PFOA in maternal milk also were fairly similar throughout lactation (means of 1.1, 2.8, and 6.2 µg/ml in the 3, 10, and 30 mg/kg/day groups, respectively) and were approximately one-tenth of the PFOA levels in the maternal plasma.

Table B-15. Placenta, Amniotic Fluid, and Embryo/Fetus PFOA Concentrations in Sprague-Dawley Rats[a] as Reported by Hinderliter et al. (2005)

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–GD 10	GD 10 – embryo	1.40 ± 0.30	3.33 ± 0.81	12.49 ± 3.50
GD 4–GD 15	GD 15 – placenta	2.22 ± 1.79	5.10 ± 1.70	13.22 ± 1.03
	GD 15 – amniotic fluid	0.60 ± 0.69	0.70 ± 0.15	1.70 ± 0.91
	GD 15 – embryo	0.24 ± 0.19	0.53 ± 0.18	1.24 ± 0.22
GD 4–GD 21	GD 21 – placenta	3.55 ± 0.57	9.37 ± 1.76	24.37 ± 4.13
	GD 21 – amniotic fluid	1.50 ± 0.32	3.76 ± 0.81	8.13 ± 0.86
	GD 21 – fetus	1.27 ± 0.26	2.61 ± 0.37	8.77 ± 2.36

Notes: GD = gestation day.
[a] Data are presented as mean ± standard deviation (µg/mL). Samples were pooled by litter and were collected 2 hours post-dosing.

Table B-16. Fetus/Pup PFOA Concentration in Sprague-Dawley Rats During Gestation and Lactation[a] as Reported by Hinderliter et al. (2005)

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–GD 21	GD 21—fetal plasma	5.88 ± 0.69	14.48 ± 1.51	33.11 ± 4.64
GD 4–LD 3	LD 3—pup plasma	2.89 ± 0.70	5.94 ± 1.44	11.96 ± 1.66
GD 4–LD 7	LD 7—pup plasma	0.65 ± 0.20	2.77 ± 0.58	4.92 ± 1.28
GD 4–LD 14	LD 14—pup plasma	0.77 ± 0.10	2.22 ± 0.38	4.91 ± 1.12
GD 4–LD 21	LD 21—pup plasma	1.28 ± 0.72	3.25 ± 0.52	7.36 ± 2.17

Notes: GD = gestation day; LD = lactation day.
[a] Data are presented as mean ± standard deviation (µg/mL). Samples were pooled by litter and were collected 2 hours post-dosing.

Table B-17. Maternal Milk PFOA Concentration in Sprague-Dawley Rat During Lactation[a] as Reported by Hinderliter et al. (2005)

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–LD 3	LD 3–milk	1.07 ± 0.26	2.03 ± 0.33	4.97 ± 1.20
GD 4–LD 7	LD 7–milk	0.94 ± 0.22	2.74 ± 0.91	5.76 ± 1.26
GD 4–LD 14	LD 14–milk	1.15 ± 0.06	3.45 ± 1.18	6.45 ± 1.38
GD 4–LD 21	LD 21–milk	1.13 ± 0.08	3.07 ± 0.51	7.48 ± 1.63
NA	Average milk	1.07 ± 0.09	2.82 ± 0.60	6.16 ± 1.06

Notes: GD = gestation day; LD = lactation day; NA = not applicable.
[a] Data are presented as mean ± standard deviation (µg/mL). Samples were from five dams/group/time point and were collected 2 hours post-dosing.

PFOA accumulation in young rats is impacted by both sex and age. Han (2003) administered groups of 4–8-week-old Sprague-Dawley rats (10 per sex per age) a single dose of 10 mg/kg/day PFOA by oral gavage. Blood samples were collected 24 hours after dosing and the plasma

concentration of PFOA was measured by HPLC-MS. In the 5- and 6-week-old female rats, the plasma PFOA concentrations were about twofold lower than in the 4-week-old rats (Table B-18). However, in the 5-week-old males, the concentration of plasma PFOA was about fivefold higher than in the 4-week-old group, suggesting a developmental change in excretion rate. PFOA plasma concentrations were 35–65-fold higher in males than in females at every age except at 4 weeks. Thus, it appears that maturation of the transport features responsible for the sex difference in PFOA elimination occurs between the ages of 4 and 5 weeks in the rat.

Table B-18. Plasma PFOA Concentrations in Postweaning Sprague-Dawley Rats[a] as Reported by Han (2003)

Age (weeks)	Males	Females
4	7.32 ± 1.01	2.68 ± 0.64
5	39.24 ± 3.89	1.13 ± 0.46
6	43.19 ± 3.79	1.18 ± 0.52
7	37.12 ± 4.07	0.57 ± 0.29
8	38.55 ± 5.44	0.81 ± 0.27

Notes:
[a] Data are presented as mean ± standard deviation (µg/mL).

Hinderliter et al. (2006b) continued the investigation of the relationship between age and plasma PFOA in male and female Sprague-Dawley rats. Immature rats at 3, 4, and 5 weeks of age were administered PFOA via oral gavage at a single dose of 10 or 30 mg/kg. Rats were not fasted prior to dosing. Two hours after dosing, five rats per sex per age group and dose group were sacrificed and blood samples were collected. The remaining five rats per sex per age and dose group were placed in metabolism cages for 24-hour urine collection. These rats were sacrificed at 24 hours and blood samples were collected.

In the male rats, plasma PFOA concentrations for either the 10 or 30 mg/kg dosage groups did not differ significantly by sample time (at 2 and 24 hours) or by animal age (3, 4, and 5 weeks), except at 2 hours for the 5-week-old group ($p < 0.01$), which showed the lowest PFOA level (Table B-19). PFOA plasma concentrations following a 30 mg/kg dose were 2–3 times higher than those following a 10 mg/kg dose. These data do not demonstrate a difference between the 5-week-old rats and the younger 3- and 4-week-old groups at 24 hours after dosing, and thus do not support the observations from the Han study (2003).

Table B-19. Plasma PFOA Concentrations in Male Sprague-Dawley Rats at 2 and 24 Hours After Oral Gavage as Reported by Hinderliter et al. (2006b)

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/mL)			
		2 Hours Post-dose		24 Hours Post-dose	
		Mean	SD	Mean	SD
3	10	41.87	4.01	34.22	7.89
4	10	39.92	4.45	42.94	5.33
5	10	26.32*	6.89	40.60	3.69
3	30	120.65	12.78	74.16	18.23

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/mL)			
		2 Hours Post-dose		24 Hours Post-dose	
		Mean	SD	Mean	SD
4	30	117.40	18.10	100.81	13.18
5	30	65.66*	15.53	113.86	23.36

Notes: SD = standard deviation.
* Statistically significantly different by sample time and animal age ($p < 0.01$).

In the female rats, plasma PFOA concentrations were significantly lower in the 5-week-old group than in the 3- or 4-week-old groups at the 24-hour time period for both doses and for the 30 mg/kg dose group at 2 hours (Table B-20). Plasma PFOA concentrations following a 30 mg/kg dose were approximately 1.5–4 times higher than those observed following a 10 mg/kg dose.

At 24 hours post-dose, plasma PFOA levels in the female rats were significantly lower than the plasma PFOA levels in male rats, especially at 5 weeks of age. The data for the 5-week-old female rats compared with the 3- and 4-week-old groups at 24 hours are consistent with the Han (2003) data in that they demonstrate a decline in plasma levels compared with their earlier measurements. Thus, the developmental change is one that appears to be unique to the female rat.

Table B-20. Plasma PFOA Concentrations in Female Sprague-Dawley Rats at 2 and 24 Hours After Oral Gavage as Reported by Hinderliter et al. (2006b)

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/mL)			
		2 Hours Post-dose		24 Hours Post-dose	
		Mean	SD	Mean	SD
3	10	37.87	5.77	13.55[b]	3.83
4	10	29.88	12.15	18.98[b]	7.01
5	10	33.23	7.41	1.36[a, b]	0.87
3	30	84.86	10.51	51.43[b]	13.61
4	30	80.67	14.10	28.01[b]	9.90
5	30	56.90 [a]	29.66	3.42[a, b]	1.95

Notes: SD = standard deviation.
[A] Statistically significantly different from the 3- and 4-week values ($p < 0.01$).
[b] Statistically significantly different from 2-hour values ($p < 0.01$).

The data demonstrate that both dose and sex influence plasma levels. Post-dosing clearance (CL) is slow for both doses at 2 and 24 hours in males and females at PNW 3 and 4. At 5 weeks, however, the plasma levels after 24 hours are greater than those at 2 hours in males. In females, for the high dose at 2 hours, plasma levels are similar to those in males, while at 24 hours they are only 3% of the value for males. This suggests that uptake from the intestines is similar while the rate of excretion at 5 weeks and beyond is considerably greater for female rats than males. They are comparable for PNW 3 and 4.

In a supplemental study to determine the effect of fasting (Hinderliter et al., 2006b), 4-week-old rats, four rats per sex, were administered 10 mg/kg PFOA via oral gavage. Animals (two per sex) were fasted overnight for 12 hours before dosing with PFOA. All the rats were sacrificed at 24 hours post-dosing and blood was collected for analysis of PFOA in plasma. Plasma PFOA concentrations in male rats were 64.95 and 30.00 μg/mL for the fasted and nonfasted animals, respectively. Plasma PFOA concentrations in the female rats were 68.16 and 26.54 μg/mL for the fasted and nonfasted animals, respectively. Given the consistency in the 4-week-old rat plasma PFOA concentrations, the authors concluded that age-dependent changes in female PFOA elimination are observable between 3 and 5 weeks of age. PFOA uptake was greater in the fasted animals than the fed animals, suggesting competition for uptake in the presence of food components that share common transporters and/or decreased contact of PFOA with the intestinal epithelium in the presence of dietary materials. This is consistent with the finding that dietary fat may negatively impact absorption (Li et al., 2015).

An oral two-generation reproductive toxicity study of PFOA in rats was conducted (Butenhoff et al., 2004a). Five groups of rats (30 sex/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, or 30 mg/kg/day. At scheduled sacrifice, after completion of the cohabitation period in F_0 male rats and on lactation day (LD) 22 in F_0 female rats, blood samples were collected. Serum analysis for the F_0 generation males showed that PFOA was present in all samples tested, including low levels in controls (0.0344 ± 0.0148 μg/mL). Levels of PFOA were similar in the two male dose groups (51.1 ± 9.30 and 45.3 ± 12.6 μg/mL, respectively, for 10 and 30 mg/kg/day dose groups). In the F_0 female controls, serum PFOA was below LOQ (0.00528 μg/mL). Levels of PFOA found in female sera were lower than in males but increased between the two dose groups; treated females had an average concentration of 0.37 ± 0.0805 and 1.02 ± 0.425 μg/mL, respectively, for the 10 and 30 mg/kg/day dose groups.

B.2.4.2.2 Mice

Fenton et al. (2009) orally dosed pregnant CD-1 mice (n = 25/group) with 0, 0.1, 1, or 5 mg PFOA/kg on GD 17. On GD 18, five dams/group were sacrificed and trunk blood, urine, amniotic fluid, and the fourth and fifth mammary glands were collected Additionally, one fetus from each dam was retained for whole-pup analysis. The remaining dams were allowed to litter and samples (excluding amniotic fluid) also were collected on postnatal day (PND) 1, 4, 8, and 18. At each time point, a single pup was euthanized and retained for whole-pup analysis. Blood from the remaining pups was collected and pooled. Milk was collected from dams on PND 2, 8, 11, and 18 following a 2-hour separation of the pups from the dam.

PFOA levels in mice during gestation and lactation in selected fluids and tissues are summarized in Table B-21. The concentrations of PFOA in dam serum were approximately twice that detected in amniotic fluid. Compared with the amniotic fluid, concentrations of PFOA in the fetuses were increased by 2.3-, 3.1-, and 2.7-fold at 0.1, 1, and 5 mg/kg, respectively. The highest concentration of PFOA was detected in the serum of nursing dams. In the dams, the PFOA serum concentrations exhibited a U-shaped response curve over time; the lowest serum concentrations were observed at the time of peak lactation. Dam mammary tissue and milk PFOA concentrations showed a U-shaped response which mirrored that found in dam serum. The concentrations of PFOA in pup serum were significantly higher than PFOA concentrations in dam serum and appeared to decrease as the time for weaning approached. When pup PFOA concentrations were calculated with consideration for pup body weight gain, PFOA body burden

increased through the peak of lactation and began to decrease by PND18, showing an inverse U-shaped response curve. The authors hypothesized that the U-shaped curve was observed for the lactating dams because of hydro-dilution; essentially, the increases in blood volume and milk volume at the time of peak lactation led to lower PFOA concentrations during this particular time.

Table B-21. Select Fluids and Tissues PFOA Concentrations in CD-1 Mice During Gestation and Lactation[a] as Reported by Fenton et al. (2009)

Tissue	Day	Dose		
		0.1 mg/kg	1 mg/kg	5 mg/kg
Dam Serum[b]	GD 18	143 ± 19	1,697 ± 203	7,897 ± 663
	PND 1	217.5 ± 35	1,957.0 ± 84	9,845.6 ± 1,478
	PND 4	110.0 ± 12	1,269.4 ± 235	6,776.6 ± 561
	PND 8	46.7 ± 21	360.8 ± 98	1,961.8 ± 414
	PND 18	123.3 ± 41	1,035.2 ± 305	5,156.5 ± 1,201
Amniotic Fluid[b]	GD 18	99.0 ± 28	865.3 ± 191	3,203.8 ± 492
Dam Urine[b]	GD 18	21.9 ± 8.6	104.9 ± 69.7	666.7 ± 169
	PND 1	7.7 ± 1.7	116.8 ± 64	492.3 ± 119
	PND 4	8.4 ± 6.4	53.5 ± 15	401.5 ± 117
	PND 8	0.8 ± 0.22	11.6 ± 6.2	40.1 ± 17
	PND 18	1.8 ± 1.1	18.7 ± 8.6	91.7 ± 49
Mammary Gland[c]	GD 18	18.9 ± 1.9	307.2 ± 30.4	1429 ± 186
	PND 1	27.4 ± 6.8	343.8 ± 53	1,933.5 ± 194
	PND 4	9.6 ± 8.4	239.2 ± 53	1,461.8 ± 267
	PND 8	2.4 ± 3.8	71.7 ± 22	411.8 ± 78
	PND 18	17.1 ± 10	239.9 ± 76	1,372.8 ± 240
Milk[b]	PND 2	32.5 ± 12	716.7 ± 145	1,236.6 ± 1,370
	PND 8	11.6 ± 8.1	77.4 ± 19	245.1 ± 26
	PND 11	5.4 ± 1.0	42.3 ± 9.1	282.5 ± 162
	PND 18	43.5 ± 19	251.8 ± 147	909.8 ± 308
Whole Pup[c]	GD 18	136.3 ± 15	1,665.8 ± 213	6,256.5 ± 751
	PND 1	150.9 ± 21	1,606.9 ± 288	7,134.5 ± 1,097
	PND 4	91.8 ± 8.9	1,183.2 ± 187	5,071.4 ± 267
	PND 8	60.9 ± 16	729.0 ± 92	3,118.5 ± 424
	PND 18	17.5 ± 11	251.9 ± 112	1,391.5 ± 118
Pup Serum[b]	PND 1	324.7 ± 36	3,926.8 ± 480	16,286.4 ± 1,372
	PND 4	267.6 ± 47	3,020.8 ± 223	11,925.2 ± 1,077
	PND 8	260.2 ± 56	2,548.2 ± 245	9,215.8 ± 594
	PND 18	111.8 ± 30	1,124.8 ± 236	5,894.3 ± 743

Notes: GD = gestation day; PND = postnatal day.
[a] Animals were exposed to PFOA dose on GD17.
[b] Data are presented as mean ± standard deviation (ng/mL).

[c] Data are presented as mean ± standard deviation (ng/g).

Macon et al. (2011) gavage-dosed CD-1 mice with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD 1 to GD 17 or with 0, 0.01, 0.1, or 1.0 mg PFOA/kg from GD 10 to GD 17. As shown in Table B-22, at the lowest dose, PFOA concentrations in the serum peaked at or before PND 7 but peaked around PND 14 for the two higher doses. Calculated blood burdens, which take into account the increasing blood volumes and body weights for females, showed an inverted U-shaped curve peaking at PND 14 for all doses. In the liver, PFOA concentrations decreased over time with the highest concentration observed at PND 7. Lower concentrations of PFOA were detected in the brain of the offspring on PND 7 and PND 14. As shown in Table B-23, after exposure to low doses of PFOA from GD 10 to GD 17, serum PFOA concentration in the female offspring declined from PND 1 through the end of the experiment. Calculated blood burden showed a gradual increase from PND 1 to PND 14, followed by a decline through PND 21.

Table B-22. Serum, Liver, and Brain PFOA Concentration in Female CD-1 Mouse Pups After GD 10−17 Exposure[a] as Reported by Macon et al. (2011)

Tissue	Day	Dose		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Serum[a]	PND 7	4,980 ± 218	11,026 ± 915	20,700 ± 3,900
	PND 14	4,535 ± 920	16,950 ± 3,606	26,525 ± 2,446
	PND 21	1,194 ± 394	377 ± 607	8,343 ± 1,078
	PND 28	630 ± 162	1,247 ± 208	4,883 ± 1,378
	PND 42	377 ± 81	663 ± 185	2,058 ± 348
	PND 63	55 ± 17	176 ± 85	–
	PND 84	16 ± 5	71 ± 8	125
Liver[b]	PND 7	2,078 ± 90	8,134 ± 740	16,700 ± 749
	PND 14	972 ± 124	4,152 ± 483	10,290 ± 1,028
	PND 21	1,188 ± 182	1,939 ± 637	2,339 ± 1,241
	PND 28	678 ± 130	2,007 ± 560	7,124 ± 1,081
	PND 42	342 ± 87	617 ± 145	1,145 ± 274
	PND 63	118 ± 22	320 ± 113	417 ± 160
	PND 84	43 ± 12	55 ± 12	235 ± 79
Brain[b]	PND 7	150 ± 26	479 ± 41	1,594 ± 162
	PND 14	65 ± 12	241 ± 20	650 ± 44
	PND 21	<LOQ[c]	31 ± 5	133 ± 23
	PND 28	<LOQ	<LOQ	62 ± 93
	PND 42	<LOQ	<LOQ	<LOQ
	PND 63	<LOQ	<LOQ	<LOQ
	PND 84	<LOQ	<LOQ	<LOQ

Notes: GD = gestation day; LOQ = limit of quantification; PND = postnatal day; – = not measured.
[a] Data are presented as mean ± standard deviation (ng/mL).
[b] Data are presented as mean ± standard deviation (ng/g).
[c] LOQ: serum full gestation = 10−20 ng/g; liver = 35 ng/g; brain = 35 ng/g; late gestation serum = 5 ng/mL.

Table B-23. Serum PFOA Concentrations in Female CD-1 Mouse Pups After GD 10−17 Exposure as Reported by Macon et al. (2011)

Tissue	Day	Dose		
		0.01 mg/kg	0.1 mg/kg	1.0 mg/kg
Serum[a]	PND 1	284.5 ± 21.0	2,303.5 ± 114.4	16,305.5 ± 873.5
	PND 4	184.1 ± 12.1	–	–
	PND 7	150.7 ± 20.9	1,277.8 ± 122.6	11,880.3 ± 1,447.6
	PND 14	80.2 ± 13.9	645.4 ± 114.2	6,083.7 ± 662.6
	PND 21	16.5 ± 2.1	131.7 ± 24.5	2,025.1 ± 281.9
Blood Burden (calculated)[b]	PND 1	15.2 ± 1.7	114.3 ± 5.4	926.0 ± 47.6
	PND 4	20.6 ± 0.1	–	–
	PND 7	27.3 ± 3.8	221.7 ± 24.9	1965.9 ± 256.7
	PND 14	27.0 ± 4.6	218.5 ± 39.8	2033.6 ± 293.5
	PND 21	7.9 ± 1.0	66.4 ± 12.8	984.7 ± 142.8

Notes: PND = postnatal day.
[a] Data are presented as mean ± standard deviation (ng/mL).
[b] Blood burden determined by (body weight × (58.5/1000) × serum × 0.55).

White et al. (2011) measured serum PFOA concentrations in three generations of CD-1 mice (Table B-24). Pregnant mice (F_0, n = 10–12 dams/group) were gavage-dosed with 0, 1, or 5 mg PFOA/kg from GD 1 to GD 17. A separate group of pregnant mice (n = 7–10 dams/group) were gavage-dosed with either 0 or 1 mg PFOA/kg from GD 1 to GD 17 and received drinking water containing 5 parts per billion (ppb) PFOA beginning on GD 7 and continuing until the end of the study for their offspring – except during breeding and early gestation – to simulate a chronic low-dose exposure. Increases in serum PFOA concentrations were observed in the control + 5 ppb PFOA groups of the F_1 and second (F_2) generations and in the 1 mg/kg + 5 ppb PFOA group of the F_2 generation. Decreases were observed for the remaining groups.

Table B-24. Serum PFOA Concentration in CD-1 Mice Over Three Generations[a] as Reported by White et al. (2011)

Generation/ Day	Dose			
	0 mg/kg + 5 ppb	1 mg/kg	1 mg/kg +5 ppb	5 mg/kg
Dams at Weaning				
F_0/PND 22	74.8 ± 11.3	6,658.0 ± 650.5	4,772.0 ± 282.4	26,980.0 ± 1,288.2
F_1/~PND 91	86.9 ± 14.5	9.3 ± 2.6	173.3 ± 36.4	18.7 ± 5.2
Offspring				
F_1/PND 22	21.3 ± 2.1	2,443.8 ± 256.4	2,743.8 ± 129.7	10,045 ± 1,125.6
F_1/PND 42	48.9 ± 4.7	609.5 ± 72.2	558.0 ± 55.8	1,581.0 ± 245.1
F_1/PND 63	66.2 ± 4.1	210.7 ± 21.9	187.0 ± 24.1	760.3 ± 188.3
F_2/PND 22	26.6 ± 2.4	4.6 ± 1.2	28.5 ± 3.7	7.8 ± 1.9
F_2/PND 42	57.4 ± 2.9	0.4 ± 0.0	72.8 ± 5.8	0.4 ± 0.0
F_2/PND 63	68.5 ± 9.4	1.1 ± 0.5	69.2 ± 4.3	1.2 ± 0.5

Notes: F_0 = parent generation; F_1 = offspring generation 1; F_2 = offspring generation 2; PND = postnatal day.
Data are presented as mean ± standard deviation (ng/mL).

To examine the effect of PFOA on the embryo-placenta unit, Blake et al. (2020) exposed CD-1 mice to PFOA at 0, 0.1, or 5 mg/kg-day from embryonic day (E) 1.5 to 11.5 or 17.5 via oral gavage. PFOA levels in the maternal serum, amniotic fluid, and whole embryo are presented in Table B-25. The mean concentration of PFOA in whole embryo is approximately 7 times higher on E 17.5 than E 11.5 for both the 1 and 5 mg/kg/day dose groups. At E 11.5, the levels of PFOA in maternal serum is approximately 5.5 times the levels observed in the amniotic fluid for the 1 mg/kg/day group and 13 times the levels observed in the 5 mg/kg/day group. Dosimetry for amniotic fluid was not reported for the mice examined at E 17.5.

Table B-25. Maternal Serum, Amniotic Fluid, and Whole Embryo PFOA Concentrations in CD-1 Mice Exposed During Gestation Day 1.5–17.5 as Reported by Blake et al. (2020)

Biological Matrix	Gestational Age	Dose	
		1 mg/kg/day	5 mg/kg/day
Maternal serum[a]	E 11.5	25.4 ± 3.7	117.3 ± 20.6
	E 17.5	18.7 ± 3.2	95.1 ± 14.1
Amniotic fluid[a]	E 11.5	4.6 ± 2.8	8.8 ± 2.7
	E 17.5	NR	NR
Whole embryo[b]	E 11.5	0.80 ± 0.10	2.34 ± 0.27
	E 17.5	5.78 ± 0.71	16.4 ± 1.75

Notes: E = embryonic day; NR = not reported; SD = standard deviation.
[a] Data are presented as mean ± standard deviation (ng/mL).
[b] Data are presented as mean ± standard deviation (ng/g).

Transfer of PFAS via lactation does not appear to correlate with lipophilicity (Fujii et al., 2020). Lactating FVB/NJcl mice were given a single IV dose of PFOA and other PFCAs chemicals with chain lengths from C8 to C13 on PND 8–PND 13. Maternal blood and milk were collected from the dam 24 hours after administration. The milk/plasma (M/P) concentration ratio for PFOA was 0.32. Ratios exhibited a U-shaped curve with increasing chain length: 0.30 for C9, 0.17 for C10, 0.21 for C11, 0.32 for C12, and 0.49 for C13. While the M/P concentration ratio did not correlate to lipophilicity of PFCAs, the estimated relative daily intake increased with chain length: 4.16 for PFOA (C8), 8.98 for C9, 9.35 for C10, 9.51 for C11, 10.20 for C12, and 10.49 for C13. These findings suggest that the amount transferred from mothers to pup during lactation may also relate to chain length-dependent clearance.

B.2.5 Volume of Distribution Data

B.2.5.1 Human Studies

Several researchers have attempted to characterize PFOA exposure and intake in humans through PK modeling (Lorber and Egeghy, 2011; Thompson et al., 2010). As an integral part of model validation, the parameter for the volume of distribution (V_d) of PFOA within the body was calibrated from available data. In the models discussed in the Toxicity Assessment (U.S. EPA, 2024b), V_d was defined as the total amount of PFOA in the body divided by the blood or serum concentration.

Two groups of researchers defined a V_d of 170 mL/kg body weight for humans for use in a simple, single compartment, first-order PK model (Lorber and Egeghy, 2011; Thompson et al., 2010). The models developed by these groups were designed to estimate intakes of PFOA by young children and adults (Lorber and Egeghy, 2011) and the general population of urban areas on the east coast of Australia (Thompson et al., 2010). In both models, the V_d was calibrated using human serum concentration and exposure data from two contaminated U.S. communities and assumes that most PFOA intake is from contaminated drinking water. Thus, in using the models to derive an intake from contaminated water, the V_d was calibrated so that model prediction of elevated blood levels of PFOA matched those seen in residents.

The assignment of V_d values used in several modeling studies is shown in Table B-26. The value of 170 mL/kg is frequently used when considering both males and females. Mondal et al. (2014) assigned a value 198 mL/kg for breastfeeding females. Shin et al. (2011) assigned values by sex (181 mL/kg for males and 198 mL/kg for females). Gomis et al. (2017) used a higher V_d of 200 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 nonpregnant females, and across serum, plasma, and whole blood fractions.

Table B-26. Summary of PFOA Volume of Distribution Values Assigned in Human Studies

Study	Population	Sex	Compartment	V_d	AUC or Mean/Median Concentration Measured in Compartment	Steady-state Considerations
Mondal et al. (2014)	Adult, breastfeeding	Females	Maternal serum	198 mL/kg	GM Breastfeeding :18.32 ng/mL (95% CI: 16.36, 20.50) GM Non-breastfeeding: 19.26 (16.80, 22.08)	NR
Zhang et al. (2015a)	Adult	Males and females	Whole blood	170 mL/kg	Mean: 2.71; GM: 2.47	Steady state assumed
	Adult, pregnant	Females	Whole blood	170 mL/kg	Mean: 3.36; GM: 3.09	Steady state not assumed due to variable PFAS levels during pregnancy
Worley et al. (2017)	>12 yr	Males and females	Blood (2016)	170 mL/kg bodyweight	Mean: 11.7 µg/L (95 CI: 8.7–14.6)	NR
	>12 yr	Males and females	Blood (2010)	170 mL/kg bodyweight	Mean: 16.3 (95 CI: 13.2–19.6)	NR
Fu et al. (2016)	Adult, occupational	Males and females	Serum	170 mL/kg	Mean: 1,052 ng/mL Median: 427 ng/mL	NR
Zhang et al. (2013c)	Adults	Males and females	Serum and whole blood	170 mL/kg	Mean: 3.1 ng/mL	NR
Shin et al. (2011)	Adult, nonoccupational	Males	Serum	181 mL/kg	Median predicted: 13.7 ppb; observed 23.5 ppb (updated values in Erratum) (Shin et al., 2013)	NR
	Adult, nonoccupational	Females	Serum	198 mL/kg	Median predicted: 13.7 ppb; observed 23.5 ppb (updated values in Erratum) (Shin et al., 2013)	NR
Gomis et al. (2017)	Human and animals	Males and females	Serum	200 mL/kg	Reports an average of human and animal V_d values	Authors note that due to declining values in U.S. and Australian populations, steady state was not achieved in the past decade.

Notes: AUC = area under curve; CI = confidence interval; GM = geometric mean; NR = not reported; U.S. = United States; V_d = volume of distribution; yr = years.

B.2.5.2 Animal Studies

In Fujii et al. (2015), PFOA distribution in male and female FVB/NJcl mice (8–10 weeks of age) administered by IV (0.31 µmol/kg) or gavage (3.13 µmol/kg) was determined using a two-compartment model. Serum PFOA concentrations varied linearly by dose regardless of route. The V_d after IV injection was calculated as dose/C(0). As shown in Table B-27, the V_d of PFOA was low in mice after IV injection and exhibited no differences between sexes. The low serum V_d was consistent with the high percentage (32.3%) of administered dose calculated for serum. The measured percentage of administered dose was higher in the liver (47.4%) although V_d for this compartment was not calculated.

In this study, the authors examined PFCAs with chain lengths between 6 and 14 and observed that V_d increased as a function of chain length in both males and females. The authors suggested that this may be linked to the lipophilicity of PFCAs and their increasing affinity for serum and liver fatty acid binding proteins. For PFOA, V_d corresponded to the volume of extracellular water. Interestingly, V_d values corresponded to different compartments based on chain length, specifically the total volume of blood for C7 and the volume body water for C11 and C12).

Table B-27. PFOA Volume of Distribution in Serum of FVB/NJcl Mice as Reported by Fujii et al. (2015)

Route	Dose (µmol/kg)	Sex	V_d l kg−1 a	AUC µmol l−1 hour (0 to 24 hours)a
IV	0.313	Male	0.18 ± 0.04	42.2 ± 9.9
IV	0.313	Female	0.15 ± 0.04	49.5 ± 11.9
Oral[b]	3.13	Male	NR	348 ± 76
Oral[b]	3.13	Female	NR	495 ± 64

Notes: AUC = area under curve; IV = intravenous; NR = not reported; Vd = volume of distribution.
[a] V_d and AUC reported as means ± standard deviation.
[b] Steady state achieved 8 days after initial dose (oral).

Two recent studies (Dzierlenga et al., 2019; Kim et al., 2016b) measured toxicokinetic parameters in rats, including V_d. In the Kim et al. (2016b) study, V_d values were calculated as Dose × AUMC/(AUC0-∞) 2, where AUMC is the area under the first moment curve (Table B-28). Similar to the Fujii et al. (2015) study in mice, V_d values were similar in males and females. While organ specific V_d values were not determined, the liver and kidney exhibited partition coefficients greater than 1 in males (2.31 ± 0.38 for liver and 1.18 ± 0.47 for kidney). While the partition coefficients in females for the kidney (1.23 ± 0.39) were similar to males, they were significantly lower in the livers of females (0.81 ± 0.36) compared with males. Partition coefficients were similar in males and females for the heart, lung, and spleen. Although V_d values were not significantly different between males and females, the differential partition coefficients in liver and kidney may relate to the higher V_d values calculated for females compared with males.

Dzierlenga et al. (2019) calculated the apparent volume of central (V_1) and peripheral (V_2) distribution in rats. In this study, the plasma concentration-time profiles were best described using one-compartment models in males and a two-compartment model in females. As detailed in Table B-28, males and females were administered different doses that were higher than those used in the Kim et al. (2016b) study. Females were administered 40–320 mg/kg compared with 6–48 mg/kg in males. Several observations were apparent for V_d in males. V_d values were

substantially lower in the peripheral compartment compared with the central compartment, and V_ds were substantially lower in the peripheral compartment after IV administration relative to oral administration. V_ds were similar after oral dosing at 6 and 12 mg/kg (159 ± 12 and 154 ± 11 mL/kg, respectively) and only increased at the highest dose of 48 mg/kg (202 ± 18 mL/kg). In contrast to males after IV dosing, female V_d values were similar in central and peripheral compartments (108 ± 24 and 98.7 ± 39.8 mL/kg, respectively) although the dose in females of 40 mg/kg was substantially higher than the 6 mg/kg dose in males.

In females, both peripheral and central V_ds were calculated after oral dosing at all doses. Peripheral V_d values were dramatically lower than central V_d values at all doses by the oral route (Table B-28). These trends are consistent with the observations that peak tissue levels were reached readily in both males and females. However, while tissue levels in males were steady over the course of several days, tissue levels in females dropped quickly (in the span of hours), which likely reflects the shorter half-life in females.

In a third study (Iwabuchi et al., 2017), PFOA was administered to male Wistar rats as a single bolus dose (BD) and V_d was measured as BD/elimination rate constant (ke) × plasma concentration (AUC). V_d values were calculated for blood, serum, and several tissues. The whole blood V_d (0.42 kg tissue volume/kg BW) was almost threefold higher than the serum V_d. Organ V_d values were highest in the brain (9.0 kg tissue volume/kg BW) and spleen (2.3 kg tissue volume/kg BW). V_ds were 1–2 orders of magnitude lower in the heart, kidney, and liver (0.91, 0.27, and 0.083 kg tissue volume/kg BW, respectively). An interesting observation from this analysis is that, for PFOA, 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.

A single study examined V_d in primates. Butenhoff et al. (2004b) calculated a V_d from noncompartmental PK analysis of data from cynomolgus monkeys. Three males and three females were administered a single IV dose of 10 mg/kg, and serum PFOA concentrations were measured in samples collected up to 123 days post-dosing. The V_d of PFOA at steady state ($V_d$ss) was similar for both sexes at 181 ± 12 mL/kg for males and 198 ± 69 mL/kg for females.

Table B-28. Summary of PFOA Volume of Distribution Calculations in Rats

Study	Method of V_d Calculation	Route	Dose	Strain	Age	Sex	V_d	Compartment	Concentration Measured in Compartment[a]	C_{max}
Kim et al. (2016b)	Dose × AUMC/(AUC0-∞)^2	Oral	1 mg/kg	Sprague-Dawley	8–12 wk	Males	106.4 ± 8.90 mL/kg	Blood plasma	AUC: 24.81 ± 1.41 μg day/mL	7.55 ± 0.51 μg/mL
						Females	153.83 ± 9.19 mL/kg	Blood plasma	AUC: 1.39 ± 0.06 μg day/mL	5.41 ± 0.38 μg/mL
		IV	1 mg/kg	Sprague-Dawley	8–12 wk	Males	112.12 ± 29 0.41 mL/kg	Blood plasma	AUC: 21.10 ± 1.51 μg day/mL	8.92 ± 2.34 μg/mL
						Females	171.37 ± 11 0.19 mL/kg	Blood plasma	AUC: 1.63 ± 0.09 μg day/mL	5.84 ± 0.38 μg/mL
Dzierlenga et al. (2019)	Standard equations (Gabrielsson and Weiner, 2000)	Oral	6 mg/kg	Sprague-Dawley	8 wk	Males	159 ± 12 mL/kg	Peripheral	AUC: 39.37 ± 2.42 mM·h	0.089 ± 0.007 mM
			12 mg/kg	Sprague-Dawley	8 wk	Males	154 ± 11 mL/kg	Peripheral	AUC: 69.79 ± 3.86 mM·h	0.185 ± 0.013 mM
			48 mg/kg	Sprague-Dawley	8 wk	Males	202 ± 18 mL/kg	Peripheral	AUC: 178.4 ± 12.1 mM·h	0.560 ± 0.048 mM
			40 mg/kg	Sprague-Dawley	8 wk	Females	73.6 ± 20.6 mL/kg	Central	AUC: 5.217 ± 0.507 mM·h	0.580 ± 0.060 mM
							5.55 ± 1.62 mL/kg	Peripheral	AUC: 5.217 ± 0.507 mM·h	0.580 ± 0.060 mM
			80 mg/kg	Sprague-Dawley	8 wk	Females	130 ± 24 mL/kg	Central	AUC: 8.066 ± 0.869 mM·h	0.961 ± 0.118 mM
							19.9 ± 12.9 mL/kg	Peripheral	AUC: 8.066 ± 0.869 mM·h	0.961 ± 0.118 mM
			320 mg/kg	Sprague-Dawley	8 wk	Females	272 ± 1990 mL/kg	Central	AUC: 57.00 ± 7.97 mM·h	2.06 ± 0.61 mM
							69.9 ± 1849 0.1 mL/kg	Peripheral	AUC: 57.00 ± 7.97 mM·h	2.06 ± 0.61 mM
		IV	6 mg/kg	Sprague-Dawley	8 wk	Males	114 ± 5 mL/kg	Central	AUC: 28.0 ± 1.69 mM·h	0.127 ± 0.006 mM

Study	Method of V_d Calculation	Route	Dose	Strain	Age	Sex	V_d	Compartment	Concentration Measured in Compartment[a]	C_{max}
							39.2 ± 14.5 mL/kg	Peripheral	AUC: 28.0 ± 1.69 mM·h	0.127 ± 0.006 mM
			40 mg/kg	Sprague-Dawley	8 wk	Females	108 ± 24 mL/kg	Central	AUC: 2.87 ± 0.31 mM·h	0.893 ± 0.196 mM
							98.7 ± 39.8	Peripheral	AUC: 2.87 ± 0.31 mM·h	0.893 ± 0.196 mM
Iwabuchi et al. (2017)	Dose / $k_e \times$ plasma concentration (AUC)	Oral	100 µg/kg, single dose	Wistar	7–9 wk	Males	9.0 kg tissue volume/kg BW	Brain	160 µg/kg tissue volume – day	8.77 µg/kg
							0.91 kg tissue volume/kg BW	Heart	1,500 µg/kg tissue volume – day	108 µg/kg
							0.083 kg tissue volume/kg BW	Liver	35,000 µg/kg tissue volume – day	1,270 µg/kg
							2.3 kg tissue volume/kg BW	Spleen	630 µg/kg tissue volume – day	49.2 µg/kg
							0.27 kg tissue volume/kg BW	Kidney	6,600 µg/kg tissue volume – day	624 µg/kg
							0.42 kg tissue volume/kg BW	Whole blood	4,300 µg/kg tissue volume – day	265 µg/kg
							0.15 kg tissue volume/kg BW	Serum	9,200 µg/kg tissue volume – day	759 µg/kg

Notes: AUC = area under curve; AUMC = area under first moment curve; BW = body weight; C_{max} = maximum plasma concentration; IV = intravenous; k_e = elimination rate constant; NR = not reported; Vd = volume of distribution; wk = weeks.
[a] Presented as AUC or Mean/Median.

B.3 Metabolism

A summary of studies that provide information on PFOA metabolism from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD is shown in Figure B-3.

Evidence Stream	Grand Total
Animal	2
Human	1
In Vitro	
Grand Total	3

Figure B-3. Summary of PFOA Metabolism Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOA HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

PFOA does not appear to be metabolized in mammals. In a recent study, Gannon et al. (2016) investigated the metabolism of PFOA in vivo and in vitro using rodent models. Specifically, male and female mice (Crl:CD1(ICR)) and rats (Sprague-Dawley) were exposed to a single oral dose of PFOA at 3 mg/kg and 30 mg/kg, respectively. Urine samples collected from both rodent species were analyzed by high-performance liquid chromatography. The authors subsequently screened for metabolites using the control-comparison tool, IntelliExtractTM. Only the anionic form of PFOA was detected. There was almost complete recovery of the dose in the urine, confirming that PFOA is not metabolized. In addition, normal and heat-inactivated rat hepatocytes (5×10^6 cells/mL) were exposed to 50 µM of PFOA in a 3 mL suspension. No differences in clearance rate were found and no metabolites were detected. Similarly, no evidence of metabolism was observed using fluorine-19 nuclear magnetic resonance (NMR) spectroscopy in male Fischer 344 rats administered a single i.p. injection of a very high dose of PFOA (150 mg/kg) (Goecke et al., 1992). Gomis et al. (2016) examined the contribution of direct uptake of PFOA compared with indirect uptake of 8:2 fluorotelomer alcohol (FTOH) and metabolism using a dynamic one-compartment pharmacokinetic (PK) model applied to six ski wax technicians. The model calculated an average metabolism yield of 0.003 (molar concentration basis; uncertainty range: 0.0006−0.01) for transformation of 8:2 FTOH to PFOA.

B.4 Excretion

A summary of studies that provide information on PFOA excretion from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD is shown in Figure B-4.

Evidence Stream	Grand Total
Animal	16
Human	29
In Vitro	7
Grand Total	48

Figure B-4. Summary of PFOA Excretion Studies

Interactive figure and additional study details available on HAWC.
[a] Figure does not include studies discussed in the 2016 PFOA HESD or those that solely provided background information on toxicokinetics.
[b] Select reviews are included in the figure but are not discussed in the text.

B.4.1 Urinary and Fecal Excretion

B.4.1.1 Human Studies

The majority of human studies predominantly consider PFOA excretion after oral exposure, either implicitly or explicitly. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the sex/age of the individual. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains. Fewer studies have examined excretion through the fecal route. Animal studies suggest that sex and competing PFAS compounds influence fecal excretion.

Several major studies highlight the urinary excretion of PFOA in humans. Zhang et al. (2015b) derived estimates for PFOA's urinary excretion rate using paired urine and blood samples from 54 adults (29 male, 25 female, ages 22–62) in the general population and 27 pregnant females (ages 21–39) in Tainjin, China. Urinary excretion was calculated by multiplying detected PFOA concentration in first-draw morning urine samples by the predicted urinary volume (1,600 mL/day for males and 1,200 mL/day for females). PFOA was detected in the blood samples for all participants but for only 76% of the urine samples from the general population and 30% for the pregnant females. Total daily PFOA intake was modeled for the general population and used to estimate a daily urinary excretion rate of 25% but was higher in males than in females (31% and 19%, respectively). In contrast to the estimates relating to PFOS, there was little difference in urine:blood ratio between nonpregnant females age 21–50 and those age 51–61, although the urine:blood ratio was found to be lower for pregnant females than nonpregnant females (0.0011 and 0.0029, respectively), suggesting the placenta and cord blood as possible elimination pathways. There was a direct correlation between the PFOA concentrations in blood and creatinine adjusted urine ($r = 0.348$ $p = 0.013$) for the general population but not for the pregnant females. When limited to the eight females who had

detectable levels in both blood and urine, there was a significant correlation ($r = 0.724$, $p = 0.042$).

Zhang et al. (2013c) calculated median renal clearance rates of 0.16 mL/kg/day in young women and 0.19 mL/kg/day in men and older women for total PFOA. In a later study, Fu et al. (2016) determined the renal clearance half-lives of PFOA in 302 occupational workers (213 male, 89 female) from one of the largest producers of PFAS-related compounds in China. Paired serum and urine samples were collected. The participants were subdivided based on their work assignment. Serum PFOS and PFHxS were highest in workers of the sulfonation department and the serum PFOA levels were highest in workers from the electrochemical fluorination department.

Serum PFOA concentrations were in the ranges of 2.52–32,000 ng/mL (median 424 ng/mL). The average concentrations of serum PFOA was significantly higher in males (1,215 ± 2,936) ng/mL than in females (659 ± 743) ng/mL. The median urine concentration for all workers was 4.3 ng/mL (range: LOD – 53.6 ng/mL). The correlation coefficient of PFOA concentrations in paired serum and urine samples of 0.64 was found to be highly statistically significant, ($p < 0.01$), suggesting that urine concentrations could serve as effective bioindicators for PFOA exposure in occupational settings. Daily renal clearance was calculated for each PFAA as follows:

$$\frac{Urine\ PFAA\ Concentrations\ Daily\ \times\ Daily\ urine\ excretion\ volume}{Serum\ PFAA\ concentrations\ \times\ Body\ weight}$$

Urine excretion volumes were assigned as 1.4 L/day and 1.2 L/day for males and females, respectively, and body weight as reported in questionnaires. The daily renal clearance was the highest for PFOA (geometric mean: 0.067 mL/day/kg) followed by PFOS (geometric mean: 0.010 mL/day/kg). The high efficiency of PFOA renal clearance was reflected in the relative abundance of PFOA from 12% in the serum samples to 42% in the urine samples. Sex did impact daily renal clearance values, which were significantly lower in males compared with females ($p < 0.01$).

A single case report study demonstrated fecal excretion of PFOA in humans. Fecal PFOA was measured in an exposed man before and after treatment with bile sequestering agents (Genuis et al., 2010). Before treatment, his urine and stool levels of PFOA levels were 3.72 ng/mL and below detectable limits (0.5 ng/g), respectively. After treatment with cholestyramine, PFOA measurements in stool increased to 0.96 ng/g in the first weeks after treatment and to 1.19 ng/g several months later after subsequent treatments with saponins.

Urinary clearance of PFCAs in humans was observed to decrease with increasing alkyl chain lengths, while biliary clearances increased (Fujii et al., 2015). In these studies, paired bile-serum and urine-serum were obtained from the archived samples in the Kyoto University Human Specimen Bank. Bile samples were taken by nasobiliary drainage, percutaneous transhepatic biliary drainage or percutaneous transhepatic gallbladder drainage for 24 hours. Blood samples were taken from the same patients on the same day. Blood and urine were also collected from healthy volunteers. 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-hour period with the serum concentration. Fecal clearance was calculated using the estimated biliary resorption rate.

The authors estimated that total human clearance was 0.096 mL/kg/day and was 50−100 times smaller than those clearances estimated in mice after oral gavage dosing. In humans, 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 that PFOA could only be excreted into the urine and feces via the bile).

Interestingly, PFCAs with chain lengths of C6 and C7 were rapidly excreted into urine, whereas PFOA and PFCAs with longer chain lengths were deposited mainly in the liver. Thus, chain length for PFCAs may be a major determinant of bioaccumulation as well as excretion rate and route. These authors also conducted a toxicokinetics analysis in mice (discussed in the next section). They ascertained that human urinary clearances for PFCAs were more than 200 times smaller than those in mice. Fecal clearances in humans were also an order of magnitude lower than those estimated in mice after oral gavage and IV dosing (ranging from 1.1 to 4.3 mL/day/kg) also differed by one order of magnitude, indicating the other membrane transporters in the liver may also be involved.

Although no data were identified on urine or fecal excretion of PFOA following inhalation exposures in humans, the Hinderliter study (2006a) provides evidence of clearance following single and repeated inhalation exposures in Sprague-Dawley rats. Plasma PFOA concentrations following a single exposure to 1, 10, or 25 mg/m^3 PFOA declined 1 hour after exposure in females and 6 hours after exposure in males. In females, the elimination of PFOA was rapid at all exposure levels and, by 12 hours after exposure, their plasma levels had dropped below the analytical LOQ (0.1 µg/mL). In males, the plasma elimination was much slower and, at 24 hours after exposure, the plasma concentrations were approximately 90% of the peak concentrations at all exposure levels. In the repeated exposure study, male and female rats were exposed to the same concentrations for 6 hours per day, 5 days per week for 3 weeks. Steady-state plasma levels were reached in males by 3 weeks, but plasma PFOA levels in females returned to baseline within 24 hours of each dose.

No data were identified on excretion following dermal exposures. Minimal fecal excretion is anticipated for the dermal route of exposure although the biliary pathway can be a route for excretion of material absorbed through the skin, distributed to the liver, and discharged to the gastrointestinal tract.

B.4.1.2 Animal Studies

Butenhoff et al. (2004b) studied the fate of PFOA in cynomolgus monkeys in a 6-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at DRs of 0, 3, 10, and 30/20 mg/kg for 6 months, with urine and fecal samples collected at 2-week intervals. All dosed groups reached steady-state urine PFOA levels after four weeks, which were 53 ± 25, 166 ± 83, and 181 ± 100 µg/mL, respectively. Two monkeys exposed to 10 mg/kg and three monkeys exposed to 20 mg/kg were monitored for 21 weeks (recovery

period) following dosing. Within two weeks of recovery, urine PFOA concentrations were < 1% of the value measured during treatment and decreased slowly thereafter. Lower amounts were excreted in feces. These results are consistent with both renal and biliary excretion in male monkeys.

There have been a number of studies of excretion in rats because of the sex differences noted in serum levels. Hinderliter et al. (2006b) investigated the relationship between age and urine PFOA concentrations in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered PFOA via oral gavage as a single dose of 10 or 30 mg/kg, and urine was collected for 24 hours.

Urine PFOA concentrations differed significantly ($p < 0.01$) with age, dose, and sex. For all doses and ages, urinary excretion of PFOA was substantially higher in females than in males, and this difference increased with age, as female excretion increased and male excretion decreased. In both sexes, urine PFOA was higher (2.5 to 6.5 times) at the 30 mg/kg dose as compared with the 10 mg/kg dose (Table B-29).

Table B-29. Urine PFOA Concentrations in Male and Female Sprague-Dawley Rats, 24 Hours After Oral Gavage[a] as Reported by Hinderliter et al. (2006b)

Age (weeks)	Dose (mg/kg)	Urine PFOA			
		Male		Female	
		Mean	SD	Mean	SD
3	10	9.57	4.86	21.17	8.95
4	10	4.53	2.45	23.26	15.27
5	10	4.03	2.36	49.77	24.64
3	30	51.76	28.86	94.89	26.26
4	30	28.70	18.84	104.12	28.97
5	30	15.65	6.24	123.16	51.56

Notes: SD = standard deviation.
[a] Data are presented as mean ± standard deviation (µg/mL)

Kim and colleagues (2016b) extended the study of male and female Sprague-Dawley rats to evaluate fecal excretion. They also compared oral and intravenous administration of PFOA, giving a single 1 mg/mL dose by either pathway. Urine and feces were measured daily for 12 days in males and females after dosing. Like previous studies, the highest concentrations were found in urine under all conditions. In males, the levels detected in urine and feces were very similar from both oral and intravenous exposure. By the oral route, 26.42 ± 2.64 µg was detected in urine versus 23.60 ± 9.45 µg in feces. Levels were even more similar in male rats dosed intravenously (22.47 ± 1.94 µg in urine versus 21.13 ± 12.31 µg in feces). In contrast, females excreted much higher levels in urine compared with males and compared with feces. After oral administration, urine and fecal levels were 124.95 ± 6.38 µg and 24.60 ± 4.18 µg, respectively. The values measured after intravenous administration were similar to those observed after oral dosing (131.87 ± 6.82 µg in urine vs. 18.04 ± 1.35 µg in feces). The differences between males and females in amounts detected in urine and feces translated to significant differences in the

estimated half-life values (1.64 and 1.83 days in males vs. 0.15 and 0.19 days in females by the oral and intravenous routes).

Kudo et al. (2007) injected male Wistar rats with [^{14}C]PFOA via femoral vein at the doses of 0.041, 0.41, 2.07, 4.14, 12.42, and 16.56 mg/kg BW. Blood, urine, and bile samples were withdrawn at several time points between 0 and 300 minutes after injection and renal and biliary clearance rates were calculated. The biliary clearance value was calculated as 0.07 mL/hr/kg BW at the lowest dose (0.041 mg/kg BW) and increased in a dose-dependent manner that was not statistically significant. Similarly, renal clearance rates were not significantly different at the different doses even though they ranged between 0.1 and 0.4 mL/hr/kg BW.

Other studies comparing urinary and fecal excretion following PFOA administration by gavage among male Sprague-Dawley rats have found much higher excretion rates from urine than from feces (Cui et al., 2010; Benskin et al., 2009). Benskin et al. (2009) gave single doses of 0.5 mg PFOA/kg to each rat and monitored for 38 days, while Cui et al. (2010) gave 0, 5, or 20 mg/kg/day over 28 days and monitored for the duration. Among the single-dose rats, 91%–95% of the daily excreted PFOA was eliminated in the urine after the initial 24 hours. On day 3, the mean PFOA concentration in urine and feces were 265 ng/g and 28 ng/g. The half-life for elimination from plasma in male rats was 13.4 days (Benskin et al., 2009). Vanden Heuvel et al. also examined excretion via urinary and biliary routes in male and female Sprague-Dawley rats exposed to a single i.p. dose of [^{14}C]PFOA of 4 mg/kg (Vanden Heuvel et al., 1991). In female rats, 90% of PFOA was eliminated in urine within the first 24 hours. In contrast, elimination in males was roughly equivalent by the urinary and fecal routes. However, ligation of the kidneys for a 6-hour period resulted in equal elimination in bile in males and females providing evidence that the sex-specific differences relate to differential renal excretion of male and female rats. These finding correlated with the observation that 0.95% of the dose/g in males versus 2.0% dose/g in females was present in the kidneys at 2 hours post-treatment.

Among the repeated dose rats, a sharp increase in urinary and fecal excretion expressed as percent of dose/day was observed during week 1 in rats of both dose groups. The excretion rate leveled off at about 50% for the low-dose animals for the remainder of the 28 days. In the case of the high-dose animals, the urinary excretion remained level at about 80% for the second and third weeks and then increased sharply to about 140% at 28 days. The fecal excretion rates followed an upward trend throughout the 28 days with the terminal percent/day about 25% for the low-dose group and 40% for the high-dose group.

Studies on male and female CD rats have similar findings to those done in Sprague-Dawley rats; namely, that females excreted PFOA more efficiently than males, excretion rates increased with higher dosages, and both sexes excreted more PFOA by urine than by feces. Hundley et al. (2006) examined excretion of PFOA in one male and one female CD rat, giving each a single dose of 10 mg/kg [^{14}C]PFOA and collecting urine and feces at 12−24 hour intervals for five days post-dose (Table B-30). Kemper (2003) gave either single or repeat doses ranging from 1–25 mg/kg (Table B-31) and collected urine and feces for 7 or 28 days for females and males, respectively. Hundley et al. found that the female rat had excreted almost all dosed [^{14}C]PFOA within 48 hours, with urinary excretion accounting for about 2.65 times the amount of fecal excretion. In the male rat, PFOA was excreted from urine at a similar rate relative to fecal excretion, but much slower overall; only about 19% had been excreted after 48 hours, and only 34% after 120 hours. Kemper (2003) found that after 28 days, singly dosed male rats excreted

47%–68% of the initial dose; interestingly, while the females consistently excreted more of the PFOA than males, none of the dose groups were found to eliminate 100% of the [^{14}C]PFOA after 7 days.

Table B-30.Cumulative Percent [^{14}C]PFOA Excreted in Urine and Feces by Male and Female CD Rats[a] as Reported by Hundley et al. (2006)

Rat	Hours After Dosing					
	12	24	48	72	96	120
Male	0.6	8.7	19.2	23.4	30.2	34.3
Female	52.5	96.4	99.8	100.0	100.0	100.0

Notes: [^{14}C]PFOA = [^{14}C]Radiocarbon perfluorooctanoic acid.
[a] Data are presented in % total dose administered.

Table B-31. Percentage of Dose Excreted in Urine and Feces of Male and Female Sprague-Dawley Rats Exposed to [^{14}C]PFOA via Oral Gavage as Reported by Kemper (2003)

Dose and Regimen	Sex	Urine[a]	Feces[b]
Single Dose 1 mg/kg	Male	43.238 ± 3.015	14.055 ± 4.003
	Female	75.872 ± 4.066	2.169 ± 2.923
Single Dose 5 mg/kg	Male	62.201 ± 3.656	5.568 ± 1.779
	Female	77.867 ± 6.034	5.886 ± 5.387
Single Dose 25 mg/kg	Male	53.265 ± 8.490	12.490 ± 4.153
	Female	84.381 ± 12.023	1.868 ± 2.546
Repeated Dose 1 mg/kg/day	Male	52.430 ± 7.959	19.841 ± 6.620
	Female	68.537 ± 16.631	12.384 ± 15.775

Notes: [^{14}C]PFOA = [^{14}C]Radiocarbon perfluorooctanoic acid; SD = standard deviation.
[a] Data are presented as mean ± standard deviation (µg/mL).
[b] Data are presented as mean ± standard deviation (µg/g).

Dose is an important variable that impacts excretion. Rigden et al. (2015b) exposed groups of five male Sprague-Dawley rats to doses of 0, 10, 33, and 100 mg/kg/day for 3 days and maintained them for 3 additional days; overnight urine was collected and body weight was measured daily. Of greatest interest relative to the limitations on renal resorption, is the dose-related increase in urine PFOA concentration and urine PFOA concentration per mg creatinine for the 33 and 100 mg/kg/day groups compared with the 10 mg/kg/day group. The peak in PFOA excretion normalized to creatinine occurred on day 3 after the cessation of dosing. The concentration at 33 mg/kg/day was 500 times greater than that at 10 mg/kg/day. At the 100 mg/kg/day dose, the peak concentration was about 3,200 times greater than for the low dose. The low-dose excretion was only slightly greater than the controls. The urine results support the renal resorption hypothesis concept and suggest that there is a threshold limit on resorption that, once exceeded, dramatically increases PFOA loss in urine. As a consequence, half-life for continuous low-dose exposures will be longer than for single or short-term high-dose exposures.

Another study (Gao et al., 2015) also compared concentrations in urine and feces of male and female Wistar rats. A mixture of PFOA/PFNA/PFOS were administered to the rats by drinking water for 90 days, with each compound at doses of 0, 0.05, 0.5, and 5 mg/L. While the focus of

this study was measuring concentrations in the hair of animals (discussed below under Other Routes of Excretion), the authors measured concentrations of each PFAA in urine and feces samples by collecting excreta in standard metabolism cages overnight for 24-hour intervals on day 84 (week 12). The intake for each compound was calculated as the drinking volume multiplied by water concentration of 0.05, 0.5, and 5 mg/L. These translated to intake values for PFOA, PFNA and PFOS of 0.15 and 0.12 mg/kg BW, 1.52 and 1.22 mg/kg BW, and 13.6 and 17.7 mg/kg BW for female and male rats, respectively. At the high dose of 5 mg/L, there were higher levels of PFOA in urine and feces of males and females. However, and in contrast to that observed by others, there were far higher levels of PFOS in feces compared with urine for both males and females. It is unclear whether the higher levels of PFOS in feces reflects rat strain or dose differences among the various studies or is driven by differential excretion pathways in rats exposed to a mixture of PFNAs. Hundley et al. (2006) examined excretion of PFOA in CD mice, BIO-15.16 hamsters, and New Zealand white rabbits. One male and one female of each species was given a single dose of 10 mg/kg [^{14}C]PFOA and housed in metabolism cages. Urine and feces were collected at 12−24-hour intervals for five days post-dose. Additional samples were collected from rabbits at 144 and 168 hours post-dose.

Over 120 hours, both mice excreted similar amounts of PFOA, although the male mouse excreted a greater proportion in feces (3.4% [^{14}C]PFOA in urine and 8.3% [^{14}C]PFOA in feces), and the female mouse excreted more via urine (6.7% [^{14}C]PFOA in urine and 5.7% [^{14}C]PFOA in feces). The male hamster excreted far more than the female, although both excreted more via urine than by feces; the male excreted 90.3% and 8.2% [^{14}C]PFOA in urine and feces, respectively, and the female hamster excreted 45.3% and 9.3% [^{14}C]PFOA. Over 168 hours, both rabbits excreted most of the original dose, and both predominantly excreted via urine (76.8% and 4.2% [^{14}C]PFOA from the male, and 87.9% and 4.6% [^{14}C]PFOA from the female in urine and feces, respectively). The cumulative percentages of [^{14}C]PFOA excreted are shown in Table B-32.

Table B-32. Cumulative Percent [^{14}C]PFOA Excreted in Urine and Feces in Mouse, Hamster, and Rabbit[a] as Reported by Hundley et al. (2006)

Species	Sex	Hours After Dosing						
		12	24	48	72	96	120	168
Mouse	Male	0.4	4.1	6.7	8.6	9.1	10.8	–
	Female	0.2	4.1	6.5	8.4	9.0	11.0	–
Hamster	Male	67.3	84.5	96.1	97.4	98.2	98.4	–
	Female	11.3	24.6	36.4	43.9	50.1	54.0	–
Rabbit	Male	77.8	80.2	80.4	80.4	80.4	80.4	80.4
	Female	86.7	90.5	92.0	92.2	92.7	92.9	93.0

Notes: [^{14}C]PFOA = [^{14}C]Radiocarbon perfluorooctanoic acid.
[a] Data are presented in % of total dose administered.

Fujii and colleagues (2015) compared elimination in humans and mice exposed to using a two-compartment model. Toxicokinetics and clearance was investigated in FVB/NJcl mice exposed by oral gavage and intravenous administration of PFCAs with carbon chain lengths between C6 and C10. At 24 hours after exposure, urine and feces were collected in metabolic cages. In mice, the short-chained PFCAs (C6 and C7) were rapidly eliminated in the urine, whereas long-chain

PFCAs (C8 to C14) accumulated in the liver and were excreted slowly in feces. For PFOA administered IV, urinary clearance was higher in males (13.1 mL/day/kg) compared with females (9.8 mL/day/kg). PFOA administered by oral gavage was also higher in males (9.2 mL/day/kg) compared females (6.6 mL/day/kg), but clearance was significantly lower than rates measured after IV administration.

Fecal clearance of PFOA after IV administration was higher in females (2.0 mL//day/kg) compared with males (1.1 mL/day/kg). After gavage administration, the opposite was observed with higher rates observed in males (4.0 mL/day/kg) compared with females (2.4 mL/kg/day). The feces clearance after 24 hours of gavage administration represents PFOA contained in the bile and unabsorbed PFCAs that passed through the gut, and this likely accounts for the higher fecal clearance after gavage dosing. The actual fecal clearances of PFCAs were represented by the fecal clearances of IV-administrated PFCAs. In contrast to urinary clearance, fecal clearance rates were still lower than urinary clearance rates by both dosing routes.

Interestingly, these authors also estimated urinary and fecal clearance rates in humans, which were 1–2 orders of magnitude lower than rates estimated in mice. This study illustrates chain length, sex, and species have dramatic impacts on the rate and route of PFOA excretion.

Studies in 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 through the fecal route in animals and humans and through hair in animals. Most studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. Excretion through the fecal route appears to be more efficient in males compared with females and in rodents compared with humans. Also, exposures to mixtures of PFNAs may also alter the relative amounts of PFOA excreted through the fecal route, quite possibly due to differential lipophilicity and cellular uptake as well as differential affinities for transporters associated with chain length and branching. 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.

B.4.2 Physiological and Mechanistic Factors Impacting Excretion

B.4.2.1 Renal Resorption

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats. Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs located in the proximal portion of the descending tubule. OATs are found in other tissues as well and were discussed earlier for their role in absorption and distribution. In the kidney, they are responsible for delivery of organic anions (including a large number of medications) from the serum into the kidney tubule for excretion, as well as reabsorption of anions from the glomerular filtrate. The transporters are particularly important in excretion of PFOA because it binds to surfaces of serum proteins (particularly albumin), which makes much of it unavailable for removal during glomerular filtration. Other transporter families believed to be involved in renal excretion are the OATPs and the MRPs. However, they have not been evaluated as extensively as the OATs for their role in renal excretion.

OATs are located on both the basolateral (serum interface) and apical surfaces of the brush boarder of the proximal tubule inner surface. At the basolateral surface, the OATs transport the perfluorooctanoate anion from the serum to the tubular cells (Klaassen and Aleksunes, 2010; Nakagawa et al., 2009; Cheng and Klaassen, 2008; Klaassen and Lu, 2008; Nakagawa et al., 2008; Anzai et al., 2006). OAT1, 2, and 3 are located on the basolateral membrane surface. OAT4 and OAT5 are located on the apical surface of the tubular cells, where they reabsorb the PFOA anions from the glomerular filtrate. Figure B-5 diagrams the flow of organic anions such as the PFOA anion from serum to the glomerular filtrate for excretion and resorption of organic acids from the glomerular filtrate with transport back to serum. OATs can function for uptake into the cell across both the basolateral and apical surfaces.

Several MRP transporters also appear to function in the kidney and move organic anions in and out of cells at both the basolateral surface (e.g., MRP2/4) and the apical surface (e.g., MRP1) as well as one or more OATPs on each surface (Klaassen and Aleksunes, 2010; Cheng and Klaassen, 2009; Kusuhara and Sugiyama, 2009; Yang et al., 2009b; Klaassen and Lu, 2008; Launay-Vacher et al., 2006). Bidirectional movement of PFOA across both the basolateral and apical surfaces is driven by concentration gradients and/or active transport. Far more data exist on PFOA and OATs in the kidneys than on OATPs and MRPs. Abbreviations for individual transporters on the basolateral and apical surfaces differ across publications. The accepted convention is to use uppercase letters to refer to human transporters and lowercase letters to refer to animal transporters. For this report, the data are not reported by species but by transporter family and the uppercase letters are used.

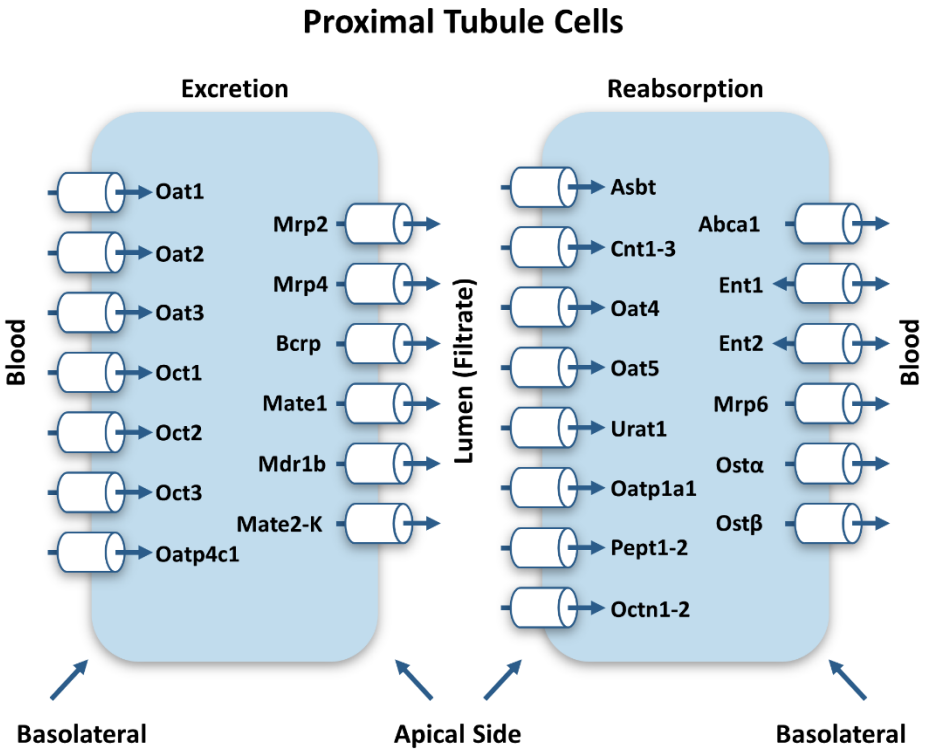


Figure B-5. Localization of Transport Proteins

Adapted from Klaassen and Aleksunes (2010).

Knowledge about specific OAT, OATP, and MRP transporters in the kidneys is rapidly evolving. A low membrane density or blockage of basolateral OATs will decrease PFOA excretion while low membrane densities or blockage of apical OATs will increase excretion because they decrease resorption of anions from the glomerular filtrate.

The earliest studies of the impact of sex on PFOA urinary excretion were conducted on male and female Holtzman rats by Hanhijarvi et al. (1982) using probenecid, an inhibitor of renal excretion of organic acids that has since been found to specifically inhibit OAT1–6 and OAT8. The female rats that had not received the probenecid excreted 76% of the administered dose of PFOA over a 7-hour period, while males excreted only 7.8% of the administered dose over the same period. The authors concluded that the female rat possesses an active secretory mechanism that rapidly eliminates PFOA from the body that male rats do not possess.

Kudo et al. (2002) examined the role of sex hormones and OATs on the renal clearance (CL_R) of PFOA. Gonadectomy alone caused an increase in CL_R of PFOA in both male and female rats (14-fold and twofold, respectively). Treatment with testosterone reduced the PFOA CL_R in castrated males and intact females. Conversely, treatment with estradiol increased the CL_R of PFOA in intact male rats but reduced that of ovariectomized female rats back to normal values. Studies by Vanden Heuvel et al. (1992) support a role for testosterone in limiting PFOA elimination in male Sprague-Dawley rats. Intact and castrated rats were administered an i.p. injection (4 mg/kg [^{14}C]PFOA). Castration increased the elimination of [^{14}C]PFOA in feces and urine. Administration of testosterone, but not estradiol reduced elimination to the same level as rats with intact testes. In female rats, neither ovariectomy nor ovariectomy plus testosterone altered PFOA urinary elimination. Early studies from Kudo et al. (2002) and Cheng et al. (2006) found that intact males were found to express less OAT2, more OATP1a1, and more OATP3a1 than their female counterparts. Castration was found to increase OAT2 and decrease OATP1a1. Ovariectomy increased OAT3 in female rats but did not affect OATP1a1, which was already virtually absence from intact female mice. Treatment with estradiol increased OAT2 in intact male rats, while 17-β estradiol decreased OATP1a1 in both castrated and ovariectomized mice but did not affect OATP3a1. Finally, treatment with testosterone increased OAT2 in castrated rats, while 5α-dihydroxy-testosterone increased both OATP1a1 and OATP3a1 in castrated and ovariectomized mice. Multiple regression analysis of the data suggested that OAT2 and OAT3 are responsible for urinary elimination of PFOA in the rat; however, the possibility of a resorption process mediated by OATP1 was mentioned as a possible factor in male rat retention of PFOA. OAT2 and OAT3 are located on the basolateral cell surface. OATP1 is located on the apical surface of the renal tubule cells (Kudo et al., 2002).

Katakura et al. (2007) examined PFOA clearance in male and female Wistar rats and in Eisai hyperbilirubinemic rats (EHBR) lacking the MRP2 transporter. Renal clearance rates were higher in female Wistar rats (12.82 ± 4.15 mL/hr/kg BW) compared with males (1.02 ± 0.59 mL/hr/kg BW). However, PFOA clearance in MRP2-deficient EHBR rats did not differ from wild-type rats, indicating MRP2 is not involved in renal transport.

As suggested by Hinderliter et al. (2006b), 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. This was evidenced by changes in measured PFOA in plasma and

urine, such that maturing females experienced decreased plasma PFOA and increased urine PFOA, while the opposite was seen in males. Taken together with previous information, the change in female rats seems to involve excretion-promoting OATs (Kudo et al., 2002) while the change in males seems to involve excretion-reducing OATPs (Cheng et al., 2006).

Numerous in vitro studies using human embryonic kidney cells (HEK 293) and Chinese hamster ovary (CHO), time- and concentration-dependent studies as well as competition studies with known transporters have been utilized to evaluate the role of various transporters in the renal excretion of PFOA. For example, Yang et al. (2010) examined cellular uptake of PFOA by OATP1A2 in CHO and HEK293 cells transfected with OATP1A2 plasmid DNA or vector DNA (control). PFOA uptake in OATP1A2-transfected HEK293 cells was no different from uptake in control cells. Uptake of estrone-3-sulfate (E3S), a known substrate of OATP1A2, was inhibited ~30% in the presence of 100 μM PFOA (C8). Inhibition varied by PFAS of different chain lengths (~62% by C9, ~70% by C10, ~42% by C11, and ~18% by C12). E3S uptake was not inhibited by C4–C7.

Other studies observed Michaelis-Menten kinetics in transporter-transfected cells compared with passive diffusion in control (vector only) cells, and several transporters have been identified as having PFOA renal transport activity, including OAT1, OAT3, OAT4, OATP1a1, and URAT1 (Yang et al., 2010; Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). Limited data suggest possible roles for OAT2 and OAT1PA2 in uptake of PFOA.

Yang et al. (2009b) investigated the role of OAT polypeptide 1a1 (OATP1a1) in PFOA uptake. In time-dependent uptake experiments using transfected CHO cells, uptake of PFOA by OATP1a1-transfected cells increased proportionally to time during the first 2 minutes of incubation. Vector-transfected cells had a significant level of uptake of PFOA attributed to nonspecific passive diffusion. In the concentration-dependent uptake experiments, while saturation levels were not reached in OATP1a1-transfected cells, active PFOA uptake could be derived from the difference between the uptake of the OATP1a1 cells and the passive diffusion of the vector-transfected cells. Given the results of the uptake and additional inhibition experiments, the authors suggested that passive diffusion could be an important route of PFOA distribution and that renal reabsorption in the male rat could be mediated by OATP1a1

Katakura et al. (2007) measured uptake of [^{14}C]PFOA in Xenopus oocytes transfected with oatp1, OAT3, and Npt2 plasmid constructs. OAT3 and oatp1, but not Npt2, enhanced PFOA transport. However, feeding rats a low phosphate diet that increases Npt2 expression in renal proximal tubules, decreased renal clearance of PFOA in both male and female rats by 50%. It was unclear whether the low phosphate diet altered expression of other transporters in that then mediate PFOA transport in the rat kidney.

In vitro studies were supported by in vivo analysis of OATPs gene and protein expression in rat kidneys (Yang et al., 2009b). OAT polypeptide 1a1 (OATP1a1), located on the apical side of proximal tubule cells and could be the mechanism for renal reabsorption of PFOA in rats. The level of mRNA of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney, OATP1a1 protein expression is higher in male rat kidneys, and it is regulated by sex hormones. One of its known substrates is estrone-3-sulfate (E3S). A substantial presence of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate and reduce excretion.

Limited evidence exists for a role for OAT and OATP1A2 in PFOA uptake. In transformed HEK 293 cells transfected with OAT 2, prostaglandin F2α uptake by OAT2 was inhibited moderately by PFOA, 75%–85% of control at 10 µmol PFOA, and 65% of control at 100 µmol PFOA (Nakagawa et al., 2008). However, in the same study, the authors observed that HEK 293 cells or S2 (cells derived from proximal tubule) transfected with OAT failed to take up radiolabeled µmol [^{14}C]PFOA. Similarly, Yang et al. (2010) observed that PFOA uptake in OATP1A2-transfected HEK293 cells was no different from uptake in control cells though they did observe inhibition of E3S uptake. At 100 µmol, E3S uptake was inhibited ~30% by PFOA (C8), ~62% by PFNA (C9), ~70% by PFDA (C10), ~42% by PFUnDA (C11), and ~18% by (PFDoDA) C12. E3S uptake was not inhibited by C4–C7 perfluorocarboxylates.

The kinetic response of the OAT1, OAT3, and OATP1a1 transporters to increasing concentrations of selected perfluorinated carboxylates was also evaluated by Weaver et al. (2010). The change in transport velocity (ng/mg protein/min) with increasing concentrations of the perfluorinated carboxylate exhibited a Michaelis-Menten-type response. The kinetic data were analyzed to determine the K_m and V_{max}, and the results are summarized in Table B-33.

Table B-33. Kinetic Parameters of Perfluorinated Carboxylate Transport by OAT1, OAT3, and OATP1a1 as Reported by Weaver et al. (2010)

Transporter	PFAS	K_m (µmol)	V_{max} (nmol/mg protein/min)
OAT1	PFHpA (C7)	50.5 ± 13.9	2.2 ± 0.2
	PFOA (C8)	43.2 ± 15.5	2.6 ± 0.3
OAT3	PFOA (C8)	65.7 ± 12.1	3.8 ± 0.5
	PFNA (C9)	174.5 ± 32.4	8.7 ± 0.7
OATP1a1	PFOA (C8)	126.4 ± 23.9	9.3 ± 1.4
	PFNA (C9)	20.5 ± 6.8	3.6 ± 0.5
	PFDA (C10)	28.5 ± 5.6	3.8 ± 0.3

Notes: K_m = Michaelis constant; OAT = Organic Anion Transporter; PFAS = Per- and polyfluoroalkyl substances; PFHpA = Perfluoroheptanoic acid; PFNA = perfluorononanoic acid; V_{max} = maximum rate of transport.

The Michaelis-Menten kinetic data (K_m and V_{max} (maximum initial rate of an enzyme catalyzed reaction)) indicate that there are substantial differences in the affinity of the perfluorinated carboxylate with 8 and 9 carbon chains for OAT3, with PFOA (C8) favored over PFNA (C9). OAT3 is an export transporter located on the basolateral side of the tubular cells; thus, when present in a mixture consisting of comparable concentrations of both, renal tubular excretion of PFOA would tend to decrease excretion of PFNA. For OATP1a1, a resorption transporter located on the apical side of the renal tubular cells, PFNA and PFDA (C10) have a greater affinity for the transport protein than PFOA. The kinetic data suggest that the net impact of these relationships would be to favor excretion of PFOA (C8) over PFNA (C9) and possibly PFDA (C10) when all three fluorocarbons are present in the exposure matrix at approximately equal concentrations. There were minimal kinetic differences between transport of PFHpA (C7) and PFOA (C8) by OAT1, an export transporter on the basolateral surface of the renal tubular cells.

Sakolish and colleagues developed a 3D microphysiological in vitro model using RPECs designated as a "kidney tubule chip" of the human proximal tubule (Sakolish et al., 2020). The kidney tubule chip results for reabsorption were combined with a physiologically based "parallel

tube model" (Janků, 1993) that was used to model overall renal clearance kinetics in humans in vivo. When compared with reported in vivo renal clearance (in vivo data were obtained from Reece et al. (1985)) the kidney tubule chip combined with a physiologically based kinetic model qualitatively and quantitatively recapitulated in vivo kinetics in the kidney.

PFOA, used as the positive control in this study, exhibited a low but measurable amount of reabsorption. The ratio of renal clearance using the combined chip and PBPK model for PFOA was estimated to be 0.40 μM at the low dose (0.01 μM) and 0.32 μM at the higher dose (1.0 μM). In contrast this ratio for creatinine (used as a negative control for resorption) was 0.54 mM and 1.17 mM for doses of 0.1 and 1.0 mM, respectively. The authors suggest the lower than expected levels of PFOA resorption may be due to one of the following factors: (1) the high degree of protein binding of PFOA in vivo actually is the primary driver of slow renal clearance as long as the unbound fraction is ≤0.01, with reabsorption contributing to a lesser degree; (2) the lack of a vascular channel in the tissue chip limits resorption (e.g., tubular secretion is not accounted for); and (3) basal OAT4 expression in the RPTECs used in the PFOA experiments was relatively low based on immunohistochemistry observations (Sakolish et al., 2020).

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. Males have a higher rate of resorption than females for the smaller amount they can transport into the glomerular filtrate via OATP1a1 in the apical membrane.

Much work remains to be done to explain the sex differences between male and female rats and to determine whether it is relevant to humans. The broad range of half-lives in human epidemiology studies suggests a variability in the unbound fraction of PFOA in serum or in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variations in human OATs and OATPs are described in a review by Zaïr et al. (2008).

Cheng and Ng (2017) attempted to incorporate transport functions into a PBPK model by including parameters for PFOA cellular uptake and efflux via both passive diffusion and transport. Specific parameters included uptake into hepatocytes mediated by Na+/taurocholate cotransporting polypeptide (Ntcp) and renal efflux by organic solute transporter (Ost). This model also included a number of additional mechanistic parameters, including association with serum albumin in circulatory and extracellular spaces and association with intracellular proteins in liver and kidney. All the PFOA-related parameters used in the model were derived from in vitro assays. Model results were compared with experimental data from several studies (Kim et al., 2016b; Kudo et al., 2007; Kemper, 2003). This model was moderately successful in predicting toxicokinetics and tissue distribution of PFOA in rats, although several outputs either over- or under-estimated data relative to experimental observations. Also, there are limitations related to the quantity and quality of in vitro-derived inputs. Nevertheless, further refinements of this or related models may provide new insights into the roles of specific transporters and serum and tissue binding proteins in PFOA toxicokinetics and may further eliminate the role of transporters in mediating sex-specific elimination rates.

B.4.2.2 Enterohepatic Resorption

In animals, the impact of PFOA on several membrane transporter systems linked to biliary transport was studied by Maher et al. (2008) as part of a more detailed study of PFDA. A dose of 80 mg/kg by intraperitoneal (IP) injection (propylene glycol: water vehicle) was found to significantly increase ($p < 0.05$) the expression of MRP3 and MRP4 in the livers of C57BL/6 mice 2 days after treatment. MRP3 and MRP4 are believed to protect the liver from accumulation of bile acids, bilirubin, and potentially toxic exogenous substances by promoting their excretion in bile. There were significant increases in serum bilirubin and bile acids after PFDA exposure, signifying increased export. Conversely, Western Blot analysis and messenger ribonucleic acid (mRNA) measurements showed significant decreases ($p < 0.05$) in the protein levels for OATP1a1, OATP1a4, and OATP1b2 following exposure to 40 mg PFOA/kg (Cheng and Klaassen, 2008). There was no significant impact on NTCP protein or the serum levels of bile acids. The OATPs are transporters responsible for the uptake of bile acids and other hydrophobic substances such as steroid conjugates, ecosinoids, and thyroid hormones into the liver.

These studies, all by the same laboratory, were carried out at high, single-dose exposures, which limit their value in extrapolating to low- and repeat-dose scenarios. The results suggest a decrease in the uptake of favored substrates into the liver and an increase in removal of favored substrates from the liver via bile. Upregulation of MRP3 and MRP4, coupled with decreased OATp levels, could be beneficial due to increased biliary excretion of bile acids, bilirubin, and potentially toxic exogenous substances, including PFOA. Given the results with the more extensive evaluation of PFDA including mouse strains null for several receptors (PPARα, constitutive androstane receptor (CAR), pregnane X receptor (PXR), and farnesoid X receptor (FXR)), the authors concluded that the changes in receptor proteins were primarily linked to activation of PPARα.

Gastrointestinal elimination of PFOA was reported in a case history of a single human male with high serum levels of perfluorinated chemicals that 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, his serum PFOA concentration lowered from 5.9 ng/g serum to 4.1 ng/g serum 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.

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 CHO and HEK-293 cells transfected with transporter cDNA, as well as CHO Flp-In cells expressing human OATP2B, and compared with wild-type control cells transfected with vector only. 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). The authors suggest that these results may relate to the longer serum elimination half-lives of these 2 PFCAs.

In summary, relatively few studies have investigated resorption through enterohepatic routes. The transporters involved in PFOA resorption through these routes may include MRP3 and MRP4 as well as OATP1A1, OATP1A4, OATP1B1, OATP1B2, OAT2B1, and OAT1B3. Preliminary evidence suggests 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.

B.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

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 B.4.4, females clearly eliminate PFOA through routes not available to males.

The total daily elimination of PFOA in pregnant females was estimated to be 11.4 ng/day, lower than the 30.1 ng/day estimated for PFOS (Zhang and Qin, 2014). The distribution of PFOA from maternal serum to the fetus and infants is discussed in detail above (Section. B.2.4). A study by Zhang et al. (2013b) exemplifies the routes and amounts of PFOA eliminated by pregnant females. Paired maternal whole blood and cord blood samples were analyzed from 32 females from Tianjin, China. The maternal blood concentration of PFOA was 3.35 ng/mL. 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. Thus, pregnant females may eliminate PFOA through cord blood, placenta, and amniotic fluids. Blood loss during childbirth could be another source of excretion.

The elimination of PFOA in pregnant women corresponds to an increase in concentrations in the placenta. Mamsen et al. (2019) observed an increase in PFOA accumulation from gestational age 50 to 300 days, with male placentas showing higher levels of than female placentas. The authors estimated a placenta PFOA accumulation rate of 0.11% increase per day during gestation.

Mamsen and colleagues measured placental samples and fetal tissues in relation to maternal plasma levels of 5 PFAS in 39 Danish women who underwent legal termination of pregnancy before gestational week 12 (Mamsen et al., 2017). All PFAS were transferred from mother to fetus albeit with different efficiencies and a significant positive correlation was observed for fetal age (exposure duration) and for fetal:maternal plasma ratios for all PFAS compounds. Fetal organ levels of PFOA were lower than maternal blood. The average concentration of PFOA was 0.17 ng/g in fetal tissues compared with 0.23 ng/g in placenta and 2.1 ng/g in maternal plasma. The increasing fetal PFOA level with fetal age finding suggest that the rate of elimination of PFAS from mother to fetus may increase through the gestational period.

The same group (Mamsen et al., 2019) measured PFOA accumulation in fetal tissues across the three trimesters from 78 pregnant women who underwent elective pregnancy terminations and from cases of IUFD. Fetal tissues (placenta, liver, lung, heart, CNS and adipose) were collected for 38 first-trimester pregnancies, 18 second-trimester pregnancies and 22 third-trimester pregnancies. PFOA was above LOQ in 100% of maternal serum samples, in 82% of placenta samples and 70% of fetal organs. In general, the concentrations of PFOA in fetal tissue increased from first trimester to third trimester except for liver and heart which showed highest levels in the second trimester compared with the third trimester. Analysis of the placenta:serum ratio of PFOA revealed a 5.6% higher ratio in male fetuses than in female fetuses ($p < 0.05$). These studies support the placenta and fetus as important routes of PFOA elimination in pregnant women and suggests that the magnitude of elimination may be influenced by the sex of the fetus.

Underscoring the importance of pregnancy as a lifestage when excretion is altered, Zhang et al. (2015b) observed that the partitioning ratio of PFOA concentrations between urine and whole blood in pregnant women (0.0011) was significantly lower ($p = 0.017$) than the ratios found in nonpregnant women (0.0028) and may be affected by the increase in blood volume during pregnancy (Pritchard, 1965).

After birth, women can also eliminate PFOA via lactation. Tao and colleagues (2008) measured 45 human breast milk samples collected in 2004 from Massachusetts and PFOS (mean 131 ng/L) and PFOA (mean 43.8 ng/L) were the predominant PFAS compounds measured. Elimination through breast was more recently measured in 293 samples collected from 127 mothers in the Children's Health and Environmental Chemicals in Korea (CHECK) Cohort (Lee et al., 2017). Results were stratified by age, parity, body mass, delivery method, and infant sex. The median PFOA concentrations in breast milk across all samples was 38.5 ng/L (range of 25.1–61.5 ng/L) and the median concentration for all PFAS chemicals measured was 151 ng/L (range of 105–212 ng/L). Only PFOS concentrations were higher than PFOA with a median concentration of 47.4 ng/L (36.4–63.8 ng/L).

In this study, pooled breast milk samples were measured to follow the time course of PFOA in breast milk after birth. Concentrations in breast milk measured 30 days after birth were significantly higher (ANOVA, $p < 0.05$) than those measured prior to 7 days after birth. These findings contrast with results of other studies. Thomsen et al. (2010) reported that breast milk levels of PFOA and PFOS decreased by 7% and 3.1%, respectively, during the first month after birth. PFOA levels significantly decreased in breast milk over a 4-month lactation period (Kang et al., 2016). Demographic factors, maternal diets, sample sizes, the lactational periods measured may account for these discrepancies.

Lower PFOA levels in the breast milk of multiparous women provides further evidence for pregnancy and lactation as elimination pathways. Lee and colleagues (2017) observed that primiparous mothers showed higher levels of PFOA in breast milk with a median concentration of 46.0 ng/L compared with 33.4 ng/L for mothers giving birth to more than one child ($p < 0.05$). In another study, multivariable models estimated that parous women had 40% lower PFOS (95% CI: −56 to −17%) and 40% lower PFOA (95% CI: −54, −23%) concentrations compared with nulliparous women (Jusko et al., 2016). These authors also measured concentrations in colostrum. The geometric mean concentration in was 35.3 ng/L for PFOS and 32.8 ng/L for PFOA.

PFOA was also measured in maternal serum, cord serum and breast milk from 102 female volunteers hospitalized between June 2010 and January 2013 for planned cesarean delivery in Toulouse, France (Cariou et al., 2015). Mean PFOA concentrations were 1.22, 0.9191 and 0.041 ng/mL in maternal serum, cord serum and breast milk, respectively. The observed ratios of cord and maternal serum for PFOA was 0.78 in this study. However, the ratio between breast milk and maternal serum was 0.038 ± 0.013 suggesting a low transfer from maternal blood to breast milk relative to maternal blood to cord blood.

Studies in animals support elimination through pregnancy and lactation observed in humans. Fujii and colleagues (2020) used the M/P concentration ratio as a measure of chemical transferability in FVB/NJcl mice. On PND 8 to PND 13, dams ($n = 12$) were given a single administration of PFOA by tail vein injection (3.13 μmol/kg). To facilitate milking, dams were

administered 4.0 U/kg oxytocin and milk was collected from all dams by aspirating with pulsations using a novel apparatus. After milking, maternal blood was collected to obtain plasma. Maternal plasma PFOA concentrations were significantly higher than milk (13.78 vs. 4.38 μmol/L, $P < 0.05$) and the M/P ratios was 0.32. The M/P ratios were similar for PFOA (C8), PFNA (C9), PFDoDA (C12), and PFTriDA (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 age.

B.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 clearance rate 0.029 mL/day/kg. The link between menstruation and PFOA elimination is based on several observations. First, males and older females have longer PFOA elimination half-lives than young females (i.e., females of childbearing age) (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 examined the 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 this data (Ruark et al., 2017) suggested that this association could be explained by reversed causality and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Furthermore, Lorber et al. (2015) compared individuals who had undergone blood removal treatments for medical reasons to menstruating females. Measurements showed lower PFOA and PFOS concentrations in the groups experiencing regular blood loss. Estimated concentrations based on a one-compartment model were consistent with measured serum concentrations. Overall, this study provides data and modeling that support the initial hypothesis that ongoing blood loss explains lower PFAA concentrations in humans. These authors 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. Curiously, studies providing direct measurements of PFOA in menstrual blood were not identified. However, for PFOA 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) examined the possibility that hair could be a potential route of PFAS elimination. They exposed adult male and female Wistar rats to 0, 0.05, 0.5, and 5 mg/L of PFOA, PFNA, and PFOS via drinking water for 90 days. The hair samples were cleaned, sonicated, dried, and alkaline digested to extract PFAAs. PFOA, PFNA, and PFOS were detected in all the hair samples of treated groups. A dose-dependent increase in hair PFOA concentration was observed in all exposed animals. The mean hair concentrations of PFOA ranged from 3.31 to 444 ng/g, suggesting that hair may be a potential route for PFOA elimination. Interestingly, the 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.

Gao et al. (2015) also measured the composition of the mixture excreted in in urine, feces and hair after administration of 0.5 or 0.05 mg/L. As summarized in Table B-34, at the lower dose of 0.05 mg/mL, PFOA was not detected in urine of males, and made up a smaller proportion of total mixture excreted in hair but not feces. In females however, PFOA was the predominant constituent excreted in urine, but made up the minority constituent excreted in feces and especially in hair. These findings underscore the impact of mixtures and sex on PFOA excretion.

Table B-34. Estimated Percentage of the Sum of PFOS, PFNA, and PFOA in Excreta and Serum of Male and Female Wistar Rats[a] as Reported by Gao et al. (2015)

Sex	PFAA	Serum	Urine	Feces	Hair
Males	PFOS	24.6	89.0	20.8	30.0
	PFNA	59.9	11.0	53.0	45.4
	PFOA	15.6	ND	26.1	24.6
Females	PFOS	89.0	ND	62.4	78.0
	PFNA	11.0	38.9	21.7	18.0
	PFOA	ND	61.1	16.1	4.2

Notes: ND = not detected; PFAA = perfluoroalkyl acids; PFNA = perfluorononanoic acid; PFOS = Perfluorooctanesulfonic acid.
[a] Data are presented in % total PFAAs administered. Animals exposed to 0.05 mg/L in Gao et al. (Gao et al., 2015)

Excretion of PFOA through sweat was measured in one study (Genuis et al., 2013). Sweat samples were collected during sauna or exercise from 20 human adult subjects. While another chemical class was readily detected in sweat (polychlorinated biphenyls (PCBs)) no appreciable levels of PFOA or other PFAS chemicals investigated were detected in sweat despite their detection in serum. The authors conclude that sweating does not facilitate clearance of PFHxS, PFOS, or PFOA. In a case report study (Genuis et al., 2010), excretion through sweat was also measured in a single male subject exposed to perfluorinated chemicals via inhalation exposure and subjected to treatment with bile sequestrants. With the exception of PFHxS, no other PFAS chemicals, including PFOA, were detected in sweat.

Thus far, no single study has conducted a comparative analysis of elimination of PFOA through all possible routes of excretion. A comprehensive analysis stratified by age and sex would be necessary to advance the understanding PFOA excretion by all possible routes, and to establish factors that influence the proportion of PFOA excreted through urine versus other excreta matrices.

B.4.5 Half-life Data

B.4.5.1 Overview

In general a half-life represents elimination by all routes, which includes metabolism for other chemicals, but because PFOA/PFOS are not metabolized, it can be interpreted for excretion (after correction for BW changes). The calculation of PFOA half-lives reported in the literature vary considerably, which poses challenges in predicting both the routes and rates of excretion.

Several interrelated physiological and mechanistic factors impacting excretion are summarized here:

- The capacity of PFOA to be reabsorbed via renal and enterohepatic routes of excretion and binding affinities to relevant transporters including OATs, OATPs, MRPs, and sodium-dependent transporters involved in bile acid transport including NTCP and the apical sodium-dependent bile acid transporter. Exposures to high levels of PFOA under acute conditions (e.g., contaminated drinking water) or in occupational settings may result in saturation of resorption transporters and increased excretion.
- Binding affinity to serum proteins may limit the concentration of the unbound fraction available for resorption through renal or enterohepatic transporters. Moreover, binding to serum proteins may limit passive diffusion of perfluorinated chemicals across the placental barrier.
- Phospholipid lipid binding affinity (phospholipophilicity) can further reduce the unbound fraction of PFOA as well as uptake into cells. As reported by Sanchez Garcia et al. (2018), phospholipophilicity shows the highest correlation to cellular accumulation data compared with other measures of lipophilicity, raising the possibility that phospholipid binding affinity could distinguish between high and low accumulating compounds as well as half-life measures.
- Chain length and branching. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains. Interactions with transporters also vary by chain length.
- Exposure to mixtures of perfluorinated compounds with differential binding affinities to transporters, serum binding proteins and phospholipids could impact both the rate and route of PFOA excretion.
- Sex and species can influence both the rate and route excretion. First, several elimination pathways are specific to females including menstruation, pregnancy, and lactation. Second, sex-specific hormones can impact expression of transporters involved in resorption. Furthermore, elimination half-lives vary dramatically by species, with much longer half-lives calculated in humans compared with animals.

B.4.5.2 Human Studies

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. Using a linear mixed model, Bartell et al. (2010) determined an average half-life of 2.3 years based on a study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District in Washington, West Virginia, and the Little Hocking Water Association (LHWA) in Ohio.

The results of this assessment showed a 26% decrease in PFOA concentration per year after adjustment for covariates and a half-life of 2.3 years (confidence interval (CI) = 2.1–2.4). The only potential confounders determined to be significant were the treatment plant ($p = 0.03$) and homegrown vegetable consumption ($p < 0.001$). This confounder, as well as changes in the source of drinking water during the study could also have impacted the results.

In another study, the drinking water supply was contaminated with a mixture of perfluorinated chemicals when a soil-improver mixed with industrial waste was applied upriver to agricultural lands in Arnsberg, Germany (Brede et al., 2010). The PFOA levels in the finished drinking water were measured as 500–640 ng/L in 2006. PFOS and PFHxS were also present. The estimate for the human half-life was 3.26 years (geometric mean; range 1.03–14.67 years). Regression analysis of the data also suggested that the elimination rate might have been greater in younger subjects and older subjects.

Seals et al. (2011) determined half-life estimates for 602 residents of Little Hocking, Ohio, and 971 residents of Lubeck, West Virginia, who were part of the C8 study but had relocated to a different area of the country. The half-life estimates for Little Hocking ranged from 2.5 to 3.0 years (average 2.9 years) and for Lubeck ranged from 5.9 to 10.3 years (average 8.5 years).

Given their analysis, the authors suggested that, if their assumptions were correct, a simple first-order elimination model might not be appropriate for PFOA given that the rate of elimination appeared to be influenced by both concentration and time. There was a difference in the CL for the two locations even though the range of years elapsed since relocation was the same for both communities. The authors identified three potential limitations of their analysis: the cross-sectional design, the assumption that exposure was uniform within a water district, and a potential bias introduced by exclusion of individuals with serum values <15 ng/mL.

3M (3M, 2002, 2000) conducted a half-life study on 26 retired fluorochemical production workers from their Decatur, Alabama, (n = 24) and Cottage Grove, Minnesota, (n = 3) plants. The mean serum elimination half-life of PFOA in these workers was 3.8 years (1,378 days; 95% CI: 1,131, 1,624 days) and the median was 3.5 years (Olsen et al., 2005). No association was reported between the serum elimination half-life and with initial PFOA concentrations, age, or sex of the retirees, the number of years retired or working at the production facility, or medication use or health conditions.

Harada et al. (2005) studied the relationship between age, sex, and serum PFOA concentration in residents of Kyoto, Japan. They found that females in the 20–50-year-old age group (all with regular menstrual cycles) had serum PFOA concentrations that were significantly lower than those in females over age 50 (all post-menopausal). Harada et al. (2005) also estimated the CL_R rate of PFOA in humans and found it to be only about 0.001% of the GFR. There was no significant difference in CL_R of PFOA with respect to sex or age group, and the mean value was 0.03 ± 0.013 mL/day/kg.

Zhang et al. (2013c) determined half-lives for PFOA isomers based on paired serum samples and early morning urine samples collected from healthy volunteers in two large Chinese cities. Half-lives were determined using a one-compartment model and an assumption of first-order CL. The mean half-life for the sum of all PFOA isomers in younger females (n = 12) was 2.1 years (range 0.19–5.2 years) while that for all males and older females (n = 31) was 2.6 (range 0.0059–14 years); the medians were 1.8 and 1.7 years, respectively. The mean values for the four branched-chain isomers of PFOA were lower than the value for the linear chain, suggesting that resorption transporters might favor uptake of the linear chain over the branched-chain isomers. Older females and males have longer half-lives than young females, suggesting the importance of monthly menstruation as a pathway for excretion (Zhang et al., 2013c).

The rate of serum PFOA decline was measured in residents of two communities exposed to contaminated municipal drinking water contaminated in Bleking County, Sweden in 2013 (Li et al., 2018b). A biomonitoring program ensued between 2014 and 2016 for residents exposed to contaminated water and an unexposed community. A subset of residents (ager range of 15–50 year) were included in a panel study to estimate PFOA half-lives. Drinking water PFOA levels were 100 ng/L prior to closure of the waterworks facility and 1.0 ng/L in the unexposed community. The mean serum levels among the 106 participants 6 months after the end of exposure was 21.1 ± 14.7 ng/mL. The average decrease in PFOA was 26% of its previous value each year. The excretion rate constant after the end of exposure was 0.26 (95% CI: 0.24, 0.28) and was higher in females (0.29) than males (0.25) but this did not reach significance. The mean half-life was 2.7 years and was also shorter in females (2.4 years) than in males (2.8 years). There was a high level of inter-individual variation in half-lives.

Fu et al. (2016) determined the half-live of PFOA in 302 occupational workers from one of the largest producers of PFOS-related compounds in China. The half-lives of PFAAs in workers were estimated by daily clearance rates and annual decline rates of PFAAs in serum by a first-order model based on fasting blood and urine samples collected over a period of five years. Mean and median urine concentrations for PFOA among all workers were 4.3 and 1.9 ng/mL, respectively, whereas in serum, mean and median PFOA were 1,052 and 427 ng/mL. The renal clearance rate for PFOA ranged from 0.00009 to 2.4 mL/kg/day (geometric mean of 0.067 mg/kg/day).

Half-lives were calculated by Ln2/k using two approaches. In the first approach, k was defined as Cl_{total}/V_d, where V_d stands for the volume of distribution of PFAAs in the human body and Cl_{total} represents the total daily PFAAs clearance in the human body. Cl_{total} was defined as renal clearance for men and women older than 50, and as the sum of menstrual and renal clearance in young women. V_d of PFOA was set at 170 mL kg−1 and 230 mL kg−1 for PFOS. In the second approach, k was defined as the average annual decline rates of PFAAs In workers who participated in this study.

The half-life of PFOA estimated using daily clearance rate was 4.1 years (geometric mean value) and 4.0 years (geometric median value). However, when measured by annual decline rate, the half-life of PFOA was estimated to be 1.7 years. The geometric mean values of the half-lives of PFOA and PFOS for men here were 4.7 and 60.9 years (range 0.44–3,663 years), respectively, while those in females were 3.1 and 8.0 years (range 0.76–30,475 years). The authors suggest that half-lives estimated by the limited clearance route information could be considered as the upper limits for PFAAs and that the unrealistically long half-lives determined using urine clearance values may indicate that other clearance play important roles in elimination of PFAAs in humans including fecal elimination. Another possibility is that the apparent half-lives of PFAAs calculated through annual decline rates could be affected by the high ongoing levels of exposure.

Worley and colleagues (2017) calculated PFOA half-lives in subjects living near a PFAS manufacturer in Alabama that had discharged waste into a local wastewater treatment plant. Sewage sludge from this plant was applied to local agricultural fields. In 2010, ATSDR collected blood samples from subjects and followed up with blood and urine measurements in 2016. Biological half-lives were estimated for PFOA using a one-compartment pharmacokinetic model.

Geometric mean serum PFOA concentrations were significantly higher in subjects ($p \leq 0.0001$) in both 2010 (16.3 ng/L) and 2016 (11.7 ng/L) relative to national averages reported by NHANES (3.07 ng/L in 2009–2010 and 1.94 ng/L in 2013–2014). Interestingly, the authors observed a non-significant relationship between PFOA serum and urine concentrations in women ($n = 23$, Pearson's $r = 0.35$) and a significant strong linear relationship in men ($n = 22$, Pearson's $r = 0.75$).

The half-life for PFOS was estimated to be 3.3 years, similar to the 3.9 years estimated for PFOA. For these calculations, the V_d values were scaled to bodyweight (values of 170 mL/kg bodyweight for PFOA and 230 mL/kg bodyweight for PFOS were assigned) When the authors varied the V_d and intake values by 20%, half-life values varied by several months (half-life estimates for PFOS ranged from 3.0 to 3.6 years). The authors suggest these parameters have a significant impact on half-life estimates.

Xu et al. (2020c) estimated the half-life of PFAS by sampling urine (4 times) and blood (5 times) from 26 airport employees between 2 weeks to 5 months after the end of a 2-month exposure to PFAS-contaminated drinking water. The levels of PFOA in the airport's contaminated water were about 1,000 times higher than those in the municipal communities (300 ng/L at airport vs. 0.3 ng/L in municipal water). Specific gravity adjusted urine median PFOA concentrations were PFOA was 0.031 ng/mL, with a range of 0.010–0.13 ng/mL as determined from the second to the fifth sampling periods.

The median PFOA concentration in the first serum sample taken from all 26 employees was 9.1 ng/mL and the serum/water ratio was reported as 30. PFOA median concentrations measured in paired serum and urine samples obtained from the second to the fifth sampling were reported as 10 ng/mL and 0.031 ng/mL respectively with an average urine/serum ratio of 0.0032. The significant difference between the serum/water ratio and the urine/serum ratio is suggestive of the influence of the clearance rate on the overall serum levels (lower the clearance rate and higher serum levels correlate to longer the half-lives). Similar to Fu and colleagues (2016), the half-life of PFOA was estimated as 1.77 years.

Gomis et al. (2016) examined the contribution of direct uptake of PFOA compared with indirect uptake of 8:2 fluorotelomer alcohol (FTOH) and metabolism investigated using a dynamic one-compartment pharmacokinetic (PK) model applied to six ski wax technicians. This model estimated an average intrinsic elimination PFOA half-life of 2.4 years (1.8−3.1 years accounting for variation between technicians and model uncertainty).

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., 2017; Fu et al., 2016; Zhang et al., 2013d) (Table B-35). PFOA half-life values among these four 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 non-urinary 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., 2017). 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.

Table B-35. Summary of PFOA Concentration in Blood and Urine in Relation to Half-life Values in Humans

Study	Number of Subjects	Age Range	Primary Exposure Route	Exposure	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half-Life	Considerations
Xu et al. (2020c)[a]	26 19 Males 7 Females	22–62 yr	Oral, drinking water	210 ng/L (linear) 88 ng/L (branched) 300 ng/L Total**	median: 10 ng/mL (4.1–28 ng/mL)	median: 0.031 ng/mL range: 0.010–13 ng/mL (not creatinine adjusted)	1.77 yr	• 1 woman was previously pregnant 2018 during sampling year • PFOA also measured in the private well of one airport employee living near the airport (PFOA concentration in well was lower than the airport at 0.53 ng/L linear and <0.3 ng/L branched)
Worley et al. (2017)	153 (2010) 63 males 90 females 45 (2016) 22 males 23 females	2010: mean 52.0 2016: mean 62.6	Oral, drinking water	NR	2010: GM[1] 16.3 ng/mL (13.2–19.6 95% CI) 2016: GM 11.7 ng/mL (8.7–14.6, 95% CI)	2016 Creatinine adjusted: mean 0.031 ng PFAS/g creatinine median 0.024)[b] 2016 not adjusted for creatinine: mean 0.027 ng/mL median 0.022 ng/mL	3.9 yr	• LOD was 0.01 µg/L, detection rate 95.6% • Clearance rate was not reported
Fu et al. (2016)	302 213 males 89 females	Males: 19–65 median 41 Females: 19–50 median 37	Occupational	NR	mean: 1,052 ng/mL median 427 ng/mL, (2.5–32,000 ng/mL)	mean: 4.3 ng/mL median 1.9 ng/mL (LOD-53.6 ng/mL) (not creatinine adjusted)	Male: 4.7 yr Females: 3.1 yr Overall: 4.1 yr	• Urinary samples were only taken from 274 participants while there were serum samples for every participant • For half -life calculation for females, menstrual clearance was added to renal clearance •Clearance rate for PFOA = 0.062 mL/kg-day
Zhang et al. (2013c)	86 47 males 37 females	22–68	Unspecified	NR	mean 3.1 ng/mL median 2.3 ng/mL (0.26–29 ng/mL)	mean 122 ng/g creatinine median 23 ng/g creatinine,	Young females: 2.1 yr Males and older females: 2.6 yr	• All participants had paired (whole blood/serum and urine). For young females, menstrual clearance was

Study	Number of Subjects	Age Range	Primary Exposure Route	Exposure	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half-Life	Considerations
						(3.5–1869 ng/g creatinine)		estimated and added to renal clearance. • Renal clearance rate for total PFOA: mean 0.30 mL/day/kg (young female), 0.77 mL/day/kg (male and older) female)

Notes: CI = confidence interval; GM = geometric mean; LOD = limit of detection; NR = not reported; yr = years.

[a] Measured concentrations in drinking water at airport before and after mitigation measures. Authors state, "The geometric mean and median value for PFHxS, PFOA, and PFOS were 14.7 and 11.7, 4.1 and 4.0, 32.6 and 21.6 years, respectively, by the daily clearance rates, and they were 3.6, 1.7, and 1.9 years estimated by annual decline rates. The half-lives estimated by the limited clearance route information could be considered as the upper limits for PFAAs, however, the huge difference between two estimated approaches indicated that there were other important elimination pathways of PFAAs other than renal clearance in human."

[b] ng/g reported in methods but in results reported as μg/g creatinine.

All human PFOA half-life values identified in the recent literature review are provided in Table B-36. PFOA half-life values fell within a range from 0.53 years for a branched PFOA in young females (Zhang et al., 2013c) to 22 years in a study of primiparous women in Sweden (Glynn et al., 2012). Second, half-life values varied by geographical region. Using a population model, Gomis et al. (2017) derived shorter half-life values for Americans relative to Australians. Because elimination should be the same at the population level, this variation may reflect the shorter time frame of biomonitoring data in Australia relative to the NHANES dataset. Third, age and sex difference in PFOA half-lives have not been rigorously evaluated, though estimates in males are generally longer than those in females (Li et al., 2018b; Gomis et al., 2017; Fu et al., 2016) and exhibit an age-related increase (Genuis et al., 2014; Zhang et al., 2013c). While most studies were conducted in adults and/or adolescents, at least one study examined PFOA half-lives in a Newborn Screening Programs (Spliethoff et al., 2008). Whole blood was collected as dried spots on filter paper from almost all infants born in the United States. One hundred and ten of the NSPs collected in the state of New York from infants born between 1997 and 2007 were analyzed for PFOA. The study authors determined the half-life of PFOA using the regression slopes for natural log blood concentrations versus the year 2000 and after. The calculated half-life for PFOA was 4.4 years. Fourth, linear isomers exhibit longer half-lives than branched isomers (Zhang et al., 2013c).

Table B-36. Summary of Human PFOA Half-Life Values

Study	Number of Subjects	Age Range[a]	Estimated Half-Life (years)	Subjects
Bartell et al. (2010)	200 100 males 100 females	54.5 ± 15	2.3 yr	Study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District in Washington, West Virginia, and the Little Hocking Water Association (LHWA) in Ohio. Source waters for these systems had become contaminated with PFAS from the DuPont Works Plant in Washington, West Virginia, between 1951 and 2000.
Brede et al. (2010)	20 children 22 adult females 23 adult males	Children: 7.4–8.3 Females: 27–49 Males: 32–71	3.26 yr	Subjects exposed to contaminated drinking water supply in Arnsberg, Germany.
3M (2002)	9 7 males 2 females	61 (55–64)	4.37 yr (range 1.50 to 14.49 yr)	Second interim report with nine retired fluorochemical production workers from the 3M Decatur, Alabama.
Costa et al. (2009)	53 males	20–63	5.1 yr (range 2.6–9.7 yr)	53 males working in a PFAA production facility in Italy from 1978 to 2007.
Fu et al. (2016)	302 213 males 89 females	Males: 19–65 median 41	based on daily clearance rate Male: 4.7 yr	Occupationally exposed subjects working in one of the largest fluorochemical plants (Henxin

Study	Number of Subjects	Age Range[a]	Estimated Half-Life (years)	Subjects
		Females: 19–50 median 37	Females: 3.1 yr Overall: 4.1 yr based on annual decline rate Overall: 1.7 yr	Chemical Plant) in Yingcheng, Hubei province, China.
Genuis et al. (2014)	53 Father 47 Mother 22 first male child 19 second female child 17 third male child 16 fourth male child 3	16–53	Father: 2.61 Mother: 2.61 First male child: 2.03 Second female child: 1.85 Third male child: 1.80 Fourth male child: 1.59	A family (6 patients) identified to have elevated serum concentrations of PFAAs, likely through repeated commercial spraying of their home carpets with stain-repellents. Patients were treated by intermittent phlebotomy over a 4–5-yr period.
Glynn et al. (2012)	413 females	19–41	22 yr	Primiparous women 3 wk after delivery in Uppsala County, Sweden 1996−2010 (the POPUP study; Persistent Organic Pollutants in Uppsala Primiparas).
Gomis et al. (2016)	6	35–60	2.4 yr	six occupationally exposed ski waxers for whom direct and indirect exposures via inhalation were characterized.
Gomis et al. (2017)	Australia: A total of 24–84 pools per survey containing between 30 and 100 individual samples. USA: 2,000 individuals were sampled throughout the USA	12 + (USA) <16–>60 (Australia)	Australian men: 2 yr American men: 2.4 yr Australian women: 1.8 yr American women: 2.1 yr	Population based model using Australian biomonitoring studies from 2009–2014 (Toms et al. 2014, 2009) and the National Health and Nutrition Survey (NHANES) from 2003–2011 in the USA. A total of 24–84 pools per survey were obtained, with each pool containing between 30 (2007) and up to 100 individual samples (2003, 2009 and 2011) Study reports intrinsic elimination half-lives.
Li et al. (2018b)	50 Males: 20 Females 30	15–50	Males: 2.8 yr Females: 2.4 yr	Subjects in Ronneby, Sweden, exposed to contaminated water through a municipal water source.
Seals et al. (2011)	602 residents of Little Hocking OH: 602 Lubeck WV: 971	<20 20–29 30–39 40–49 50–59 60–69 >70	2.9 yr (Little Hocking) 8.5 yr (Lubeck)	602 residents of Little Hocking, Ohio, and 971 residents of Lubeck, West Virginia, who were part of the C8 study but had relocated to a different area of the country.
Splitehoff et al. (2008)	240	Newborn infant (1–2 d)	4.4 yr	New York State newborn screening program bloodspot specimens from newborn infants.

Study	Number of Subjects	Age Range[a]	Estimated Half-Life (years)	Subjects
Worley et al. (2017)	153 (2010) 63 males 90 females 45 (2016) 22 males 23 females	2010: mean 52.0 2016: mean 62.6	3.9 yr	Residentially exposed population from Lawrence, Morgan and Limestone Counties, Alabama recruited by ATSDR.
Xu et al. (2020c)	26 19 males 7 females	22–62 yr	1.77 yr	Subjects in Arvidsjaur, Sweden exposed to contaminated drinking water occupationally (working at the airport) and through residential drinking water.
Zhang et al. (2013c)	86 47 males 37 females	22–68	Young females: 2.1 yr Males and older Females: 2.6 yr n-PFOA young females: 2.3 males and older females: 2.8 iso-PFOA young females: 1.4 males and older females: 2.5 4m-PFOA young females: 0.64 males and older females: 1.4 5m-PFOA young females: 0.53 males and older females: 1.3	Healthy volunteers in Shijiazhuang and Handan, Hebei province, China, in April−May 2010.

Notes: LHWA = Little Hocking Water Association; NHANES = National Health and Nutrition Examination Survey; OH = Ohio; PFAA = perfluoroalkyl acids; PFAS = per- and polyfluorinated alkyl substances; PFOA = perfluorooctanoic acid; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; USA = United States of Amercia; WV = West Virginia; yr = years.

[a] Data on age range presented in years (mean ± standard deviation, where applicable).

B.4.5.3 Animal Studies

B.4.5.3.1 Nonhuman Primates

Butenhoff et al. (2004b) looked at the elimination half-life in monkeys treated for 6 months with 0, 3, 10, and 20 mg/kg/day via capsules. Elimination of PFOA from serum after cessation of dosing was monitored in recovery monkeys from the 10 and 20 mg/kg dose groups. For the two monkeys exposed to 10 mg/kg, serum PFOA elimination half-life was 19.5 ($r^2 = 0.98$) days and indicated first-order elimination kinetics. For three monkeys exposed to 20 mg/kg, serum PFOA elimination half-life was 20.8 days ($r^2 = 0.82$) and also indicated first-order elimination kinetics, although dosing was suspended at different time points because of weight loss.

B.4.5.3.2 Rats

Kemper (2003) examined the plasma concentration profile of PFOA following gavage administration in sexually mature Sprague-Dawley rats. Male and female rats (four per sex per group) were administered single doses of PFOA by gavage at DRs of 0.1, 1, 5, and 25 mg PFOA/kg. After dosing, plasma was collected for 22 days in males and 5 days in females.

Plasma concentration versus time data were then analyzed using noncompartmental PK methods (Table B-37, Table B-38). To further characterize plasma elimination kinetics, animals were given oral PFOA at a rate of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (extended time).

Plasma elimination curves were linear with respect to time in male rats at all dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. To further characterize plasma elimination kinetics, particularly in male rats, animals were given oral PFOA at a dose of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (2,016 hours in males). The estimated plasma elimination half-life in this experiment was approximately 277 hours (11.5 days) in male rats.

Plasma elimination curves were biphasic in females at the 5 mg/kg and 25 mg/kg dose levels. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. The estimated plasma elimination half-life in the extended time experiment was approximately 3.4 hours in females. Kemper et al. (2003) reported half-lives of 6–8 days for male Sprague-Dawley rats (Table B-37) and 3–16 hours for females (Table B-38).

Table B-37. PK Parameters in Male Sprague-Dawley Rats Following Administration of PFOA as Reported by Kemper et al. (2003)

Parameter	Dose					
	0.1 mg/kg	**1 mg/kg**	**5 mg/kg**	**25 mg/kg**	**1 mg/kg (IV)**	**0.1 mg/kg Extended Time**
T_{max} (hr)	10.25 (6.45)	9.00 (3.83)	15.0 (10.5)	7.5 (6.2)	NA	5.5 (7.0)
C_{max} (µg/mL)	0.598 (0.127)	8.431 (1.161)	44.75 (6.14)	160.0 (12.0)	NA	1.08 (0.42)
Lambda z (1/hr)	0.004 (0.001)	0.005 (0.001)	0.0041 (0.0007)	0.0046 (0.0012)	0.004 (0.000)	0.0026 (0.0007)
$T_{1/2}$ (hr)	201.774 (37.489)	138.343 (31.972)	174.19 (28.92)	157.47 (38.39)	185.584 (19.558)	277.10 (56.62)
AUC_{INF} (hr·µg/mL)	123.224 (35.476)	1,194.463 (2,15.578)	6,733.70 (1,392.83)	25,155.61 (7,276.96)	1,249.817 (113.167)	206.38 (59.03)
AUC_{INF}/D (hr·µg/mL/mg·kg)	1,096.811 (310.491)	1,176.009 (206.316)	1,221.89 (250.28)	942.65 (284.67)	1,123.384 (100.488)	2,111.28 (586.77)
Cl_p (mL·kg/hr)	0.962 (0.240)	0.871 (0.158)	0.85 (0.21)	1.13 (0.31)	0.896 (0.082)	0.51 (0.17)

Notes: AUC_{INF} = area under the plasma concentration-time curve, extrapolated to infinity; AUC_{INF}/D = AUC_{INF} normalized to dose; Cl_p = plasma clearance; C_{max} = maximum plasma concentration; IV = intravenous; Lambda z = terminal elimination constant; $T_{1/2}$ = terminal elimination half-life; T_{max} = time to C_{max} = NA = Not applicable.
Data presented as mean ± (standard deviation)

Table B-38. PK Parameters in Female Sprague-Dawley Rats Following Administration of PFOA as Reported by Kemper et al. (2003)

Parameter	Dose					
	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg (IV)	0.1 mg/kg Extended Time
T_{max} (hr)	0.56 (0.31)	1.13 (0.63)	1.50 (0.58)	1.25 (0.87)	NA	1.25 (0.50)
C_{max} (µg/mL)	0.67 (0.07)	4.782 (1.149)	20.36 (1.58)	132.6 (46.0)	NA	0.52 (0.08)
Lambda z (1/hr)	0.231 (0.066)	0.213 (0.053)	0.15 (0.02)	0.059 (0.037)	0.250 (0.047)	0.22 (0.07)
$T_{1/2}$ (hr)	3.206 (0.905)	3.457 (1.111)	4.60 (0.64)	16.22 (9.90)	2.844 (0.514)	3.44 (1.26)
AUC_{INF} (hr·µg/mL)	3.584 (0.666)	39.072 (10.172)	114.90 (11.23)	795.76 (187.51)	33.998 (7.601)	3.34 (0.32)
AUC_{INF}/D (hr·µg/mL/mg·kg)	31.721 (5.880)	38.635 (10.093)	20.78 (2.01)	29.54 (6.92)	30.747 (6.759)	34.39 (3.29)
Cl_p (mL·kg/hr)	32.359 (6.025)	27.286 (7.159)	48.48 (4.86)	35.06 (.88)	34.040 (9.230)	29.30 (3.06)

Notes: AUC_{INF} = area under the plasma concentration-time curve, extrapolated to infinity; AUC_{INF}/D = AUC_{INF} normalized to dose; Cl_p = plasma clearance; C_{max} = maximum plasma concentration; IV = intravenous; Lambda z - terminal elimination constant; $T_{1/2}$ = terminal elimination half-life; T_{max} = time to C_{max}; NA = not applicable.
Data presented as mean ± (standard deviation)

Gibson and Johnson (1979) administered a single dose of [^{14}C]PFOA averaging 11.4 mg/kg by gavage to groups of three male 10-week-old CD rats. The elimination half-life of [^{14}C]PFOA from the plasma was 4.8 days.

Toxicokinetic parameters informing half-lives were derived by comparing oral IV dosing in rats (Kim et al., 2016b). Sprague-Dawley rats were administered 2 mg/kg PFOA by either the IV or oral route. Urine and feces were collected weekly, and blood was collected at 10 time points over the first day and then up to 70 days after exposure. Half-lives in females and males were similar. In females, half-lives of 23.50 ± 1.75 and 24.80 ± 1.52 days were estimated after oral and IV dosing, respectively. In males, values were slightly longer (26.44 ± 2.77 and 28.70 ± 1.85 after oral and IV dosing, respectively). Half-life estimates were substantially longer than those observed by Kemper (2003) in Sprague-Dawley rats, as well in CD rats reported by Gibson and Johnson (1979). As shown in Table B-39, Sex differences were also observed for other TK parameters including C_{max}, T_{max}, AUC (calculated from time 0 to infinity) and V_d indicating more rapid clearance of PFOA in females relative to males.

Table B-39. PK Parameters in Male and Female Sprague-Dawley Rats Following Oral and IV Administration of PFOA as Reported by Kim et al. (2016b)

Parameter	1 mg/kg			
	Oral		IV	
	Male	Female	Male	Female
T_{max} (hr)	2.07 ± 0.21*	0.06 ± 0.004	8.92 ± 2.34	5.84 ± 0.38
C_{max} (µg/mL)	7.55 ± 0.51	5.41 ± 0.38	NA	NA
AUC (µg-day/mL)	24.81 ± 1.41	1.39 ± 0.06	21.10 ± 1.51*	1.63 ± 0.09
$T_{1/2}$ (day)	1.83 ± 0.47	0.15 ± 0.01	1.64 ± 0.44*	0.19 ± 0.01
V_d	106.40 ± 8.90	153.83 ± 9.19	112.12 ± 29.41	171.37 ± 11.19

Notes: AUC = area under curve; C_{max} = maximum plasma concentration; IV = intravenous; NA = not applicable; $T_{1/2}$ = terminal elimination half-life; T_{max} = time to C_{max}; V_d = volume of distribution.
Data presented as mean ± standard deviation.
*p < 0.05 between male and female.

Lou et al. (2009) determined values of 21.7 days (95% CI: 19.5–24.1) for male CD1 mice and 15.6 days (95% CI: 14.7–16.5) for females for use in their pharmacokinetic model.

Depending on the experimental conditions, half-lives in rats ranged from 0.03 days in the initial period of high-dose (40 mg/kg) exposure in females (Dzierlenga et al., 2019) 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 (Kim et al., 2016b Dzierlenga, 2019, 5916078). Similar to humans and mice, half-life estimates were shorter in female rats compared with male rats.

In Sprague-Dawley rats exposed to a single dose of [^{14}C]PFOA via the i.p. route, tissue elimination rates were slightly slower in the liver of male rats compared with other tissues. The PFOA half-life averaged 11 days in liver compared with an average of 9 days in extrahepatic tissues (Vanden Heuvel et al., 1991). In female rats, elimination rates were substantially shorter with an average half-life of 3 hours in both liver and extrahepatic tissues. The whole-body elimination half-life was calculated as <1 day in females versus 15 days for males and was attributed to sex differences in the excretion of PFOA by the kidney.

B.4.5.3.3 Mice

Half-life estimates (15.6 to 21.7 days) in the single mouse study (Lou et al., 2009) were generally longer than those measured in rats.

A summary of animal half-life values identified in animals is shown in Table B-40. Values in both primates and rodents were much shorter than those estimated in humans as exemplified by values 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 the initial period of high-dose (40 mg/kg) exposure in females (Dzierlenga et al., 2019) 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., 2019; Kim et al., 2016b). Similar to humans and mice, half-life estimates were shorter in female rats compared

with male rats. In contrast, female half-life values exceeded male values in cynomolgus monkeys suggesting species-specific factors impacting elimination across sexes. Similar to results in humans, PFOA isomers exhibited shorter half-lives compared with linear forms.

Table B-40. Summary of Animal PFOA Half-life Values Identified in the Literature Review

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex	Dose	Estimated Half-Life
Butenhoff et al. (2004b)	Monkey, cynomolgus	IV	3–4 yr	Male	10 mg/kg	13.6, 13.7, and 35.3 for three males
				Female	10 mg/kg	26.8, 29.3, and 41.7 for three females
Lou et al. (2009)	Mice, CD-1	Oral	70–80 d	Male	1 and 10 mg/kg	21.7
				Female	1 and 10 mg/kg	15.6
Benskin et al. (2009)	Rat, Sprague-Dawley	Oral	Adult (429 g)	Male	0.4 mg/kg n-PFOA (0.5 mg/kg PFOA)	n-PFOA: 13.4 iso-PFOA: 8.11 4m-PFOA: 4.32 5m-PFOA: 3.95 3m-PFOA: 6.26 tb-PFOA: 2.25 5,3/5,4m2-PFOA: 1.79 4,4m2-PFOA: 1.28 B8-PFOA: 9.10
Dzierlenga et al. (2019)	Rat, Sprague-Dawley	IV	8 wk	Male	6 mg/kg – T1/2 initial phase	2.8 ± 1.4
					6 mg/kg – T1/2 terminal phase	10.3 ± 1.2
					6 mg/kg – T1/2 overall	6.4 ± 0.5
				Female	40 mg/kg – T1/2 initial phase	0.03 ± 0.02
					40 mg/kg – T1/2 terminal phase	0.22 ± 0.01
		Oral	8 wk	Male	6 mg/kg – T1/2 overall	12.5 ± 0.7
					12 mg/kg – T1/2 overall	10.8 ± 0.5
					48 mg/kg – T1/2 overall	8.96 ± 0.42

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex	Dose	Estimated Half-Life
				Female	40 mg/kg – T1/2 initial phase	0.11 ± 0.02
					40 mg/kg – T1/2 terminal phase	1.23 ± 0.4
					40 mg/kg – T1/2 overall	0.11 ± 0.03
					80 mg/kg – T1/2 initial phase	0.16 ± 0.02
					80 mg/kg – T1/2 terminal phase	1.82 ± 1.13
					80 mg/kg – T1/2 overall	0.16 ± 0.03
					320 mg/kg – T1/2 initial phase	0.06 ± 1.09
					320 mg/kg – T1/2 terminal phase	0.75 ± 0.11
					320 mg/kg – T1/2 overall	0.58 ± 4.20
Kemper (2003)	Rat, Sprague-Dawley	Oral	Sexually mature	Male	0.1 mg/kg	8.4
					1 mg/kg	5.8
					5 mg/kg	7.3
					25 mg/kg	6.6
					1 mg/kg (IV)	5.8
					0.1 mg/kg extended	11.5
				Female	0.1 mg/kg	0.1
					1 mg/kg	0.1
					5 mg/kg	0.2
					25 mg/kg	0.7
					1 mg/kg (IV)	0.1
					0.1 mg/kg extended	0.1

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex	Dose	Estimated Half-Life
Kim et al. (2016b)	Rat, Sprague-Dawley	IV	8–12 wk	Male	1 mg/kg	1.64 ± 0.44
				Female	1 mg/kg	0.19 ± 0.01
		Oral	8–12 wk	Male	1 mg/kg	1.83 ± 0.47
				Female	1 mg/kg	0.15 ± 0.01
Kudo et al. (2002)	Rat, Wistar	IV	9 wk	Male	48.63 mol/kg body weight	5.68 ± 0.99
				Female	48.63 mol/kg body weight	0.08 ± 0.03
Vanden Heuvel et al. (1991)	Rat, Sprague-Dawley	IP	6 wk	Male	4 mg/kg	15

Notes: d = days; IV = intravenous injection; IP = intraperitoneal injection; wk = weeks; yr = years.
[a] Data presented in mean days ± standard deviation unless otherwise noted.

Appendix C. Nonpriority Health System Evidence Synthesis and Integration

C.1 Reproductive

The U.S. Environmental Protection Agency (EPA) identified 64 epidemiological and 16 animal studies that investigated the association between perfluorooctanoic acid (PFOA) and reproductive effects. Of the 22 epidemiological studies addressing male reproductive endpoints, 2 were classified as *high* confidence, 15 as *medium* confidence, 4 as *low* confidence, and 1 was considered *uninformative* (Section C.1.1). Of the 52 epidemiological studies addressing female reproductive endpoints, 5 were classified as *high* confidence, 25 as *medium* confidence, 20 as *low* confidence, and 2 were considered *uninformative* (Section C.1.1). Of the animal studies, 4 were classified as *high* confidence, 11 as *medium* confidence, and 1 was considered *low* confidence (Section C.1.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.1.1 Human Evidence Study Quality Evaluation and Synthesis

C.1.1.1 Male

C.1.1.1.1 Introduction

The 2016 Health Advisory (U.S. EPA, 2016a) and Health Effects Support Document (HESD) (U.S. EPA, 2016c) reports identified limited evidence of effects of PFOA on reproductive effects in men and boys. One study (Joensen et al., 2009) of Danish men in the military (n = 105) showed non-significant inverse associations with serum PFOA and semen volume, sperm concentration, sperm count, sperm motility, and sperm morphology. Comparing men with combined perfluorooctanoic acid/perfluorooctane sulfonic acid (PFOA/PFOS) serum levels revealed significantly ($p < 0.05$) less morphologically normal sperm in those men with higher PFOA/PFOS levels compared with those with low PFOA/PFOS levels. No associations were observed for serum sex hormones in this study. In healthy young Danish males Joensen et al. (2014) observed no associations with reproductive hormones. Semen parameters were also assessed in men from the Longitudinal Investigation of Fertility and the Environment Study (LIFE) cohort (Buck Louis et al., 2015), and significant associations were observed for a few morphological parameters, including fewer coiled tails, increased curvilinear velocity, and a larger acrosome area of the head. One prospective birth cohort study (Vested et al., 2013) followed offspring for approximately 20 years after mothers provided a third trimester blood sample. Regarding prenatal PFOA exposure, a significant negative trend was observed for total sperm count with 34% reductions in total count for each of the highest two tertiles compared with the lowest PFOA tertile. Additionally, prenatal PFOA exposure was associated with higher follicle stimulating hormone (FSH) (responsible for stimulating testicular growth) and luteinizing hormone (LH) (responsible for stimulating testosterone production) concentrations in these men after 20 years. Three occupational studies (Costa et al., 2009; Sakr et al., 2007a; Olsen et al., 1998) observed minimal evidence of reproductive effects in male employees. A study (Olsen et

al., 1998) on male employees (n = 111) at a Minnesota PFOA production plant (1993–1995) observed non-significant elevated estradiol (E2) in the highest PFOA exposure group; however, the study authors suggest this may have been confounded by a high correlation between E2 and BMI. A study (Sakr et al., 2007a) of employees at a DuPont facility in West Virginia observed associations for serum E2 and testosterone, but they did not address circadian variations in hormone levels and concluded the biological significance of the result was unclear. No other associations were observed in occupational studies evaluating males.

For this updated review, 21 studies (22 publications)[5] report on the association between PFOA and male reproductive effects since the 2016 document. There were several pairs of studies investigating the same population, including the Biopersistent Organochlorines in Diet and Human Fertility (INUENDO) cohort (Leter et al., 2014; Kvist et al., 2012), the Odense Child Cohort (Jensen et al., 2020b; Lind et al., 2017a), the Genetic and Biomarkers study for Childhood Asthma (GBCA) (Zhou et al., 2017c; Zhou et al., 2016), and a cross-sectional sample of men from a reproductive medical center in Nanjing, China (Cui et al., 2020; Pan et al., 2019). One pair of studies assessed populations from related cohorts belonging to the Hokkaido study on the Environment and Children's Health (Goudarzi et al., 2017a; Itoh et al., 2016).

Eleven studies were in children and adolescents (Jensen et al., 2020b; Liu et al., 2020b; Di Nisio et al., 2019; Ernst et al., 2019; Wang et al., 2019a; Goudarzi et al., 2017a; Lind et al., 2017a; Zhou et al., 2017c; Itoh et al., 2016; Lopez-Espinosa et al., 2016; Zhou et al., 2016), and the remainder of the publications were on the general population. Different study designs were utilized, including four cohort studies (Jensen et al., 2020b; Ernst et al., 2019; Goudarzi et al., 2017a; Itoh et al., 2016) with the remainder of the studies following a cross-sectional design. All observational studies measured PFOA in blood components (i.e., blood, plasma, or serum); however, PFOA in semen was additionally measured in four studies (Cui et al., 2020; Di Nisio et al., 2019; Pan et al., 2019; Song et al., 2018b). The studies were conducted in different study populations including populations from Australia, China, Denmark, the Faroe Islands, Greenland, Italy, Japan, Poland, Taiwan, Ukraine, and the United States. While most studies evaluated the relationship between exposure to PFOA and sex hormone concentrations, other male reproductive outcomes investigated included: sex-hormone-related steroid hormones (e.g., dehydroepiandrosterone (DHEA)), pubertal markers (e.g., voice break), semen analysis, genomic effects in sperm (e.g., DNA methylation), and anthropometric measurements (e.g., anogenital distance (AGD), penis length).

C.1.1.1.2 Study Quality

There are 22 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 male reproductive effects. Study quality evaluations for these 22 studies are shown in Figure C-1.

Of the 22 studies identified since the 2016 assessment, two studies were classified as *high* confidence, 15 studies as *medium* confidence, four studies as *low* confidence, and one study (Song et al., 2018b) was determined to be *uninformative*. Publications from the GBCA (Zhou et al., 2017c; Zhou et al., 2016) were considered *low* confidence because of concerns of selection

[5] Zhou et al. (2016) and Zhou et al. (2017c) use differing methods to analyze participants from the same population using the same health outcome.

bias and confounding. Cases and controls in Zhou et al. (2017c) were drawn from separate sources resulting in some concern for selection bias by recruiting individuals from different catchment areas. One *low* confidence study (Di Nisio et al., 2019) adjusted results only for age, resulting in concerns about potential for residual confounding by socioeconomic status (SES). One National Health and Examination Survey (NHANES) study (Lewis et al., 2015) did not adjust for the participant sampling design in the analysis which contributed to a *low* confidence rating. Song et al. (2018b) only reported bivariate correlations between exposure levels and semen parameters with no accounting for potential confounders which contributed to the study being classified as *uninformative*.

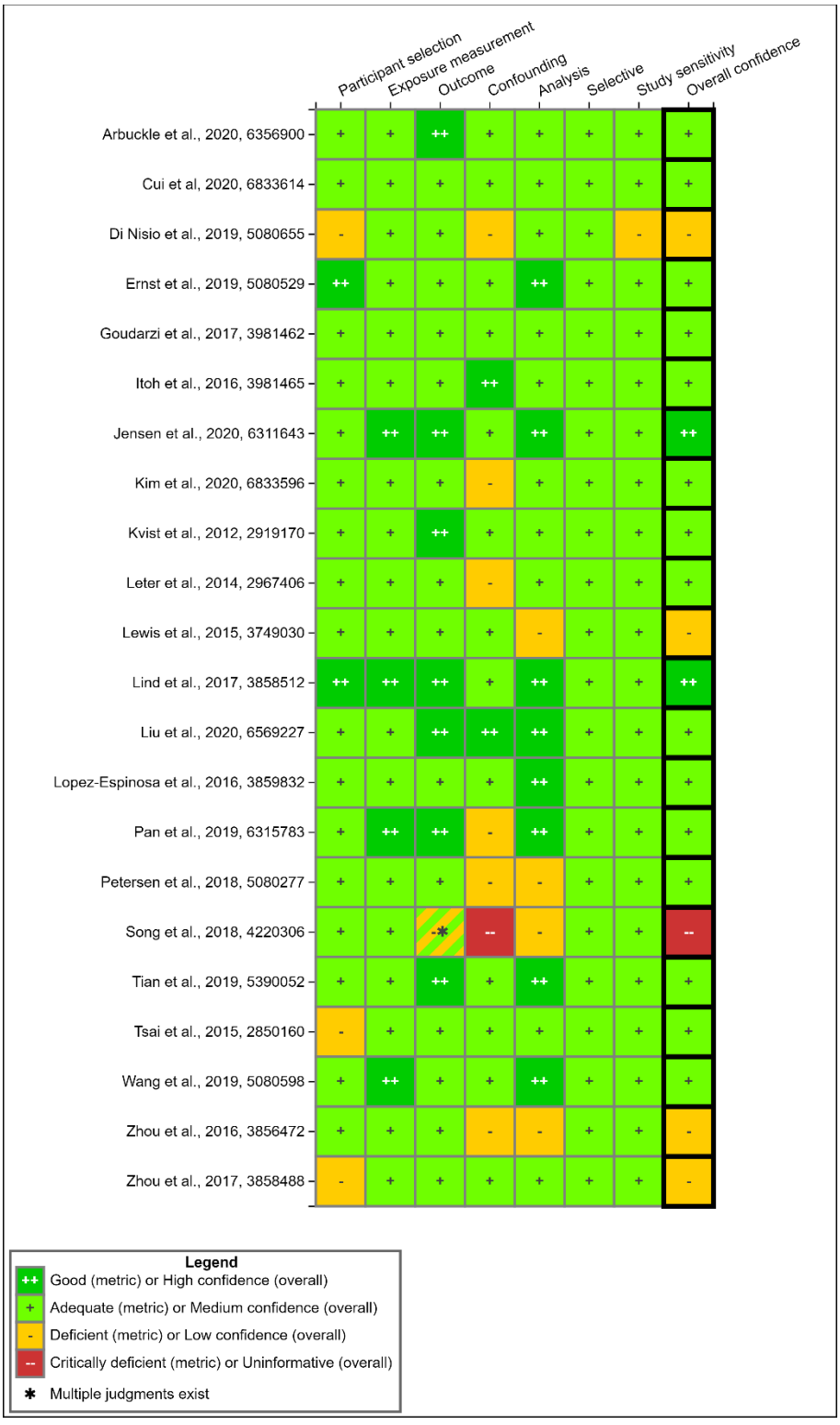


Figure C-1. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Male Reproductive Effects

Interactive figure and additional study details available on HAWC.

C.1.1.1.3 Findings From Children and Adolescents

Sex hormone levels and related steroid hormone levels were examined in nine studies (Jensen et al., 2020b; Liu et al., 2020b; Di Nisio et al., 2019; Wang et al., 2019a; Goudarzi et al., 2017a; Zhou et al., 2017c; Itoh et al., 2016; Lopez-Espinosa et al., 2016; Zhou et al., 2016) and five observed significant effects (Appendix D). A *high* confidence study (Jensen et al., 2020b) in boys from to the Odense cohort observed a borderline significant positive association between prenatal PFOA and FSH at four months ($p = 0.06$), but no associations for other serum sex and steroid hormones (i.e., androstenedione, 17-hydroxyprogesterone (17-OHP), and dehydroepiandrosterone sulfate (DHEAS)). A *medium* confidence study (Goudarzi et al., 2017a) examined male children from the Sapporo cohort, in the Hokkaido Study on the Environment and Children's Health and observed a significant inverse association ($p = 0.025$) with DHEA in cord blood. Associations were not observed among other androgenic hormones. Results from an overlapping *medium* confidence study (Itoh et al., 2016) from the Hokkaido cohort were largely non-significant except for a significant increase in inhibin B in cord blood. Quartile analyses supported this association, but the trend did not reach significance ($p = 0.063$). A *medium* confidence study (Liu et al., 2020b) in male infants in China observed a significant positive association with progesterone in cord blood.

A *medium* confidence cross-sectional study (Lopez-Espinosa et al., 2016) of boys (6–9 years) recruited from residents residing near the Mid-Ohio Valley DuPont chemical plant (C8 Health Project) observed a significant inverse association with testosterone, and a significant inverse trend (p for trend = 0.030) by quartiles of PFOA. In contrast, a cross-sectional study (Di Nisio et al., 2019) in Italian high school students examined associations between PFOA levels and possible risk factors for diseases of the male reproductive system and observed significantly increased semen PFOA levels, testosterone, and LH ($p = 0.003$) in exposed individuals compared with unexposed controls. These studies report effects in opposite directions; however, the significance of this conflicting evidence is not entirely clear as each population had reached different points in pubertal development. Additionally, Di Nisio et al. (2019) only controlled for age in all analyses, which may result in some residual confounding by SES or smoking.

Pubertal development and semen parameters were examined in two studies (Di Nisio et al., 2019; Ernst et al., 2019) and effects were seen in one (Appendix D). One *medium* confidence study (Ernst et al., 2019) observed no associations between prenatal PFOA exposure from first trimester maternal serum samples and pubertal stages (i.e., Tanner stages) and pubertal landmarks (e.g., acne, voice break, or first ejaculation. Comparisons of semen analysis in Italian high school students (Di Nisio et al., 2019), observed significantly increased semen levels and a reduced number of sperm with normal morphology ($p < 0.001$) and a slight increase in semen pH ($p = 0.005$) in exposed individuals compared with controls.

Anthropometric measurements of male reproductive organs were examined in four studies (Arbuckle et al., 2020; Di Nisio et al., 2019; Tian et al., 2019b; Lind et al., 2017a) and three observed effects (Appendix D). A *high* confidence Danish study (Lind et al., 2017a) in children from the Odense cohort observed non-significant smaller AGD and penile width at three months of age with increasing PFOA. Children from the Shanghai-Minhang Birth Cohort Study (Tian et al., 2019b) were evaluated at birth, six months, 12 months of age for changes in AGD. At six months of age, significant decreases were observed for the second lowest quartile. The effect was consistent in direction for higher quartiles of PFOA exposure but did not reach significance.

At 12 months of age, associations were positive, but none were significant. Di Nisio et al. (2019) reported smaller AGD in exposed compared with unexposed adolescents ($p = 0.019$). Significant differences ($p < 0.001$) were also observed for penile and testicular measurements in adolescents, including smaller testicular volume, shorter penis length, and smaller penis circumference. A smaller borderline significant pubis-to-floor distance was also observed ($p = 0.064$).

C.1.1.1.4 Findings From the General Adult Population

Serum sex hormones were examined in four studies (Cui et al., 2020; Petersen et al., 2018; Lewis et al., 2015; Tsai et al., 2015) and two observed effects (Appendix D). A *medium* confidence study (Cui et al., 2020) evaluated serum hormone concentrations in men with fecundity issues and men from couples with female factor infertility. Serum and semen PFOA were significantly correlated (Spearman's $r = 0.646$, $p < 0.01$). Total and free testosterone were inversely associated ($p < 0.05$) with serum and with semen PFOA levels. E2 and the total testosterone-LH ratio were inversely associated ($p < 0.05$) with semen PFOA, but not with serum PFOA levels. Analyses by quartile agreed and showed significant inverse trends for all outcomes with significant associations in continuous analyses. Analyses stratified by age showed these associations remained in participants 30 years old or younger but were not observed in those participants over 30 years of age. A *medium* confidence cross-sectional study (Petersen et al., 2018) on Faroese men also observed a decrease in free testosterone with increasing serum PFOA levels, however, the association was borderline significant ($p = 0.05$). The free testosterone-E2 ratio was inversely associated ($p = 0.02$) with PFOA levels in this sample. One study (Lewis et al., 2015) analyzed sex hormone concentrations among NHANES participants, but no clear patterns or significant effects were observed.

Semen characteristics and genomic effects in sperm were examined in five studies (Pan et al., 2019; Petersen et al., 2018; Song et al., 2018b; Leter et al., 2014; Kvist et al., 2012) and three observed effects (Appendix D). A *medium* confidence study (Pan et al., 2019) in men from Nanjing, China observed significant positive associations ($p < 0.05$) with sperm concentration, total sperm count, and the sperm DNA fragmentation index (DFI) – a measure of the percentage of sperm with damaged DNA. In analyses by quartiles, significant associations were observed for sperm concentration and for the second and fourth quartiles, however, the trend was not significant. Positive associations were observed for sperm DFI among the two highest quartiles of exposure, and the trend was significant (p for trend = 0.03). A significant inverse association ($p = 0.03$) was observed with progressive motility with a significant decreasing trend (p for trend = 0.02). Related motility measures, such as sperm curvilinear velocity and sperm straight-line velocity, did not have significant inverse trends in continuous analyses, however, an inverse association was observed for the highest quartile of exposure for each outcome. No other consistent trends for semen parameters were identified using semen concentrations of PFOA, and no associations were observed with serum PFOA.

One *medium* confidence study (Kvist et al., 2012) evaluating men from the INUENDO cohort from Greenland, Poland, or Ukraine, observed a significant positive association ($p = 0.05$) with the Y:X-chromosome ratio in sperm when pooling data across study countries. This association was also observed in the Ukraine subset of the cohort but not in other country-specific analyses. Chromosomal changes were further characterized in another INUENDO study (Leter et al., 2014) using a sperm DNA global methylation assay. Methylation of the LINE-1 loci was significantly increased ($p < 0.05$) in men from Ukraine, but no effect was observed in other

INUENDO communities or in the pooled analysis. The LINE-1 loci are a non-transposonic repetitive satellite DNA sequence generally observed in or adjacent to every centromere and was used as a surrogate marker of global DNA methylation.

C.1.1.2 Female

C.1.1.2.1 Introduction

Reproductive health outcomes of interest in females vary by stage of biological maturity and by pregnancy status. Of interest across the life stages, reproductive hormone levels, such as prolactin, FSH, LH, testosterone, and E2, are commonly examined as indicators of reproductive health. Additional reproductive health outcomes of interest include timing of puberty among children and adolescents; fertility indicators, impacts to menstruation, and occurrence of menopause among nonpregnant adult females; and gestational hypertension, preeclampsia, and breastfeeding duration among pregnant females.

The 2016 PFOA HESD (U.S. EPA, 2016c) concluded that there was suggestive evidence of an association with risk of pregnancy-induced hypertension or preeclampsia based on studies in highly exposed (C8 Health Project) populations (Darrow et al., 2013; Savitz et al., 2012a; Savitz et al., 2012b; Stein et al., 2009). There was conflicting evidence from two studies on altered female pubertal onset, and there were suggestive data from two studies on reduced fecundity and fertility. Limited suggestive findings on age at menarche or onset of menopause were hampered by the potential for reverse causation due to PFOA excretion via menstruation. One study examined female reproductive hormone levels in the C8 Health Project (Knox et al., 2011) and found no association between PFOA and E2 levels.

For this updated review, 49 studies (53 publications) report on the relationships between PFOA exposure and female reproductive outcomes.[6] Of these, 21 were cohort studies, 20 cross-sectional studies, and 12 case-control studies. Twenty-one studies were conducted in adults, six were in children and adolescents, 11 were in both adults and children, and 15 were conducted in pregnant women. Most studies assessed exposure to PFOA using biomarkers in blood. Others used amniotic fluid and follicular fluid.

C.1.1.2.2 Study Quality

There are 52 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 female reproductive effects. Study quality evaluations for these 52 studies are shown in Figure C-2, Figure C-3, and Figure C-4.

Among the 52 publications available for review, five were classified as *high* confidence, 25 as *medium* confidence, 20 as *low* confidence, and two were considered *uninformative*. Because menstruation is a primary route of PFOA excretion, reverse causality was a specific concern for cross-sectional studies that measured blood PFOA and reproductive hormones with known menstrual fluctuations that failed to report sample collection timing (Heffernan et al., 2018; Zhang et al., 2018b). Several *low* confidence studies lacked an appropriate strategy for identifying potential confounders (Mccoy et al., 2017; Zhou et al., 2017a) or failed to adjust for

[6] Singular studies with two associated publications include Avanasi et al. (2016a) and Avanasi et al. (2016b); Dhingra et al. (2016a) and Dhingra et al. (2017); Wang et al. (2019c) and Wang et al. (2019a); Zhou et al. (2017c) and Zhou et al. (2017a).

key confounders, such as age and SES (Heffernan et al., 2018; Zhou et al., 2016). The *low* confidence studies had deficiencies in participant selection (Heffernan et al., 2018; Zhang et al., 2018b), exposure measurement methods (Avanasi et al., 2016a, b; Campbell et al., 2016), reliance on self-reporting for exposure, outcome, or covariate information (Avanasi et al., 2016a, b; Campbell et al., 2016), and small sample size (Heffernan et al., 2018; Mccoy et al., 2017). Maekawa et al. (2017) was considered *uninformative* for this assessment because of lack of information on participant selection and lack of adjustment for key confounders in the analysis. Lee et al. (2013) was also considered *uninformative* due to lack of consideration of key confounders in analyses.

In the evidence synthesis below, *high* and *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association. Commonly assessed effects were pregnancy-related outcomes (e.g., preeclampsia, gestational hypertension), menstrual dysfunction (e.g., endometriosis, cycle irregularity), female fertility indicators, and female reproductive hormone levels (e.g., E2, testosterone, sex hormone binding globulin (SHBG)). Other female reproductive outcomes discussed in this review include breastfeeding duration, genital tract infection rate, and female pubertal milestones.

Figure C-2. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects

Interactive figure and additional study details available on HAWC.

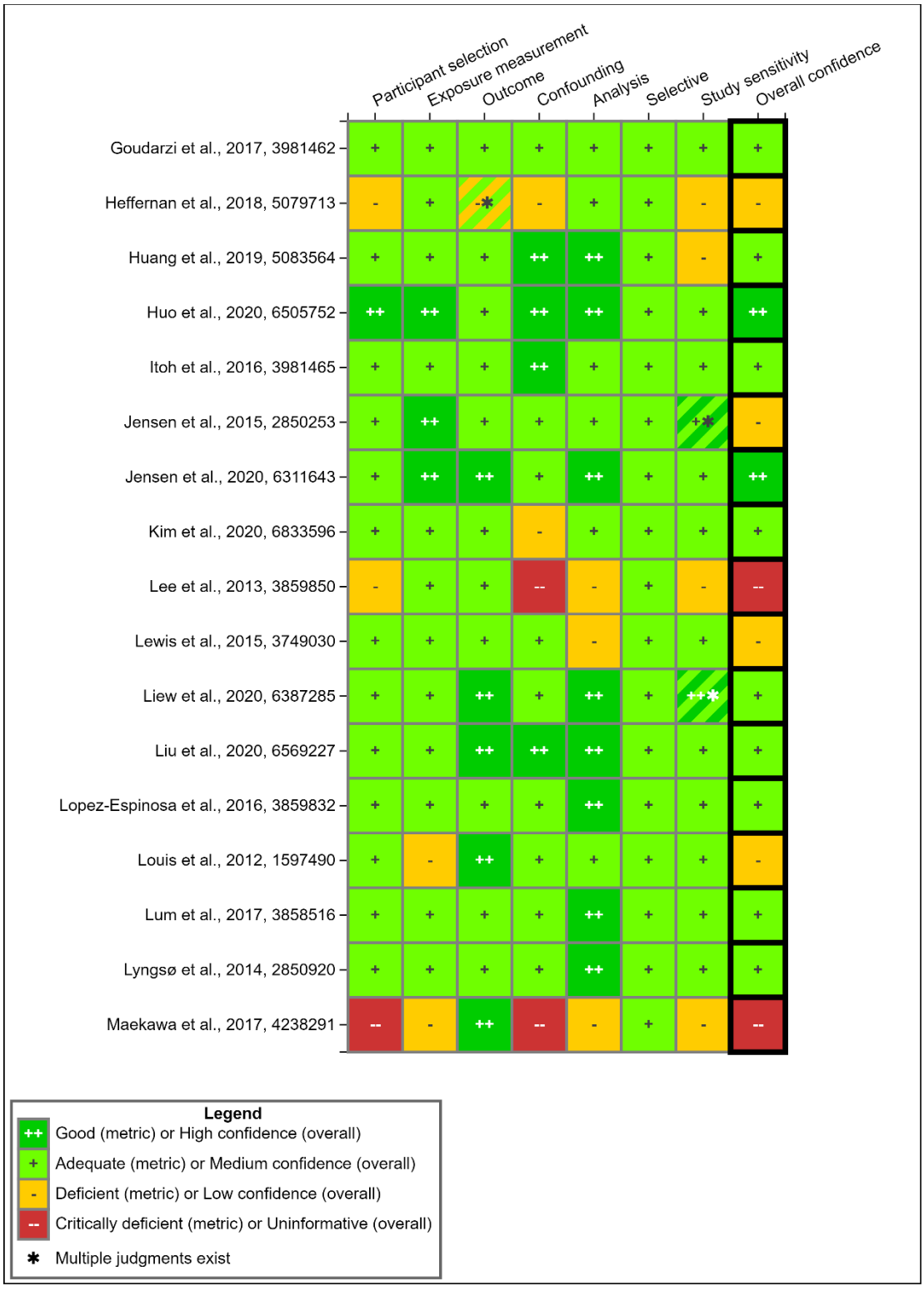


Figure C-3. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects (Continued)

Interactive figure and additional study details available on HAWC.

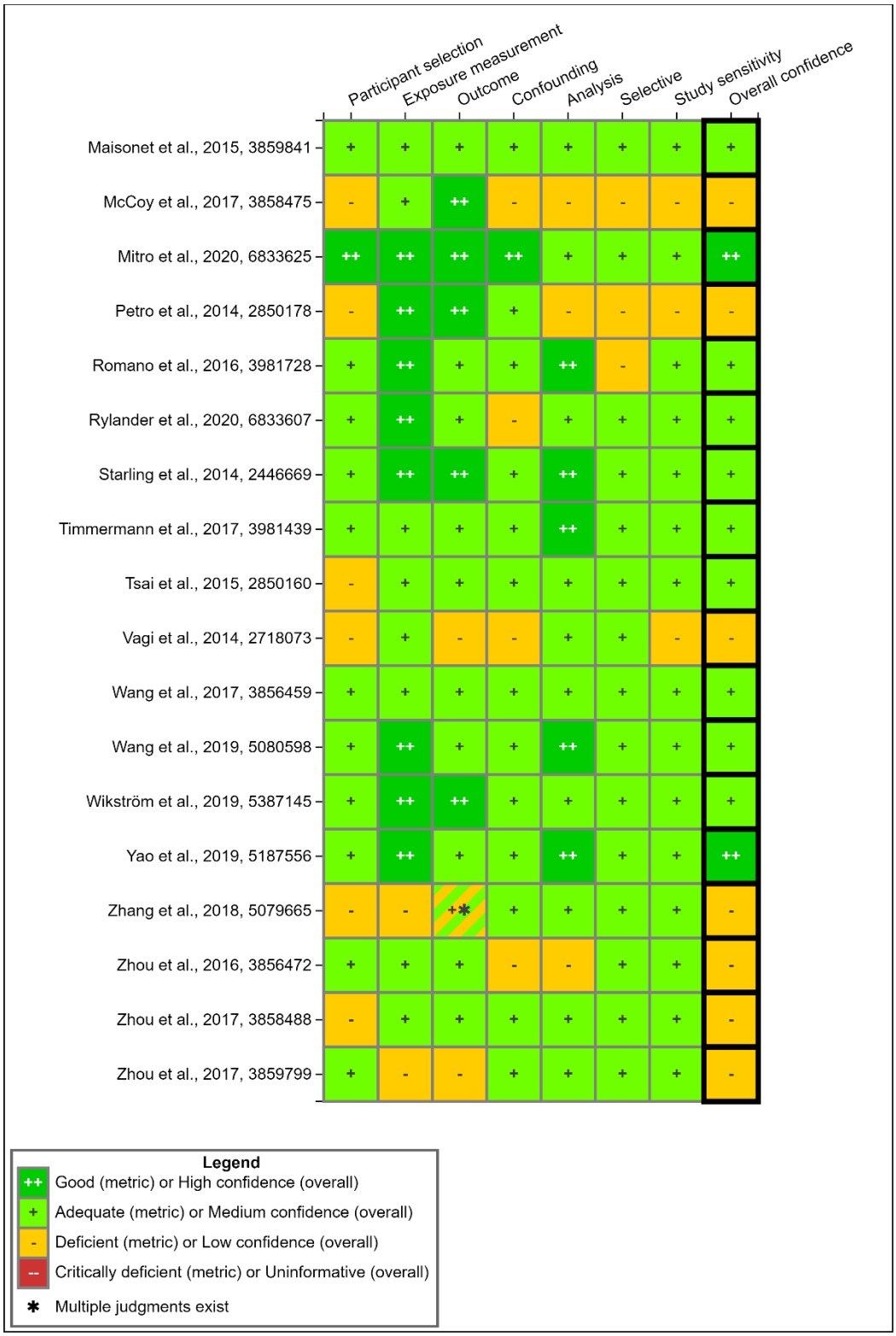


Figure C-4. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Female Reproductive Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.1.1.2.3 Findings From Children and Adolescents

Two *high* confidence, eight *medium* confidence, and two *low* confidence studies assessed relationships between PFOA exposure and female reproductive outcomes in children and adolescents (Appendix D). Studies in infants primarily focused on reproductive hormone levels, while studies in adolescents focused on reproductive hormone levels as well as pubertal milestones.

Two *high* confidence (Jensen et al., 2020b; Yao et al., 2019) and four *medium* confidence (Liu et al., 2020b; Wang et al., 2019a; Goudarzi et al., 2017a; Itoh et al., 2016) studies examined the association between PFOA exposure and female reproductive hormones in female infants. One *medium* cross-sectional analysis reported a significant positive association between cord blood PFOA and cord blood estriol in female infants (beta: 0.29, 95% CI: 0.02, 0.56) (Wang et al., 2019a). Two *high* (Jensen et al., 2020b; Yao et al., 2019) and three *medium* confidence studies (Liu et al., 2020b; Goudarzi et al., 2017a; Itoh et al., 2016) observed no significant associations between maternal serum or cord blood PFOA levels and reproductive hormones, such as 17-OHP, DHEA, FSH, and LH (Jensen et al., 2020b), E2, testosterone, or testosterone-to-E2 ratio (Yao et al., 2019) progesterone (Liu et al., 2020b), prolactin, SHBG, testosterone, DHEA, androstenedione (Goudarzi et al., 2017a; Itoh et al., 2016).

Three *medium* confidence studies and one *low* confidence study examined the effects of PFOA exposure on female reproductive hormone levels in female adolescents with mixed results. Two *medium* confidence studies observed positive associations with E2 in a high-exposed population (Lopez-Espinosa et al., 2016) and testosterone (Maisonet et al., 2015a). As part of the C8 Health Project, Lopez-Espinosa et al. (2016) observed significantly increased E2 levels in serum PFOA quartile 2 compared with quartile 1 (percent difference = 12.6; 95% CI: 3.0, 23.1), but smaller non-significant, positive associations were observed for girls in the two highest PFOA quartiles. In daughters from the Avon Longitudinal Study of Parents and Children (ALSPAC), Maisonet et al. (2015a) reported a positive association for total testosterone at age 15 when analyzed by maternal serum PFOA tertiles (beta for maternal PFOA tertile 2 vs. tertile 1: 0.15, 95% CI: −0.02, 0.32; beta for tertile 3 vs. tertile 1: 0.24, 95% CI: 0.05, 0.43). Maternal serum PFOA was not significantly associated with daughter's SHBG levels. No associations were observed for follicular stimulating hormone or SHBG in a *medium* confidence study (Tsai et al., 2015) or for E2 or testosterone in a *low* confidence study (Zhou et al., 2016).

One *medium* confidence study and one *low* confidence study reported no evidence of an association between prenatal PFOA exposure and pubertal milestones in female adolescents. Breast development, pubic hair development, axillary hair development, and age at menarche were not associated with maternal blood PFOA during pregnancy in 555 adolescent girls from the Danish National Birth Cohort (DNBC) (Ernst et al., 2019). Zhou et al. (2017a) reported positive associations between PFOA and risk of hypomenorrhea (OR for PFOA quantile 3 (Q3) vs. quantile 1 (Q1): 2.68, 95% CI: 1.24, 5.78), irregular menstrual cycle (OR for PFOA quantile 4 (Q4) vs. Q1: 1.99, 95% CI: 1.22, 3.24; OR per log increase PFOA: 1.52, 95% CI: 1.08, 2.15), and long menstrual cycle (OR for PFOA Q4 vs. Q1: 1.95, 95% CI: 1.21, 3.14; OR per log increase PFOA: 1.5 (1.06, 2.1) among female adolescents aged 10–15 years. However, the analyses were not adjusted for key confounders in this *low* confidence study.

C.1.1.2.4 Findings From Pregnant Women

Seven studies examined the relationship between PFOA exposure and preeclampsia (Appendix D). Of these, six observed positive non-significant associations (Borghese et al., 2020; Rylander et al., 2020; Huang et al., 2019; Wikström et al., 2019; Avanasi et al., 2016a, b) and one observed a negative non-significant association (Huo et al., 2020a). Huo et al. (2020a), a *high* confidence cohort study of 3,220 pregnant women, observed non-significant decreased odds of preeclampsia in women with higher serum PFOA levels (OR for women in the 80th percentile or higher for serum PFOA (ln-ng/mL) vs. women below the 80th percentile = 0.92; 95% CI: 0.5, 1.7; OR per unit increase in serum PFOA (ln-ng/mL) = 0.89; 95% CI: 0.5, 1.57). All four *medium* confidence studies observed, positive non-significant associations between PFOA exposure and preeclampsia, in cross-sectional (Huang et al., 2019), case-control (Rylander et al., 2020) and cohort studies (Borghese et al., 2020; Wikström et al., 2019). One *low* confidence study re-analyzed data from a study reviewed in the 2016 PFOA HESD, Savitz et al., 2012, and observed non-significant, positive associations between modeled serum PFOA levels and odds of preeclampsia (Avanasi et al., 2016a, b).

One *high* confidence study (Huo et al., 2020a) and two *medium* confidence studies examined the relationship between PFOA exposure and gestational hypertension reporting non-significant mixed effects. Huo et al. (2020a), a *high* confidence cohort study of 3,220 pregnant women, observed non-significant increased odds of gestational hypertension in women with higher serum PFOA levels. Similarly, Borghese et al. (2020) found non-significant increased odds of gestational hypertension for women in plasma PFOA tertile 3 vs. tertile 1 and per $\log_2$-ng/mL unit increase in plasma PFOA. In contrast, Huang et al. (2019) reported non-significant reduced odds of gestational hypertension with increasing maternal plasma PFOA levels in both tertile and continuous analyses. When exploring the association between PFOA exposure and impacts on blood pressure, Borghese et al. (2020) found a significant positive association between first trimester plasma PFOA (µg/L) and systolic blood pressure (SBP) (beta: 0.82; 95% CI: 0.23, 1.42; p = 0.006) and diastolic blood pressure (DBP) (beta: 0.64; 95% CI: 0.24, 1.05; p = 0.002). A significant relationship was also observed between continuous plasma PFOA (µg/L) measured at delivery and SBP (beta: 1.52; 95% CI: 0.52, 2.50; p = 0.002) as well as DBP (beta: 1.11; 95% CI: 0.44, 1.78; p = 0.001). Results were less consistent when stratified by infant sex.

Two *medium* confidence studies assessed the relationship between serum PFOA levels in pregnancy and breastfeeding duration and both reported significant, inverse associations between the two (Timmermann et al., 2017b; Romano et al., 2016). Using data from two Faroese birth cohorts (N = 1,130), one study observed significant, negative associations between maternal serum PFOA (ng/mL) and both exclusive (regression coefficient per doubling of serum PFOA (ng/mL): −0.5 months; 95% CI: −0.7, −0.3 months) and total (regression coefficient per doubling of serum PFOA (ng/mL): −1.3 months; 95% CI: −1.9, −0.7 months) breastfeeding duration (Timmermann et al., 2017b). These observations were supported by a prospective birth cohort study which observed a consistent, positive trend between increasing serum PFOA quartile and relative risk of breastfeeding duration at three and six months postpartum. Relative risk of breastfeeding termination at three months postpartum was significantly increased for women in serum PFOA quartiles 3 (risk ratio (RR) = 1.63; 95% CI: 1.16, 2.28) and 4 (RR = 1.77; 95% CI: 1.23, 2.54) compared with quartile 1. Relative risk of breastfeeding termination at six months postpartum was also significantly increased for women in serum PFOA quartiles 3 (RR = 1.38; 95% CI: 1.06, 1.79) and 4 (RR = 1.41; 95% CI: 1.06, 1.87) compared with quartile 1.

One *high* confidence study examined SHBG measured 3 years postpartum in 812 women enrolled in the Project Viva birth cohort (Mitro et al., 2020). The study observed a negative non-significant association between early pregnancy plasma PFOA and SHBG. These findings were consistent in analyses stratified by age at pregnancy (<35 years vs. ≥35 years).

One *medium* confidence study (Lyngsø et al., 2014) examined the effects of serum PFOA levels on pre-pregnancy menstruation. The study reported significantly increased odds of long menstrual cycles for women in the highest PFOA tertile compared with the lowest (OR: 1.8, 95% CI: 1.0, 3.3) and when analyzing PFOA as a continuous variable (OR: 1.5, 95% CI: 1.0, 2.1). Significant results persisted when analyses were restricted to nulliparous women.

C.1.1.2.5 Findings From the General Adult Population

One *high* confidence, eight *medium* confidence, and 11 *low* confidence studies assessed relationships between PFOA exposure and female reproductive outcomes in nonpregnant adult women (Appendix D). Assessed outcomes included various fertility indicators, age at natural menopause, and reproductive hormone levels.

Five *medium* confidence studies and eight *low* confidence studies examined female fertility indicators and no clear associations or dose-response trends were observed. A cohort study of 501 couples attempting to conceive observed positive significant associations but no trend across baseline serum PFOA tertiles for day-specific probability of pregnancy or menstrual cycle length (Lum et al., 2017). Crawford et al. (2017) observed positive association with cycle-specific time to pregnancy and anti-Müllerian hormone (AMH), a biomarker of ovarian reserve, and a negative association with day-specific time to pregnancy, but the associations were non-significant. A *low* confidence study examining time to pregnancy (Bach et al., 2018) reported a positive association. Another study of AMH examined levels in female adolescents in the ALSPAC and found a significant positive association between maternal serum PFOA during pregnancy and AMH concentration (beta: 0.05; 95% CI: 0.01, 0.09). This association was not significant after missing data imputation (Donley et al., 2019). A *low* confidence study investigated PFOA exposure and premature ovarian insufficiency (POI), reporting no significant associations (Zhang et al., 2018b), while another *low* confidence study found positive associations between the highest PFOA tertile and polycystic ovary syndrome when compared with the lowest PFOA tertile (Vagi et al., 2014). Wang et al. (2017) observed no associations and no trend in odds of endometriosis-related infertility across plasma PFOA tertiles. Campbell et al. (2016) reported increased odds of endometriosis only for the third PFOA exposure quartile compared with the lowest PFOA quartile (OR: 5.45; 95% CI: 1.19, 25.04), while another *low* confidence study did not observe an association with endometriosis diagnosis (Louis et al., 2012). Kim et al. (2020b) observed a positive non-significant association between PFOA in follicular fluid and fertilization rate. Other *low* confidence studies examining fertility-related outcomes reported non-significant positive associations between PFOA exposure and percent fertilization (Mccoy et al., 2017), minimal correlation with expression of nuclear receptors when examined by fertility status (Caserta et al., 2013), and no association between maternal serum PFOA and infertility (Bach et al., 2015).

The two studies (3 publications) examined age at natural menopause, and all observed positive associations. A *high* confidence study of premenopausal women aged 45–56 in the Study of Women's Health Across the Nation (SWAN) cohort (Ding et al., 2020) reported a significantly

increased risk of natural menopause for women in the highest exposure tertile (HR = 1.31; 95% CI: 1.04, 1.65), but no significant association per doubling of serum PFOA. A *medium* confidence study (2 publications) (Dhingra et al., 2017; Dhingra et al., 2016a) of women ages 30–65 years in the high-exposed Mid-Ohio Valley cohort assessed associations between both measured and modeled PFOA exposure and self-reported menopause). Menopause was significantly associated with serum PFOA (p-trend = 0.04), but not modeled PFOA exposure (p-trend = 0.90) (Dhingra et al., 2017). However, the findings might be hampered by reverse causation, likely due to reduced kidney function, as urine is a primary route of PFOA excretion.

One *medium* confidence study and four *low* confidence studies assessed the relationship between serum PFOA levels and reproductive hormone levels in nonpregnant adult women. In the *medium* confidence study, no clear dose-response trends were observed for either FSH or SHBG across quartiles by age category (Tsai et al., 2015). While one *low* confidence study observed mixed associations between PFOA levels and increased testosterone, with a significant positive association reported for controls (Heffernan et al., 2018), another (Zhang et al., 2018b) observed no significant associations between PFOA and any female reproductive hormone outcomes, including E2, prolactin, testosterone, LH, and FSH. Two other *low* confidence studies, Lewis et al. (2015) and Petro et al. (2014), reported no association for total testosterone or E2, respectively.

C.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are four studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 12 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and reproductive effects. Study quality evaluations for these 16 studies are shown in Figure C-5.

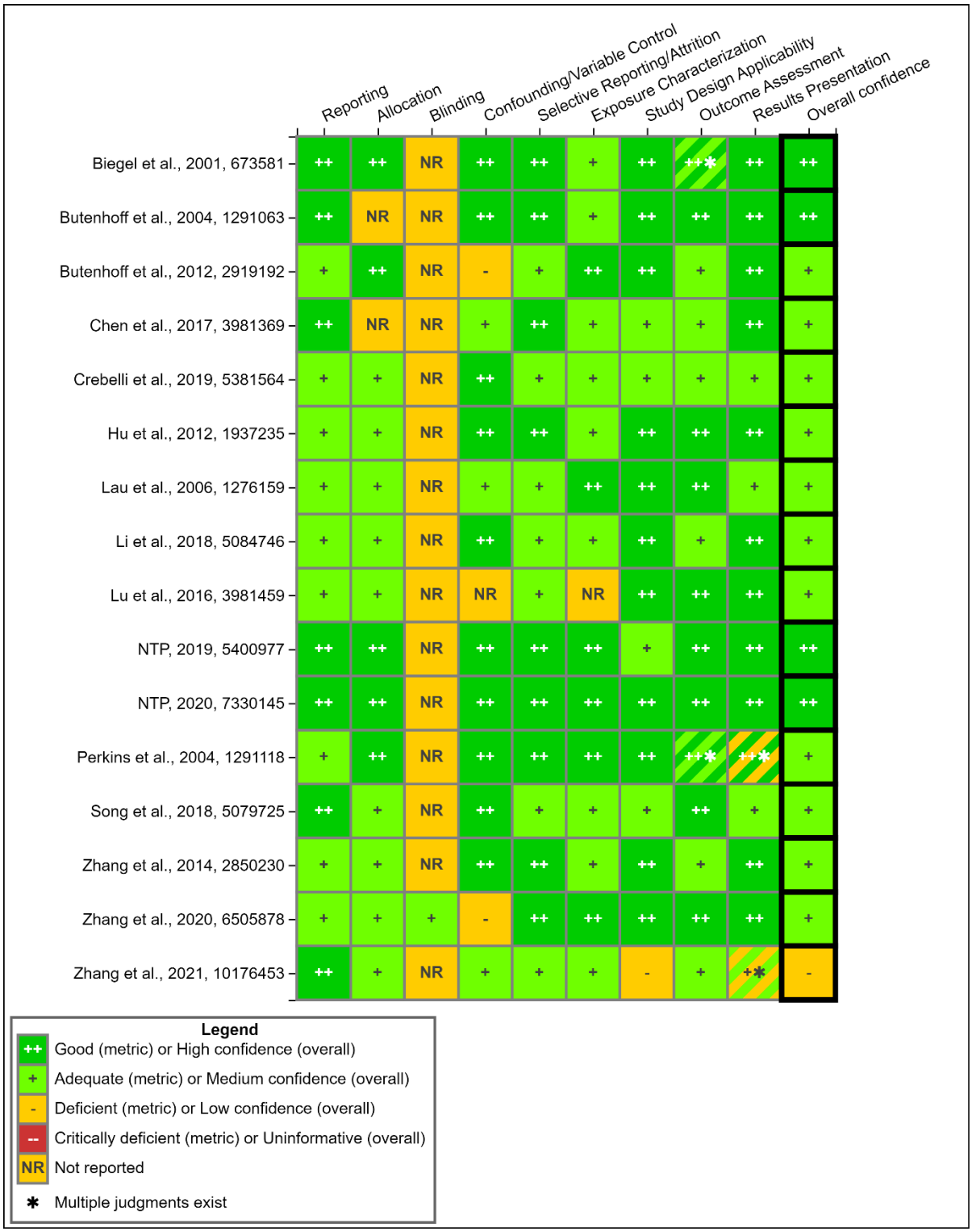


Figure C-5. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Reproductive Effects

Interactive figure and additional study details available on HAWC.

Several animal studies report significant effects on reproductive endpoints following oral exposure to PFOA; however, the evidence is not consistent across species with effects observed in mice more frequently than in rats or monkeys. In addition, the effects were observed at dose levels that have been shown to reduce growth and body weight in several studies, which may explain the effects observed on reproductive endpoints. Effects observed in male rodents include reduced fecundity (mice only), decreased epididymal weights, decreased sperm count and quality, and morphological changes in the testes and epididymides. Female rodents exposed to PFOA have displayed prolonged diestrus and reduced number and size of corpora lutea compared with vehicle controls. In addition, alterations in reproductive hormone levels have been observed in male and female rodents. Oral studies in mice and rats report effects in altered puberty (delayed vaginal opening in females and altered preputial separation in males). Developmental studies in mice have reported adverse effects on the weight and histopathology of the placenta (see Toxicity Assessment, (U.S. EPA, 2024b)), and there have been cancers observed in reproductive organs that are discussed in (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.1.2.1 Reproductive Performance

One standard two-generation reproduction study is available for PFOA that reported no effects on mating or fertility in rats administered PFOA by gavage for 10 weeks prior to mating with doses ranging from 1 to 30 mg/kg/day (York et al., 2010; Butenhoff et al., 2004a). Reproductive endpoints including number of days in cohabitation, fertility index, pregnancy, implantation, and length of gestation were not affected in either generation. Although F_1 pups exposed to 30 mg/kg/day had decreased birth weight and survival (see Toxicity Assessment, (U.S. EPA, 2024b)), no effects were observed on reproductive performance or fertility in these animals as adults. Reproductive outcomes in WT mice dosed orally from GD 1–17 with 0.1, 0.3, 0.6, 1, 3, 5, 10 and 20 mg/kg were examined. In the WT mice, the number of implantation sites, number of live and dead pups per litter and maternal weight were not affected by PFOA. However, the incidence of full litter resorption was significantly increased at doses of 5 mg/kg/day or higher (Abbott et al., 2007). Similarly, the number of pups per litter in CD-1 mice exposed to 0.1 and 1 mg/kg PFOA from GD 1.5–17.5 did not significantly differ from control groups (Cope et al., 2021).

Information on the reproductive performance of mice exposed to PFOA prior to and during mating is available from two studies. Fecundity was decreased in male BALB/c mice following exposure to 5 mg/kg/day PFOA by gavage for 28 days when mated to untreated females, shown by reductions in the numbers of mated females per male mouse and pregnant females per male mouse (Lu et al., 2016a). The authors did not measure body weight or sperm parameters in the treated males and did not report if any clinical signs of toxicity were observed, therefore it is difficult to interpret the toxicological significance of the effect on reproductive performance. In contrast, Hu et al. (2012) administered PFOA (0.02, 0.2, or 2 mg/kg/day) to female C57BL/6N mice by daily gavage from the day they were paired with untreated males through weaning of offspring. On average, females were dosed for 12.9 (±7.3) days prior becoming pregnant. No effects were observed in the number of days to pregnancy or the number of dams that became pregnant between treated groups and controls (Hu et al., 2012).

C.1.2.2 Sperm Parameters

Sperm parameters were quantitatively measured in two studies in rats (NTP, 2019a; York et al., 2010; Butenhoff et al., 2004a) and two studies in mice (Zhang et al., 2014; Li et al., 2011). Overall, the findings were not consistent between rats and mice and therefore do not provide clear evidence of an adverse effect on spermatogenesis.

In a short-term study by NTP, male Sprague-Dawley rats were administered 0.625, 1.25, 2.5, 5, or 10 mg/kg/day PFOA by gavage for 28 days and sperm parameters were evaluated in the control and three highest dose groups at the end of the treatment period (sample size n = 10) (NTP, 2019a). Cauda epididymal sperm count was significantly decreased (24%) in the high-dose group compared with controls, but when normalized to sperm count per gram of cauda epididymis, the difference was no longer statistically significant. No effects were observed on epididymal sperm motility or testicular spermatid counts. Histopathological examination of the epididymis revealed hypospermia and exfoliated germ cells in one rat each in the 5 and 10 mg/kg/day groups, though the findings were not significantly different from the control group. Body weight was significantly reduced in males treated with dose levels ≥2.5 mg/kg/day and the highest dose group weighed 19% less than controls at necropsy. This could explain the reduction in sperm count observed at that dose level. A two-generation reproduction study in Sprague-Dawley rats with doses up to 30 mg/kg/day PFOA found no treatment-related effects on epididymal sperm count, density, motility, or morphology, as well as testicular spermatid count or density (sample size n = 28–30) (York et al., 2010; Butenhoff et al., 2004a). The incidences of hypospermia and exfoliated germs cells in the epididymis were slightly higher for P_0 males treated with 30 mg/kg/day versus controls (2/14 vs. 0/13 for each finding); however, it is not clear if statistical analyses were performed for those results.

Zhang et al. (2014) administered 0.31, 1.25, 5, or 20 mg/kg/day PFOA to adult male BALB/c mice by gavage for 28 days, but sperm parameters were only evaluated in the control and 5 mg/kg/day groups (sample size n = 5). At the end of the treatment period, epididymal sperm count was significantly decreased (32%) in the 5 mg/kg/day group compared with controls. Sperm motility and progressiveness were also significantly reduced. In addition, the rates of head and neck teratosperm were significantly increased as was the overall rate of teratosperm.[7] Body weights were not reported in this study, and it is unclear whether the mice in the 5 mg/kg/day group experienced concurrent systemic toxicity.

Li et al. (2011) also evaluated sperm parameters in a study designed to examine the involvement of mouse and human PPARα in male reproductive effects induced by PFOA. Adult male wild-type, PPARα-humanized, and PPARα-null mice of a 129/Sv background were administered 1 or 5 mg/kg/day PFOA by daily gavage for 6 weeks. At the end of the treatment period, body weights did not differ between the control and treated groups. Epididymal sperm count and motility were unaltered by treatment (sample size n = 8–10); however, the percentage of sperm abnormalities was significantly increased in both treated groups of wild-type and humanized

[7] The text of Zhang et al. (2014) reports that sperm motility and progressiveness were both significantly reduced and the overall rate of teratosperm was significantly increased in treated rats, but the results in figures 1D(b), (c), and (d) show the opposite effects. It appears that the figures are mislabeled, and the results were switched. The corresponding author was contacted for clarification, but no response was received.

PPARα mice, but not in PPARα-null mice. Therefore, the effects observed in this particular study are potentially related to PPARα.

The overall evidence is suggestive of an effect of PFOA on spermatogenesis, but there are several limitations with the dataset that make interpretation difficult. The studies that observed adverse effects on sperm parameters did not evaluate fertility or fecundity, while the only study that found an effect on fecundity did not measure sperm parameters or report if overt toxicity occurred in the males. Furthermore, the studies in mice used relatively small sample sizes (n = 5–10), while a comprehensive two-generation study in rats with large sample sizes (n = 28–30) observed no effects on sperm parameters or male fertility (York et al., 2010; Butenhoff et al., 2004a). Epididymal sperm concentration was reduced by 24% in rats treated with 10 mg/kg/day (NTP, 2019a) and by 32% in mice treated with 5 mg/kg/day (Zhang et al., 2014); however, the reduction observed in rats was negated when normalized to weight of the cauda epididymis (NTP, 2019a). The study in mice did not normalize sperm count to organ weight to determine if the effect remained significant. Furthermore, body weights of rats were significantly reduced at the same dosage that caused reduced sperm concentration, which could explain the effect on sperm. Body weights were not reported by Zhang et al. (2014) to determine if that was also a confounding factor in mice. Increased rates of sperm abnormalities were reported in two studies with mice (Zhang et al., 2014; Li et al., 2011), but not observed in the two-generation study in rats (York et al., 2010). In summary, it is unclear whether the effects on spermatogenesis observed in mice are the result of direct toxicity to reproductive processes or a reflection of PFOA's effects on body weight or other systemic effects. Figure C-6 summarizes the effects of PFOA on sperm counts observed in animal studies.

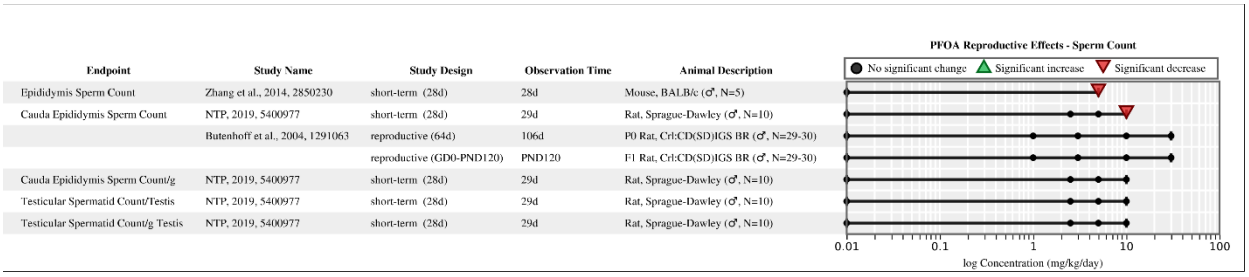


Figure C-6. Sperm Counts in Rodents Following Exposure to PFOA (Logarithmic Scale)

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; F_1 = first generation; PND = postnatal day; d = day.

C.1.2.3 Estrous Cyclicity and Ovarian Function

A small number of studies have evaluated estrous cyclicity and effects on corpora lutea following oral exposure to PFOA, and some significant effects have been observed.

A tendency toward prolonged diestrus was reported in one study with rats (NTP, 2019a) and in one study with mice (Zhang et al., 2020b). In the study by NTP, adult female rats were treated for 28 days with doses up to 100 mg/kg/day and estrous cyclicity was evaluated daily during the last 16 days of treatment. The cycles of treated rats were observed to be mostly similar to controls; however, rats dosed with 100 mg/kg/day spent around 20% more time in diestrus than controls (62.5% vs. 51.9% of the cycle). Markov analyses indicated that high-dose females had a

higher probability than control animals to transition from a regular cycle to a cycle with prolonged diestrus ($p < 0.001$). No effects were observed in the mean estrous cycle length or the lengths of time spent in other estrous stages. The body weights of females were not significantly altered by treatment (NTP, 2019a).

A two-generation reproduction study in rats (Butenhoff et al., 2004a) found no evidence of extended diestrus in P_0 or F_1 female rats, but the doses were lower than the NTP study and the authors did not specifically evaluate the proportion of time spent in diestrus. The study authors observed a significant increase in the number of estrous stages per 21 days in the high-dose (30 mg/kg/day) F_1 females compared with controls (5.4 vs. 4.7 estrous stages/21 days); however, there were no significant differences observed in the incidences of rats displaying prolonged diestrus or estrus (defined as > 6 days for each), and no significant changes were observed in the estrous cycles of females in the P generation. The slight increase observed in number of estrous stages per 21 days was most likely due to the different stages the rats entered the measurement period and was probably not related to PFOA treatment.

A study conducted with mice observed significant effects on the estrous cycle at doses much lower than those causing alterations in the NTP study in rats. Zhang et al. (2020b) administered 0.5–5 mg/kg/day PFOA to adult female mice for 28 days by gavage and monitored daily vaginal cytology throughout the study (sample size n = 8). The number of days spent in diestrus was significantly increased in females treated with 2 or 5 mg/kg/day, and the authors noted that the mice in those groups were rarely observed to enter the estrus phase of the cycle after the second week of exposure to PFOA; however, the durations of estrus and proestrus were not significantly altered by treatment. Body weight was significantly reduced in the 5 mg/kg/day group on days 24 and 28 (by 11%) but not significantly affected in the 2 mg/kg/day group.

In the same study, the numbers of corpora lutea were significantly reduced in mice administered 2 or 5 mg/kg/day PFOA for 28 days; however, no effects were observed on the antral follicle count per ovary (sample size n = 8) (Zhang et al., 2020b). Decreases in the number and size of corpora lutea were also observed in pregnant mice administered PFOA (2.5, 5 or 10 mg/kg/day) beginning on GD 1 (sample size n = 6) (Chen et al., 2017c). The numbers of corpora lutea were significantly decreased in the low- and mid-dose groups on GD 7 and in the mid- and high-dose groups on GD 13. The ratio of corpora lutea to ovarian areas was also significantly decreased at both time points in a dose-dependent manner. The results of this study suggest that PFOA treatment can significantly impair ovarian function during pregnancy and the authors also found evidence of increased oxidative stress and apoptosis in the ovaries of treated mice. Maternal body weights were not reported in this study.

The overall evidence for adverse effects of PFOA on ovarian function is suggestive but inconclusive because the effects were mainly observed in mice and in studies with small sample sizes (n = 6–8). It is likely that prolonged diestrus and reduced corpora lutea observed in mice were treatment-related effects because they followed a clear dose response, and the effects were observed at dose levels lower than those causing decrements in body weight (when reported). Rats also demonstrated a slight increase in the time spent in diestrus, but only at a relatively high dosage (100 mg/kg/day) (NTP, 2019a). Only one study was identified that evaluated effects on corpora lutea in rats (Staples et al., 1984), and that study found no difference between the number of corpora lutea in control rats and those treated with 100 mg/kg/day PFOA from GD 6–GD 15.

C.1.2.4 Altered Pubertal Timing

Lau et al. (2006) reported a slight but significant delay in vaginal opening at 20 mg/kg/day; in contrast, significant accelerations in sexual maturation were observed in males, with preputial separation occurring 4 days earlier than controls at 1 mg/kg/day and 2–3 days earlier at 3, 5, and 10 mg/kg/day, whereas preputial separation in the 20 mg/kg/day group was slightly but significantly delayed compared with controls.

A two-generation study in Sprague-Dawley rats reported significantly delayed sexual maturation (i.e., vaginal opening and preputial separation) in F_1 males and females at 30 mg/kg/day (Butenhoff et al., 2004a). 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 for 5 days/week for 4 weeks. Vaginal opening was significantly delayed in BALB/c mice dosed with 1 mg/kg/day and did not occur at all at 5 or 10 mg/kg/day. In C57BL/6 mice, vaginal opening was delayed at 5 mg/kg/day and did not occur at 10 mg/kg/day.

C.1.2.5 Reproductive Hormone Levels

C.1.2.5.1 Males

Several studies have reported significant alterations in reproductive hormone levels in male animals following oral exposure to PFOA, but the results are not consistent across species or study durations. Figure C-7 summarizes the effects of PFOA on reproductive hormone levels observed in male rodents.

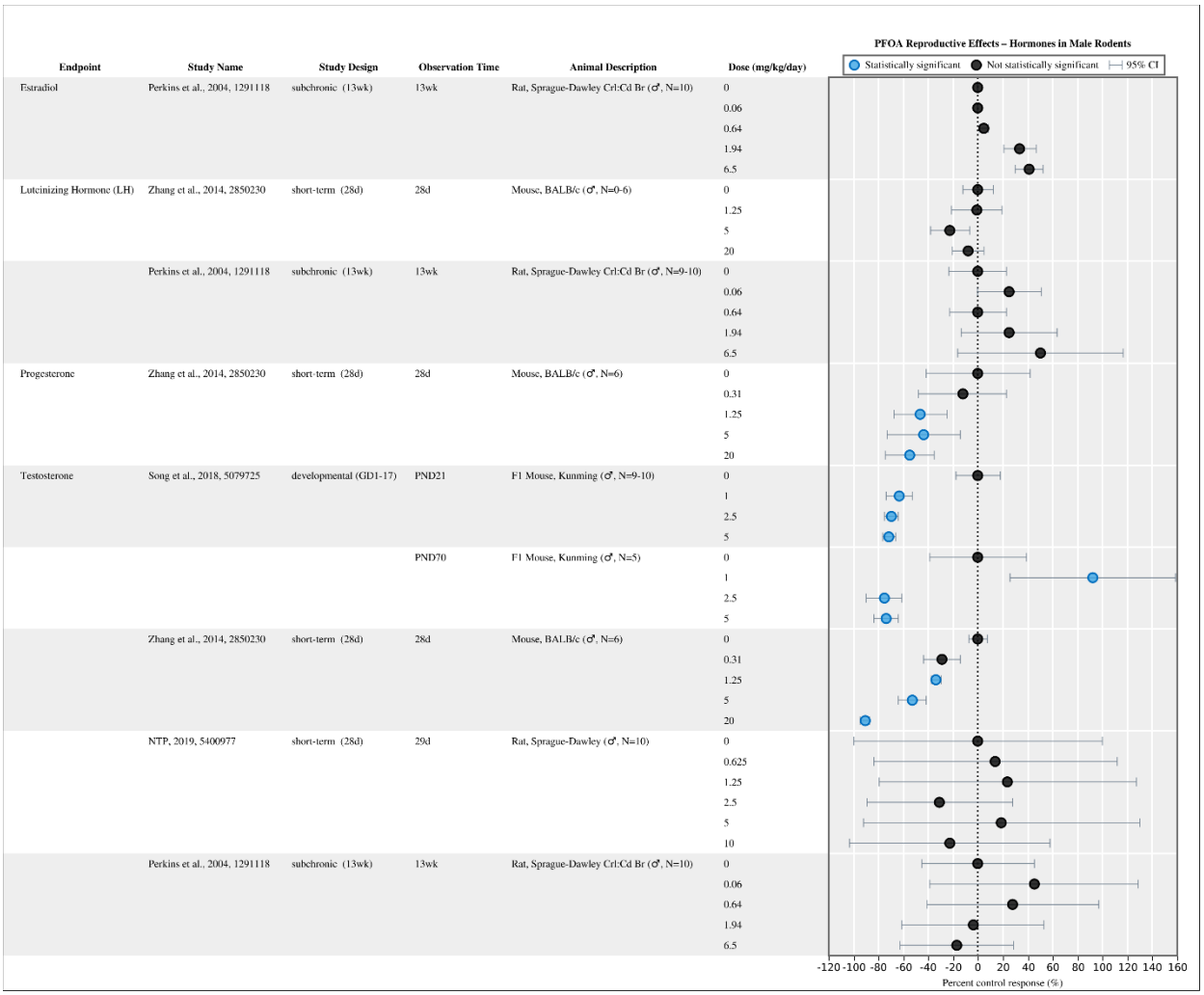


Figure C-7. Percent Change in Male Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
F_1 = first generation; PND = postnatal day; d = day; wk = week.

Testosterone levels were measured in several studies, and significant reductions were reported in two-week studies in rats (Biegel et al., 1995; Cook et al., 1992) and several studies in mice (Song et al., 2018a; Zhang et al., 2014; Li et al., 2011). However, a 28-day study in rats (NTP, 2019a), a 13-week study in rats (Perkins et al., 2004), and a 6-month study in male monkeys (Butenhoff et al., 2002) all observed no significant effects or consistent patterns of alterations in testosterone levels during or after exposure to PFOA. Several studies reported increased serum E2 concentrations in male rats during or after exposure to PFOA (Biegel et al., 2001; Liu et al., 1996; Biegel et al., 1995; Cook et al., 1992). However, another study in rats (Perkins et al., 2004) and one study in monkeys (Butenhoff et al., 2002) found no significant effects of PFOA on male E2 levels. The results for other male reproductive hormones measured in serum shown no clear dose-related trends in LH (Zhang et al., 2014; Perkins et al., 2004).

NTP (NTP, 2019a) administered 0.625–10 mg/kg/day PFOA for 28 days to male rats and found no significant differences in serum testosterone levels between treated groups and controls at the end of the treatment period. The high-dose group had serum testosterone levels 22% lower than controls, but the difference did not attain statistical significance. Likewise, a subchronic dietary study in rats found no significant treatment-related alterations in serum testosterone, E2, or LH levels measured after 4, 7, and 13 weeks of exposure with up to 100 ppm PFOA in the diet (equivalent to 6.5 mg/kg/day) (Perkins et al., 2004).

Biegel et al. (2001) measured hormones at 3-month intervals in male rats fed 300 ppm PFOA for 2 years (equivalent to 13.6 mg/kg/day), and no apparent treatment-related trends were observed in serum testosterone, prolactin, LH, or FSH levels. Serum FSH and testosterone were significantly increased only at 6 months, prolactin decreased significantly at 3 and 6 months, and LH was significantly increased at 6 and 18 months; however, serum E2 levels were consistently increased at the 1-, 3-, 6-, 9-, and 12-month time points compared with controls.

Serum testosterone was significantly reduced in the male offspring of Kunming mice administered PFOA (1, 2.5, or 5 mg/kg/day) from GD 1–GD 17 (Song et al., 2018a). On PND 21, serum testosterone levels were reduced in a dose-dependent fashion in all treated groups (by 63%–71%); however, on PND 70, there was no clear dose-response trend (serum testosterone was increased by 92% in the low-dose group and decreased in the mid- and high-dose groups by 74%–75%). Zhang et al. (2014) administered 0.31, 1.25, 5, or 20 mg/kg/day PFOA to adult male mice for 28 days, and no significant differences were observed in serum LH levels. Testicular testosterone and progesterone concentrations were both significantly reduced at dose levels ≥1.25 mg/kg/day at the end of the treatment period. Testicular testosterone was decreased by 34–91% in a dose-dependent manner, and testicular progesterone was decreased by 44%–55%. In addition, intratesticular cholesterol was significantly reduced (by 39%–44%) at ≥5 mg/kg/day.

In the 6-week mechanistic study by Li et al. (2011), plasma testosterone levels measured at the end of treatment were decreased in wild-type mice administered 1 mg/kg/day (by 37%), and significantly decreased in wild-type mice administered 5 mg/kg/day (by 57%) compared with controls. Plasma testosterone was also significantly decreased in low- and high-dose humanized PPARα mice (by 29% and 31%, respectively). In PPARα-null mice, plasma testosterone was slightly reduced in a dose-related manner, but statistical significance was not attained.

Overall, there are no clear treatment-related trends in male reproductive hormone levels across species and study durations. Serum, plasma, or intratesticular testosterone levels were all decreased in treated mice (Song et al., 2018a; Zhang et al., 2014; Li et al., 2011), but similar effects on testosterone were not observed in rats after 28 days or longer exposures. Testosterone in males is pulsatile and can display large random peaks, therefore studies measuring hormones at various time points over the course of a study are more useful for determining treatment-related effects than studies that measured concentrations at a single time point, for example at necropsy. The studies that measured male hormone levels at various times throughout treatment reported no consistent changes in testosterone (Perkins et al., 2004; Biegel et al., 2001). Two studies reporting reduced testosterone in mice also observed adverse effects on sperm concentration and/or quality following exposure to PFOA (Zhang et al., 2014; Li et al., 2011); however, because of the limited number of studies available and the lack of reproducibility in rats, no firm conclusions can be made about the adversity of these findings. The 22% decrease in testosterone that was observed in high-dose male rats of the 28-day study by NTP (2019a) was

not large enough to be considered adverse given the inherent variability in testosterone levels with a male and between males.

Serum E2 levels were consistently increased at multiple time points in one chronic study in male rats (Biegel et al., 2001); however, the concentrations were very low (in the range of pg/mL), and it has been shown that estrogen levels are too low to be accurately measured using radioimmunoassay kits, which was the method used in that study. Therefore, no firm conclusions can be made about the relevance of those findings as well.

C.1.2.5.2 Females

Figure C-8 summarizes the effects of PFOA on reproductive hormone levels observed female rodents.

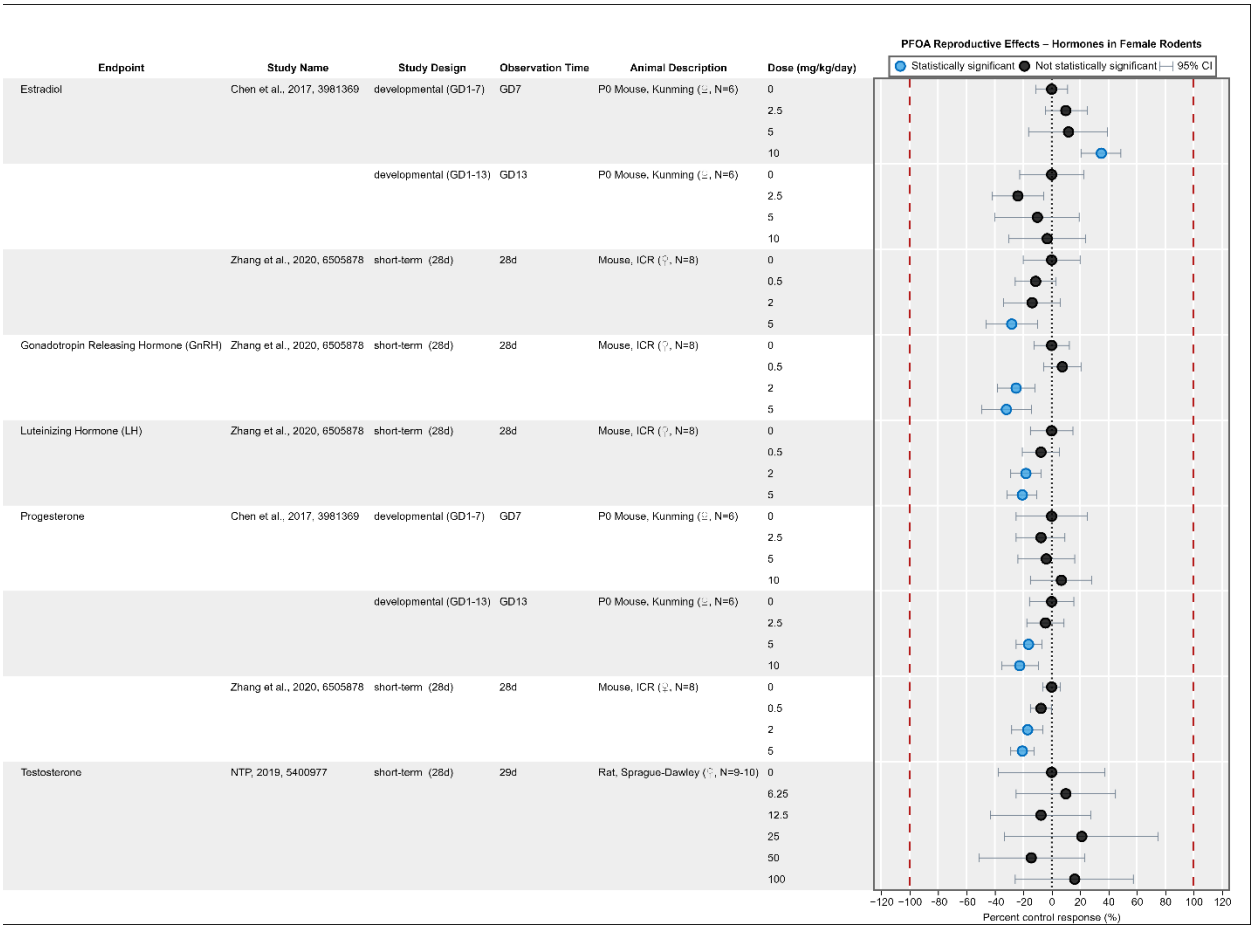


Figure C-8. Percent Change in Female Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
P_0 = parental generation; GD = gestation day; d = day.

Only three studies measured female reproductive hormones following oral exposure to PFOA, and the only effect observed in more than one study was slightly reduced progesterone levels (Zhang et al., 2020b; Chen et al., 2017c).

No significant differences were observed in serum testosterone levels of adult female rats administered 6.25–100 mg/kg/day PFOA for 28 days (NTP, 2019a), but no other reproductive hormones were measured in that study. A 28-day study in adult female mice observed significant reductions in several hormone levels following administration of 2 or 5 mg/kg/day, including reduced serum progesterone (17%–21%), gonadotrophin-releasing hormone (GnRH) (25%–32%), and LH (18%–21%). Serum E2 was also significantly reduced (28%) at 5 mg/kg/day (Zhang et al., 2020b). In contrast, when pregnant female mice were administered PFOA beginning on GD 1 (Chen et al., 2017c), serum E2 was slightly increased on GD 7 but unaltered on GD 13. Meanwhile, serum progesterone was unaltered on GD 7 but was significantly reduced on GD 13 at 5 and 10 mg/kg/day (by 16%–22%).

Because of the small dataset and the small percent changes from controls, no firm conclusions can be made about the effects of PFOA on female reproductive hormones in animals.

C.1.2.6 Reproductive Organ Weights and Histopathology

C.1.2.6.1 Males

Some studies in rats and mice indicate that PFOA exposure can result in changes in the normal structure of the testes and epididymides; however, the overall body of evidence is inconsistent with several other studies reporting no histological changes in male reproductive organs.

Absolute weights of the testes were either significantly decreased (NTP, 2020; Zhang et al., 2014) or unaltered (Crebelli et al., 2019; NTP, 2019a; Butenhoff et al., 2012; Butenhoff et al., 2004a) in adult male rodents following exposure to PFOA. Meanwhile, relative weights of the testes were either significantly increased (NTP, 2020, 2019a; Butenhoff et al., 2004a; Biegel et al., 2001) or unaltered (Zhang et al., 2014; Butenhoff et al., 2012). The decreases observed in absolute testicular weights in conjunction with unaltered or increased relative weights appear to be secondary to body weight changes and therefore unrelated to treatment with PFOA.

Several studies observed no histological changes in the testes, including a 28-day study in rats (NTP, 2019a), a 13-week dietary study in rats (Perkins et al., 2004), a two-generation reproduction study in rats (Butenhoff et al., 2004a), a 26-week study in monkeys (Butenhoff et al., 2002), and a 2-year study in rats (see Toxicity Assessment, (U.S. EPA, 2024b)) (NTP, 2020). However, there is some evidence in mice that suggests developmental exposure can alter the normal structure of the testes. Song et al. (2018a) exposed pregnant mice to 1, 2.5, or 5 mg/kg/day PFOA from GD 1–GD 17 and evaluated testicular weights and histopathology in the male offspring on PND 21 and PND 70. Absolute testis weights were significantly increased in the high-dose group on PND 21, but the effect was not observed on PND 70. There were no significant differences in relative testis weights at either time point and the change in absolute weight appeared to be related to increased body weights also observed in the high-dose group. Histopathological examination revealed significant changes in the testes of the 2.5 and 5 mg/kg/day groups on both PND 21 and PND 70. Effects that were reported quantitatively were decreased numbers of Leydig cells on PND 21 (by 25%–27%) and PND 70 (by 17%–25%) and increased intercellular substance areas on PND 21 (by 105%–111%) and PND 70 (by 9%–13%). Other microscopic changes were reported qualitatively only and included atrophy of the spermatogenic epithelium, reduction in spermatogenic cells, vacuolization of Sertoli cells and decrease or disappearance of spermatozoa at 5 mg/kg/day. With increasing dose to the dam, the degree of damage to the testes was noted to increase. From 2.5 to 5 mg/kg/day, the intercellular

substance in the testes of offspring became larger and the interstitial cells gradually decreased. The spermatogenic cells of all levels were arranged in an irregular pattern; however, vacuolization was not observed on PND 70 indicating some recovery had occurred since PND 21.

Zhang et al. (2014) also reported damage to the testes in adult male mice treated for 28 days, but results were reported qualitatively without incidence data. The findings in rats treated with 5 or 20 mg/kg/day included atrophy of the seminiferous tubule epithelia, lack of germ or Sertoli cells between basal membrane and adluminal portions, and detached germ cells sloughed off into the tubular lumen. In the 6-week mechanistic study in mice by Li et al. (2011), histopathological examination of the testes revealed abnormal seminiferous tubules with vacuoles or lack of germ cells in wild-type and humanized PPARα mice administered 5 mg/kg/day (reported qualitatively without incidence data), but these changes were not observed in PPARα-null mice. Necrotic cells in testes and significantly reduced weights of the epididymis and seminal vesicle plus prostate gland were also observed in the 5 mg/kg/day wild-type mice only.

At the 1-year sacrifice of a chronic dietary study in rats (Butenhoff et al., 2012), testicular tubular atrophy with marked aspermatogenesis was observed in in 2/15 (13%) of high-dose (300 ppm; 14.2 mg/kg/day) males but not in any of the controls (statistical significance not reported). At the terminal evaluation, there were no significant differences in the incidences of tubular atrophy, but the incidence of vascular mineralization in the testes was significantly increased in high-dose males. The incidences of the lesion in the control, 30, and 300 ppm (0, 1.3, and 14.2 mg/kg/day) groups were 0%, 6%, and 18%, respectively. In contrast, a 2-year dietary study conducted by NTP (NTP, 2020) found no treatment-related effects in the testes of rats fed PFOA at concentrations up to 300 ppm (32 mg/kg/day) for 16 weeks or 80 ppm (4.6 mg/kg/day) for 2 years (including groups that were also exposed during gestation; see Toxicity Assessment, (U.S. EPA, 2024b)).

Effects on the epididymis have also been observed following PFOA exposure. Absolute weights of the epididymis or cauda epididymis were significantly reduced in a few studies (NTP, 2019a; Lu et al., 2016b; Butenhoff et al., 2004a), and relative epididymis weight was also significantly reduced in one of those studies (Lu et al., 2016b).

In the two-generation reproduction study in rats, absolute weights of several male reproductive organs were significantly decreased in the high-dose males of the P_0 (i.e., right and left epididymis, cauda epididymis, seminal vesicles with and without fluid, and prostate) while the relative weights of those organs were all significantly increased (except for the prostate) (York et al., 2010; Butenhoff et al., 2004a). The patterns observed were consistent with significant decrements in body weights that were also observed in male groups treated with ≥1 mg/kg/day, and there were no treatment-related changes observed in histopathology of those organs.

NTP (2019a) observed hypospermia and exfoliated germ cells in the epididymis of one rat each in the 5 and 10 mg/kg/day groups following 28 days of oral exposure, although the incidences were not statistically different from controls (n = 10 per group evaluated). This coincided with significantly reduced absolute weights of the left cauda epididymis (≥5 mg/kg/day) and left epididymis (10 mg/kg/day) as well as reduced epididymal sperm count (10 mg/kg/day). However, relative epididymal weights were not reported in this study. No treatment-related effects were observed in the testes, seminal vesicles, or accessory sex glands.

In a 28-day study in mice, absolute weights of the epididymis were reduced in mice treated with 5 or 20 mg/kg/day and relative epididymis weights were also reduced at 20 mg/kg/day (Lu et al., 2016b). Histopathological examination revealed empty spaces in the tubules of cauda epididymis of mice treated with 5 or 20 mg/kg/day and a lack of normal sperm (reported qualitatively without incidence data). In addition, the levels of triglycerides in the epididymis were significantly reduced at 5 and 20 mg/kg/day and the cholesterol content of the epididymis was significantly reduced at 20 mg/kg/day.

In contrast to the results observed in 28-day studies, chronic studies have reported no treatment-related changes in the epididymis or accessory sex glands of treated rats or monkeys (NTP, 2020; Butenhoff et al., 2012; Butenhoff et al., 2002).

Overall, the evidence for adverse effects on the male reproductive system is inconsistent for PFOA. Some studies have reported damage to the testes including atrophy of the seminiferous tubule epithelia (Song et al., 2018a; Zhang et al., 2014; Butenhoff et al., 2012); however, two comprehensive studies conducted by NTP (NTP, 2020, 2019a) and a two-generation reproduction study (Butenhoff et al., 2004a) all reported no significant changes in the histopathology of male reproductive organs and glands. The 2-year study by Butenhoff et al. (2012) reported a small but statistically significant increase in the incidence of vascular mineralization in the testes of high-dose males. The toxicological significance of that finding is unclear as the study authors did not evaluate any parameters related to fertility including any hormone levels nor did they see any effects on testes weights. In addition, this lesion was not observed in another chronic rat study (NTP, 2020) or in any of the shorter duration mouse studies where there were suggestive effects on sperm parameters and fecundity. When mice were exposed to PFOA in utero, the numbers of Leydig cells in the testes were decreased and there was evidence of dose-dependent testicular damage on PND 21 and PND 70 (Song et al., 2018a). Leydig cells are the primary site of testicular steroidogenesis in males (Huhtaniemi and Toppari, 1995). BWTs of the pups and growth during the lactation period were not reported; therefore, it is unclear whether these effects reflect a specific toxicity to the testes or if they resulted from delayed growth and systemic toxicity. Body weights were not reduced compared with control on PND 21 or PND 70; therefore, a direct effect on the developing testes cannot be ruled out.

Reduced epididymal weights were reported in two studies along with reduced epididymal sperm concentration and/or observations of hypospermia (NTP, 2019a; Lu et al., 2016b). It is also unclear whether these effects resulted from a specific toxicity to the epididymis or from concurrent systemic toxicity as effects were observed in conjunction with decrements in body weight (NTP, 2019a) or body weights were not reported (Lu et al., 2016b).

C.1.2.6.2 Females

Histopathological changes in the uterus and ovary have been observed following exposure to PFOA; however, comprehensive studies with chronic exposure durations do not provide evidence of increased nonneoplastic lesions in female reproductive organs.

Li et al. (2018a) administered PFOA (1, 5, 10, 20, or 40 mg/kg/day) to pregnant Kunming mice from GD 1–GD 17 and measured apoptosis in the uterine tissue on GD 18. The number of apoptotic cells was significantly increased for females dosed with 5 mg/kg/day or higher in a dose-dependent manner compared with controls, and embryo survival was significantly decreased at doses ≥10 mg/kg/day (see Toxicity Assessment, (U.S. EPA, 2024b)). The uterus

was examined in several other studies with no significant changes reported in organ weight or incidences of nonneoplastic lesions, including a 28-day study in rats (NTP, 2019a), a two-generation reproduction study in rats (Butenhoff et al., 2004a) and a 2-year dietary study in rats (Butenhoff et al., 2012). No significant differences in uterine weights were observed at the 16-week interim evaluation of the NTP 2-year dietary study in rats (see Toxicity Assessment, (U.S. EPA, 2024b))(NTP, 2020); however, the terminal evaluation found that females treated with PFOA had a higher incidence of uterine adenocarcinoma that may have been related to exposure (see Toxicity Assessment, (U.S. EPA, 2024b)). The incidences of nonneoplastic lesions of the uterus were not significantly increased in any of the PFOA exposure groups (NTP, 2020).

As mentioned above, Chen et al. (2017c) and Zhang et al. (2020b) both observed significant changes in the ovaries of adult female mice administered PFOA, including reductions in the number of corpora lutea and the ratio of corpora lutea to ovarian areas. However, the NTP chronic dietary study (NTP, 2020) and a two-generation reproduction study (Butenhoff et al., 2004a) both found no treatment-related effects in the ovaries of treated rats. Butenhoff et al. (2012) observed a significant, dose-related increase in the incidences of ovarian tubular hyperplasia in rats exposed for 2 years to PFOA in the diet. The incidences of this lesion in the control, 30, and 300 ppm groups were 0%, 14%, and 32%, respectively. The tissues were subjected to a pathology peer review using updated diagnostic nomenclature and no statistical differences were found between treated groups and controls (Mann and Frame, 2004).

C.1.3 Mechanistic Evidence Synthesis

Mechanistic evidence linking PFOA exposure to adverse reproductive outcomes is discussed in Sections 3.2.2, 3.2.7, 3.3.3, 3.3.4, and 3.4.3 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 56 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 reproductive effects. A summary of these studies is shown in Figure C-9. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to reproductive effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	1	2
Big Data, Non-Targeted Analysis	2	0	5	6
Cell Growth, Differentiation, Proliferation, Or Viability	11	0	23	29
Cell Signaling Or Signal Transduction	10	1	24	32
Extracellular Matrix Or Molecules	0	0	3	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	3	0	2	4
Hormone Function	9	1	22	28
Inflammation And Immune Response	2	0	1	2
Oxidative Stress	3	0	6	9
Xenobiotic Metabolism	1	0	6	6
Other	0	0	1	1
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	23	2	41	56

Figure C-9. Summary of Mechanistic Studies of PFOA and Reproductive Effects

Interactive figure and additional study details available on HAWC.

C.1.4 Evidence Integration

C.1.4.1 Reproductive Effects in Males

There is *slight* evidence for an association between PFOA exposure and male reproductive effects based on inverse associations with testosterone in male children and adults, and decreased AGD in children. Negative effects were observed for some semen characteristics (e.g., semen motility, DNA fragmentation), but positive associations were also observed (e.g., sperm concentration). There was inconsistent evidence for the relationship between PFOA exposure and testosterone in cross-sectional studies (Di Nisio et al., 2019; Lopez-Espinosa et al., 2016) in children and young adults. Inconsistent associations were observed in populations at different stages of pubertal development, and one positive association was observed in a *low* confidence study (Di Nisio et al., 2019). One *medium* confidence study (Liu et al., 2020b) observed a positive association for progesterone in male infants. Studies in adolescents did not observe effects on pubertal development, but negative associations were observed for testicular volume, penis length, penis circumference, and number of sperm with normal morphology (Di Nisio et al., 2019). In adults, there was evidence in two studies (Cui et al., 2020; Petersen et al., 2018) of inverse associations between serum PFOA and testosterone (total and free), and these associations were also observed using semen PFOA. Inverse associations were also observed for E2 and the total testosterone-LH ratio. For semen and sperm characteristics in adults, associations were observed for several parameters in analyses of semen PFOA, including increased sperm concentration and total sperm count, decreased motility and number of morphologically normal sperm, and increased sperm DNA fragmentation. Other results for markers of genotoxic effects (e.g., sperm Y:X-chromosome ratio, sperm DNA methylation) in

sperm were inconsistent. Overall, these studies provide additional evidence of potential effects on testosterone levels in adult males.

The animal evidence for an association between PFOA exposure and reproductive toxicity in males is *slight* based on several *high* or *medium* confidence animal studies; however, the evidence from animal studies is similarly inconsistent as in epidemiological studies. Despite this, some studies observed significant alterations in reproductive hormone levels and adverse effects on sperm parameters. Exposure during development or for short durations in adult rodents has resulted in changes in the normal structure of the testis and epididymis (NTP, 2019a; Song et al., 2018a; Lu et al., 2016b; Zhang et al., 2014; Li et al., 2011). Chronic exposure studies generally found limited histological changes in the testes that included increased incidence of vascular mineralization (Butenhoff et al., 2012) and Leydig cell hyperplasia (Biegel et al., 2001). However, these findings were not observed in another 2-year study by NTP (2020). EPA concluded that the observed changes in the testes and epididymis represent toxicities possibly relevant to humans. In particular, alterations in Leydig cell structure or physiology may be driving the reductions in testosterone and effects on sperm parameters seen in both humans and animals (Zirkin and Papadopoulos, 2018).

C.1.4.2 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause reproductive effects in males under relevant exposure circumstances (Table C-1). This conclusion is based primarily on effects on inverse associations with testosterone in male children and adults, and decreased AGD in children observed in studies in humans exposed to median PFOA ranging from 1.4 to 34.8 ng/mL. Although there is some evidence of negative effects of PFOA exposure on semen and sperm characteristics in adults, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. For male reproductive toxicity, the conclusion is based primarily on observed changes in hormonal parameters and in the normal structure of the testis and epididymis in animal models following exposure to doses as low as 1 mg/kg/day PFOA. However, findings from animal studies are similarly inconsistent as in epidemiological studies.

Table C-1. Evidence Profile Table for PFOA Reproductive Effects in Males

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 C.1.1)					⊕⊙⊙ ***Evidence Suggests***
Male reproductive hormones 1 *High* confidence study 8 *Medium* confidence studies 4 *Low* confidence studies	Results from studies in children were inconsistent regarding measures of testosterone. Significant increases (1/9) and significant inverse associations (1/9) with total testosterone were observed, but the remaining studies reported imprecise results (6/9). Increases in estrogenic hormones (i.e., estrone, estradiol, and estriol) were observed in children (2/4), but only one result was significant. Significant increases in LH (1/9) and progesterone (1/9), and inverse associations with androgen hormones, such as DHEA and androstenedione (2/9) were observed in children. Significant inverse associations with free testosterone (2/4) and total testosterone (1/4) were observed in adults. Inverse associations in LH, FSH, and SHBG were also observed (1/4).	• *High* and *medium* confidence studies • *Coherence* of findings between changes in androgenic and estrogenic sex hormones	• *Low* confidence studies • *Imprecision* of findings • Potential for *residual confounding* by SES and smoking status	⊕⊙⊙ *Slight* Evidence for male reproductive effects is based on several *medium* confidence studies reporting consistent and precise associations. More evidence is available for associations with male testosterone levels, male pubertal development, and male anthropometric measurements compared with other effects. Other findings were either inconsistent or are reported in a limited number of studies.	*Primary basis*: Human evidence indicted effects on inverse associations with testosterone in male children and adults, and decreased AGD in children observed in studies in humans exposed to median PFOA. Although there is some evidence of negative effects of PFOA exposure on semen and sperm characteristics in adults, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. Animal evidence indicated changes in hormonal parameters and in the normal structure of the testis and epididymis in animal models following exposure to PFOA. However, findings from animal studies are similarly inconsistent as in epidemiological 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**	
Semen parameters 4 *Medium* confidence studies 1 *Low* confidence study	The only *low* confidence study evaluating adolescents observed significant inverse associations with sperm concentrations and progressive motility and increased semen pH with higher exposure. In four *medium* confidence studies of adults, results were mixed, with one study finding evidence of significantly increased sperm concentration and count and significant inverse associations with measures of motility and morphology (1/4). Other studies reported inverse associations with semen parameters (3/4), with one result for progressive motility reaching significance (1/3).	• *Medium* confidence studies • Consistent direction of effects	• *Low* confidence study • *Imprecision* of most findings • Potential for *residual confounding* by SES and smoking status		*Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Anthropometric measurements of male reproductive organs 1 *High* confidence study 2 *Medium* confidence studies 1 *Low* confidence study	In children and adolescents, one *medium* and one *low* confidence study reported significant effects for anthropometric measurements of male reproductive organs (2/4). In a *medium* confidence study, children from the Shanghai-Minhang Birth	• *High* and *medium* confidence studies • *Consistent direction* of effects • *Coherence* of findings	• *Low* confidence study • *Imprecision* of some findings • Potential for *residual confounding* by SES and smoking status		

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**	
	Cohort study reported significant inverse associations with AGD. A *low* confidence study reported smaller AGD in exposed compared with unexposed children, and significant differences in testicular measurements, such as smaller testicular volume and shorter penis length. A *high* confidence study reported inverse associations with AGD that did not reach significance.				
Male pubertal development 1 *Medium* confidence study	Findings for changes in timing of pubertal development milestones were non-significant. Voice break was observed to occur earlier for those in the highest exposure tertile, but the association was not significant.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.1.2)					
Organ weights 4 *High* confidence studies 6 *Medium* confidence studies	Several rodent studies have shown changes in testis or epididymis weight following PFOA exposure (6/10). However, evidence is not consistent as one mouse study (1/10) and several rat studies (3/10) show no effect of PFOA on the weight of male	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across studies and species • Changes in body weight may limit ability to interpret these responses	⊕⊙⊙ *Slight* Evidence was based on 11 *high* and *medium* confidence studies. Changes in male reproductive organs, such as organ weight or	

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**	
	reproductive organs. Absolute testis weights were mostly unchanged in rats (4/6) and mice (1/3), although relative testis weight was increased in rats (3/5) and unchanged in mice (2/2). Absolute epididymis weight was decreased in two studies (2/3), with one in mice and one in rats. The study in mice also reported decreased relative epididymis weight (1/1).			structural changes, were observed. However, these results were inconsistent among studies. Effects observed in male rodents include decreased epididymal weights, delayed sexual maturation, decreased sperm count and quality, alterations in reproductive hormone levels, and morphological changes in the testes and epididymides.	
Histopathology 4 *High* confidence studies 5 *Medium* confidence studies	Several studies in rats and mice found changes in the structure of the testes and epididymides (6/9). In rats, nonneoplastic changes in the testes were noted (2/6) including increased Leydig cell hyperplasia and vascular mineralization. A short-term rat study found a slight increase in exfoliated germ cells in the epididymis. In mice, one short-term study found changes in the epididymis including empty spaces in the tubules of cauda epididymis and a lack of normal sperm. Another mouse study observed	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across 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**	
	increased tubular degeneration and atrophy of the seminiferous tubules in the testes. A third mouse study found decreased numbers of Leydig cells and increased intercellular area in the testes of pups exposed in utero. Two chronic rat studies found no changes in the testis, epididymis, prostate, or seminal vesicles.				
Male reproductive hormones 2 *High* confidence studies 3 *Medium* confidence studies	Testosterone was decreased following PFOA exposure (2/5), but only in male mice. In rats, testosterone was either increased (1/3) or showed no difference (2/3). Decreases in progesterone were observed in male mice (1/1) and in prolactin for male rats (1/1). LH was decreased in one rat study (1/3). Estradiol was consistently increased in one male rat study (1/2). No changes were observed in FSH (0/1) in male rats.	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects among studies and species • *Limited number* of studies examining specific outcomes		
Sperm parameters 2 *High* confidence studies 1 *Medium* confidence study	Sperm count was decreased following PFOA exposure in two studies (2/3), including one study in mice and one short-term	• *High* and *medium* confidence studies	• *Limited number* of studies evaluating endpoint • *Inconsistent direction* of effects between species		

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**	
	study in rats. However, a two-generation reproduction study in rats found no effects on sperm count. Sperm motility was decreased in one mouse study (1/3), but not in two rat studies.		• *Incoherence* of findings between decreased sperm count and lack of effects on fertility		
Male pubertal development 1 *High* confidence study 1 *Medium* confidence study	The timing of preputial separation in males was altered (2/2). One rat study found delayed preputial separation after PFOA exposure. One mouse study found that preputial separation occurred earlier at low doses but later at the highest dose.	• *High* and *medium* confidence study	• *Limited number* of studies evaluating endpoint		
Male mating and fertility 1 *High* confidence study	One two-generation reproduction study reported no effects on mating or fertility in rats administered PFOA for 10 wk prior to mating (1/1).	• *High* confidence study	• *Limited number* of studies evaluating endpoint		

Notes: AGD = anogenital distance; DHEA = dehydroepiandrosterone; FSH = follicle stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone binding globulin; SES = socioeconomic status; wk = weeks.

C.1.4.3 Reproductive Effects in Females

There is *slight* evidence for an association between PFOA exposure and female reproductive effects in humans based on observed infertility effects across a limited number of epidemiological studies, observed in populations with high exposure levels and at levels typical in the general population.

Results for female fertility are mixed. In the 2016 Health Assessment (U.S. EPA, 2016c), two studies reported correlations between higher PFOA levels and infertility (Vélez et al., 2015; Fei et al., 2009). Studies published since the 2016 PFOA HESD have observed no clear dose-response trends or directionality for a potential relationship (Kim et al., 2020b; Crawford et al., 2017; Lum et al., 2017; Wang et al., 2017). However, Kim et al. (2020b) did observe some non-significant, positive associations between follicular fluid PFOA and fertility etiology factors for other gynecologic pathologies, including endometriosis, polycystic ovarian syndrome (PCOS), genital tract infections, and idiopathic infertility.

There is limited evidence of an inverse association between serum PFOA levels in pregnancy and breastfeeding duration. Timmermann et al. (2017b) observed negative associations between PFOA exposure and exclusive and total breastfeeding duration, while Romano et al. (2016) observed increased relative risk of breastfeeding termination with increasing PFOA exposure.

Evidence of a relationship between PFOA exposure and the female reproductive milestones of age at menarche and menopause is mixed. In the 2016 Health Assessment (U.S. EPA, 2016c), Kristensen et al. (2013) reported a positive association between prenatal PFOA exposure and later age at menarche, while Christensen et al. (2011) reported no association between the two. Since the 2016 Health Assessment, Ernst et al. (2019) observed a non-significant, negative association between prenatal PFOA exposure and age at menarche. Other studies have investigated relationships between the menarche as well as menopause and concurrent PFOA exposure. In the 2016 Health Assessment, Lopez-Espinosa et al. (2011) observed a positive association between concurrent PFOA exposure and age at menarche. More recently, Ding et al. (2020) observed an inverse relationship between PFOA levels and age at menopause. However, findings from studies concurrently assessing menstruation events and PFOA levels in blood must be interpreted with caution due to potential reverse causality, as menstruation is a primary route of PFOA excretion for people who menstruate.

Since the 2016 PFOA Health Assessment (U.S. EPA, 2016c), 11 studies have assessed relationships between PFOA exposure and various female reproductive hormones, nine of which studied female infants and adolescents. Most studies did not report significant associations or consistent trends between PFOA exposure and reproductive hormones including 17-OHP, DHEA, E2, FSH, SHBG, and testosterone. *Medium* confidence studies have observed significant, positive associations between cord blood PFOA and estriol in female infants (Wang et al., 2019a), concurrent PFOA exposure and serum E2 in female adolescents (Lopez-Espinosa et al., 2016), and maternal serum PFOA during pregnancy and AMH concentrations in adolescent daughters (Donley et al., 2019). There were few studies assessing relationships between PFOA exposure and female reproductive hormone levels in adult women (both pregnant and nonpregnant), and those identified did not report consistent evidence of relationships between PFOA exposure and these outcomes. Evidence of relationships between PFOA exposure and human female reproductive hormonal outcomes remains inconsistent.

The recent review observed evidence of an association between PFOA and preeclampsia and gestational hypertension; there is conflicting evidence on altered puberty onset and limited data suggesting reduced fertility and fecundity. The associations are inconsistent across reproductive hormone parameters, and it is difficult to assess the adversity of these alterations.

The animal evidence for an association between PFOA exposure and female reproductive toxicity is *slight* based on several *high* and *medium* confidence animal studies; however, it is often unclear whether alterations seen in animal studies reflect specific toxicity to the reproductive system or if they result from concurrent systemic toxicity. Despite this, some studies observed significant alterations in reproductive hormone levels and ovarian physiology which were not confounded by alterations in body weight. Specifically, effects of PFOA on the ovary included altered estrous cyclicity and number of corpora lutea. In female mice, effects on the estrous cycle (lengthened diestrus phase) were observed at doses that did not significantly reduce body weight (Zhang et al., 2020b). These results in mice are supported by a study in female rats that similarly found slightly lengthened diestrus phase, though with a much higher PFOA dose (NTP, 2019a). Altered ovarian physiology was also evidenced by two studies (short-term and gestational) in adult female mice showing reduced numbers of corpora lutea with increasing PFOA doses (Zhang et al., 2020b; Chen et al., 2017c) and one study in female rats (chronic) showing increased tubular hyperplasia of the ovarian stroma (Butenhoff et al., 2012).

C.1.4.4 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause reproductive effects in females under relevant exposure circumstances (Table C-2). This conclusion is based primarily on effects on infertility, female reproductive milestones, and female reproductive hormonal outcomes observed in studies in humans exposed to median PFOA ranging from 3.7 to 30.1 ng/mL. There is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. For female reproductive toxicity, the conclusion is based primarily on alterations in ovarian physiology and hormonal parameters in adult rodents following exposure to doses as low as 1 mg/kg/day PFOA. However, findings from animal studies are similarly inconsistent as in epidemiological studies.

Table C-2. Evidence Profile Table for PFOA Reproductive Effects in Females

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 C.1.1)					⊕⊙⊙ ***Evidence Suggests***
Female reproductive hormones 3 *High* confidence studies 10 *Medium* confidence studies 7 *Low* confidence studies	In 12 studies of female children and adolescents, 4 studies reported significant associations. Positive associations were reported for estriol in infants in a *medium* confidence study (1/4). Both E2 and total testosterone levels had positive associations reported in both a *medium* and a *low* confidence study (2/4). Results from 9 studies of adults, rarely met significance, though one *low* confidence study reported increased testosterone relative to controls, and another *low* confidence study reported increased levels of prolactin. There were no significant results for SHBG.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Imprecision* of most findings • Potential for *selection bias* and *residual confounding* by age and SES	⊕⊙⊙ *Slight* Evidence for female reproductive effects is based on several studies reporting effects on sex hormones and increased odds of preeclampsia. There was also evidence for changes in age at natural menopause. Uncertainties remain regarding mixed findings in studies of sex hormones, and a limited number of studies examining outcomes such as female reproductive milestones and anthropometric measurements.	*Primary basis*: Human evidence indicted effects on infertility, female reproductive milestones, and female reproductive hormonal outcomes observed in studies in humans exposed to PFOA. There is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. Animal evidence indicated alterations in ovarian physiology and hormonal parameters in adult rodents following exposure to PFOA. However, findings from animal studies are similarly inconsistent as in epidemiological studies. *Human relevance, cross-stream coherence, 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**	
Preeclampsia and gestational hypertension 1 *High confidence* study 5 *Medium* confidence studies 3 *Low* confidence studies	Seven studies examined preeclampsia in pregnant women (7/9). None reported significant results, though of *medium* and *low* confidence studies, 6 reported positive associations (6/6) and two reported negative associations (2/6) for at least one exposure group or for continuous analyses. Of the three studies examining gestational hypertension (3/9), two reported increased odds but neither reached significance (2/3). However, after observing non-significant increased odds of gestational hypertension, one *medium* confidence study reported increased DBP and significantly increased SBP.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Imprecision* of all findings • Potential for *reverse causality*		
Female reproductive milestones 1 *High* confidence study 3 *Medium* confidence studies	Three studies examined reproductive milestones related to menstruation, two in adolescent populations and one in an adult population. Two	• *High* and *medium* confidence studies • *Consistent direction* of effects • *Coherence* of findings	• *Low* confidence study • Potential for *residual confounding* by not identifying confounders		

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**	
1 *Low* confidence study	studies, one *low* confidence study in adolescents (1/2) and one *medium* confidence study in adults (1/1), reported significant increases in long menstrual cycles. The study in adolescents also reported increased risk of hypomenorrhea and irregular menstruation. There were no significant effects with other pubertal milestones. Two studies of *medium* and *high* confidence evaluated age at natural menopause. Both observed significant positive associations, though only among the highest exposure group in the *high* confidence study.				
Fertility indicators 6 *Medium* confidence studies 7 *Low* confidence studies	Examinations of fertility indicators include fecundability, fertilization rate, and measures of ovarian health, such as anti-Müllerian hormone levels or endometriosis. Thirteen studies evaluated fertility	• *Medium* confidence studies	• *Low* confidence studies • *Imprecision* of most findings • Potential for *residual confounding* by not identifying confounders		

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**	
	indicators in nonpregnant women with mixed results. Five reported significant positive associations (5/13) with anti-Müllerian hormone, a marker of ovarian reserve, in adolescents (1/5), and increased odds of endometriosis (2/5) and ovarian syndromes (2/5). Other studies did not report significant associations for these measures and some observed inverse associations.				
Breastfeeding 2 *Medium* confidence studies	Two *medium* confidence cohort studies reported significant inverse associations with breastfeeding duration (2/2).	• *Medium* confidence studies • *Consistent direction* of effects • *Precision* of findings	• *Limited number* of studies examining outcome		
Anogenital distance 1 *High* confidence study 1 *Medium* confidence study	Two studies examined measures of anogenital distance, including anoclitoris and anofourchette distances, in female infants. A *medium* confidence study reported non-significant increases in anoclitoris distance for	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent* direction of 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**	
	all exposure groups and in continuous analysis. Results for anofourchette distances were non-significant and mixed. A *high* confidence study observed non-significant mixed results for both measures.				
Evidence From In Vivo Animal Studies (Section C.1.2)					
Organ weights 3 *High* confidence studies 3 *Medium* confidence studies	Several rodent studies show a lack of evidence of changes in female reproductive organ weights following PFOA exposure (5/6). Only one mouse study found decreased absolute and relative gravid uterus weight following gestational PFOA exposure; however, concurrent decreases in maternal body weight and in embryo survival and body weight make these results difficult to interpret. Otherwise, there were no changes in uterus weight (4/5) or ovary weight (2/2) among mouse or rat studies.	• *High* and *medium* confidence studies	• Changes in body weight may limit ability to interpret these responses	⊕⊙⊙ *Slight* Evidence is based on 8 *high* and *medium* confidence studies. Changes in female reproductive organs, such as organ weight or structural changes, were observed. However, these results were inconsistent among studies. Effects observed in female rodents include morphological changes in the uterus, delayed sexual maturation, alterations in reproductive hormone levels, and alterations in ovarian physiology and structure including effects on the estrous cycle (prolonged diestrus), reduced number	

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**	
Histopathology 3 *High* confidence studies 2 *Medium* confidence studies	For nonneoplastic effects on uterus, one study found evidence of effects following PFOA exposure (1/5). This study in mice found dose-related increases in the number of apoptotic cells in the uterine tissue of pregnant mice on GD 18. For nonneoplastic effects on ovaries, three rat studies found no exposure-related effects on the ovaries (3/4). However, one rat study observed a dose-related increase in ovarian tubular hyperplasia after 2 yr of PFOA exposure.	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Inconsistent direction* of effects among studies	and size of corpora lutea in the ovaries, and increased tubular hyperplasia of the ovarian stroma.	
Female reproductive hormones 1 *High* confidence study 2 *Medium* confidence studies	Progesterone was slightly decreased in female mice following PFOA exposure (2/2). No effects on serum testosterone levels were reported in a short-term study in female rats (1/1). One mouse study found that estradiol increased in dams after gestational PFOA exposure (1/2).	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent direction* of effects across 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**	
	However, another mouse study in adults (1/2) found decreases in E2, along with decreases in LH and in GnRH.				
Estrous cyclicity 2 *High* confidence studies 1 *Medium* confidence study	Exposed rats and mice spent more time in diestrus (i.e., prolonged diestrus) in two studies (2/3). However, a two-generation rat study did not find evidence for prolonged diestrus. No changes in estrous cycle length were noted (3/3).	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects among studies • *Limited number* of studies examining outcome		
Ovarian function 2 *Medium* confidence studies	Decreases in the number of corpora lutea in the ovaries were observed in mice following PFOA exposure (2/2).	• *Medium* confidence studies • *Consistent direction* of effects	• *Limited number* of studies examining outcome		
Female pubertal development 1 *High* confidence study 1 *Medium* confidence study	Delayed vaginal opening was observed in female rats and mice following PFOA exposure (2/2).	• *High* and *medium* confidence study • *Consistent direction* of effects	• *Limited number* of studies examining outcome		
Female mating and fertility 1 *High* confidence study 1 *Medium* confidence study	No effects on female mating or fertility parameters were observed in one- and two-generation reproduction studies in rats with PFOA exposure	• *High* and *medium* confidence study • *Consistent direction* of effects	• *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**	
	beginning 10 wk prior to mating (2/2).				

Notes: E2 = estradiol; SHBG = sex hormone binding globulin; SES = socioeconomic status; DBP = diastolic blood pressure; SBP = systolic blood pressure; GD = gestational day; LH = luteinizing hormone; GnRH = gonadotropin-releasing hormone; wk = weeks.

C.2 Endocrine

EPA identified 34 epidemiological and 9 animal studies that investigated the association between PFOA and endocrine effects. Of the epidemiological studies, 4 were classified as *high* confidence, 15 as *medium* confidence, 9 as *low* confidence, 3 as mixed (1 *high*/*medium*, 1 *medium*/*low*, and 1 *medium*/*uninformative*) confidence, and 3 were considered *uninformative* (Section C.2.1). Of the animal studies, three were classified as *high* confidence, and six were considered *medium* confidence (Section C.2.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.2.1 Human Evidence Study Quality Evaluation and Synthesis

C.2.1.1 Introduction

Thyroid disease encompasses conditions such as hypothyroidism and hyperthyroidism, and it is more common in females than in males. Hypothyroidism is characterized by elevated thyroid stimulating hormone (TSH) and concurrently low T4 concentrations, while subclinical hypothyroidism is characterized by elevated TSH in conjunction with normal T4 and triiodothyronine (T3) levels. Hyperthyroidism is characterized by elevated T4 and low TSH, and subclinical hyperthyroidism is characterized by low levels of TSH with normal T4 and T3 levels.

The 2016 Health Advisory (U.S. EPA, 2016a) and HESD (U.S. EPA, 2016c) identified limited evidence of endocrine effects of PFOA for thyroid disease, hypothyroidism, and hypothyroxinemia. Evidence from occupational cohorts and from general population studies was mixed. An analysis of an occupational cohort in Minnesota (Olsen et al., 1998) showed elevated TSH ($p = 0.002$) levels in a single exposure group (10–30 μg/mL serum PFOA); however, this increase was not observed for those with greater exposure (>30 μg/mL serum PFOA). Pooled occupational analyses, combing the Minnesota cohort with cohorts from Belgium and Alabama (Olsen and Zobel, 2007; Olsen et al., 2003), showed a negative association for free T4, and a positive association was found for T3. Two studies on participants from the C8 Health Project showed positive associations between estimated PFOA exposure (cumulative and yearly) and all incident self-reported thyroid disease in women (Winquist and Steenland, 2014b), and thyroid disease in children examining modeled in utero PFOA exposure and concurrent PFOA serum concentrations (Lopez-Espinosa et al., 2012). As a result of these findings, the C8 Science Panel concluded that a probable link exists between PFOA and thyroid disease (C8 Science Panel, 2012). In general population studies, positive associations were found with T4 in older adults (Shrestha et al., 2015), with T3 (free and total) in females (Wen et al., 2013), and between prenatal PFOA (cord blood) and T4 concentrations in thyroid disease-free girls (de Cock et al., 2014b). Other studies did not observe significant associations in adults and children (Lin et al., 2013b; Bloom et al., 2010). Most results in studies on pregnant women were not significant except for small positive associations with TSH (Berg et al., 2015), especially in pregnant women with elevated TPOAb (Webster et al., 2014).

For this updated review, 32 studies (33 publications)[8] report on the association between PFOA exposure and endocrine effects. Six publications were studies in pregnant women (Aimuzi et al., 2020; Dreyer et al., 2020; Inoue et al., 2019; Itoh et al., 2019; Reardon et al., 2019; Shah-Kulkarni et al., 2016), and the remainder of the publications were on the general population. One study was a controlled trial (Convertino et al., 2018), six were cohort studies (Lebeaux et al., 2020; Reardon et al., 2019; Blake et al., 2018; Liu et al., 2018a; Preston et al., 2018; Crawford et al., 2017), six were cohort and cross-sectional studies (Dreyer et al., 2020; Kim et al., 2020a; Itoh et al., 2019; Xiao et al., 2019; Kato et al., 2016; Wang et al., 2014) two case-control studies (Kim et al., 2016a; Predieri et al., 2015), one case-control and cross-sectional study (Zhang et al., 2018b), and 18 cross-sectional studies (Abraham et al., 2020; Aimuzi et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Inoue et al., 2019; Jain and Ducatman, 2019b; Byrne et al., 2018; Dufour et al., 2018; Heffernan et al., 2018; Kang et al., 2018; Khalil et al., 2018; Li et al., 2017b; Tsai et al., 2017; Christensen et al., 2016b; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Lewis et al., 2015; Jain, 2013). All observational studies measured PFOA in blood components (i.e., blood, plasma, or serum). Two studies (Itoh et al., 2019; Kato et al., 2016) belonged to the same cohort, the Hokkaido Study on the Environment and Children's Health. While most studies evaluated the relationship between exposure to PFOA and thyroid hormone concentrations, other endocrine outcomes examined included: thyroid disease, thyroid antibodies (thyroglobulin antibodies (TgAb) and thyroid peroxidase antibody (TPOAb)), and thyroid hormone-associated proteins (e.g., thyroglobulin, T4-binding globulin).

C.2.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies. First, timing of exposure and hormone concentration measurements was important. Several studies on mother-child dyads examined relationships between maternal serum PFOA measurements and thyroid hormones in both mothers (i.e., a cross-sectional analyses) and in cord blood or children's serum (i.e., a longitudinal analyses). Longitudinal comparisons between maternal PFOA concentrations measured during pregnancy and thyroid hormone levels in cord blood or the child's blood attenuate any concerns for potential reverse causality. Measuring PFOA and thyroid hormone concentrations concurrently in maternal serum 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., 2018b), current blood concentrations are expected to correlate well with past exposures. Second, timing of thyroid hormone assessment was a recurring concern due to the diurnal variation in thyroid hormones. Thyroid hormone outcome misclassification due to timing of blood collection is non-differential, however, study sensitivity may be impacted in cases where timing of collection was uncontrolled.

There are 34 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 endocrine effects. Study quality evaluations for these 34 studies are shown in Figure C-10 and Figure C-11.

Of the 34 studies identified since the 2016 assessment, 4 studies were classified as *high* confidence, 15 as *medium* confidence, 9 as *low* confidence, 3 as *mixed* (1 *high/medium*, 1

[8] Itoh et al. (2019) reports thyroid-related hormone levels in a population overlapping with Kato et al. (2016).

medium/low, and 1 *medium/uninformative*) confidence, and 3 were considered *uninformative* (Abraham et al., 2020; Seo et al., 2018; Kim et al., 2016a).

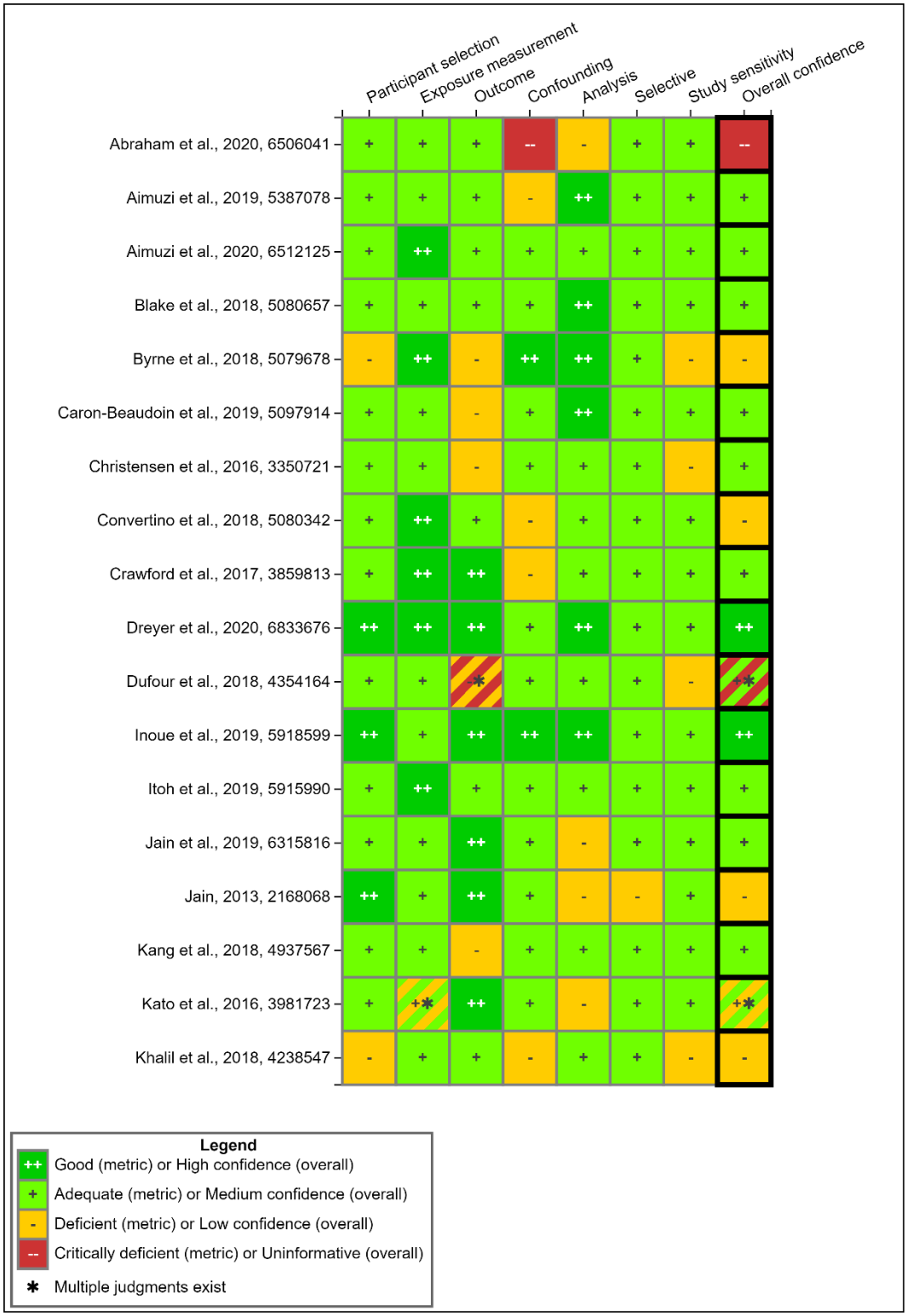


Figure C-10. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Endocrine Effects

Interactive figure and additional study details available on HAWC.

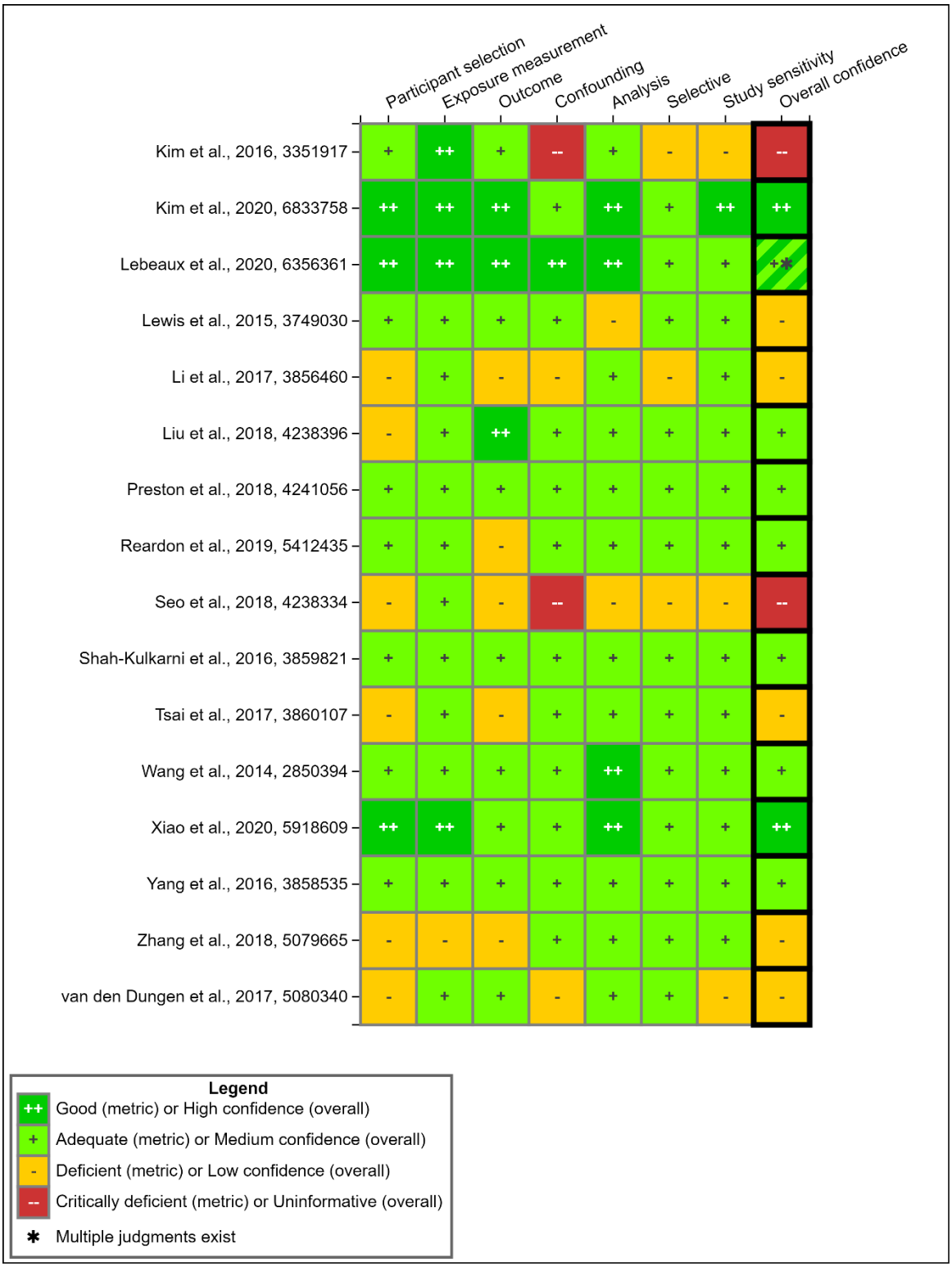


Figure C-11. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Endocrine Effects (Continued)

Interactive figure and additional study details available on HAWC.

These differences resulted in *high* confidence (Lebeaux et al., 2020) and *medium* confidence (Dufour et al., 2018; Kato et al., 2016) for infant or child analyses. For maternal analyses which tend to be cross-sectional in nature, the uncertainty regarding temporality resulted in *medium* confidence (Lebeaux et al., 2020), *low* confidence (Kato et al., 2016), or *uninformative* (Dufour et al., 2018) ratings.

Studies rated as *low* confidence or *uninformative* had deficiencies including lack of accounting for population sampling methods (Lewis et al., 2015), or residual confounding (Abraham et al., 2020; Convertino et al., 2018; Kim et al., 2016a; Predieri et al., 2015), or lack of information on allocation of participants to treatment levels (Convertino et al., 2018), participant recruitment and case definitions (Kim et al., 2016a; Predieri et al., 2015) or small sample sizes (Kim et al., 2016a; Predieri et al., 2015).

C.2.1.3 Findings From Children

One *high* confidence study (Kim et al., 2020a) observed no association with subclinical hypothyroidism in children 6 years of age. Congenital hypothyroidism (CH) was assessed in South Korean infants in a very small case-control study (Kim et al., 2016a). PFOA concentrations were significantly higher in infants with CH compared with controls (means 5.4 and 2.12 ng/mL, respectively, p-value < 0.01) (Appendix D). However, the study was considered *uninformative* because of potential key confounding factors were not controlled for in the analysis, and the small sample size.

Thyroid hormone levels were examined in 19 studies (Abraham et al., 2020; Kim et al., 2020a; Lebeaux et al., 2020; Aimuzi et al., 2019; Caron-Beaudoin et al., 2019; Itoh et al., 2019; Xiao et al., 2019; Dufour et al., 2018; Kang et al., 2018; Khalil et al., 2018; Preston et al., 2018; Tsai et al., 2017; Kato et al., 2016; Kim et al., 2016a; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Predieri et al., 2015; Wang et al., 2014) and four observed significant effects (Appendix D). One *high* confidence study (Xiao et al., 2019) observed a large positive association between maternal third trimester PFOA and cord serum TSH. The effect size for TSH was similar after stratification by infant sex, but no longer significant. Additionally, sex-stratified analyses showed positive associations between maternal PFOA and measures of T4 (total T4 and free T4 index (FTI)) in cord blood from female infants. No other significant associations were observed for TSH among other studies on children. Another *high* confidence study (Kim et al., 2020a) showed positive associations between serum PFOA concentrations and free T4 levels at age 6. After stratifying by child sex, the association remained among boys but was not observed in girls. This effect was also observed in a *medium* confidence cross-sectional study in newborns (Aimuzi et al., 2019), which reported significant positive associations with free T4 in cord blood. When stratified by sex, the effect persisted in male newborns, but was not seen in female newborns. These three studies report consistent, significant positive associations with T4 in children; however, the effect was not consistent between boys and girls in different populations. Similarly, a *medium* confidence cross-sectional study (Kang et al., 2018) showed a borderline significant positive association between serum PFOA and free T4 ($p = 0.075$). Analyses of children from the Hokkaido Study (Itoh et al., 2019; Kato et al., 2016) did not observe significant associations with thyroid hormones. The remaining studies that did not observe significant effects.

Thyroid antibody (TA) levels were examined in one study (Itoh et al., 2019) which found significant effects (Appendix D). A *medium* confidence study on children from the Hokkaido Study on the Environment and Children's Health (Itoh et al., 2019) showed mixed associations between maternal PFOA concentrations and thyroglobulin antibody levels. An inverse association was found for TgAb levels among boys born to TA-negative mothers; no effects were seen among all boys or boys born to TA-positive mothers. The opposite trend was seen in girls; a positive association for TgAb levels was observed for girls born to TA-positive mothers. No effects were observed in all girls or girls born to TA-negative mothers.

C.2.1.4 Findings From Pregnant Women

Thyroid hormone levels were examined in five studies (Aimuzi et al., 2020; Inoue et al., 2019; Itoh et al., 2019; Reardon et al., 2019; Shah-Kulkarni et al., 2016) and two observed significant effects (Appendix D). A *medium* confidence study (Preston et al., 2018) in pregnant women showed a significant decrease in the FTI with increasing first trimester serum PFOA concentrations. Associations with other thyroid hormones were not observed among the whole study sample. However, analyses stratified by TPOAb status showed a borderline significant ($p = 0.08$) inverse effect of PFOA on TSH among TPOAb-positive women; no effects were seen in TPOAb-negative women. Another *medium* confidence study (Aimuzi et al., 2020) observed a positive association between serum PFOA and early pregnancy free T4, but this effect was not seen when stratified by TPOAb status.

Thyroid hormone antibodies were examined in one study (Itoh et al., 2019) which found a significant effect. A negative association was observed for TPOAb levels in first trimester serum among mothers in the Hokkaido Study. One cross-sectional study (Dufour et al., 2018) on mother-child dyads showed evidence of a large increased risk of hypothyroidism in mothers (OR Q4 vs. Q1 (95% CI): 5.62 (1.64–26.11)), however, there was a great deal of uncertainty in regard to timing of outcome ascertainment and the method of disease classification, which diminish confidence in the findings for maternal hypothyroidism.

One *high* confidence study examined adrenal hormones among pregnant women in the Odense Child Cohort (OCC) and showed a significant decrease in serum cortisol with twofold increases in serum PFOA concentrations (Dreyer et al., 2020). However, diurnal urinary (dU) -cortisol, dU-cortisone, and dU-cortisol/cortisone showed non-significant decreases.

C.2.1.5 Findings From the General Adult Population

One study examined thyroid disease among male anglers (age > 50 years) and observed a non-significant increase in odds of self-reported thyroid disease with increasing serum PFOA concentrations (Christensen et al., 2016b).

Thyroid function was examined in 12 studies (Lebeaux et al., 2020; Jain and Ducatman, 2019b; Blake et al., 2018; Byrne et al., 2018; Convertino et al., 2018; Liu et al., 2018a; Seo et al., 2018; Zhang et al., 2018b; Crawford et al., 2017; Li et al., 2017b; Lewis et al., 2015; Jain, 2013) and seven observed significant effects (Appendix D). A *low* confidence case-control study (Zhang et al., 2018b) examined women with and without POI found a positive association among controls (i.e., women without POI) for TSH concentrations with increasing plasma PFOA concentrations. Similarly, TSH levels were elevated in women with POI which was accompanied by a concomitant negative association with free T4 concentrations. The thyroid hormone

concentrations were within normal ranges in both cases and controls. Another *low* confidence case-control study (Heffernan et al., 2018) on women with and without PCOS found a similar increase in TSH among cases. However, findings need to be interpreted with caution, since both studies were considered *low* confidence due to a lack of information on the control recruitment and selection process.

Results were mixed in three overlapping NHANES studies (Jain and Ducatman, 2019b; Lewis et al., 2015; Jain, 2013). One *low* confidence study (Lewis et al., 2015) showed several significant and borderline significant results among NHANES (2011–2012) participants including an inverse association with total T4 in men aged 40 to 60 years, increased total T4 and decreased TSH in women aged 12 to 20 years, increased free T3 in women aged 20 to 40 years, and concurrent increases in free and total T3 among women aged 60 years or older. However, there is no evidence NHANES complex sampling design was accounted for in the analysis which contributed to a *low* confidence rating. Jain (2013), another *low* confidence study, found a significant increase in TSH levels among those NHANES (2007–2008) participants in the highest tertile (≥5.1 ng/mL) of PFOA exposure compared with the lowest (≤3.3 ng/mL). A *medium* confidence follow-up study (Jain and Ducatman, 2019b) on NHANES (2007–2012) participants investigated associations with serum PFOA and thyroid hormone concentrations, stratified by glomerular function (GF) status (GF1, GF-2, GF-3A, and GF-3B/4). Few significant and borderline significant results were observed; however, the direction of association was inconsistent across increasing glomerular filtration groups and did not suggest an interaction with glomerular filtration status. Associations between PFOA and thyroid hormones were inconsistent across NHANES studies. Lewis et al. (2015) and Jain (2013) found significant effects in opposite directions for TSH, however, these effects were in observed in different NHANES cycles and among different subpopulations. In the 2011–2012 NHANES participants, Lewis et al. (2015) found consistent effects for T3 in women of different ages, but other results were inconsistent between age and sex groupings.

Inverse associations with TSH and T4 were also observed in a *medium* confidence study (Blake et al., 2018) in individuals residing near a uranium processing facility in an area with per- and polyfluoroalkyl substances- (PFAS-)contaminated drinking water (Fernald Community Cohort). One additional *low* confidence, cross-sectional study (Byrne et al., 2018) on Alaska natives found a significant positive association for TSH among all participants and an inverse association with total T3 in men; however, this population was relatively small (total n = 85; male n = 38) with low exposure levels (median: 1.01 ng/mL (25th–75th percentile: 0.753–1.44 ng/mL)).

In a controlled trial (Convertino et al., 2018) in which subjects were administered ammonium perfluorooctanoate (APFO) doses ranging 50–1,200 mg for six weeks, (Convertino et al., 2018) report an increase in the average rate of change in free T4. A dose-dependent increase was also demonstrated by grouping subjects into three treatment bins and showing increasing mean and median free T4 concentrations. This study, however, was rated as *low* confidence because potential confounders were not considered during participant allocation to treatment groups or in the statistical analysis.

C.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and six studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and endocrine effects. Study quality evaluations for these nine studies are shown Figure C-12.

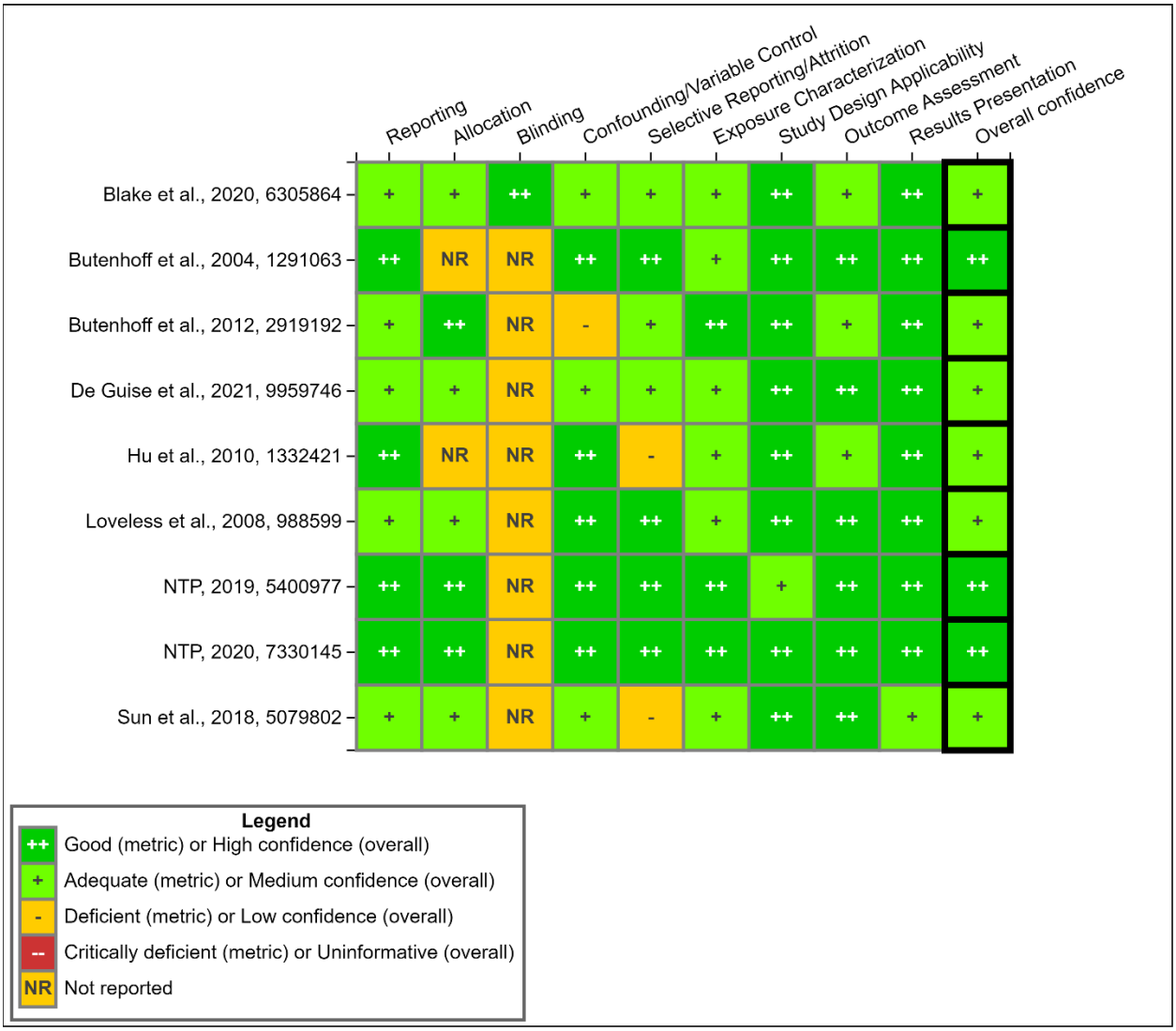


Figure C-12. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Endocrine Effects

Interactive figure and additional study details available on HAWC.

Available animal toxicity data suggest that PFOA exposure can interfere with male and female endocrine systems. Overall, studies have reported endocrine organ weight changes, hormone fluctuations, and organ histopathology across studies of varying durations of oral exposure to PFOA. Effects typically exhibit a sex-bias depending on the species, endpoint, and exposure paradigm, likely due to known toxicokinetic differences (see Toxicity Assessment, (U.S. EPA,

2024b)). The thyroid gland and thyroid hormones appear to be affected by PFOA exposure. Effects of PFOA on gonads and placenta and on reproductive hormones are described in detail in (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.2.2.1 Organ Weight Changes

Significant changes in absolute and relative endocrine organ weights have been observed in monkeys (Goldenthal et al., 1978) and rats (NTP, 2019a; Butenhoff et al., 2012; Butenhoff et al., 2004a) following oral exposure to PFOA, often with a male-bias in response (Figure C-13).

Absolute and relative thyroid gland weight was quantified as part of a short-term exposure study conducted by NTP (2019a). In that study, male Sprague-Dawley rats received 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg/day PFOA and females received 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day via gavage for 28 days. Absolute thyroid weight was only significantly increased in males of the 2.5 mg/kg/day exposure group. Thyroid gland weight relative to body weight was elevated in males administered > 1.25 mg/kg/day PFOA by the end of the study, which may be related to reductions in mean body weights that were observed in males but not females (Section C.3.2.2), though body weight in males of the 1.25 mg/kg/day dose group was only modestly reduced by 4.6% compared with controls. No statistically significant effects were observed in females at any dose and no effects were observed on absolute or relative adrenal gland weight in either sex (NTP, 2019a).

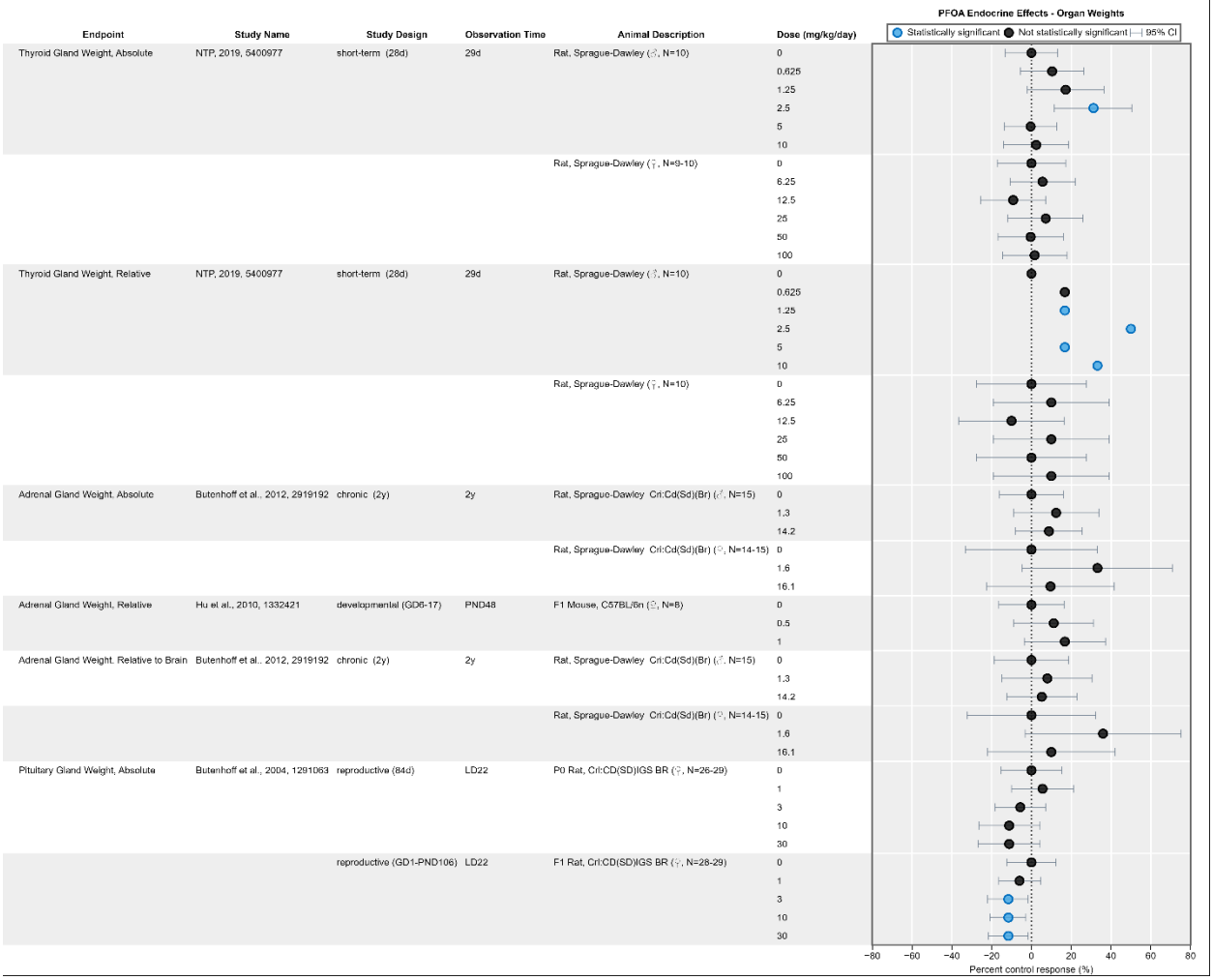


Figure C-13. Percent Change in Endocrine Organ Weights Relative to Controls in Rodents Following Exposure to PFOA[a]

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; y = year.
[a] CIs for some studies may be too narrow to view at this scale.

Relative pituitary gland weight was elevated in male rhesus monkeys exposed to 3 mg/kg/day via gavage for 90 days. Changes in body weight were similar to controls for these animals (Goldenthal et al., 1978). In male Sprague-Dawley (Crl:COBS@CD(SD)BR) rats, pituitary weights (absolute and relative to brain or body weight) were reduced following a year-long dietary exposure to 300 ppm PFOA, which is equivalent to 14.2 mg/kg/day (Butenhoff et al., 2012). The decrease was consistent across all measures despite slight (i.e., <10%) non-significant decreases in both body weight and absolute brain weight. Decrements in pituitary gland weight were not observed in female rats given the same 300 ppm exposure for 1 year (16.1 mg/kg/day equivalent) (Butenhoff et al., 2012). Another study by Butenhoff et al. (2004a) in Sprague-Dawley rats described female-specific reductions in pituitary gland weight following a multi-lifestage PFOA exposure paradigm. In this study, absolute pituitary gland weights were reduced in adult F_1 females (on lactational day 22 of the F_2 generation) following oral exposure to 3, 10,

or 30 mg/kg/day PFOA from GD 0–PND 127 (Butenhoff et al., 2004a). Although relative pituitary weights were not provided, there were not significant changes in body weights at sacrifice nor absolute brain weights (Section C.4.2), which implies the reduction in absolute pituitary weight may reflect a specific effect on the pituitary gland. F_1 pup weight was only reduced in the 30 mg/kg/day group during development, indicating that slower pup growth is not an explanation for the reduced absolute pituitary weights.

Male-specific reductions in absolute adrenal gland weight and relative to brain weight were observed by Butenhoff et al. (2012) after 1 year of exposure to 300 ppm PFOA (equivalent to 14.2 mg/kg/day) but was not observed after 2 years (Butenhoff et al., 2012). A developmental exposure study by Hu et al. (2010) examined relative body weight of adrenal glands in PND 48 F_1 female C57BL/6N mice following maternal exposure to 0, 0.5, or 1 mg/kg PFOA from GD 6−17. There was an apparent dose-related trend, but none of the adrenal weights of exposed groups were significantly different from the control and the study authors did not conduct a trend test.

C.2.2.2 Hormone Fluctuations

Several studies have described fluctuations in the levels of hormones secreted from the adrenal, pituitary, and thyroid glands following short-term exposure to rats and mice (NTP, 2019a; Sun et al., 2018b), exposure during pregnancy to mice (Blake et al., 2020), and chronic exposure to nonhuman primates (Butenhoff et al., 2002).

In the aforementioned 28-day rat study conducted by NTP (2019a), male-specific reductions in T4, FT4, and T3 were observed in almost all exposure groups (Table C-3; Figure C-14); T3 was not significantly affected in the 10 mg/kg/day group, though statistically significant reductions were observed in all lower dose groups. Notably, these effects in males occurred at doses lower than those that resulted in decreased body weight, which may be confounding with hormone responses, as shown in dietary restriction studies in rats (Laws et al., 2007). T4 and FT4 were significantly reduced in females from the 100 mg/kg/day exposure group (NTP, 2019a). Opposing effects of TSH were observed between the sexes. Although female TSH concentrations were increased in all exposure groups (6.25–100 mg/kg/day), male TSH was reduced in the 5 and 10 mg/kg/day exposure groups when compared with controls (NTP, 2019a).

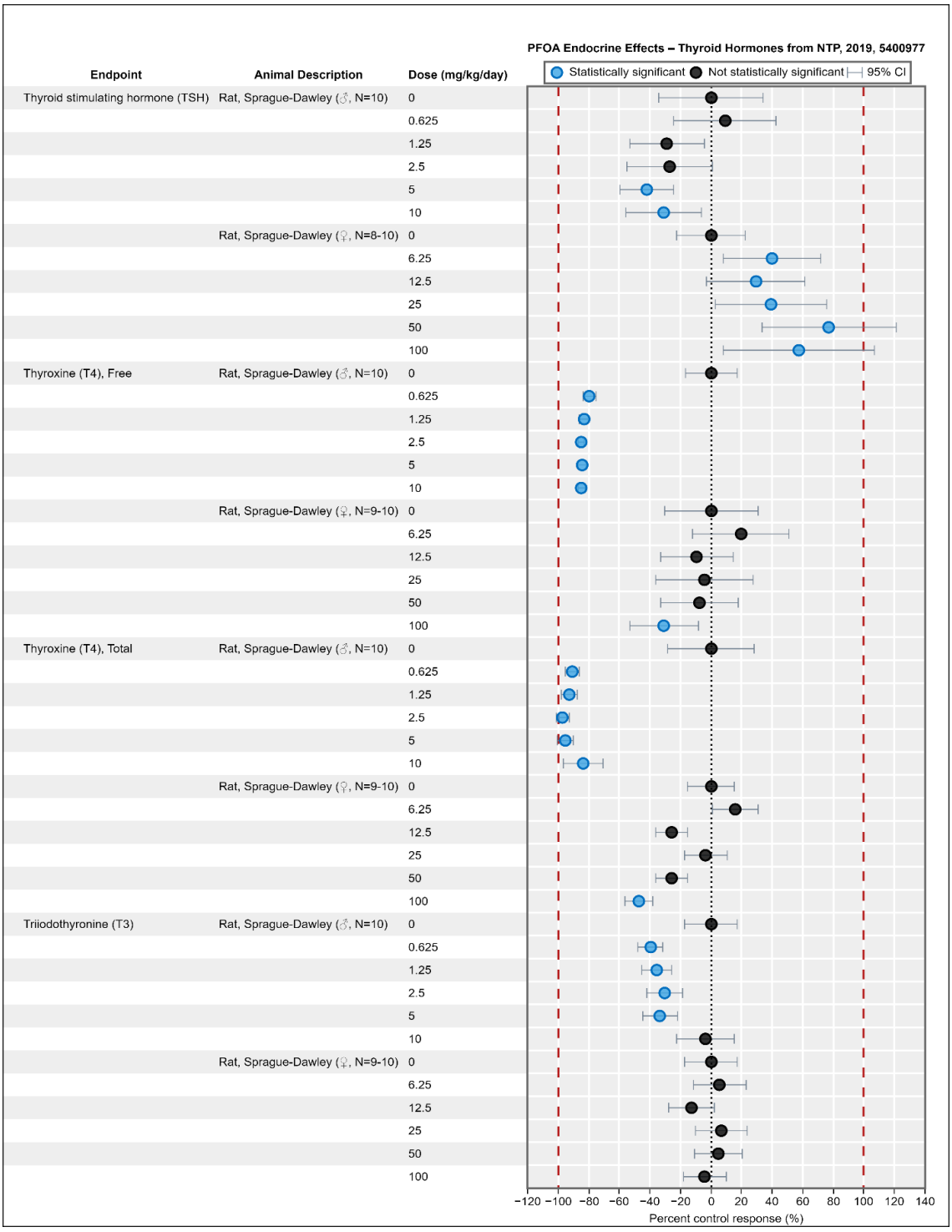


Figure C-14. Percent Change in Thyroid and Thyroid-Related Hormone Levels of Male and Female Rats Exposed to PFOA for 28 Days as Reported by NTP (2019a)[a,b]

Interactive figure and additional study details available on HAWC.
TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; CI = confidence interval.
[a] Some hormone measurements in male rats were below or approaching the limit of quantifications for FT4 (0.3 ng/dL), T4 (0.5 µg/dL), and T3 (50 ng/dL).
[b] The red dashed lines indicate a 100% increase or 100% decrease from the control response.

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 17.5. On GD 17.5, levels of thyroid hormones in male and female placentas were determined, including T4, T3, 3,3',5'-triiodothyronine (reverse T3, rT3), ratio of T3 to T4 (T3:T4), and ratio of rT3 to T4 (rT3:T4). There were no significant effects of PFOA exposure on rT3, T3, T4, T3:T4, or rT3:T4 ratio.

In a chronic exposure study by Butenhoff et al. (2002), male cynomolgus monkeys were given 0, 3, 10, or 30/20 mg/kg PFOA per day for 26 weeks. The "30/20" notation reflects a reduction from 30 to 20 mg/kg/day at day 22 of the study due to toxicity in this exposure group. Only two animals from the 30/20 mg/kg/day group survived until sacrifice, which introduces uncertainty to the results of this dose group, though they are discussed here. Although no change in TSH was noted in the highest dose group, it was significantly elevated in both the 3 and 10 mg/kg/day exposure groups by the end of the study, at day 182 (increases of 63% and 118% changes, respectively). In the lowest exposure group (3 mg/kg/day), T4 was reduced across multiple timepoints, and decreases reached significance in all three dose groups (33%, 29%, and 32% decreases, respectively) at the conclusion of the study. A dose-dependent decrease in FT4 was also observed across multiple time points, with decreases at day 182 of 33%, 38%, and 42% in the 3, 10, and 30/20 mg/kg/day dose groups, respectively, compared with control levels. Similar trends were seen in T3 and free triiodothyronine (FT3) levels throughout the study. By day 182, total and free T3 levels were decreased by 15%, 14%, and 34% and 13%, 17%, and 40%, respectively, with increasing dose levels.

Prior to this updated assessment, the available literature measuring thyroid hormones was limited and acute studies were discussed in the 2016 PFOA HESD (U.S. EPA, 2016c). One such study in adult male Sprague-Dawley rats given a single oral exposure of PFOA (20 mg/kg) reported an 80% reduction in T4 and FT4, and a 25% reduction in serum T3 (Martin et al., 2007). This single-dose study supports the thyroid hormone level perturbations, specifically, the sensitivity of T4 and FT4, that are observed in the current literature update.

Table C-3. Associations Between PFOA Exposure and Thyroid and Thyroid-Related Hormone Effects in Rodents and Nonhuman Primates

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
TSH	NTP (2019a)	Sprague-Dawley rat	28 d	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 5–10 mg/mg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↑ 6.25–100 mg/kg/day
	Butenhoff et al. (2002)	Cynomolgus monkeys	26 wk	0, 3, 10, or 30/20 mg/kg	M	↑ 3–10 mg/kg/day
T3 (Total)	Martin et al. (2007)	Sprague-Dawley rat	Single dose	20 mg/kg/day	M	↓ 20 mg/kg/day
	NTP (2019a)	Sprague-Dawley rat	28 d	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–5.0 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	n.s.
	Butenhoff et al. (2002)	Cynomolgus monkeys	26 wk	0, 3, 10, 30/20 mg/kg/day	M	↓ 30/20 mg/kg/day

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
	Blake et al. (2020)	CD-1 mice	Developmental (GD1.5–17.5)	0, 1, 5 mg/kg/day	M	n.s.
				0, 1, 5 mg/kg/day	F	n.s.
FT3	Butenhoff et al. (2002)	Cynomolgus monkeys	26 wk	0, 3, 10, 30/20 mg/kg/day	M	↓ 30/20 mg/kg/day
rT3	Blake et al. (2020)	CD-1 mice	Developmental (GD1.5–17.5)	0, 1, 5 mg/kg/day	M	n.s.
				0, 1, 5 mg/kg/day	F	n.s.
T4 (Total)	Martin et al. (2007)	Sprague-Dawley rat	Single dose	20 mg/kg/day	M	↓ 20 mg/kg/day
	NTP (2019a)	Sprague-Dawley rat	28 d	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–10 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↓ 100 mg/kg/day
	Butenhoff et al. (2002)	Cynomolgus monkeys	26 wk	0, 3, 10, or 30/20 mg/kg	M	↓ 3–30/20 mg/kg/day
	Blake et al. (2020)	CD-1 mice	Developmental (GD1.5–17.5)	0, 1, 5 mg/kg/day	M	n.s.
				0, 1, 5 mg/kg/day	F	n.s.
FT4	Martin et al. (2007)	Sprague-Dawley rat	Single dose	20 mg/kg/day	M	↓ 20 mg/kg/day
	NTP (2019a)	Sprague-Dawley rat	28 d	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–10 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↓ 100 mg/kg/day
	Butenhoff et al. (2002)	Cynomolgus monkeys	26 wk	0, 3, 10, or 30/20 mg/kg	M	↓ 10–30/20 mg/kg/day

Notes: d = days; F = female; FT3 = free triiodothyronine; FT4 = free thyroxine; GD = gestational day; M = male; n.s. = non-significant; rT3 = reverse T3; T4 = thyroxine; T3 = triiodothyronine; TSH = thyroid stimulating hormone; wk = weeks.

Perturbations in adrenal and pituitary hormone levels have been described primarily in rodent studies (Table C-4). Loveless et al. (2008) reported elevations in serum corticosterone in male Crl:CD(SD)IGS BR rats and male Crl:CD-1(ICR)BR mice exposed to 10 or 30 mg/kg/day PFOA for 29 days, although statistically significant effects were only noted at the 10 mg/kg/day dose in mice. Increases in rats of the 10 and 30 mg/kg/day groups were 35% and 96% changes, respectively and in mice were 129% and 131% changes, respectively (Loveless et al., 2008). Two studies in mice support that PFOA exposure is associated with elevations in corticosterone in both males and females. De Guise et al. (2021) found that serum corticosterone was significantly higher in female B6C3F1 mice exposed to 1.88 or 7.5 mg/kg/day PFOA for 28 days (72%- and 158%-fold increases, respectively). Sun et al. (2018b) found that serum corticosterone was elevated in male BALB/c mice exposed to 5 or 20 mg/kg/day PFOA for 28 days (146% and

175% changes, respectively). This study also quantified adrenocorticotropic hormone (ACTH). A dose-dependent reduction in ACTH was observed, however significant effects were only observed at the 20 mg/mg/day dose (−26% and −58% changes in the 5 and 20 mg/kg/day groups, respectively) (Sun et al., 2018b).

Table C-4. Associations Between PFOA Exposure and Adrenocortical Hormone Effects in Rodents

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
CORT	De Guise et al. (2021)	B6C3F1	28 d	0, 1.88, 7.5 mg/kg/day	F	↑ 1.88 and 7.5 mg/kg/day
	Sun et al. (2018b)	BALB/c	28 d	0, 1.25, 5, 20 mg/kg/day	M	↑ 5 and 20 mg/kg/day
	Loveless et al. (2008)	Sprague-Dawley rat	29 d	0, 0.3, 1, 10, 30, mg/kg/day	M	n.s.
		CD-1(ICR)BR mice	29 d	0, 0.3, 1, 10, 30, mg/kg/day	M	↑ 10 mg/kg/day
ACTH	Sun et al. (2018b)	BALB/c	28 d	0, 1.25, 5, 20 mg/kg/day	M	↓ 20 mg/kg/day

Notes: ACTH = adrenocorticotropic hormone; CORT = serum corticosterone; d = days; F = female; M = male; n.s. = non-significant.

C.2.2.3 Histopathology

In addition to the neoplastic lesions described in (see Toxicity Assessment, (U.S. EPA, 2024b)), several nonneoplastic lesions have been observed in the thyroid gland and adrenal glands (Figure C-15).

C.2.2.3.1 Thyroid

In the 28-day exposure study, NTP (2019a) found higher incidences (8/10, minimal severity) of thyroid follicular cell hypertrophy in female rats following exposure to 100 mg/kg/day PFOA. Three of 10 high-dose males (10 mg/kg/day) also exhibited these abnormalities. No such lesions were observed in any of the other groups. Although statistical significance was not achieved, the presence of thyroid follicular cell hypertrophy in both males and females supports that it is likely an exposure-related effect (NTP, 2019a).

In two chronic exposure studies (NTP, 2020; Butenhoff et al., 2012), male and female Sprague-Dawley rats were fed diets containing PFOA for approximately 2 years. NTP (2020) used a matrix-type exposure paradigm whereby pregnant rats were administered PFOA on GD 6 and exposure was continued in offspring postweaning for a total of 107 weeks. Tissue sections from

endocrine organs, including the thyroid gland, were analyzed for histology in both male and female offspring. Dose groups for this report are referred to as "[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]" (e.g., 300/1,000; see Toxicity Assessment, (U.S. EPA, 2024b)).

In the thyroid gland, NTP (2020) reported higher incidences of follicular cell hypertrophy in males from the 0/300 ppm group at the 16-week interim evaluation as well as the terminal evaluation. In females, higher incidences were noted in the 300/1,000 ppm group at the 16-week interim. No differences were observed between groups with combined perinatal and postweaning exposure compared with groups with postweaning exposure only (NTP, 2020). NTP (2020) suggested the elevated incidence of follicular cell hypertrophy in males could be related to lower concentrations of circulating total T4 and T3, a result that was observed in the aforementioned NTP 28-day toxicity study (NTP, 2019a) but were not assessed in the chronic study. Similarly, Butenhoff et al. (2012) observed increased incidences (13%, n = 49; compared with 2% in controls, n = 50) of thyroid c-cell hypertrophy in male rats exposed to 30 ppm PFOA for 2 years (equivalent to 1.3 mg/kg/day), although the effects did not reach statistical significance nor was there an increase in the 300 ppm males. Females had an apparent dose-dependent increase in follicular cell hypertrophy with an incidence of 0/50, 1/49, and 3/49 in the control, 30 ppm, and 300 ppm, respectively; however, the results were not statistically significant. Although there were sporadic occurrences of follicular cell hyperplasia in the males, there were no apparent treatment-related effects (Butenhoff et al., 2012).

C.2.2.3.2 Adrenal

In a chronic dietary study in rats, the incidence of adrenal gland hyperplasia was 18% (n = 50) in males exposed to 300 ppm PFOA compared with 4% in controls (n = 49), but the effect did not reach statistical significance (Butenhoff et al., 2012). A rat reproductive study by Butenhoff et al. (2004a) observed treatment-related microscopic changes in the adrenal glands of high-dose F_1 animals including cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex following exposure to 3, 10, or 30 mg/kg/day (Butenhoff et al., 2004a). In males, the cells of the adrenal glands were thicker, the zona glomerulosa was more prominent, and adrenal cortex cells were more vacuolized in 2/10 males from the 10 mg/kg/day exposure group and 7/10 males from the 30 mg/kg/day group. No effects were observed in females (Butenhoff et al., 2004a). The adrenal glands appeared normal, and no histopathology was observed in a study of male cynomolgus monkeys administered up to 30 mg/kg/day PFOA for 6 months by oral tablet (Butenhoff et al., 2002), or the 28-day and chronic rat studies conducted by NTP (2020, 2019a).

Nonneoplastic lesions in the pancreas are described in the Toxicity Assessment (U.S. EPA, 2024b).

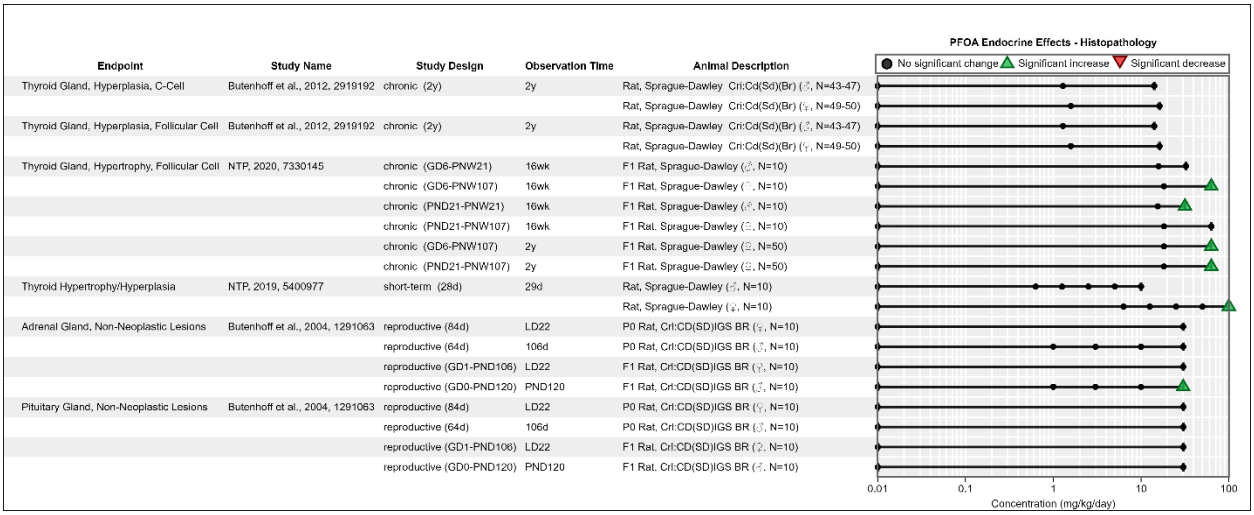


Figure C-15. Endocrine Organ Histopathology in Rodents Following Exposure to PFOA (Logarithmic Scale)

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; PNW = postnatal week; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day, wk = week; y = year.

C.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse endocrine outcomes is discussed in Sections 3.3.2, 3.3.3, 3.3.4, and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 17 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 endocrine effects. A summary of these studies is shown in Figure C-16. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to endocrine effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	1	9	10
Cell Signaling Or Signal Transduction	1	6	6
Extracellular Matrix Or Molecules	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	1	1
Hormone Function	2	11	12
Xenobiotic Metabolism	1	1	2
Other	0	2	2
Not Applicable/Not Specified/Review Article	1	0	1
Grand Total	3	15	17

Figure C-16. Summary of Mechanistic Studies of PFOA and Endocrine Effects

Interactive figure and additional study details available on HAWC.

C.2.4 Evidence Integration

There is *slight* evidence for an association between PFOA exposure and endocrine effects in humans based on studies reporting elevated levels of T4 in children and elevated levels of TSH in adults. The 2016 PFOA HESD (U.S. EPA, 2016c) included two studies reporting positive associations with thyroid disease and one study reporting negative associations. This updated review supports positive associations with thyroid disease (hypothyroidism). The most consistent thyroid hormone effects were observed in children, with four studies (2 *high* and 2 *medium* confidence) reporting positive associations for T4; however, some inconsistencies across sexes were also observed, and a large number of studies observed null effects. One study reporting significant effects on TSH in children (Aimuzi et al., 2019) conducted multi-pollutant models including other measured PFAS (i.e., PFOS, PFNA, PFDA, PFUA, PFHxS, PFDoA, and perfluorobutane sulfonate (PFBS)). PFOA was moderately correlated with other PFAS (r = 0.23–0.56) in cord blood, and estimates were found to be largely unchanged in multipollutant models. Most results in general population studies indicated positive associations for TSH. Many *high* and *medium* confidence studies generally did not observe significant associations with endocrine outcomes. Several *low* confidence studies observed associations, but the interpretation of these results is limited by several factors related to study quality. Additional uncertainty exists due to the potential for confounding by other PFAS.

The animal evidence for an association between PFOA exposure and effects in the endocrine system is considered *moderate* based evidence from eight *high* or *medium* confidence animal studies. The strongest evidence of endocrine effects is from perturbations in hormones related to the thyroid gland. Thyroid hormones appear to be sensitive to PFOA exposure but exhibit highly

complex responses depending on sex, species, and exposure duration. Perturbations were observed in both sexes, sometimes with opposite effects between the sexes (in the case of TSH). Reductions in free and total T4 as well as total T3 were noted in both rodents and chronically exposed nonhuman primates that in some cases (female rats, male nonhuman primates) coincided with compensatory increases in TSH, indicative of classical hypothyroidism. Reductions in free and total T4, as well as declines in TSH in male rats may suggest hypothyroxinemia. Elevations in thyroid gland weight were also noted (Butenhoff et al., 2012) in males, as well as increases in thyroid gland follicular cell hypertrophy in male and female rats (NTP, 2020, 2019a), however, the hormones released from the respective organs (i.e., T4 and FT4) may be more sensitive and direct indicators of toxicity. Thyroid hormones influence numerous other body systems, notably the nervous system via the hypothalamic-pituitary-thyroid (HPT) axis, thus effects on other systems may stem from thyroid-specific targets and vice versa. The available animal evidence supports evidence from human epidemiological studies indicating that PFOA exposure may affect T4 in children.

Elevations in corticosterone were noted across two animal studies (Sun et al., 2018b; Loveless et al., 2008) using male rodents, which coincided with a reduction in ACTH in one study (Sun et al., 2018b). Such effects may indicate adrenocortical toxicity, which can involve increased secretion of endogenous glucocorticoids and long-loop feedback on the hypothalamic-pituitary-adrenal (HPA) axis to reduce ACTH levels (Cox et al., 1994). However, more data on the interactions between corticosterone and ACTH are required, as well as potential histological effects in the adrenal gland, to understand the relevance of an effect of PFOA on adrenocortical hormone levels. Given the perturbations of adrenocortical hormones and thyroid hormones, it is crucial to interrogate the interaction of multiple systems in order to evaluate potential dysregulation of the HPA axis and/or HPT axis.

C.2.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause endocrine effects in humans under relevant exposure circumstances (Table C-5). This conclusion is based primarily on evidence from animal models showing alterations in circulating thyroid and adrenocortical hormone levels, increased thyroid gland weight, and increased follicular cell hypertrophy in the thyroid following exposure to doses as low as 0.625 mg/kg/day PFOA. Although a few associations between PFOA exposure and T4 in children were observed in *high* and *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistencies across sexes, age groups, and limited number of studies.

Table C-5. Evidence Profile Table for PFOA Endocrine 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 C.2.1)					⊕⊙⊙ ***Evidence Suggests***
Thyroid and thyroid-related hormones and thyroid disease 4 *High* confidence studies 17 *Medium* confidence studies 8 *Low* confidence studies	Studies in adults reported positive associations for the thyroid-related hormone TSH (3/8). Sex differences were observed in two studies, indicating increased TSH among males and decreased TSH among females. Results for thyroid hormones (i.e., T3 and T4) were generally mixed among adults; however, significant increases in total T3 were observed (3/5). One study (1/1) reported increased risk of thyroid disease in adult males, but there was minor concern for temporality due to the cross-sectional study design. Studies in children observed significant positive associations (4/19) and inverse associations (1/19) for T4, and one study observed	• *High* and *medium* confidence studies • *Coherence* of findings across multiple geographic locations	• *Low* confidence studies • *Inconsistency direction* of effect in adults which may be influenced by timing of outcome sampling (i.e., diurnal variations) • *Imprecision* of most findings in children	⊕⊙⊙ *Slight* Evidence for endocrine effects is based on increased TSH and T3 in adults, and increased T4 in children. Findings from *medium* confidence studies were frequently inconsistent or imprecise. There was limited evidence reporting effects on thyroid disease. Uncertainties remain regarding diurnal variation of thyroid hormones, differential effects in males and females, and consistency across outcome timing.	*Primary basis*: Animal evidence demonstrated alterations in circulating thyroid and adrenocortical hormone levels, increased thyroid weight, and increased follicular cell hypertrophy in the thyroid. Although a few associations between PFOA exposure and T4 in children were observed in *high* and *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistencies across sexes, age groups, and limited number of studies. *Human relevance, cross-stream coherence, 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**	
	significant positive associations for TSH. Other studies reported inconsistent or imprecise results. No clear effect for hypothyroidism in a single informative study in children. In pregnant women, positive associations were observed for TSH (4/8) and T4 (5/8).				
Thyroid hormone antibodies 1 *Medium* confidence study 1 *Low* confidence study	Studies in children observed decreased TgAb among boys born to TA-negative mothers and increased TgAb among girls born to TA-positive mothers. Among pregnant women, TPOAb levels were significantly decreased.	• *Medium* confidence study	• *Low* confidence study • *Limited number* of studies examining outcome		
Steroid and adrenal hormones 1 *High* confidence study	One study in pregnant women observed a significant decrease in serum cortisol.	• *High* confidence study	• *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.2.2)					
Thyroid and thyroid-related hormones 1 *High* confidence study 1 *Medium* confidence study	Decreased thyroid hormones were observed in male (total T4, free T4, T3) and female (total T4, free T4) rats following a 28-day exposure (1/1).	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome	(⊕⊕⊙) Moderate Evidence was based on *high* and *medium* confidence studies that	

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**	
	Sex-specific PFOA effects on TSH were observed, with increased levels in females and decreased levels in males. In a developmental study in mice (1/1), no significant effects were observed on the placental thyroid-related hormones T3, total T4, rT3, rT3:T4, or T3:T4.			demonstrated decreased thyroid hormone levels (free T4, total T4, total T3), especially in males. Alterations in adrenocortical hormone levels, such as elevated corticosterone and reduced ACTH, suggests perturbation of the HPA and/or HPT axis. Increased incidence of follicular cell hypertrophy in the thyroid gland correlated with increased thyroid gland weight.	
Adrenocortical hormones 3 *Medium* confidence studies	Corticosterone levels were increased in males (2/2) and females (1/1) following short-term exposure in rodents. One study observed a dose-dependent decrease in ACTH levels in male mice (1/1).	• *Medium* confidence studies • *Consistent direction* of effect for corticosterone levels	• *Limited number* of studies examining outcome		
Organ weights 2 *High* confidence studies 2 *Medium* confidence studies	In a 28-day rat study, increases in absolute and relative thyroid gland weights were reported in males and no significant effects were observed in females (1/1). No significant changes or transient effects were observed were observed in absolute and/or relative adrenal gland weights (4/4). Decreased	• *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**	
	absolute pituitary gland weights were observed in only female rats (1/2).				
Histopathology 3 *High* confidence studies 1 *Medium* study	Increased follicular cell hypertrophy was observed in the thyroid following short-term and chronic exposure in rats (2/3). No changes in pituitary histopathology were reported in male or female rats (2/2). No changes in adrenal histopathology were reported in female rats (2/2) but increased incidence of nonneoplastic lesions (1/1) along with a non-significant increase of benign pheochromocytoma and hyperplasia (1/1) was observed in male rats.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome		

Notes: TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; TgAb = thyroglobulin antibody; TA = thyroid antibodies; TPOAb = thyroid peroxidase antibody; rT3 = reverse T3; ACTH = adrenocorticotropic hormone; HPA = hypothalamus-pituitary-adrenal; HPT = hypothalamus-pituitary-thyroid.

C.3 Metabolic/Systemic

EPA identified 71 epidemiological and 24 animal studies that investigated the association between PFOA and systemic and metabolic effects. Of the epidemiological studies, 9 were classified as *high* confidence, 39 as *medium* confidence, 14 as *low* confidence, 5 as *mixed* (4 *medium*/*low* and 1 *medium*/*uninformative*) confidence, and 4 were considered *uninformative* (Section C.3.1). Of the animal studies, 5 were classified as *high* confidence, 17 as *medium* confidence, 1 as *low* confidence, and 1 was considered *uninformative* (Section C.3.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.3.1 Human Evidence Study Quality Evaluation and Synthesis

C.3.1.1 Introduction

Diabetes is a category of diseases caused by either insulin resistance or beta-cell disfunction, or both. Type 1 diabetes is characterized by insulin deficiency and beta-cell destruction, while type 2 diabetes is characterized by beta-cell disfunction and insulin resistance. Type 2 diabetes is more common than type 1 diabetes. Gestational diabetes commonly occurs during pregnancy and is a risk factor for developing diabetes later in life. Diabetes can lead to long-term complications in several organ systems, including micro- and macro-vascular complications.

Diagnostic criteria for diabetes include hemoglobin A1c (HbA1c) ≥6.5%, fasting plasma glucose ≥126 mg/dL, a 2-hour plasma glucose ≥127 in an oral glucose tolerance test, or a random plasma glucose ≥200 mg/dL (in patients with classic symptoms of hyperglycemia or a hyperglycemic crisis).

Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease (CVD) and diabetes, including abnormalities in triglycerides, waist circumference, blood pressure, cholesterol, and fasting glucose. It is highly prevalent in the general population of the United States. Risk factors for metabolic syndrome include insulin resistance and being overweight or obese.

The 2016 EPA Health Assessment for PFOA concluded that there is no evidence of an association between PFOA and diabetes, metabolic syndrome, or related outcomes. No associations were observed between mean serum PFOA up to 91.3–113.0 ng/mL and type 2 diabetes incidence in high-exposure (C8 Health Project) (Macneil et al., 2009) or occupational populations (Steenland et al., 2015). Additionally, the C8 Science Panel (2012), based on combined data from high-exposure and worker cohorts, concluded that there was no probable link between PFOA and type II diabetes. One general population study observed an increased risk of gestational diabetes in women with a mean pre-pregnancy serum PFOA level of 39.4 ng/mL (Zhang et al., 2015a). Serum PFOA was significantly positively associated with beta-cell function, but not associated with metabolic syndrome, metabolic syndrome waist circumference, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents from NHANES (Lin et al., 2009). No association was observed between serum PFOA concentrations (Nelson et al., 2010) and insulin resistance. Another study reported no association between PFOA and metabolic syndrome in adolescents or adults (Lin et al.,

2009). Overall, these studies show a lack of association of PFOA with diabetes, metabolic syndrome, and related outcomes.

For this updated review, 71 new epidemiologic studies (72 publications)[9] examined the association between PFOA and metabolic outcomes. Of these, 35 were cohort studies, 6 were case-control studies, 26 were cross-sectional studies, 2 were nested case-control studies, and 3 were controlled trials. Most studies measured exposure to PFOA using biomarkers in blood. One study measured exposure to PFOA using biomarkers in blood and in semen (Di Nisio et al., 2019). Biomarkers in maternal blood were used in 16 studies and cord blood was used in two studies. Shapiro et al. (2016) measured exposure to PFOA in urine and Mancini et al. (2018) estimated dietary exposure to PFOA. Most studies identified were conducted in the United States and China. Other study locations included Canada, Croatia, Denmark (including the Faroe Islands), France, Italy, Japan, Korea, Norway, Spain, Sweden, Taiwan, the Netherlands, and the United Kingdom.

Twenty-four studies examined diabetes (one in children, nine in pregnant women), and four studies examined metabolic syndrome in general adult populations. Other metabolic outcomes examined included blood glucose levels or glucose tolerance, HbA1c, insulin or insulinogenic index, insulin resistance, insulin sensitivity, adiponectin, leptin, beta-cell function, proinsulin, insulin-like factor 1, c-peptide, BMI or ponderal index, body weight, gestational weight gain, body fat, and anthropometric measurements (Appendix D).

C.3.1.2 Study Quality

Several criteria were specific to evaluating the quality of studies on metabolic outcomes. Because of concerns for potential reverse causality (where the exposure may be affected by disease status), studies evaluating diabetes were considered critically deficient if exposure and prevalent diabetes were measured concurrently, since the cross-sectional design would not allow for a reliable characterization of exposure before the onset of diabetes. Another concern is for the evaluation of insulin, Homeostatic Model Assessment of Beta-Cell Function (HOMA-B), or Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) without consideration of diabetes status, since the treatment of diabetes, particularly in those being treated with hypoglycemic medications, influences insulin production and secretion.

There are 71 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 metabolic effects. Study quality evaluations for these 71 studies are shown in Figure C-17, Figure C-18, and Figure C-19.

On the basis of the considerations mentioned, nine studies were classified as *high* confidence for all metabolic outcomes, 39 as *medium* confidence for all metabolic outcomes, two as *medium* confidence for one outcome (anthropometric measurements or diabetes) and *low* confidence for multiple other outcomes, two as *medium* confidence for one outcome (metabolic syndrome or metabolic function) and *low* confidence for one other (adiposity or insulin resistance), one as *medium* confidence for multiple outcomes and *uninformative* for one other (insulin resistance), 14 as *low* confidence for all metabolic outcomes, and 4 were considered *uninformative* for all

[9] Fassler et al. (2019) reports a cross-sectional analysis of participants from the same population as Pinney et al. (2019).

outcomes. One study (Liu et al., 2018a) was considered *uninformative* for insulin resistance, and *medium* confidence for other metabolic outcomes.

Uninformative studies had critical deficiencies in at least one domain. These deficiencies included a lack of control for confounding (Huang et al., 2018; Predieri et al., 2015; Jiang et al., 2014), lack of fasting measures for glucose measurements (Jiang et al., 2014), and treating PFOA as an outcome instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination (Jain, 2020b; Predieri et al., 2015). Other concerns leading to an *uninformative* rating included inadequate reporting of population selection (Jiang et al., 2014), small sample size, and narrow ranges for exposure (Predieri et al., 2015).

The most common reason provided for a *low* confidence rating was potential for residual confounding, particularly by SES (Fassler et al., 2019; Convertino et al., 2018; Heffernan et al., 2018; Khalil et al., 2018; Koshy et al., 2017; Christensen et al., 2016a; Lin et al., 2013a), by adiposity (Lin et al., 2013a), by age (Koshy et al., 2017), or by diabetes status (Lind et al., 2014). *Low* confidence studies presented concerns with the outcome measures including potential for outcome misclassification (He et al., 2018; Christensen et al., 2016a; Zong et al., 2016; Steenland et al., 2015), failing to account for diabetes status (Lind et al., 2014) or use of medications that would impact insulin levels or beta-cell function (He et al., 2018; Fleisch et al., 2017), analytical methods (Koshy et al., 2017), and failure to establish temporality between PFOA exposure and diabetes (Lind et al., 2014). Other concerns included selection bias (Fassler et al., 2019), which resulted from self-selection (Christensen et al., 2016a), failure to provide information on control group selection (Heffernan et al., 2018), differential recruitment for cases and controls (Lin et al., 2013a), or survival bias (Steenland et al., 2015). Small sample size was also a concern in some studies (Heffernan et al., 2018; Khalil et al., 2018; Christensen et al., 2016a). In the evidence synthesis below, *high,* and *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

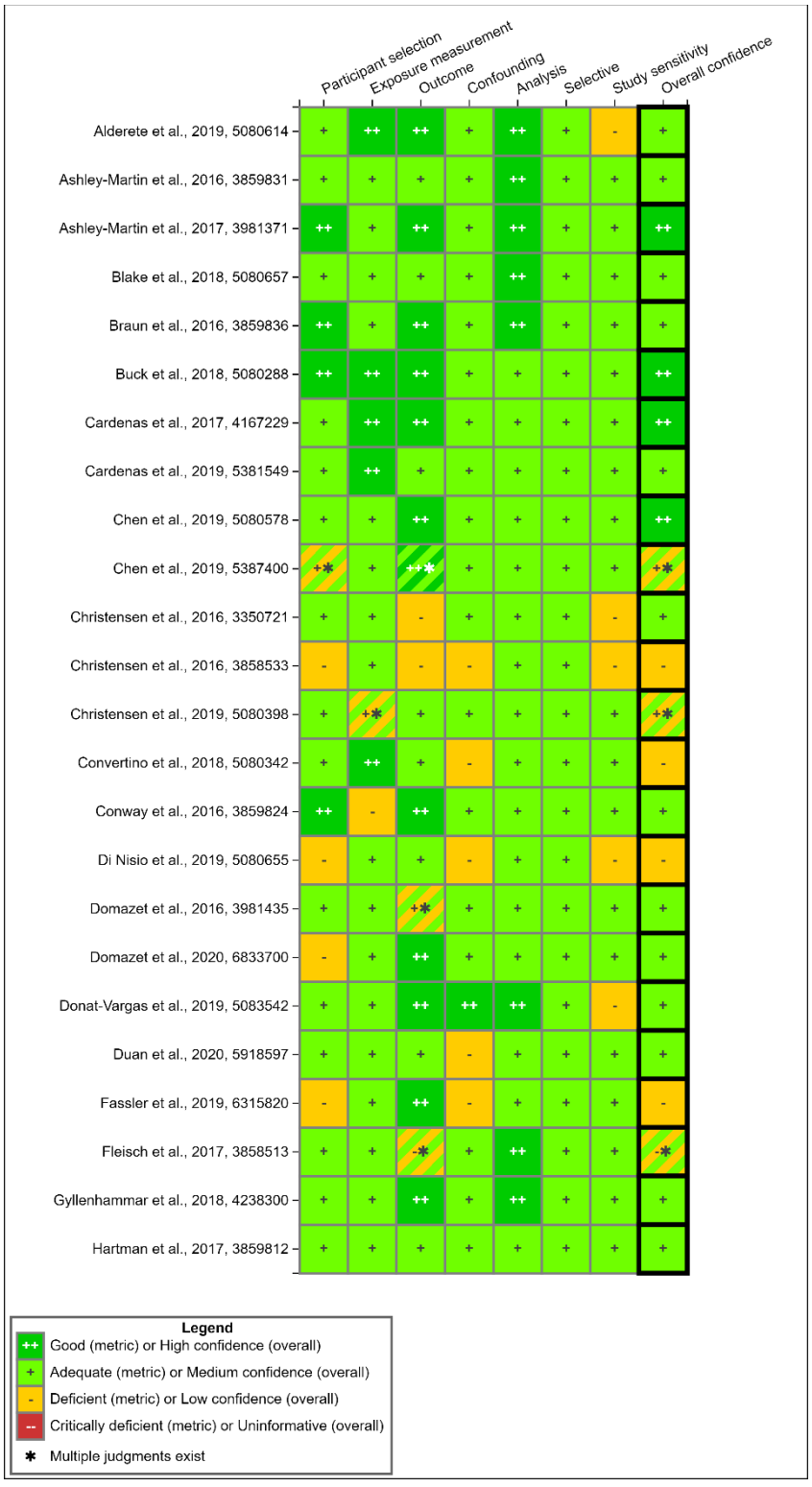


Figure C-17. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects

Interactive figure and additional study details available on HAWC.

Figure C-18. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects (Continued)

Interactive figure and additional study details available on HAWC.

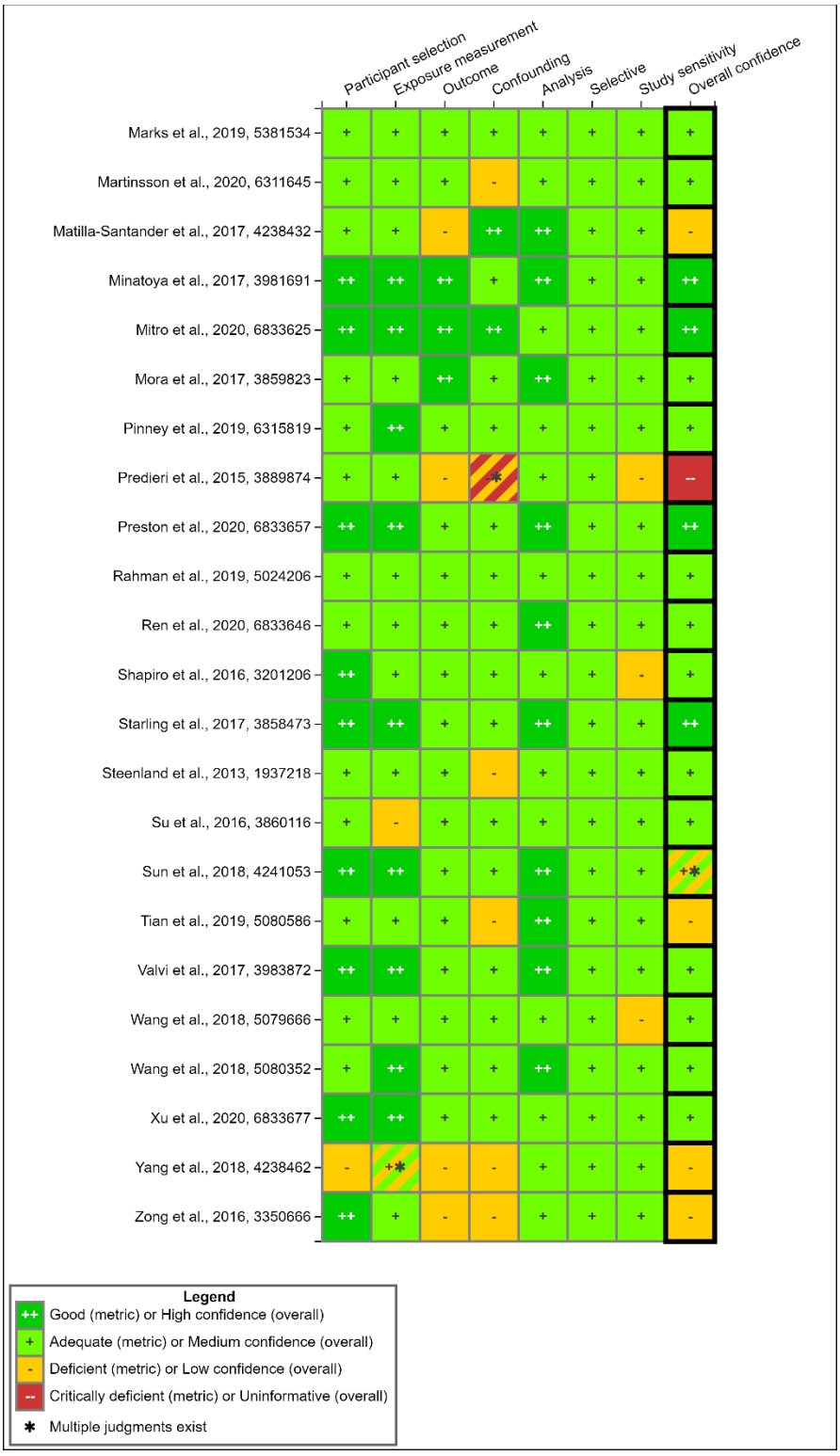


Figure C-19. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Metabolic/Systemic Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.3.1.3 Findings From Children and Adolescents

Two *medium* and two *low* confidence studies examined blood glucose in children, and only one reported a positive association with 2-hour glucose. No associations were observed for fasting glucose. Alderete et al. (2019) examined a cohort of obese Hispanic children aged 8−14, from the SOLAR Project and observed a significant association with 2-hour glucose, but no association with fasting glucose. Two cross-sectional studies reported positive non-significant associations with fasting glucose, one *medium* confidence study in 3–18-year-old Koreans (Kang et al., 2018), and one *low* confidence study in American obese 8–12 years (Khalil et al., 2018). Another cross-sectional study in girls ages 6–8 years from the Breast Cancer and Environment Research Program reported a negative, non-significant association with glucose levels (Fassler et al., 2019).

One *medium* confidence study observed positive, non-significant associations with blood glucose levels at age 15, using PFOA measured at ages 9 and at age 15 (Domazet et al., 2016). A non-significant negative association was observed between PFOA measured at age 15 and blood glucose measured at age 21 (Domazet et al., 2016).

Three studies examined the association between PFOA and insulin levels and reported no associations. One *medium* confidence study reported a positive, non-significant association with fasting insulin in obese Hispanic children aged 8–14 (Alderete et al., 2019). In contrast, two *low* confidence studies reported negative non-significant associations between PFOA and fasting insulin (Fassler et al., 2019; Khalil et al., 2018)

Insulin resistance, as described by the HOMA-IR, was examined in five studies with mixed results. Alderete et al. (2019) observed a positive, non-significant association, while four *low* confidence studies reported non-significant negative associations (i.e., decreasing insulin resistance with increasing serum PFOA) (Fassler et al., 2019; Khalil et al., 2018; Fleisch et al., 2017; Koshy et al., 2017).

A positive, but non-significant association was observed between PFOA and insulin sensitivity, measured through both the insulin sensitivity index and the CHECK Index/Quantitative Insulin Sensitivity Check Index (Fassler et al., 2019).

One *medium* confidence study reported negative associations with insulin-like growth factor 1 (IGF-1) in 6–9-year-old children in the C8 Health Project (Lopez-Espinosa et al., 2016). There was a significant negative association with IGF-1 in girls, and a significant negative association with IGF-1 in the second quartile of PFOA exposure among boys (Lopez-Espinosa et al., 2016).

Adiponectin and leptin were both examined in a *medium* confidence study from the European Youth Study, and non-significant associations were observed with adiponectin (positive), and leptin (negative) (Domazet et al., 2020). Similarly, Fleisch et al. (2017) reported a non-significant negative association with leptin in both early- and mid-childhood. Positive, non-significant association was observed between maternal blood PFOA and cord blood adiponectin (Ashley-Martin et al., 2017; Minatoya et al., 2017).

Three studies examined adiposity, and one reported a significant negative association with fat mass. One *low* confidence study observed a significant negative association with log fat mass

and fat mass percentage in girls ages 6–8 years (Fassler et al., 2019). However, concerns about selection bias and residual confounding by SES limit confidence in these results.

Chen et al. (Chen et al., 2019b) observed a positive, non-significant association with children's body fat percentage; non-significance persisted after stratification by child sex. Non-significant negative associations were observed in the third tertile of PFOA exposure for girls and in the second tertile of PFOA exposure for boys (Chen et al., 2019b). Similarly, a positive, non-significant association was observed with children's body fat mass, and non-significance persisted after stratifying by child sex (Chen et al., 2019b). In a tertile analysis, positive, non-significant associations were observed in the third tertile of PFOA exposure for all children and in the third tertile of PFOA exposure for boys; negative, non-significant associations between PFOA and body fat mass were observed in the second and third tertiles of PFOA exposure among girls (Chen et al., 2019b). A *medium* confidence cross-sectional study of 9-year-old children in the European Youth Heart Study reported a negative non-significant association with fat mass (Domazet et al., 2020).

Seven studies examined BMI measures, with mixed results. Four studies observed no associations with BMI, and two observed associations with BMI z-score.

One *high* confidence study examined the association between cord blood PFOA and age 5 BMI in the Shanghai Prenatal Cohort (Chen et al., 2019b). There was a negative but non-significant association between PFOA and BMI (i.e., decreased BMI with higher PFOA exposure levels). The effect was larger in females (beta = 0.07, 95% CI: −0.4, 0.53) than for males (beta = 0.2, 95% CI: −0.3, 0.69). Results from a tertile analysis were also non-significant, even after stratification by sex. For females, BMI increased with increasing tertiles of PFOA, while BMI decreased with increasing tertiles of PFOA in males. (Chen et al., 2019b). Two *medium* confidence studies observed positive, non-significant associations with BMI (Manzano-Salgado et al., 2017b; Braun et al., 2016). In a sex-stratified analysis, the association between maternal blood PFOA and BMI at age 7 remained positive among boys but became negative among girls (Manzano-Salgado et al., 2017b).

Of the three *low* confidence studies examining BMI, two reported positive, non-significant associations (Di Nisio et al., 2019; Koshy et al., 2017), and one reported a negative non-significant association with BMI (Khalil et al., 2018).

Six studies examined BMI z-score, two of which reported significant negative associations. Two studies from the Breast Cancer and the Environment Research Program (one *medium*, one *low* confidence) observed significant negative associations with BMI z-score in girls ages 6–8 (Fassler et al., 2019; Pinney et al., 2019). Pinney et al. (2019) observed a significant negative association with BMI z-score in girls living in the Greater Cincinnati and the San Francisco Areas. Karlsen et al. (2017) observed a non-significant negative association with BMI z-score at 18 months and age 5. In children from the POPUP study, Gyllenhammar et al. (2018b) observed a positive, significant association with BMI z-score and 3 and 4-years old children; the association with BMI z-score among 5-year-old children was positive, but not significant.

Additionally, a non-significant association was observed with BMI z-score in early- and mid-childhood (Mora et al., 2017). Another *low* confidence study reported a negative, non-significant association with BMI z-score (Koshy et al., 2017).

A *medium* confidence study reported a weak non-significant negative association between serum PFOA levels and ponderal index at birth in infants from the Hokkaido Study on Environment and Children's Health (Kobayashi et al., 2017).

No associations were observed in two *low* confidence studies examining body weight (Fassler et al., 2019) or being overweight (Koshy et al., 2017).

Four studies examined waist measurements, and two reported associations. Two studies (one *medium*, one *low* confidence) observed significant inverse associations with waist-to-height ratio (i.e., increased wait-to-height ratio as a continuous measure with higher serum PFOA exposure levels) in girls ages 6–8 (Fassler et al., 2019; Pinney et al., 2019). However, one *high* confidence study observed a positive non-significant association between cord blood PFOA and waist-to-height ratio in girls ages 5 years (Chen et al., 2019b). Inverse non-significant associations were observed for all children combined, and in boys, and a non-significant decreasing trend was observed (Chen et al., 2019b).

Two studies (one *medium* and one *low* confidence) examined waist-to-hip ratio. The *medium* confidence study observed a non-significant negative association (Pinney et al., 2019) between PFOA and waist-to-hip ratio, while the *low* confidence study reported a non-significant positive association between PFOA and waist-to-hip ratio (Fassler et al., 2019). One *medium* confidence study reported a positive, non-significant association with waist-to-hip circumference. After stratification by sex in the early childhood analysis, a non-significant negative association was observed among girls. In the mid-childhood analysis, the increase in waist-to-hip circumference ratio was greater for girls than for boys (Mora et al., 2017)

One *high*, two *medium*, and one *low* confidence study examined waist circumference and reported one association (Hartman et al., 2017). The *medium* confidence study, from the ALSPAC, assessed data from mother-daughter pairs and observed a significant decrease in female children's waist circumference (Hartman et al., 2017). Two *medium* confidence studies (Chen et al.; Mora et al., 2017) reported a positive, non-significant association between PFOA and waist circumference. After stratification by sex, non-statistical significance persisted; associations remained negative for males but were positive for females (Chen et al., 2019b). A cohort study of maternal-child pairs from the European Youth Heart Study reported a non-significant percent decrease in waist circumference at 21 years old with PFOA exposure at age 9 and age 15, and a significant percent decrease in waist circumference at 21 years old with concurrent PFOA exposure, and a non-significant percent increase in waist circumference at age 15 with age 9 PFOA exposure (Domazet et al., 2016).

In the *low* confidence study, Di Nisio et al. (2019) reported a significant difference between mean waist circumference of Italian male high school students exposed to PFOA pollution compared with those who were not exposed (Di Nisio et al., 2019).

There were three studies, each of *medium* confidence, measuring the association between PFOA and skinfold thickness. A *medium* confidence study from the SGA Study reported a non-significant positive association with tricep skinfold z-score, and a non-significant negative association with subscapular skinfold thickness z-score among 412 children (Lauritzen et al., 2018).

Another cohort study, which used a subset of data on children from the European Youth Heart Study, observed a non-significant percent increase in skinfold thickness at age 15 for increases in PFOA exposure at 9 years old, as well as a non-significant percent increase in skinfold thickness at age 21 for increases in PFOA exposure at 9 years old. However, there was a non-significant percent decrease in skinfold thickness at 21 years old with increase in PFOA exposure from 15 years old (Domazet et al., 2016).

A cohort study of mother-child pairs was used to assess the association between maternal PFOA and skinfold thickness (Mora et al., 2017) There was a positive, non-significant association between PFOA and subscapular-to-triceps skinfold thickness ratio measured in both early childhood and mid-childhood. After stratification by sex, the effect increased for females, but decreased non-significantly for males during both early- and mid-childhood. Similarly, the association between PFOA and the sum of subscapular and tricep skinfold thickness during mid-childhood decreased for males but increased for females when stratified by sex, but the sum of subscapular and tricep skinfold thickness during early childhood decreased for females and increased for males when stratified by sex (Mora et al., 2017).

C.3.1.4 Findings From Pregnant Women

Eleven studies examined gestational diabetes, and one reported a negative association between PFOA and gestational diabetes.

A *medium* confidence study of adults aged 20–60 living in Taiwan reported a significant negative association with gestational diabetes (Su et al., 2016).

In a *high* confidence cohort study from Project Viva of pregnant women, Preston et al. (2020) reported a non-significant, null association with gestational diabetes (OR = 1.0; 95% CI: 0.6, 1.6), but non-significant increased odds of gestational diabetes with increasing quartiles of PFOA (Preston et al., 2020).

Two *medium* confidence case-control studies reported increased, non-significant odds of gestational diabetes (Xu et al., 2020b; Wang et al., 2018c). In pregnant women with no family history of diabetes, Liu et al. (2018a) reported a non-significant, positive association between m-PFOA or L-PFOA and odds of gestational diabetes (Liu et al., 2019). Increased, non-significant odds of gestational diabetes were observed in the second and third tertiles of L-PFOA exposure, and in the third tertile of m-PFOA exposure; decreased, non-significant odds of gestational diabetes were observed in the second tertile of m-PFOA exposure (Liu et al., 2019). Similarly, nested case-control study conducted by Xu et al. (2020b) recruited pregnant women with no history of diabetes and reported increased, non-significant odds of gestational diabetes across quartiles of PFOA exposure and log-transformed PFOA exposure.

A study from the U.S. National Institute of Child Health and Human Development (NICHD) Fetal Growth Study reported a non-significant increased risk of gestational diabetes among all women, women with a family history of type 2 diabetes, and women with an overweight pre-pregnancy BMI (Rahman et al., 2019). A non-significant decreased risk of gestational diabetes was observed among pregnant women without a family history of type 2 diabetes and among women who did not have an overweight pre-pregnancy BMI (Rahman et al., 2019).

Three *medium* and one *low* confidence studies reported negative, non-significant associations with gestational diabetes (Wang et al., 2018a; Valvi et al., 2017; Shapiro et al., 2016; Zong et al., 2016).

Seven studies evaluated blood glucose and related measures, with mixed results. Two studies reported an association with oral glucose tolerance test results; no associations were reported for fasting glucose, impaired glucose tolerance, or hyperglycemia.

A *medium* confidence study of pregnant women with and without gestational diabetes reported increased, but non-significant odds of increased fasting blood glucose with increasing tertiles of n-PFOA (Wang et al., 2018c). Liu et al. (2019) observed positive, non-significant associations between both sum m-PFOA and L-PFOA and fasting glucose. Three *medium* confidence cohort studies observed negative, non-significant associations with fasting blood glucose (Jensen et al., 2018; Wang et al., 2018a; Starling et al., 2017).

Overall oral glucose tolerance test results were evaluated in one study (Wang et al., 2018a). When modeled continuously, there was a positive, non-significant association between PFOA and OGTT glucose. No significant difference was observed in mean oral glucose tolerance test results between tertiles of PFOA (Wang et al., 2018a).

Two *medium* confidence studies examined 1-hour blood glucose, and both reported positive significant associations. Ren et al. (2020) observed a significant increase in 1-hour plasma glucose levels and Liu et al. (2019) reported a significant positive association between serum L-PFOA and glucose homeostasis at 1-hour, and a negative, non-significant association between sum m-PFOA and 1-hour glucose.

Two *medium* confidence studies examined 2-hour blood glucose. A significant positive association was observed between L-PFOA and 2-hour glucose, but the positive association between sum m-PFOA and 2-hour glucose was not significant (Liu et al., 2019). A *medium* confidence study from the Odense Child Cohort reported a negative non-significant association between serum PFOA and 2-hour glucose among 158 women at high risk for gestational diabetes (Jensen et al., 2018).

Three studies examined impaired glucose tolerance. In a subset of women from Project Viva Preston et al. (2020) observed decreased, non-significant odds of impaired glucose tolerance. This was also observed in a tertile analysis, but the odds of impaired glucose tolerance were greater with increasing tertiles of PFOA (Preston et al., 2020). A *medium* confidence study also reported decreased odds of impaired glucose tolerance (Shapiro et al., 2016).

The single *low* confidence study observed non-significant increased odds of impaired glucose tolerance with PFOA increasing continuously, but non-significant decreased odds of impaired glucose tolerance with increasing quartiles of PFOA (Matilla-Santander et al., 2017).

One *high* confidence study examined isolated hyperglycemia in pregnant women from the Project Viva cohort (Preston et al., 2020). When analyzed continuously, increasing PFOA did not affect the odds of hyperglycemia. A quartile analysis showed non-significant decreased odds of hyperglycemia with increasing quartiles of PFOA (Preston et al., 2020).

Two studies (one *high* confidence and one *medium* confidence) evaluated blood glucose levels (Preston et al., 2020; Ren et al., 2020). Both studies reported a non-significant positive association with blood glucose levels. After stratifying by age, Preston et al. (2020) reported a non-significant negative association with blood glucose among women aged 35 and older. In the *medium* confidence study, results from an age-stratified analysis showed non-significant decreased odds of high plasma glucose for women at 20–23 gestational weeks (Ren et al., 2020).

Two studies evaluated insulin resistance measures; neither reported any associations.

There were two studies of *medium* confidence evaluating insulin levels (Jensen et al., 2018; Wang et al., 2018a). One of these studies reported a non-significant negative association with fasting insulin levels (Jensen et al., 2018), while the other observed a non-significant positive association with fasting insulin levels (Wang et al., 2018a).

Two *medium* confidence studies assessed insulin resistance. One reported a non-significant negative association (Jensen et al., 2018), while the other observed a non-significant positive association (Wang et al., 2018a) with insulin resistance. Wang et al. (2018a) reported no significant difference in mean insulin resistance between tertiles of PFOA.

One *medium* confidence study evaluated insulin sensitivity (measured using the Matsuda index) and observed a positive, non-significant association (Jensen et al., 2018).

A non-significant percent decrease in beta-cell function was observed (Jensen et al., 2018).

Adiponectin and leptin were both examined in a *high* confidence study from Project Viva, and no significant associations were observed A non-significant negative association with adiponectin and a non-significant positive association with leptin were reported (Mitro et al., 2020). After stratification by age during pregnancy, non-significant positive associations with leptin persisted; a positive, non-significant association with adiponectin was observed among women under age 35 during pregnancy (Mitro et al., 2020).

Three *medium* confidence cohort studies examined gestational weight gain, with one reporting an association. Ashley-Martin et al. (2016) used data from mother-infant pairs from the Maternal-Infant Research on Environmental Chemicals (MIREC) to estimate the odds of having high cord blood PFOA (> 0.39 ng/mL) per increase in gestational weight gain. ORs were significant for both 1 kg increase in gestational weight gain and interquartile range (IQR) increase in gestational weight gain (Ashley-Martin et al., 2016).

Jaacks et al. (2016) observed a positive, non-significant association with gestational weight gain among 218 mothers, mothers with a BMI < 25; a negative association was reported among mothers with a BMI ≥25. Increased, non-significant odds of excessive gestational weight gain were observed with increasing PFOA and decreased, non-significant odds of inadequate weight gain were reported (Jaacks et al., 2016).

Another study reported a positive, non-significant association with gestational weight gain among all women who were underweight or of normal weight and among under- or normal-weight mothers of daughters. Negative, non-significant associations with gestational weight gain were observed among overweight or obese mothers of all children, of boys, and of girls, and among normal or underweight mothers of sons (Marks et al., 2019).

One study evaluated anthropometric measurements and PFOA from the Project Viva cohort study and followed 801 pregnant women to 3 years postpartum (Mitro et al., 2020). Positive, non-significant associations were reported with 3-year postpartum arm circumference, subscapular skinfold thickness, tricep skinfold thickness, and 3-year postpartum waist circumference. After stratification by age during pregnancy, there was a significant increase in waist circumference measured at 3 years postpartum among women who were 35 or older during pregnancy (Mitro et al., 2020).

One *high* confidence cohort study evaluated BMI. A significant positive association with BMI among 786 pregnant women was reported (Mitro et al., 2020). Statistical significance did not persist after stratification by age (under 35/age 35 and older) (Mitro et al., 2020).

C.3.1.5 Findings From the General Adult Population

Eight studies investigated the relationship between PFOA and diabetes in the general population, and three reported a positive association.

A *medium* confidence study from the E3N cohort reported a non-significant increased risk of type 2 diabetes in the 7th and 8th deciles of PFOA exposure, and increased risk of type 2 diabetes was observed in the 4th–6th deciles of PFOA exposure. (Mancini et al., 2018). Another *medium* confidence study, from the Nurses' Health Study II, reported a significant association with type 2 diabetes among n female nurses (Sun et al., 2018a).

One *high* confidence cohort study from the Diabetes Prevention Program followed adults at increased risk of type 2 diabetes and observed an increased, but non-significant risk of diabetes per doubling of PFOA (Cardenas et al., 2019; Cardenas et al., 2017). After stratification by sex, a non-significant negative association was observed among men (Cardenas et al., 2017). Non-significant negative associations were also observed in analyses by tertiles (Cardenas et al., 2019).

Another *medium* confidence study reported non-significant increased odds of type 2 diabetes were observed in the second tertile of PFOA exposure, while non-significant decreased odds were observed in the third tertile of PFOA exposure (Donat-Vargas et al., 2019a).

Significant decreased odds of type 1, type 2, and uncategorized diabetes were observed in participants in the C8 Health Project (Conway et al., 2016). After stratifying by age, significant decreased odds of type 1, type 2, and uncategorized diabetes were observed among adults. significant decreased odds of type 1 diabetes were observed for children with type 1 diabetes, but non-significant increased odds of type 2 and uncategorized diabetes were observed among children (Conway et al., 2016).

Among the three *low* confidence studies, one reported a non-significant negative association with diabetes (Lind et al., 2014), while two overlapping NHANES studies reported non-significant positive associations with diabetes (He et al., 2018) and prediabetes (Christensen et al., 2016a). Significantly increased odds of diabetes were observed for males, non-significant increased odds were observed for females (Christensen et al., 2016a). *Low* confidence ratings resulted from concerns with potential for outcome misclassification (He et al., 2018; Christensen et al., 2016a), self-selection into the study, residual confounding by SES (Christensen et al., 2016a), and failure to establish temporality between exposure and outcome (He et al., 2018).

Four studies (three *medium* confidence and one *low* confidence) evaluated metabolic syndrome; one study reported an association. In an adult population of the island of Hvar (Croatia) Chen et al. (2019a) observed a positive non-significant association with risk of MetS as defined by the Adult Treatment Panel III criteria (OR = 1.89, 95% CI: 0.93, 3.86). Two *medium* confidence studies used overlapping data from NHANES and reported non-significant negative associations with metabolic syndrome. Liu et al. (2018b) observed adults aged 20 and older from the 2013–2014 NHANES cycle and Christensen et al. (2019) observed adults aged 18 and older from 2007−2014 NHANES.

A *low* confidence study observed significant increased odds of metabolic syndrome for participants with serum n-PFOA > 1.90 ng/mL compared with those with serum PFOA ≤1.90 ng/mL (Yang et al., 2018). However, concerns for selection bias, outcome misclassification, and residual confounding by SES diminish confidence in the study results.

There were five studies examining the association between PFOA and glucose, and three reported associations with fasting blood glucose, and one reported an association with 2-hour glucose.

A *medium* confidence study of adults aged 19–87 years from China reported a significant positive association with fasting blood glucose (Duan et al., 2020). Similarly, a study using NHANES data on adults from 1999 to 2014 observed a significant positive correlation between fasting glucose and serum PFOA (Huang et al., 2018). Su et al. (2016) reported a statistically significant decrease in fasting blood glucose for both increasing quartiles of PFOA and per doubling of PFOA among Taiwanese adults aged 20–60.

Another cohort study, which followed adults at high risk of type 2 diabetes, observed a positive, non-significant increase in 30-minute glucose per doubling of PFOA, while a negative, non-significant association was observed between with 2-hour glucose (Cardenas et al., 2017). A non-significant negative association with 2-hour glucose was reported per doubling in PFOA among Taiwanese adults aged 20–60, but a significant decrease in 2-hour glucose was observed for increasing quartiles of PFOA (Su et al., 2016).

One study reported non-significant decreased odds of elevated glucose with increasing tertiles of PFOA (Christensen et al., 2019). Odds were adjusted for PFDA, PFOS, PFHxS, 2-(N-methyl-PFOSA) acetate (MPAH), PFNA, perfluoroundecanoic acid (PFUnDA) simultaneously.

The association between PFOA and resting metabolic rate was assessed in the POUNDS Lost trial, a clinical trial of overweight and obese adults aged 30–70. A non-significant positive correlation between PFOA and resting metabolic rate was observed (Liu et al., 2018a). In the first 6 months of the trial, resting metabolic rate decreased non-significantly across all tertiles of PFOA exposure for both men and women. Neither the trend across tertiles nor the interaction between PFOA and sex were significant (Liu et al., 2018a). In months 6–24 of the trial, resting metabolic rate decreased significantly for males, and non-significantly for females. No statistical significance was observed for the interaction between PFOA and sex (Liu et al., 2018a).

Twelve studies examined insulin resistance measures; of these studies, one found reported significant associations with fasting insulin, insulin resistance, insulinogenic index 1, fasting

plasma insulin, 30-minute insulin, fasting proinsulin, and insulin (corrected response), and one reporting associations with the ratio of proinsulin to insulin.

The single *high* confidence study used a subset of data on adults at high risk of type 2 diabetes from the Diabetes Prevention Program (Cardenas et al., 2017). A positive, significant association was observed between PFOA and fasting insulin (Cardenas et al., 2017). Two *low* confidence studies examined fasting insulin, and both reported non-significant negative associations with fasting insulin (Chen et al., 2019a; He et al., 2018).

Two *medium* confidence studies reported negative, non-significant associations with insulin levels (Sun et al., 2018a; Domazet et al., 2016). In contrast, another *medium* confidence observed a positive, non-significant association with insulin levels (Liu et al., 2018b).

Nine studies examined insulin resistance, and one reported a significant association. A *high* confidence study of 956 adults at high risk for type 2 diabetes in the Diabetes Prevention Program reported a statistically significant, positive association with insulin resistance (Cardenas et al., 2017). A *medium* confidence study of adults in NHANES observed a non-significant increase in insulin resistance with increase in PFOA (Liu et al., 2018b). However, Donat-Vargas et al. (2019a) reported a non-significant negative association with insulin resistance in both continuous and tertile analyses. In a sensitivity analysis, a non-significant negative association was observed between insulin resistance and baseline PFOA second tertile, and between insulin resistance and PFOA measured at the end of follow-up for both the second and third tertile of PFOA exposure. A non-significant positive association with insulin resistance was reported in the third tertile of baseline PFOA exposure (Donat-Vargas et al., 2019a).

In a *medium* confidence study, a non-significant decrease in insulin resistance (measured as HOMA-IR) was observed at age 15 and 21 years old per increase in PFOA exposure from 9 years old (Domazet et al., 2016). At age 21, there was a non-significant increase in HOMA-IR per increase in PFOA measured at age 15 (Domazet et al., 2016).

Three *low* confidence studies examined the association between PFOA and insulin resistance. Non-significant negative associations between PFOA and insulin resistance were observed in continuous analyses (Chen et al., 2019a; Lind et al., 2014). In a sex-stratified tertile analysis, a non-significant negative association was observed with log-HOMA-IR among males, with non-significant increasing HOMA-IR observed with increasing quartiles of PFOA (He et al., 2018). HOMA-IR decreased non-significantly with increasing quartiles of PFOA among females (He et al., 2018). These studies were given *low* confidence ratings due to failure to account for diabetes status (Lind et al., 2014), or use of medications that impact insulin levels in HOMA-IR analyses (Chen et al., 2019a), and failure to account for the complex sampling design of NHANES in statistical analyses (He et al., 2018).

The association between plasma PFOA and insulinogenic index 1 was investigated in a *high* confidence study from the Diabetes Prevention Program. A significant positive association was observed with insulinogenic index among adults at high risk for type 2 diabetes (Cardenas et al., 2017).

In a *high* confidence study, Cardenas et al. (2017) reported significant associations were observed between PFOA and fasting plasma insulin, 30-minute insulin, fasting proinsulin, and insulin (corrected response).

In a *low* confidence study, a significant positive association was reported for the ratio of proinsulin to insulin and PFOA (Lind et al., 2014).

Five studies examined beta-cell function and two reported a significant association. A *high* confidence study from the Diabetes Prevention Program reported a significant positive association with beta-cell function (measured as HOMA-B) among adults at high risk for type 2 diabetes (Cardenas et al., 2017). A significant positive association with beta-cell function was reported in a *medium* confidence study of adults from NHANES (Liu et al., 2018b). Two *medium* confidence studies reported negative, non-significant associations with HOMA-B (Donat-Vargas et al., 2019a; Domazet et al., 2016).

One *low* confidence study reported a positive, non-significant association with HOMA-B (Chen et al., 2019a). This study was given a *low* confidence rating due to failure to exclude participants using medications that could impact beta-cell function.

Five studies examined adiponectin, and one observed an association. A *high* confidence study from the Health Outcome Measures of the Environment (HOME) study reported non-significant positive association between maternal blood PFOA and adiponectin in children (Ashley-Martin et al., 2017). In contrast, a significant negative association with adiponectin was observed among adults in the Diabetes Prevention Program (Cardenas et al., 2017). A *medium* confidence study reported a negative non-significant correlation between PFOA and plasma adiponectin (Sun et al., 2018a).

Two *high* confidence studies reported non-significant positive associations with adiponectin; no statistically significant effects were observed after stratifying by infant sex in either study (Buck et al., 2018; Minatoya et al., 2017).

Five studies examined associations with leptin. One study reported a significant association. Three *high* quality studies examined leptin (Buck et al., 2018; Ashley-Martin et al., 2017; Minatoya et al., 2017), all of which sampled mother-child pairs and observed positive, non-significant associations with children's leptin concentrations (Buck et al., 2018; Ashley-Martin et al., 2017; Minatoya et al., 2017). Two *medium* confidence studies examined leptin. One study, from the POUNDS Lost clinical trial, followed overweight and obese adults. A positive, significant correlation was observed between plasma PFOA and leptin concentrations (Liu et al., 2018a). A non-significant, slightly positive association was observed between PFOA and soluble leptin receptors (Liu et al., 2018a).

Eight studies examined hemoglobin and five reported an association. A *high* confidence study on participants in the Diabetes Prevention Program reported a significant positive association with HbA1c (Cardenas et al., 2017). Two *medium* confidence studies reported positive, non-significant associations with HbA1c (Duan et al., 2020; Sun et al., 2018a). One *medium* confidence study of PFOA and HbA1c among 10,859 NHANES participants reported a negative, significant spearman correlation between serum PFOA and plasma hemoglobin (Huang et al., 2018).

Another *medium* confidence cross-sectional study assessed the association between plasma PFOA and HbA1c among adults aged 20–60 (Su et al., 2016). A negative, non-significant association between HbA1c and continuous PFOA was reported, but a significant decrease in average HbA1c was observed with increasing quartiles of PFOA (Su et al., 2016). In the POUNDS Lost trial, a clinical trial of overweight and obese adults, negative, significant correlation was observed between PFOA and HbA1c (Liu et al., 2018a). Additionally, a *medium* confidence cross-sectional analysis of adults from NHANES reported a significant negative association with HbA1c (Liu et al., 2018b).

One *low* confidence study reported a statistically significant negative association with HbA1c among women with PCOS, and a non-significant positive association with HbA1c among women without PCOS (Heffernan et al., 2018). Another *low* confidence study reported no significant association between PFOA and glycated hemoglobin (Chen et al., 2019a). *Low* confidence ratings were given to these studies due to failure to exclude participants using medications that could impact HbA1c (Chen et al., 2019a) and concerns with participant selection and residual confounding (Heffernan et al., 2018).

Eight studies evaluated body weight measures, and six reported an association.

One study, from the POUNDS Lost clinical trial, evaluated body weight and observed a negative, non-significant association with weight loss in the first 6 months of the trial, and a positive, non-significant association with weight loss in months 6–24 of the trial (Liu et al., 2018a). A significant increase in average weight gain during months 6–24 of the trial was observed with increasing tertiles of PFOA (Liu et al., 2018a).

Seven studies evaluated being overweight and one reported a significant association. A cohort study of mothers and children from the Faroe Islands followed mother-child pairs reported an increased, significant risk of being overweight at age 5 with increase in maternal PFOA and a non-significant increased risk of being overweight at 18 (Karlsen et al., 2017). In a tertiles analysis, a non-significant negative association was observed with being overweight at 18 months, and a non-significant positive association was observed with being overweight at age 5 (Karlsen et al., 2017). A significant increased risk of being obese at age 5 was observed in the highest tertile of maternal PFOA exposure (Karlsen et al., 2017).

A *medium* confidence study reported significantly greater serum PFOA among obese adults compared with non-obese adults (Jain and Ducatman, 2019e). Five *medium* confidence studies evaluated maternal PFOA and risk of being overweight or obese in their children; these studies reported increased, non-significant risk or odds of being overweight (Martinsson et al., 2020; Lauritzen et al., 2018; Manzano-Salgado et al., 2017b; Mora et al., 2017; Braun et al., 2016). In a sex-stratified analysis, Mora et al. (2017) observed an increased, non-significant relative risk of being overweight or obese among boys, but a decreased, non-significant risk among girls.

In the *low* confidence studies, significant associations were seen between PFOA and being overweight (Tian et al., 2019c) and being obese (Yang et al., 2018). One study was given a *low* confidence rating due to concerns with BMI being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis (Tian et al., 2019c). *Low* confidence ratings were also given due to concerns with outcome misclassification and residual confounding by SES (Yang et al., 2018).

One study observed a significant negative association with weight-for-age z-score among children (Braun et al., 2016). A significant interaction between maternal PFOA and age was observed in the second tertile of maternal PFOA exposure, but not in the third tertile of maternal PFOA exposure (Braun et al., 2016).

Five studies evaluated body fat measures, and one reported an association. Four studies of *medium* confidence evaluated body fat (Liu et al., 2019; Hartman et al., 2017; Mora et al., 2017; Braun et al., 2016). A negative, non-significant association was observed between maternal plasma PFOA and body fat percentage in young girls in the ALSPAC, and this association persisted after stratification by age at menarche (Hartman et al., 2017). However, the negative association between maternal plasma PFOA and trunk fat percentage in young girls was significant (Hartman et al., 2017). Three *medium* confidence studies reported positive, non-significant associations with body fat measures (Liu et al., 2019; Mora et al., 2017; Braun et al., 2016).

Two *medium* confidence studies evaluated fat mass, and no associations were reported. Non-significant, positive associations with fat mass were reported among children (Jeddy et al., 2018) and overweight and obese adults (Liu et al., 2019).

Fifteen studies assessed BMI, and one reported a significant association.

In the HOME study, a cohort study of mother-child pairs, PFOA exposure was measured during pregnancy and BMI was recorded at age 8 (Braun et al., 2016). Significant positive associations with BMI z-score were observed in the second tertile of maternal PFOA exposure, and a negative, non-significant association was observed in the third tertile of maternal PFOA exposure (Braun et al., 2016). Additionally, significant increases in BMI z-score between ages 2 and 8 were observed in both the second and third tertile of maternal PFOA exposure (Braun et al., 2016). Two *medium* confidence studies of mother-child pairs observed positive, but non-significant association between maternal serum PFOA child's BMI z-score (Jensen et al., 2020a; Lauritzen et al., 2018).

Two *high* confidence studies and three *medium* confidence studies observed positive, non-significant associations with BMI (Chen et al., 2019a; Liu et al., 2018a; Cardenas et al., 2017; Mora et al., 2017; Domazet et al., 2016). After sex-stratification, a negative, non-significant association with BMI was observed among male children in mid-childhood (Mora et al., 2017). Domazet et al. (2016) reported a non-significant positive association between PFOA measured at age 15 and BMI at age 21.

In a *medium* confidence cohort study from the ALSPAC, a significant negative association with BMI was observed among mother-child pairs (Hartman et al., 2017). In a *medium* confidence study from the Fernald Community Cohort, a repeated-measures analysis reported a non-significant percent decrease in BMI was observed per IQR increase in PFOA, while a latent-analysis reported a non-significant percent increase in BMI per IQR increase in PFOA (Blake et al., 2018). In a sex-stratified analysis, non-significant percent decreases were observed for both males and females (Blake et al., 2018).

In the single *low* confidence study, Tian et al. (2019c) observed a statistically significant increase in BMI with increase in PFOA. In a sex-stratified analysis, a statistically significant positive

association was reported between PFOA and BMI among men; the association between PFOA and BMI among women was positive, but not significant (Tian et al., 2019c). This study was given a *low* confidence rating due to concerns with BMI being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis.

Four studies examined anthropometric measurements, and one reported significant association with waist circumference. One *medium* confidence study reported a negative, non-significant association with hip circumference (Chen et al., 2019a). Three *medium* confidence studies evaluated waist measurements and observed positive, non-significant associations with waist circumference (Chen et al., 2019a; Liu et al., 2018a; Braun et al., 2016)

A *low* confidence study from the Isomers of C8 Health project evaluated waist circumference among adults. A significant, positive association with waist circumference was observed. After stratification by sex, the association with waist circumference among men remained significant, but was not significant among women (Tian et al., 2019c). Significant increased odds of increased waist circumference were observed in the overall study population and among men; odds of increased waist circumference were increased but non-significant among women (Tian et al., 2019c). This study was given a *low* confidence rating due to concerns with waist circumference being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis.

C.3.1.6 Findings From Occupational Studies

There was one occupational study, which came from the C8 Health Project (Steenland et al., 2013). A decreased, non-significant risk of type 1 diabetes was observed in the second and fourth quartiles of PFOA exposure in both lagged and unlagged analyses were observed. A non-significant increased risk of type 1 diabetes was observed in the third quartile in both lagged and unlagged analyses (Steenland et al., 2013).

C.3.2 Animal Evidence Study Quality Evaluation and Synthesis

C.3.2.1 Metabolic Homeostasis

There is one study from the 2016 PFOA HESD (U.S. EPA, 2016c) and five studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and metabolic effects. Study quality evaluations for these six studies are shown in Figure C-20.

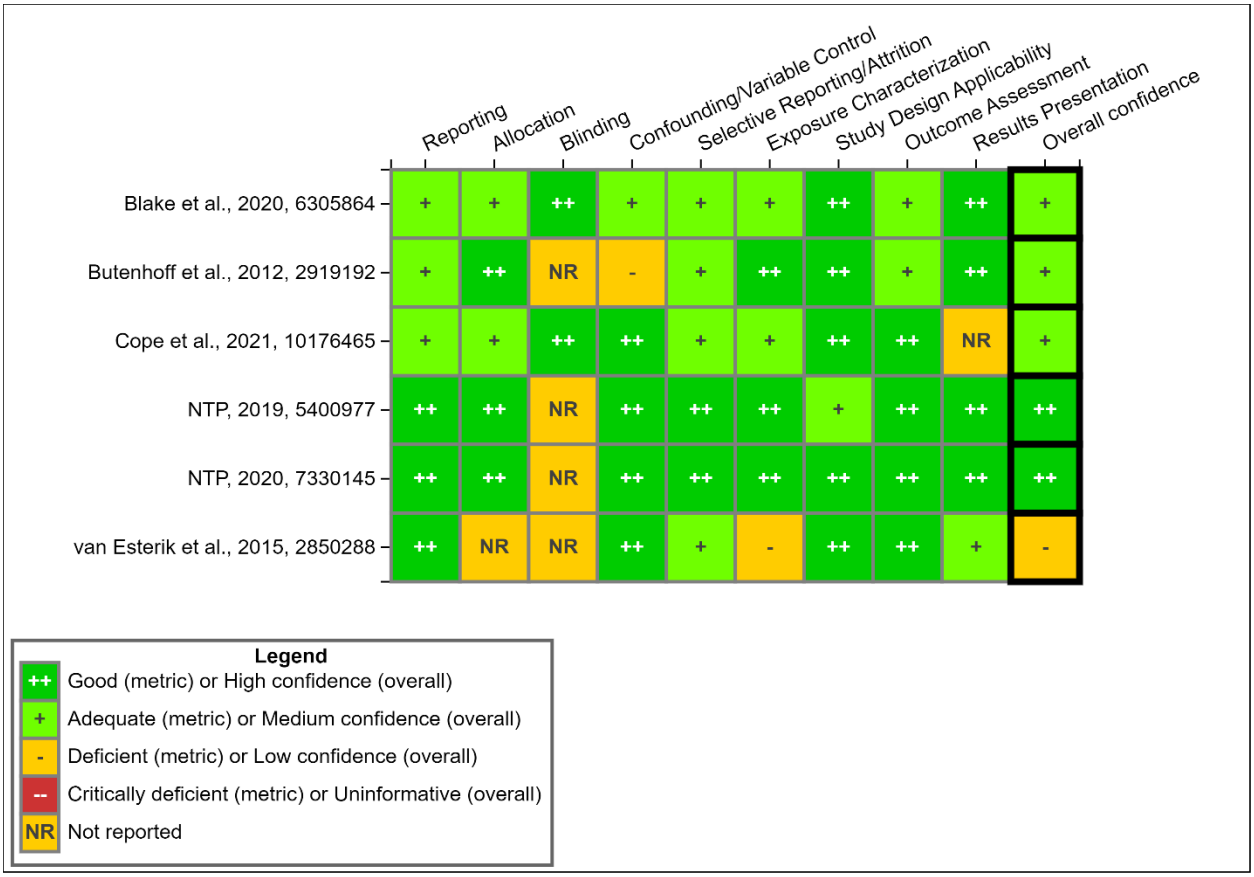


Figure C-20. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Metabolic Effects

Interactive figure and additional study details available on HAWC.

PFOA has been observed to cause perturbations in metabolic homeostasis in rodents. However, there appears to be differences in responses depending on species, length of exposure, and sex. Overall, the effects on metabolic parameters following PFOA exposure are inconclusive.

In a 28-day study conducted by NTP (2019a), glucose was significantly decreased in male Sprague-Dawley rats following exposure to ≥2.5 mg/kg/day PFOA. No significant response was observed in the female rats treated with up to 100 mg/kg/day PFOA. In a single-dose study in male Sprague-Dawley rats, Elcombe et al. (2010) similarly observed a significant decrease in serum glucose after administration of 300 ppm PFOA in feed (equivalent to approximately 19 mg/kg/day) for 28 days. However, a chronic study by Butenhoff et al. (2012) observed increases in glucose when measured beginning at 3 months. The authors exposed Sprague-Dawley rats to 30 or 300 ppm PFOA in feed for a 24-month period. Serum samples for clinical chemistry measurements were taken at 3, 6, 12, 18, and 24 months. For males in the 30 ppm group (~1.3 mg/kg/day PFOA), glucose levels were significantly higher than controls at 3, 6, and 12 months, then returned to baseline control levels at 18 and 24 months. Male rats in the 300 ppm group (~14.2 mg/kg/day PFOA) had significantly higher serum glucose levels than the control groups at the 3- and 24-month time points. In female rats, effects on serum glucose were only observed at the 6-month timepoint; in both the 30 and 300 ppm groups (~1.6 and

~16.1 mg/kg/day PFOA, respectively), serum glucose levels were significantly lower than controls. In a 2-year study conducted by NTP (2020), no effects on glucose levels were reported in male and female Sprague-Dawley rats (see Toxicity Assessment, (U.S. EPA, 2024b)).

In CD-1 mice, four independent studies investigated the effects of gestational PFOA exposure on adult offspring (Cope et al., 2021; Quist et al., 2015; Hines et al., 2009) or pregnant dams (Blake et al., 2020) and found no effect on glucose levels or glucose tolerance. Interestingly, Hines et al. (2009) observed weight gain in female offspring exposed to lower doses of PFOA (0.01, 0.1, and 0.3 mg/kg/day but not 1 mg/kg/day or controls) from GD 1–17. This weight gain was correlated with mid-life (21–33 weeks of age) increased serum insulin and leptin levels in the 0.01 and 0.1 mg/kg/day groups, but not glucose tolerance in early (15–16 weeks of age) or late (70–74 weeks of age) adulthood. These results indicate potential susceptibility to metabolic dysfunction later in life after low-dose gestational PFOA exposure. However, in a similar study, Quist et al. (2015) exposed pregnant mice to 0, 0.01, 0.1, 0.3, or 1 mg/kg/day from GD 1–17 and observed no statistical differences in serum glucose or insulin levels in female offspring at postnatal week 13 (PNW 13). Blake et al. (2020) also saw no effect on dam serum glucose with gestational exposure to 1 or 5 mg/kg/day PFOA from GD 1.5–11.5 or GD 1.5–17.5. Cope et al. (2021) exposed dams to 0.2, 1.0, or 2.0 PFOA mg/kg/day from GD 1.5–17.5 and observed a slightly elevated but non-significant fasting glucose level in male pups fed low-fat diets (LFD) and female pups fed either LFD or high-fat diets (HFD) at PND 54–58. The study also reported a dose-dependent increase in insulin levels, which caused a 43.1% decrease in QUICKI score in males pups exposed to 1 mg/kg PFOA. No significant effect on glucose tolerance was observed.

Body mass composition in male pups fed LFD was altered with significant increases in fluid mass at 0.1 mg/kg/day, and fat mass, fluid mass, and percent fluid at 1.0 mg/kg/day (Cope et al., 2021). No significant changes were observed in male pups fed with HFD at any PFOA dose group. Female pup fed LFD or HFD had no significant changes to body mass composition at any PFOA dose group.

C.3.2.2 Survival, Clinical Observations, Body Weight, and Food/Water Consumption

There are 8 studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 14 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and systemic effects. Study quality evaluations for these 22 studies are shown in Figure C-21.

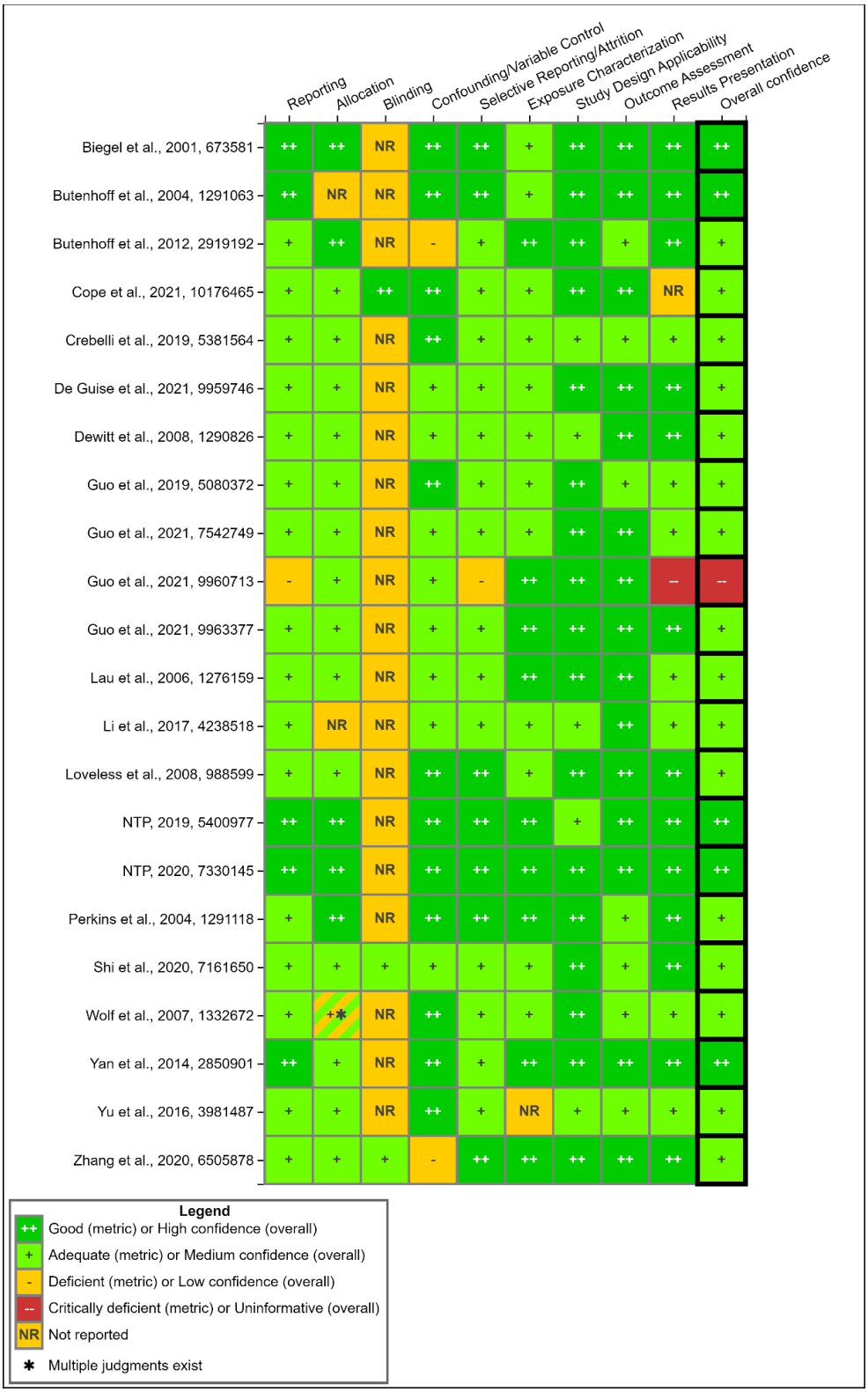


Figure C-21. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Systemic Effects

Interactive figure and additional study details available on HAWC.

Available animal toxicity data suggest that PFOA exposure can elicit whole-body toxicity, which is reflected by changes in survival, body weights, food consumption, and other clinical observations. Reductions in survival precipitated only at higher doses of PFOA in a single nonhuman primate study. Reductions in terminal body weight and reductions in weight gain are consistently observed across studies of varying durations of oral exposure to PFOA. Prior to this updated assessment, the available literature measuring clinical outcomes, food and water consumption, body weight, and survival primarily consisted of acute studies (U.S. EPA, 2016c). Many of the findings were consistent with those in more recent literature and are included herein.

C.3.2.2.1 Survival

Although one subchronic toxicity study in nonhuman primates exposed to > 30 mg/kg/day for 90 days PFOA showed reductions in survival (Goldenthal et al., 1979), survival rates were not affected in rodent studies across study durations and doses (NTP, 2020; Crebelli et al., 2019; NTP, 2019a; Perkins et al., 2004; Thomford, 2001). Interestingly, survival was increased in two studies: Butenhoff et al. (2012) and Biegel et al. (2001). Butenhoff et al. (2012) fed male Sprague-Dawley rats 0, 30, or 300 ppm PFOA via the diet (equivalent to 0, 1.3, or 14.2 mg/kg/day) for 2 years and observed that survival was increased in males at the highest dose (Figure C-22). No significant effect was observed in female rats (exposure equivalents of 0, 1.6, or 16.1 mg/kg/day) in this study (Figure C-22). Similarly, Biegel et al. (2001) observed increased survival in male Crl:CD BR (CD) rats fed 300 ppm (equivalent to 13.6 mg/kg/day) PFOA each day at the end of another 2-year study. In other studies of rats, mice, and nonhuman primates included in this updated assessment, all animals survived to the end of study (Figure C-22).

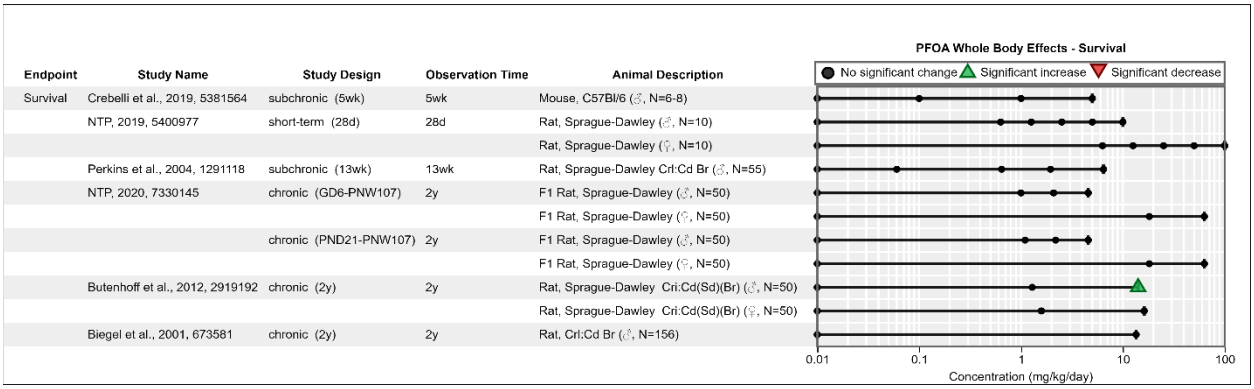


Figure C-22. Effects on Survival in Rodents Following Exposure to PFOA (Logarithmic Scale)

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; PNW = postnatal week; PND = postnatal day; F_1 = first generation; d = day; wk = week; y = year.

C.3.2.2.2 Clinical Observations

Clinical observations have been reported in some animal studies of oral exposure to PFOA. Two 28-day studies described clinical assessments following 28 days of oral PFOA exposure via gavage in Sprague-Dawley rats. Whereas NTP (2019a) did not observe any treatment-related clinical observations in 7- to 9-week-old Sprague-Dawley rats exposed to PFOA (0–10 mg/kg/day, males; 0–50 mg/kg/day, females) for 28 days, Cui et al. (2009) described adverse

clinical signs in male Sprague-Dawley rats exposed to 5 mg/kg/day, including cachexia and lethargy in the third week of study. In a 5-week study in male C57BL/6 mice exposed to 0.1–5 mg/kg/day PFOA, no signs of overt toxicity were observed (Crebelli et al., 2019).

The aforementioned study by Butenhoff et al. (2004a) reported that there were low incidences of dehydration, urine-stained abdominal fur, and ungroomed fur in at least three of the 30 P_0 male, but not female rats exposed for 70 days in the 30 mg/kg/day exposure group. No effects were noted in lower exposure groups (1–10 mg/kg/day), nor in the F_1 offspring at the end of study.

The chronic exposure study by Butenhoff et al. (2012) checked for palpable masses daily during the 2-year exposure, but the incidence was indistinguishable from controls in all exposure groups.

C.3.2.2.3 Body Weight in Adults

Reductions in body weight and/or reductions in weight gain have been observed in nonhuman primates as well as across rodent studies of varying exposure lengths (short-term, subchronic, chronic), species (rats or mice), and strains of mice.

In a short-term exposure study, Dewitt et al. (2008) found that mean body weight was reduced in female C57BL/6N mice exposed to 15 or 30 mg/kg/day PFOA in drinking water for 15 days; no effects were observed at or below 7.5 mg/kg/day (Figure C-23). Six independent studies reported body weights from BALB/c mice exposed to various doses (ranging from 0.4–20 mg/kg/day) of PFOA via gavage for 28 days; all exposures began around 6–8 weeks of age (Guo et al., 2021a; Guo et al., 2021b; Guo et al., 2019; Li et al., 2017a; Yu et al., 2016; Yan et al., 2014). Of these, Yu et al. (2016) was the only study that did not observe any changes in body weight; mice were exposed to 0.5 or 2.5 mg/kg/day PFOA (Figure C-23). Significant reductions in body weight that differed by more than 10% of control were observed only at the highest doses tested in the other studies: 2.5 mg/kg/day in Li et al. (2017a), 10 mg/kg/day in Guo et al. (2021a; 2021b; 2019), and 5 or 20 mg/kg/day in Yan et al. (2014) (Figure C-23). Two studies reported weight reductions in ICR mice exposed for approximately one month. Zhang et al. (2020b) observed that 5 mg/kg/day, but not 0.5 or 2 mg/kg/day, PFOA was sufficient to reduce body weight in female ICR mice after 28 days of exposure (Figure C-23). Males were not evaluated. Son et al. (2008) observed similar results in male ICR mice exposed to 17.63 or 47.21 mg/kg/day for 21 days. Females were not evaluated.

Another short-term exposure study by Loveless et al. (2008) in CD-1 mice administered 0, 0.3, 1, 10, 30 mg/kg/day for 28 days via gavage noted that mean terminal body weights at the end of study were 86% and 78% of control at 10 or 30 mg/kg/day, respectively. In another study, 6- to 8-week-old C57BL/6 mice were exposed to 0, 0.1, 1, or 5 mg/kg/day PFOA in drinking water for 5 weeks. Whereas untreated control mice gained an average of 5.1 ± 0.2 g over the course of the 5-week study, mice treated with 5 mg/kg/day PFOA gained significantly less weight (3.0 ± 0.1 g) (Crebelli et al., 2019). Shi et al. (2020) had similar findings for the 8-week-old C57BL/6J male mice that were dosed with 0, 0.5, 1, and 3 mg/kg/day in drinking water for 5 weeks. Mice at all dose levels (0.5, 1, or 3 mg/kg/day) were reported to show a marked decline in body weight gains starting around day 21. The highest dose group (3 mg/kg/day) was reported to have a lower body weight compared with the control group, which was demonstrated by both a significant lower body weight on day 35 and a significant difference in body weight gain over the study period. De Guise et al. (2021) exposed B6C3F1 female mice to 0, 1.88, and 7.5 mg/kg/day

PFOA via drinking water for 4 weeks. Mice in the high-dose group had significantly lower body weight compared with the mice in the control group from exposure day 14 to 28.

Five short-term studies have determined the effect of PFOA on body weight in rats. Loveless et al. (2008) applied the aforementioned exposure paradigm for CD-1 mice in male Crl:CD(SD)IGS BR rats. Mean terminal body weights at the end of the 28-day study were 10 and 25% lower than control at 10 and 30 mg/kg/day, respectively. Another study exposed male and female Sprague-Dawley rats to PFOA for 28 days (0, 0.625, 1.25, 2.5, 5, or 10 mg/kg/day for males, 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day for females) (NTP, 2019a). The mean body weights of 0.625, 1.25, and 2.5 mg/kg/day males and all treated females were within 10% of the respective vehicle control groups throughout the study. At the end of study, mean body weights of the 5 and 10 mg/kg/day males were 12% to 19% lower, respectively, than those of the vehicle control group. No effects on terminal body weight were observed in females.

The remaining three short-term PFOA exposure studies (Rigden et al., 2015a; Cui et al., 2009; Pastoor et al., 1987) in rats also suggest a decrease in body weight following PFOA exposure and are discussed in greater detail in the 2016 PFOA HESD (U.S. EPA, 2016c). Briefly, Pastoor et al. (1987) reported a 17% decrease in body weight from controls in male Crl:CD (SD) BR rats that had been exposed to 50 mg/kg PFOA for 7 days. Females were not evaluated. Cui et al. (2009) found that terminal body weight was significantly reduced in male Sprague-Dawley rats exposed to 20 mg/kg/day PFOA for 28 days, but the magnitude of this change (in comparison to controls) was less than 10%. No effects were observed at the 5 mg/kg/day group and females were not evaluated. Rigden et al. (2015a) exposed male Sprague-Dawley rats to 0, 10, 33, or 100 mg/kg/day PFOA via gavage for three days and recorded body weights each day throughout exposure as well as for four days after the end of exposure. Although body weight decreased on the last day of exposure in the 33 and 100 mg/kg/day exposure groups, growth resumed and the trajectory mirrored that of all other groups including controls during the 4 days after exposure.

In a subchronic exposure study, Perkins et al. (2004) weighed male Sprague-Dawley rats weekly over the course of a 13-week exposure to 0, 0.06, 0.64, 1.94, or 6.5 mg/kg/day. Body weight change and absolute body weight at study termination were both reduced in the highest exposure group (Figure C-23). Another subchronic study in rhesus monkeys (two per sex per group) reported reductions in body weight following exposure to 30 or 100 mg/kg/day PFOA for 13 weeks (Goldenthal et al., 1979). The reduction in weight loss preceded death in one monkey of each sex. Changes in body weight were similar to controls in the other dose groups (3 or 10 mg/kg/day).

Absolute body weights of parental (P)-generation male and female Sprague-Dawley rats were measured in a reproductive toxicity study by Butenhoff et al. (2004a); six-week-old rats were exposed to 0, 1, 3, 10, or 30 mg/kg/day PFOA via gavage for at least 70 days prior to mating and until sacrificed. During the peripubertal period (through test day 15), body weight relative to the control group was reduced in males exposed to 10 or 30 mg/kg/day. Terminal body weight was reduced in P_0 males following 106 days of exposure at dosages of 3 mg/kg/day and above, and the changes were greater than 10% in groups exposed to 10 or 30 mg/kg/day (Figure C-23). Body weights for the P_0 females were not significantly different (and generally within 10% from control) during the precohabitation period, body weights in the P_0 females at other time points are discussed in the Toxicity Assessment (U.S. EPA, 2024b).

Two chronic exposure studies reported opposing effects on body weights in male rats that were fed chow laden with 300 ppm (equivalent to 13.6 mg/kg/day) PFOA for 2 years (Butenhoff et al., 2012; Biegel et al., 2001). Whereas the Butenhoff et al. (2012) study was performed in Sprague-Dawley rats and evaluated the effects of PFOA on body weight in each sex, Biegel et al. (2001) used Crl:CD BR rats and only looked at males. Butenhoff et al. (2012) reported reduced body weights in males and females whereas Biegel et al. (2001) reported a 34% increase in body weight.

Of note, a few studies observed that the reductions in body weight and/or body weight change began around day 14–15 of exposure in BALB/c mice (Li et al., 2017a) and in Sprague-Dawley rats (NTP, 2019a). Although this observation was specific to males in one 28-day rat study (NTP, 2019a), it was common to both sexes in BALB/c mice (Li et al., 2017a). Zhang et al. (2020b) observed a trending reduction in body weight in female ICR mice at day 15 of exposure to 5 mg/kg/day PFOA, however the effect did not reach significance until day 25 and males were not tested. More data are required to understand whether the reductions in body weight are more common in a particular sex.

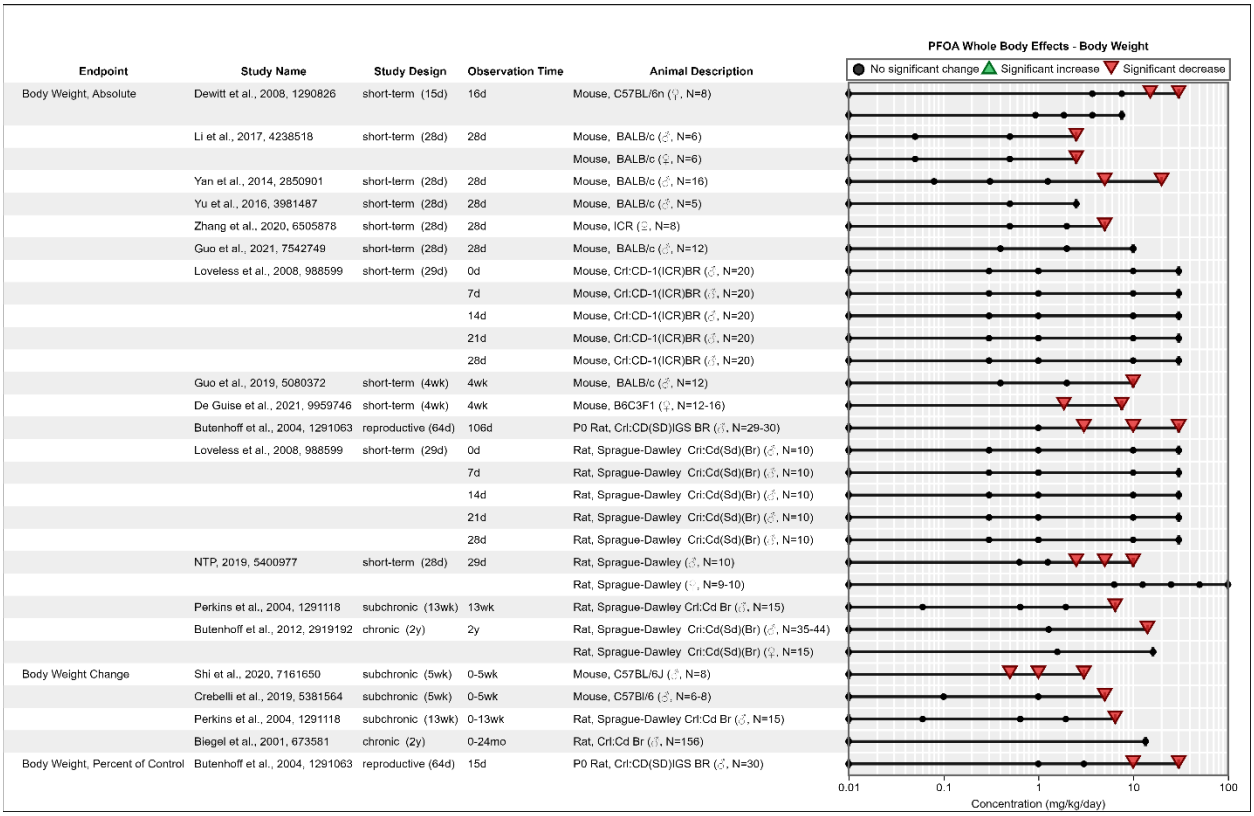


Figure C-23. Effects on Body Weight in Rodents Following Exposure to PFOA (Logarithmic Scale)

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; PNW = postnatal week; PND = postnatal day; P_0 = parental generation; d = day; wk = week; y = year.

C.3.2.2.4 Body Weight in Adults Following Developmental Exposure

Studies with animals exposed perinatally prior to weaning (i.e., up to PND 28) are described in the Toxicity Assessment (U.S. EPA, 2024b).

Several developmental exposure studies have evaluated body weight changes after weaning in CD-1 mice, Kunming mice, and Sprague-Dawley rats perinatally exposed to PFOA, most of which saw reductions in body weight (relative to litter) prior to weaning (see Toxicity Assessment, (U.S. EPA, 2024b)). Lau et al. (2006) exposed pregnant CD-1 mice to 0, 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA from GD 1–18 and weighed male and female pups at postnatal week 6.5 and 60 (PNW 6.5 and PNW 60), as well as the dams at GD 18. Weight gain in dams that carried pregnancy to term is described in the Toxicity Assessment (U.S. EPA, 2024b). Decrements in body weight of offspring were noted in the 10 mg/kg/day exposure group for PNW 6.5 male pups only. No changes in body weight were observed in PNW 6.5 females, and offspring from the 20 mg/kg/day group were precluded from the analysis due to low viability. The male-specific weight loss did not persist to PNW 60 in either sex (Figure C-24). Similarly, Song et al. (2018a) observed reduced body weights in PND 70 pups following gestational exposure to 1 mg/kg/day PFOA, where pregnant Kunming mice were exposed to 0, 1, 2.5 or 5 mg/kg/day from GD 1–17 (Figure C-24). Interestingly, this reduction was not observed in the 2.5 or 5 mg/kg/day groups, which were significantly heavier than controls at an earlier timepoint, PND 21 (see Toxicity Assessment, (U.S. EPA, 2024b)). In Cope et al. (2021), male pups of CD-1 mice exposed to 0.1 or 1 mg/kg/day PFOA did not display significant difference in body weight except for an increase in the 1 mg/kg/day PFOA group at PNW 17 in the low-fat diet group. In female pups, no significant differences were observed among any exposed group.

Absolute body weights in adult F_1-generation rats were also measured in the aforementioned study by Butenhoff et al. (2004a). P_0 male and female Sprague-Dawley rats were exposed to 0, 1, 3, 10, or 30 mg/kg/day PFOA for at least 70 days prior to mating and until sacrificed and their offspring (F_1 generation) were dosed similarly beginning at weaning. Relative body weights were reduced in F_1 male and female juvenile (PND 35) rats, as well as peripubertal F_1 (PND 56) male rats from the 30 mg/kg/day group. Additionally, male rats from the 10 mg/kg/day group had significantly reduced body weight (postweaning) beginning at PND 77 and lasting through the end of the study. A dose-dependent reduction in body weight at the end of the study (PND 120) was observed in F_1 males (Figure C-24). Effects on maternal body weight and on offspring prior to weaning are described in (see Toxicity Assessment, (U.S. EPA, 2024b)).

Two rodent studies evaluated the relative sensitivities of body weight to perinatal and/or postnatal exposure of PFOA. NTP (2020) evaluated the effects on body weight following perinatal and/or postweaning exposure to PFOA in Sprague-Dawley rats. In that study, pregnant rats were exposed to 0, 150, or 300 ppm PFOA to constitute a perinatal exposure in offspring, and postnatal exposures (0, 150, or 300 ppm for males, 0, 300, or 1,000 ppm for females) were continued during the postweaning period for 2 years (see Toxicity Assessment, (U.S. EPA, 2024b)). Body weights at the 16-week interim period tended to be lower in all F_1 gestational (GD 6–PNW 21; GD 6–PNW 107) and postweaning (PND 21–PNW 21; PND 21–PNW 107) exposure groups and reached significance in all male exposure groups. At the end of the 2-year study, there were no consistent effects of PFOA exposure on F_1 males. However, absolute body weight was reduced in F_1 females exposed during gestation plus after weaning (GD 6–PNW 107) as well as after weaning alone (PND 21–PNW 107).

Similar findings come from Wolf et al. (2007), who investigated the relative contributions of gestational and lactational exposures to PFOA in CD-1 mice. Pregnant mice were given 0 or 5 mg/kg/day PFOA at staggered intervals of gestational development (GD 7–17, 10–17, 13–17, or 15–17) and/or 0, 3, or 5 mg/kg/day during the lactational period (PND 1–22). Body weights were determined in male and female pups on PND 22 and PND 92. While no reductions in absolute body weight in any group at PND 92 were observed, an elevation in body weight was noted in PND 92 mice exposed to 3 mg/kg/day from GD 1–17, which had been significantly decreased from control when measured on PND 22 (see Toxicity Assessment, (U.S. EPA, 2024b)).

Figure C-24. Effects on Body Weight in Rodents Following Developmental 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; PNW = postnatal week; PND = postnatal day; F_1 = first generation; wk = week; y = year.

C.3.2.2.5 Food and Water Consumption

Reductions in body weight can be a consequence of reduction in food and/or water consumption, which have been reported in a few of the aforementioned rodent studies and two nonhuman primate studies following oral exposure to PFOA. Reductions in food or water consumption could not explain all the differences observed in body weight, however, and the limited number of studies that provided data on food consumption make it difficult to thoroughly evaluate the correlation between food consumption and effects on body weight.

Three drinking water studies of different durations reported food and water consumption in mice. Son et al. (2008) reported that food and water consumption was reduced in male ICR mice exposed to 250 mg/L PFOA (equivalent to 47.21 mg/kg/day) for 21 days, but not at concentrations of 50 mg/L (equivalent to 17.63 mg/kg/day) or below. Therefore, the aforementioned reductions in weight loss at 17.63 mg/kg/day were unlikely related to reductions in food consumption or dehydration. A shorter duration (15 day) in C57BL/6N mice exposed to 0, 3.75, 7.5, 15, or 30 mg/kg/day PFOA reported that water consumption per cage did not vary statistically between exposure groups and controls (Dewitt et al., 2008), despite reduced weight loss in the two highest exposure groups. Similarly, in another short-term study, there were no treatment-related effects on food and water intake in male C57BL/6N mice that were exposed to 0, 0.5, 1, or 3 mg/kg/day PFOA (Shi et al., 2020).

Studies of varying exposure durations in rats have also reported food and/or water consumption that in some cases support a relationship between reduced intake and weight loss. The study by Rigden et al. (2015a) noted a slight decrease in food consumption (data were not provided) and suggested dehydration related to decreased water consumption as an explanation for weight loss due to increased urine volume during the final two days of exposure. In another study of male Sprague-Dawley rats exposed to 0, 5, or 20 mg/kg/day PFOA for 28 days via gavage exhibited decreased food consumption at the 5 mg/kg/day dose (Cui et al., 2009) However, this level of exposure did not coincide with an effect on weight loss. Elcombe et al. (2010) also recorded food consumption (per gram basis) in male Sprague-Dawley rats fed 300 ppm PFOA for 28 days. Rats exposed to PFOA consumed less food by day 28. No differences in food consumption were observed in another study of male Sprague-Dawley rats fed 0, 1, 10, 30, or 100 ppm (equivalent to 0, 0.06, 0.64, 1.94, 6.5 mg/kg/day) for 13 weeks, despite reductions in body weight at the highest exposure level (Perkins et al., 2004). Females were not used in this study. Biegel et al. (2001) and Butenhoff et al. (2012) reported elevated food consumption in male rats exposed to 300 ppm PFOA for 2 years. Butenhoff et al. (2012) also evaluated female rats and reported inconsistent trends of reduced food consumption that appeared to be related to variations in body weight (Figure C-25).

The reproductive toxicity study in Sprague-Dawley rats by Butenhoff et al. (2004a) recorded food consumption of P_0 males as well as their male and female F_1 offspring at PND 35 following exposure to 0, 1, 3, 10, or 30 mg/kg/day PFOA via gavage. Mean absolute feed consumption (as a percent of control) of male P_0 rats was reduced in the highest exposure group for a majority of the time across 106 days of study. However, given the aforementioned reductions in body weight for these animals, feed consumption relative to body weight was actually elevated at the 3, 10, and 30 mg/kg/day doses. For F_1 males and females, absolute feed consumption was reduced at the 30 mg/kg/day dose (Figure C-25).

Two nonhuman primate studies covered in the 2016 PFOA HESD (U.S. EPA, 2016c) reported reductions in food consumption. Male cynomolgus monkeys displayed overt toxicity, including reduced food consumption, after just 12 days of oral exposure to 30 mg/kg/day PFOA. As a result, the exposure was reduced to 20 mg/kg/day on day 22 for the remainder of the 26-week study (Butenhoff et al., 2002). Male cynomolgus monkeys were used in another study that evaluated health effects including food consumption post exposure to 0, 2, or 20 mg/kg/day PFOA for 4 weeks. Low/no food consumption was observed in one male cynomolgus monkey from the 20 mg/kg/day exposure group (Thomford, 2001).

Endpoint	Study Name	Study Design	Observation Time	Animal Description
Feed Consumption, Absolute	Shi et al., 2020, 7161650	subchronic (5wk)	5wk	Mouse, C57BL/6J (♂, N=8)
	Butenhoff et al., 2004, 1291063	reproductive (64d)	1-106d	P0 Rat, Crl:CD(SD)IGS BR (♂, N=30)
		reproductive (GD0-PND120)	PND22-120	F1 Rat, Crl:CD(SD)IGS BR (♂, N=29-30)
		reproductive (GD1-PND106)	PND0-127	F1 Rat, Crl:CD(SD)IGS BR (♀, N=28-29)
	Perkins et al., 2004, 1291118	subchronic (13wk)	13wk	Rat, Sprague-Dawley Crl:Cd Br (♂, N=15)
Feed Consumption, Relative	Butenhoff et al., 2004, 1291063	reproductive (64d)	1-106d	P0 Rat, Crl:CD(SD)IGS BR (♂, N=30)

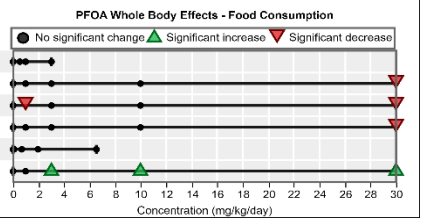


Figure C-25. Effects on Food Consumption in Rodents Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; P_0 = parental generation; F_1 = first generation; d = day; wk = week.

C.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse metabolic and systemic outcomes are discussed in Sections 3.3.2, 3.3.3, and 3.4.5 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 35 and 33 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 metabolic and systemic effects, respectively. A summary of these metabolic and systemic studies is shown in Figure C-26 and Figure C-27, respectively. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to metabolic and systemic effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	2	1	2	5
Cell Growth, Differentiation, Proliferation, Or Viability	4	0	13	16
Cell Signaling Or Signal Transduction	1	1	4	6
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	8	2	8	18
Hormone Function	3	5	3	11
Inflammation And Immune Response	2	0	1	3
Oxidative Stress	2	1	3	6
Xenobiotic Metabolism	0	0	4	4
Other	1	0	0	1
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	12	7	17	35

Figure C-26. Summary of Mechanistic Studies of PFOA and Metabolic Effects

Interactive figure and additional study details available on HAWC.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	0	0	4	4
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	8	9
Cell Signaling Or Signal Transduction	1	1	8	10
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	1	9	11
Hormone Function	0	0	1	1
Inflammation And Immune Response	0	0	3	3
Oxidative Stress	1	1	7	9
Xenobiotic Metabolism	0	1	2	3
Other	1	0	3	4
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	4	2	27	33

Figure C-27. Summary of Mechanistic Studies of PFOA and Systemic Effects

Interactive figure and additional study details available on HAWC.

C.3.4 Evidence Integration

There is *slight* evidence of an association between PFOA exposure and metabolic effects in humans based on observed effects for diabetes, gestational weight gain, leptin, and adiposity measures in *high* and *medium* confidence studies. However, there are generally imprecise and inconsistent findings across 72 epidemiological studies. Stronger evidence exists for diabetes and some adiposity measures relative to other metabolic outcomes.

The available human epidemiological evidence supports an association between PFOA and diabetes, including gestational diabetes. Five studies reported positive associations with gestational diabetes, and five studies reported positive associations with type-2 diabetes in the general population. There is evidence of a positive association with leptin in adults (fours studies) and in pregnant women (one study), while in children the findings are mixed (two studies). This suggests that age may be a factor in the association between PFOA and leptin.

Three epidemiological studies observed positive associations with gestational weight gain among pregnant women, with one association being significant. Four general population studies reported a positive association with waist circumference, and four studies of children reported non-significant positive associations with waist circumference, and one study reported inverse associations in children. There is evidence of an association between PFOA exposure and body fat and being overweight, particularly in adults, but findings are imprecise and inconsistent.

Findings for an association between PFOA exposure and metabolic syndrome were mixed in four general population epidemiological studies identified since 2016: two reported negative associations with metabolic syndrome, and two reported positive associations.

The animal evidence for an association between PFOA and systemic or metabolic effects is *indeterminate.* Although some alterations related to glucose homeostasis were reported in the five *high* or *medium* confidence studies available animal toxicity literature for metabolic effect, the results were often inconsistent when comparing between species, sexes, length of exposure, and life stages. In addition, the effects on body weight, clinical observations, and mortality from 21 *high* or *medium* confidence studies indicate that the systemic effects occur only at the high doses tested. In male rats, changes in serum glucose levels appear to be influenced by exposure duration, with short-term exposure resulting in decreased serum glucose levels and chronic exposure resulting in increased serum glucose levels. In mice, there was no evidence of altered glucose levels due to PFOA exposure and conflicting reports of changes in serum insulin levels in studies with similar exposure paradigms.

Evidence from animal studies suggests that exposure to PFOA of varying durations can elicit adverse whole-body effects, which primarily manifest as reductions in body weight that are not always explained by decreased food and/or water consumption or other clinical signs of toxicity. The effects are consistent across studies of varying exposures to PFOA, across species, and across sex. Reductions in body weight may serve as an early indicator of later PFOA toxicity because it can reflect poor health in the whole organism.

C.3.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause systemic and metabolic effects in humans under relevant exposure circumstances (Table C-6). This conclusion is based primarily on diabetes, gestational weight gain, leptin, and adiposity effects observed in *high* and *medium* confidence studies in humans exposed to median PFOA levels between 1.4 and 68 ng/mL. Although there is some evidence of negative effects of PFOA exposure on metabolic syndrome, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-6. Evidence Profile Table for PFOA Systemic and Metabolic 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 C.3.1)					⊕⊙⊙ ***Evidence Suggests***
Glucose metabolism 3 *High* confidence studies 15 *Medium* confidence studies 7 *Low* confidence studies	Significant increases in FBG (3/13), including one *high* confidence study. However, other studies reported contrasting findings, including significant decreases (1/13) in FBG levels after the OGTT, or imprecise results. Findings for FBG levels in children and pregnant women were inconsistent between studies across confidence levels.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Imprecision* of most findings • *Inconsistent direction* of effects • Potential for *selection bias* and residual confounding by SES	⊕⊙⊙ *Slight* Evidence for metabolic effects is based on increases in fasting blood glucose, increased risk of diabetes, and increases in adiposity in adults and pregnant women. Positive associations were reported for heightened glucose levels, effects on insulin regulation, diabetes, and adiposity, but many *medium* and *high* confidence studies presented non-statistically significant results, and several studies presented conflicting associations. Uncertainties remain due to mixed results, contrasting findings, and potential for residual confounding in the analysis of outcomes such as glucose metabolism, diabetes, and insulin levels.	*Primary basis*: Human evidence indicated effects on diabetes, gestational weight gain, leptin, and adiposity and there was limited animal evidence. Although there is some evidence of negative effects of PFOA exposure on metabolic syndrome, there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Diabetes (and gestational diabetes) 3 *High* confidence studies 18 *Medium* confidence studies 6 *Low* confidence studies	Findings in adults were mixed. Positive associations indicating increased risk were observed in several studies (4/11); however, significant negative associations indicating decreased risk were also	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent* direction of effect • *Imprecision* of findings • Potential for *outcome misclassification*, self-selection, residual confounding by SES, 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**	
	observed (3/11). Findings in adults (5/9) also showed reduced HbA1c, with a few reaching significance (3/9). In pregnant women, risk of diabetes or gestational diabetes was typically increased (6/10), reaching significance in one study. The only study examining diabetes in children was considered *uninformative.*		concerns about temporality		
Insulin levels 1 *High* confidence study 8 *Medium* confidence studies 7 *Low* confidence studies	In adults, studies reported HOMA-IR was significantly increased (2/10), but findings from other studies (6/10) indicated non-significant decreases. HOMA-B was also reported to be significantly increased (2/3). Findings for fasting insulin were mixed, but significant increases were observed (2/9). In pregnant women, findings indicated decreased HOMA-IR (2/3), including one significant study. In children, findings for HOMA-IR were primarily inverse (3/5), but findings were generally imprecise for fasting insulin levels.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effects • *Imprecision* of findings • Potential for *residual confounding* by diabetes status or use of medications that would impact insulin levels in some 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**	
Adiponectin and leptin 5 *High* confidence studies 3 *Medium* confidence studies 3 *Low* confidence studies	In adults, one study observed significant increased leptin (1/1) while two studies (2/2) reported decreased adiponectin, with one reaching significance. Findings in children were mixed for leptin, including one study reporting significant decreased leptin (1/3), but non-significant positive associations were observed for adiponectin. Findings in pregnant women were mixed or imprecise.	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effects • *Imprecision* of findings		
Adiposity 8 *High* confidence studies 23 *Medium* confidence studies 6 *Low* confidence studies	In adults, findings for BMI were largely positive (4/9), with two studies reporting significant increases in BMI or risk of being overweight/obese. WC was reported to be significantly increased in one study, but findings from other studies in adults were imprecise. In children, results were mixed with two studies (2/17) reporting significant positive associations with measures of BMI and two studies (2/17) reporting significant inverse associations with measures of BMI. Other	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effects • *Imprecision* of findings • Potential for residual *confounding* by SES, study sensitivity issues due to some small sample sizes		

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**	
	anthropometric measures were also mixed, however, two studies reported significant decreases in WC and waist-to-height ratio.				
Metabolic syndrome 4 *Medium* confidence studies 1 *Low* confidence study	Findings for metabolic syndrome in adults were mixed, however, two studies (2/5) observed positive associations of increased risk of MetS with large effect sizes.	• *Medium* confidence studies	• *Low* confidence study • *Imprecision* of findings • Potential for *selection bias*, outcome misclassification, and residual confounding by SES		
Evidence From In Vivo Animal Studies (Section C.3.2)					
Glucose homeostasis 2 *High* confidence studies 3 *Medium* confidence studies	Most studies reported no significant effects on glucose levels (4/5) or glucose tolerance (1/1) in rodents, however, one 28-day study in rats reported a dose-dependent decrease in glucose levels in males. One developmental mouse study observed no significant changes in insulin levels for either sex (1/1).	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Inconsistent direction* of effects across exposure durations, sex, and species • *Limited number* of studies examining outcomes	⊙⊙⊙ *Indeterminate* Alterations related to glucose homeostasis were reported in 5 *high* or *medium* confidence studies were inconclusive as there are too few studies to assess possible difference across life stages, sexes, and species and results from the existing studies are inconsistent or transient. Systemic effects (e.g., body weight, clinical observations, survival, food consumption, and water consumption) from 20 *high* or *medium* confidence studies indicate	

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**	
Body weight 5 *High* confidence studies 16 *Medium* confidence studies	Reduction in absolute body weights (19/21), body weight change (6/7), and body weight as a percentage of control (4/4) were reported following short-term, subchronic, and chronic exposure in rats and mice. In rats, body weight in males appeared to be more sensitive to the effects of PFOA.	• *High* and *medium* confidence studies • *Consistent direction* of effects	• *Confounding variables* such as decreases in food consumption	that biologically significant effects (e.g., body weight change exceeding 10% of control) tend to occur only at the highest doses tested.	
Body mass composition 1 *Medium* confidence study	One developmental mouse study reported significantly increased fluid mass, fat mass, and percent fluid mass in males only (1/1).	• *Medium* confidence study	• *Limited number* of studies examining specific outcome		
Survival and mortality 3 *High* confidence studies 3 *Medium* confidence studies	Increased survival was observed in male rats only following PFOA exposure (2/6). No significant effects on mortality were observed for females (3/3).	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent direction* of effect across studies and sex		
Clinical observations 2 *High* confidence studies 2 *Medium* confidence studies	Clinical observations were observed in rodent studies (2/4). Observations included dehydration, urine-stained abdominal fur, and/or ungroomed fur in male rats. Ataxia in females was reported in a mouse study.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcomes • *Qualitative* and *subjective* data reporting		
Food and water consumption	No significant exposure-related effect on food consumption (4/5) nor	• *High* 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**	
2 *High* confidence studies 4 *Medium* confidence studies	water consumption (2/2) was observed in rodents for either sex.	• *Consistent direction* of effects across studies			

Notes: FBG = fasting blood glucose; OGTT = oral glucose tolerance testing; SES = social economic status; HbA1c = hemoglobin A1c; HOMA-IR = homeostatic model assessment for insulin resistance; HOMA-B = homeostasis model assessment of β-cell function; BMI = body mass index; WC = waist circumference; MetS = metabolic syndrome.

C.4 Nervous

EPA identified 38 epidemiological and 11 animal studies that investigated the association between PFOA and nervous effects. Of the epidemiological studies, 3 were classified as *high* confidence, 30 as *medium* confidence, and 5 were considered *low* confidence (Section C.4.1). Of the animal studies, 3 were classified as *high* confidence, 6 as *medium* confidence, and 2 were considered *low* confidence (Section C.4.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.4.1 Human Evidence Study Quality Evaluation and Synthesis

C.4.1.1 Introduction

The 2016 Health Assessment (U.S. EPA, 2016c) reported mixed results from the literature reviewed and emphasized 2012 C8 Science Panel (2012) conclusions, which reported no probable link between PFOA exposure and neurodevelopmental disorders in children, including attention deficit hyperactivity disorder (ADHD) and learning disabilities. Among the studies reviewed for the 2016 Health Assessment, evidence of a significant positive association for child PFOA levels and parent reported ADHD was observed in children aged 12–15 in the general population (Hoffman et al., 2010), and a positive association with ADHD-like behaviors and decreased executive function in children in a highly exposed community (Stein et al., 2014). The relationship between PFOA exposure and ADHD-related behavior was also observed in a single country from the INUENDO cohort, showing a significant increase in hyperactivity among children ages 7 to 9 with elevated PFOA exposure (Høyer et al., 2015). A significant increase in risk of development of cerebral palsy in males associated with maternal PFOA was observed in a case-control study of maternal PFOA levels of participants within the DNBC (Liew et al., 2014). Studies on outcomes such as Apgar score, fine motor skills, gross motor skills, cognitive skills, behavioral problems, and coordination problems did not find significant evidence for an effect of PFOA exposure (Fei and Olsen, 2011; Fei et al., 2008a). Data interpretations within these studies were limited in some cases by use of a cross-sectional analysis (Stein et al., 2014; Hoffman et al., 2010; Fei et al., 2008a), potential random misclassification error resulting from using current PFOA levels as proxy measures of etiologically relevant exposures (Stein et al., 2014; Hoffman et al., 2010), outcomes defined by parental report (Høyer et al., 2015; Fei and Olsen, 2011; Hoffman et al., 2010; Fei et al., 2008a) or parent and teacher report (Stein et al., 2014), and limited sample sizes in some sub-analyses (Høyer et al., 2015).

For this updated review, 36 studies (38 publications)[10] investigated the association between PFOA and neurological outcomes. Two were conducted in high-exposure communities (Spratlen et al., 2020a; Stein et al., 2013). One publication (Vuong et al., 2020b) was conducted in pregnant women. The remainder were conducted on the general population. Study designs included three case-control (Shin et al., 2020; Long et al., 2019; Ode et al., 2014), 2 nested case-control (Lyall et al., 2018; Liew et al., 2015), 26 cohort (Appendix D). The studies measured PFOA in different matrices, including blood, cord blood, breast milk (Lenters et al., 2019; Forns

[10] Vuong et al. (2018b) reports score trajectories for the same population and test as Vuong et al. (2016). Vuong et al. (2020a) reports on an overlapping population with the same test as Zhang et al. (2018a).

et al., 2015), maternal serum, amniotic fluid (Long et al., 2019), and maternal plasma. Eight studies (Vuong et al., 2020b; Vuong et al., 2020a; Vuong et al., 2019; Vuong et al., 2018b; Vuong et al., 2018a; Zhang et al., 2018a; Vuong et al., 2016; Braun et al., 2014) were conducted on subsets of data from the HOME study. Two studies (Lenters et al., 2019; Forns et al., 2015) utilized data from the Norwegian Human Milk Study (HUMIS). Two studies (Liew et al., 2018; Liew et al., 2015) utilized the DNBC data. The studies were conducted in multiple locations including populations from China, Denmark, the Faroe Islands, Great Britain, Japan, the Netherlands, Norway, Sweden, Taiwan, and the United States (Appendix D). Neurological effects examined in these studies included clinical conditions such as ADHD, autism spectrum disorder (ASD), multiple sclerosis (MS), and hearing loss. Neurological function was also assessed by performance on numerous neuropsychological tests evaluating neurological domains, including development, general intelligence (i.e., intelligence quotient (IQ)), social-emotional, executive function, ADHD and attention, ASD and intellectual disability (ID), memory, and visuospatial performance.

C.4.1.2 Study Quality

There are 38 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 nervous effects. Study quality evaluations for these 38 studies are shown in Figure C-28 and Figure C-29.

Of the 38 studies identified since the 2016 assessment, three studies (Niu et al., 2019; Harris et al., 2018; Oulhote et al., 2016) were classified as having *high* confidence, 30 studies as *medium* confidence, and five as *low* confidence. Studies rated as *low* confidence had deficiencies including potential residual confounding, exposure misclassification, selection bias, and small sample size. One *low* confidence NHANES study (Berk et al., 2014) had a high likelihood of residual confounding due to the use of an insensitive marker of SES, and the analysis did not account for the population's complex sampling design. Differences in laboratory extraction methods, collection timing, and missing details on storage raised concerns for exposure misclassification in a study on children from the HUMIS cohort (Forns et al., 2015). Additionally, children were only evaluated on some, but not all, test instrument (Ages and Stages Questionnaire (ASQ)) domains, and rationale for domain selection was not provided. Concerns for Lien et al. (2016) included a high loss to follow-up, lack of detail on completion rates of ADHD questionnaires and low detection rate for PFOA. Small sample size, temporality and reporting concerns were cited as limitations in Weng et al. (2020). Finally, limitations in Ode et al. (2014) included sensitivity concerns due to the limited number of ADHD cases and potential for residual confounding due to the lack of data on other exposures potentially related to ADHD. In the evidence synthesis below, *high* and *medium* confidence studies were the focus, although *low* confidence studies were still considered for consistency in the direction of association.

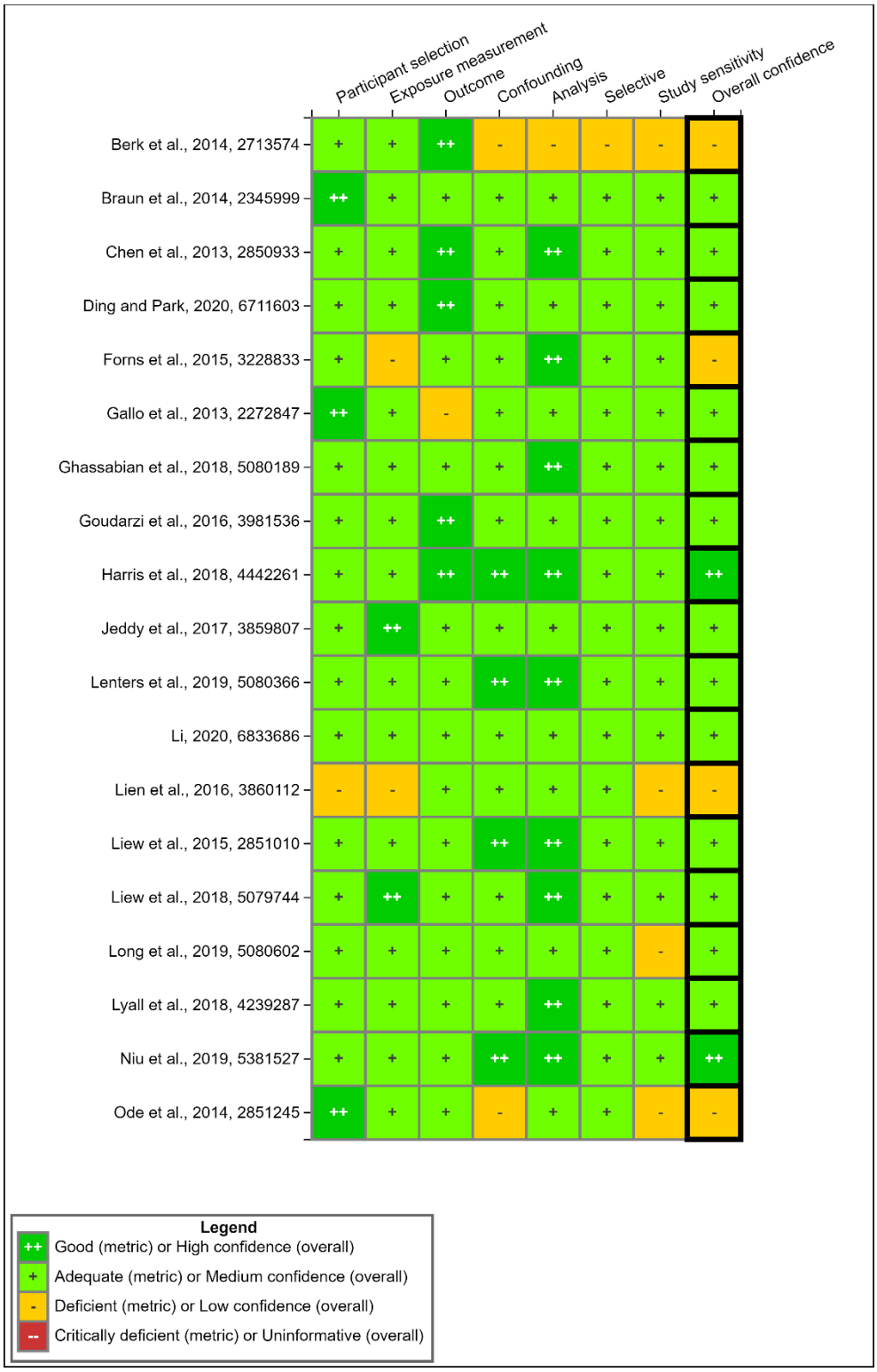


Figure C-28. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Neurological Effects

Interactive figure and additional study details available on HAWC.

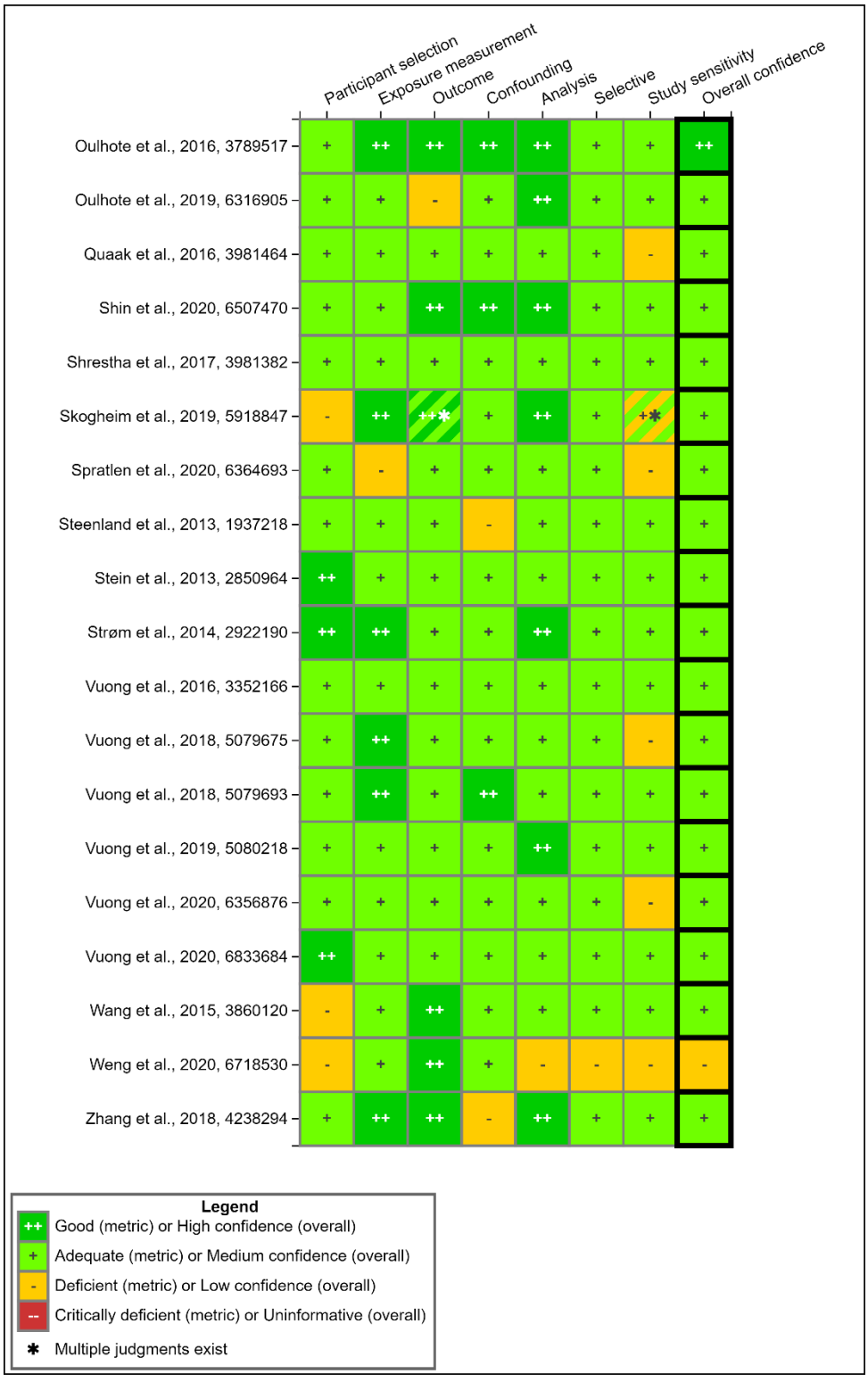


Figure C-29. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Neurological Effects (Continued)

Interactive figure and additional study details available on HAWC.

C.4.1.3 Findings From Children and Adolescents

Six cohort studies (Niu et al., 2019; Jeddy et al., 2017; Shrestha et al., 2017; Goudarzi et al., 2016b; Forns et al., 2015; Chen et al., 2013) and two high-exposure community cohort studies (Spratlen et al., 2020a; Stein et al., 2013) examined developmental outcomes in children. In a *high* confidence study (Niu et al., 2019) from the Shanghai-Minhang Birth Cohort Study (S-MBCS), maternal PFOA concentrations (median = 19.9 ng/mL) during pregnancy were consistently associated with increased risk of problems with personal-social skills in 4-year-old girls (but not in boys), as assessed by the ASQ. In boys, significant decreases in risk for problems with gross motor development were observed, and the risk of problems with problem solving skills were non-significantly elevated. Results from a *medium* confidence study (Goudarzi et al., 2016b) reported prenatal PFOA (median = 1.2 ng/mL) concentrations were associated with statistically significantly lower Mental Development Index (MDI) scores for female (but not male) infants at 6 months of age. In contrast, no apparent trends with neurodevelopmental indices from the Bayley Scales of Infant Development (BSID-II) at 1 year of age were reported in a high-exposure community study of children prenatally exposed to the WTC Disaster (Spratlen et al., 2020a). Adverse associations at 2 and 3 years were not observed, however, a significant positive association was reported for the MDI at 3 years (Spratlen et al., 2020a). A *medium* confidence study (Jeddy et al., 2017) using data from the ALSPAC observed inconsistent patterns of association between prenatal PFOA concentrations (median = 3.7 ng/mL) and neurodevelopmental indices in 15-month-olds as assessed by an adapted version of the MacArthur Communicative Development Inventories for Infants (MCDI). An inverse association was reported for intelligibility scores among 38-month-olds, but there were no associations with maternal PFOA for language or communicative scores in 38-month-olds. Results varied by maternal age at delivery, as a statistically significantly inverse association was observed for vocabulary comprehension and production scores in 15-month infants with mothers younger than 25 years of age, and a significant inverse association for intelligibility scores in children 38 months of age with mothers older than 30 years of age (Jeddy et al., 2017). Results did not suggest an adverse association between estimated or measured PFOA exposures and performance on neuropsychological tests (NEPSY-II) in a high-exposure community study of children participating in the C8 Health Project (Stein et al., 2013). In one *low* confidence study, which assessed perinatal PFOA breast milk exposures (median = 40 ng/mL) and child neuropsychological development at 6, 12 and 24 months of mother-child pairs in the HUMIS (Forns et al., 2015), no association was reported between perinatal PFOA exposures and early neuropsychological development.

Eleven studies evaluated cognitive function and IQ measures among children, with most conducted within the general population (Vuong et al., 2020a; Oulhote et al., 2019; Skogheim et al., 2019; Vuong et al., 2019; Harris et al., 2018; Liew et al., 2018; Lyall et al., 2018; Zhang et al., 2018a; Wang et al., 2015; Strøm et al., 2014) and two within high-exposure communities (Spratlen et al., 2020a; Stein et al., 2013). In a *medium* confidence study (Stein et al., 2013) of children from the C8 Health Project, girls aged 6 to 12 years with measured childhood PFOA (median = 35.0 ng/mL) exposure above the median had a 4.1 score decrease in the Wechsler Individual Achievement Test-II (WIAT-II) Numerical Operations scaled score as compared with girls below the median. A significant 4.9 score increase was observed among boys for the same measure. Overall, children in the highest versus the lowest quartile of estimated in utero PFOA (110.8–649.2 ng/mL vs. 4.5– <11.7 ng/mL) had significant increases in full-scale IQ. Across all

administered tests, no consistent adverse associations between measured childhood PFOA (median = 35.0 ng/mL) and cognitive function (Stein et al., 2013) were observed. Positive associations between prenatal PFOA (median = 5.2 ng/mL) and reading skills were reported in a *medium* confidence study in children aged 8 years utilizing data from the HOME study (Vuong et al., 2020a). Childhood serum PFOA concentrations at ages three and eight were statistically significantly associated with higher children's reading scores at ages 5 and 8 years, respectively in a *medium* confidence prospective study of data within the HOME study (Zhang et al., 2018a). No significant associations between prenatal PFOA and offspring scholastic achievement were reported in a *medium* confidence prebirth cohort study of children (up to age 20) participants within the Danish Fetal Origins Cohort (Strøm et al., 2014). Maternal prenatal PFOA (median = 3.3 ng/mL) concentrations were statistically significantly associated with lower cognitive function as assessed by the Boston Naming Test with cues in a *medium* confidence study of children aged 7 years (Oulhote et al., 2019).

Skogheim et al. (2019) examined cognitive dysfunction in preschool children from the Norwegian Mother, Father, and Child Cohort Study (MoBa) and evidence was inconsistent. Significant decreases in nonverbal working memory were observed only in the highest quintile and significant increases in verbal working memory only in the third quintile od PFOA prenatal exposure (median = 2.5 ng/mL) (Skogheim et al., 2019). No adverse associations between prenatal (geometric mean = 5.2 ng/mL) and childhood (geometric mean = 2.4 ng/mL) PFOA and cognitive function at 8 years were reported, and a statistically significant increase of 4.1 points in working memory associated with an increase in prenatal PFOA was reported in a *medium* confidence study utilizing data from the HOME study (Vuong et al., 2019). Child sex modified the positive association (Vuong et al., 2019), with higher full-scale IQ in female children, and no association in male children. In another *medium* confidence study in a highly exposed community study, statistically significant sex-specific trends between exposures and some cognitive outcomes (verbal and full-scale IQ) at four and 6 years were observed, suggesting stronger positive associations for females compared with males (Spratlen et al., 2020a). No consistent associations between prenatal PFOA and child IQ at 5 years of age were reported in a *medium* confidence study of children from the DNBC (Liew et al., 2018). Data from a *medium* confidence study (Wang et al., 2015) on the Taiwan Maternal and Infant Cohort Study showed no consistent associations between maternal serum PFOA (median = 2.5 ng/mL) and IQ measurements in children 5 or 8 years of age.

Six studies examined the potential relationship between PFOA and social-emotional and behavioral regulation problems (Weng et al., 2020; Oulhote et al., 2019; Ghassabian et al., 2018; Vuong et al., 2018a; Oulhote et al., 2016; Quaak et al., 2016). The relationship between prenatal PFOA (median = 870.0 ng/L) exposures and behavioral development at age 18 months using the Child Behavior Checklist 1.5–5 (CBCL 1.5–5) was explored in a *high* confidence study utilizing data from the Dutch cohort Linking Maternal Nutrition to Child Health (LINC) (Quaak et al., 2016). Results indicated prenatal exposure to PFOA was statistically significantly negatively associated with externalizing behavior problems in boys, indicating less problems. Statistically significant associations between serum PFOA (median = 4.1 µg/L) in children aged 5 years and total Strengths and Difficulties Questionnaire (SDQ) behavioral survey scores assessed at age seven were reported in a *high* confidence study (Oulhote et al., 2016). Maternal prenatal PFOA concentrations (median = 3.3 ng/mL) were positively associated with total SDQ scores, indicating more behavioral problems, in a *medium* confidence study of children 7 years of age

(Oulhote et al., 2019). Higher newborn PFOA levels (median = 1.1 ng/mL) in dried blood spots were associated with difficulties in prosocial behavior, but not total behavioral difficulties, as assessed by the maternal completed SDQ at age 7 in another *medium* confidence study (Ghassabian et al., 2018). Evidence was mixed and insufficient to support an overall association between prenatal PFOA (median = 5.2 ng/mL) and inattention, impulsivity as assessed by the Connors' Continuous Performance Test-II (CCPT-II) in a *medium* confidence study (Vuong et al., 2018a). A *low* confidence study on adolescents reported no significant correlations between prenatal PFOA levels (mean = 2.9 ng/mL) and brain activity in regions associated with impulsive behavior as assessed by MRI imaging in teenage offspring (Weng et al., 2020).

One *medium* confidence study (Strøm et al., 2014) from the Danish Fetal Origins Cohort examined the association between prenatal PFOA exposure and depression among offspring with 20 years of follow-up. No significant association was observed between clinical depression and maternal PFOA (3.8 ng/mL) levels.

Two *medium* confidence studies (Vuong et al., 2018b; Vuong et al., 2016) examined the relationship between PFOA concentrations and executive function in children with mixed results. Executive function was assessed with the parent-rated Behavior Rating Inventory of Executive Function (BRIEF) in both studies (Vuong et al., 2018b; Vuong et al., 2016) among HOME study participants at 5 and 8 years of age. Higher BRIEF scores indicate executive function impairments. No associations were observed between prenatal PFOA levels and executive function (Vuong et al., 2016). In analyses using childhood (8 years old) serum PFOA levels (Vuong et al., 2018b), results indicated higher PFOA levels were significantly associated with increased odds of being at risk of having clinical impairments – specifically for the metacognition index at age eight.

Six *medium* confidence studies among the general population (Lenters et al., 2019; Skogheim et al., 2019; Quaak et al., 2016; Liew et al., 2015; Strøm et al., 2014), and one in a high-exposure community (Stein et al., 2013), examined ADHD and measures of attention in children. A *medium* confidence study of participants in the C8 Health Study observed consistently lower Clinical Confidence Index scores, indicating less probability of ADHD, on the CCPT-II in children (mean age = 9.9 years) associated with increased estimated in utero PFOA levels (median = 43.7 ng/mL) and increased measured childhood PFOA (median = 35.0 ng/mL) (Stein et al., 2013). Strøm et al. (2014) investigated the association between prenatal PFOA exposure and ADHD among offspring (follow-up to age 20) of participants within the Danish Fetal Origins Cohort. No association between prenatal PFOA and offspring ADHD was reported in this *medium* confidence study. A *medium* confidence nested case-control study (Liew et al., 2015) within the framework of the DNBC examined prenatal PFOA exposures (case median = 4.1 ng/mL; control median = 4.0 ng/mL) and ADHD in children. No consistent evidence was observed to suggest that prenatal PFOA exposures increase the risk of ADHD. Quaak et al. (2016) explored the relationship between prenatal PFOA exposures and parent reported ADHD using the CBCL 1.5–5. This *medium* confidence study utilized data from the Dutch cohort LINC. No significant associations were observed between prenatal PFOA exposures and ADHD scores in the whole population as well as within the sex-stratified analyses. One *medium* confidence study (Lenters et al., 2019) examined early-life high PFOA exposures in breast milk in relation to ADHD among children (range: 7.2–14.1 years old) from

the HUMIS and observed positive non-significant associations with odds of ADHD (OR: 1.35, 95% CI: 0.87, 2.11), but not consistently in various models.

Two *low* confidence studies (Lien et al., 2016; Ode et al., 2014) examined ADHD and ADHD-related measures, but no significant associations were observed. Lien et al. (2016) evaluated the association between cord blood PFOA (mean = 1.6 ng/mL) exposures and neurobehavioral symptoms related to ADHD among 7-year-old participants from the Taiwan Birth Panel Study and the Taiwan Early-Life Cohort. No significant associations or trends were observed; however, the direction of association was primarily negative. Ode et al. (2014) investigated the association in a case-control study between cord blood PFOA (median = 1.8 ng/mL for cases; 1.83 ng/mL for controls) exposures and ADHD diagnosis in childhood (age range 5–17 years), but no consistent pattern was observed. Deficiencies identified in these studies included the reliability of exposure measures, limited study sensitivity, and potential for residual confounding.

Six *medium* confidence studies evaluated PFOA exposures in relation to autism, autistic behaviors, and ID (Shin et al., 2020; Long et al., 2019; Lyall et al., 2018; Oulhote et al., 2016; Liew et al., 2015; Braun et al., 2014). A twofold increase in serum PFOA (median = 4.06 μg/L) at age five was associated with significantly higher SDQ autism screening scores at age seven in a *high* confidence study (Oulhote et al., 2016). In a *medium* confidence study from the HOME study, increasing maternal serum PFOA concentrations (median = 5.5 μg/L) were non-significantly associated with fewer autistic behaviors in children 4 to 5 years of age as assessed by maternal completed Social Responsiveness Scale (SRS) scores (Braun et al., 2014). No consistent evidence of an association between maternal plasma PFOA (median = 3.9 ng/mL for cases; 4.0 ng/mL for controls) and diagnosed childhood autism was reported in a *medium* confidence study of mother-child pairs with an average of 10 years of follow-up within the DNBC (Liew et al., 2015). No association was observed in a *medium* confidence case-control study of amniotic fluid PFOA (median = 0.3 ng/mL for cases; 0.3 ng/mL for controls) and diagnosed ASD, with cases identified as born 1982–1999 within the Danish Psychiatric Central Registry (Long et al., 2019). Prenatal maternal serum PFOA (geometric mean = 3.6 ng/mL for ASD cases; 3.3 ng/mL for ID cases; 3.7 ng/mL for controls) was inversely associated with diagnosed ASD and ID in a *medium* confidence study of children aged 4.5–9 years (Lyall et al., 2018). No significant association was observed in a *medium* confidence study of modeled prenatal maternal PFOA (median = 1.1 ng/mL for ASD cases; 1.2 ng/mL for controls) and clinically confirmed ASD among children (age 2–5 years) in the Childhood Autism Risk from Genetics and Environment (CHARGE) study (Shin et al., 2020).

The effects on visuospatial performance were evaluated in one *high* confidence study (Harris et al., 2018) which observed associations, and one *medium* confidence study (Vuong et al., 2018a) which observed no associations. In participants from Project Viva (Harris et al., 2018) observed that children scored consistently lower on visual-motor tests (Wide Range Assessment of Visual Motor Abilities) with increasing prenatal PFOA exposure. No clear patterns were observed using early childhood (median age = 3.2 years) test performance, but significant inverse associations for mid-childhood (median age = 7.7 years) test performance were observed for the second (4.1–5.6 ng/mL) and fourth (>7.7 ng/mL) quartiles of prenatal PFOA exposure. Participants from the HOME study were assessed using the Virtual Morris Water Maze (VMWM), but no significant effects were observed (Vuong et al., 2018a).

C.4.1.4 Findings From the General Adult Population

The effects of PFOA on general intelligence and IQ test outcomes were examined in a *medium* confidence study (Shrestha et al., 2017) of adults (ages 55–74 years) in New York State. Findings indicated a significant association between serum PFOA and performance on tests for memory and learning corresponding to a 6% higher (better memory and learning) mean score.

Findings of a *medium* confidence study (Shrestha et al., 2017), described above, indicated higher serum PFOA in adults was associated with significantly better performance executive function measured by the Wisconsin Card Sorting Test (WCST).

Two studies (Shrestha et al., 2017; Berk et al., 2014) examined the effects of PFOA exposure on depression among adults. No associations were reported in a *medium* confidence study of depression, assessed by the Beck Depression Inventory (BDI), and serum PFOA (median = 8.1 ng/mL) in a cross-sectional study of adults aged 55 to74 years (Shrestha et al., 2017). One *low* confidence NHANES study (Berk et al., 2014) observed a lower prevalence of depression with increasing PFOA exposure as assessed by the nine-item depression module of the Patient Health Questionnaire (PHQ-9).

Only one *medium* confidence study (Vuong et al., 2020b) examined social-emotional effects in pregnant women. No evidence was reported to support an adverse relationship between serum PFOA during pregnancy and maternal depressive symptoms assessed by the Beck Depression Inventory-II (BDI-II) from pregnancy to 8 years postpartum.

Two *medium* confidence studies explored the relationship between PFOA and memory impairment (Shrestha et al., 2017; Gallo et al., 2013) and observed mixed effects. Gallo et al. (2013) observed statistically significant inverse associations with memory impairment in adults from the C8 Health Project. However, no adverse effects of PFOA on memory impairment were observed in adults (ages 55–74 years) in New York State (Shrestha et al., 2017).

Two *medium* confidence cross-sectional studies investigated PFOA and hearing impairment in analyses of adult NHANES participants and observed mixed effects. Li (2020) reported significant positive associations between PFOA and hearing impairment, while Ding and Park (2020) reported no significant associations.

C.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and nervous effects. Study quality evaluations for these 11 studies are shown in Figure C-30.

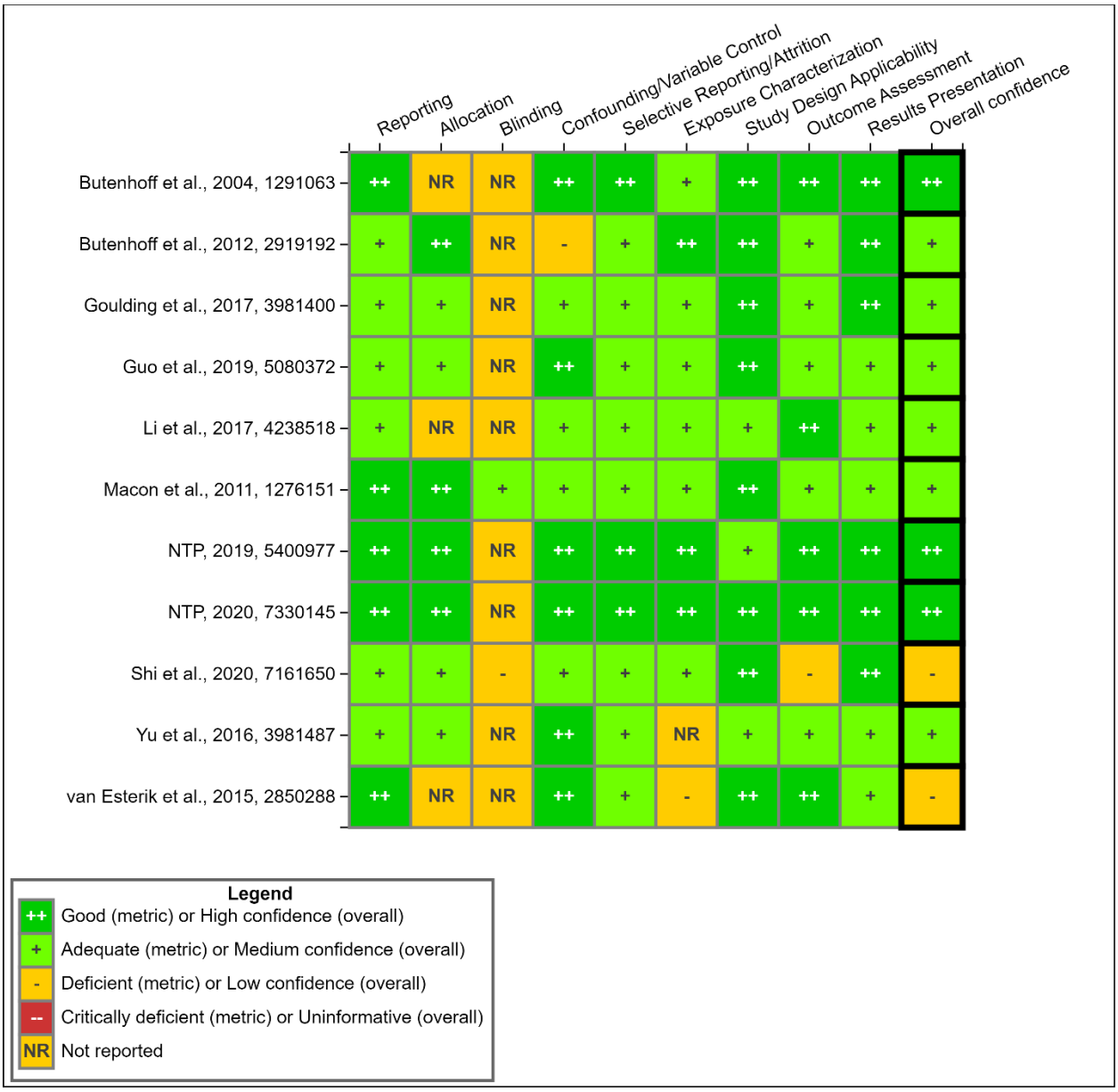


Figure C-30 Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Nervous Effects

Interactive figure and additional study details available on HAWC.

There are few studies available that evaluate neurotoxicity, including neurodevelopmental toxicity, with short-term, chronic, or gestational exposure to PFOA in experimental models. From the available literature, there is little evidence of morphological changes or damage that can be attributed to PFOA exposure. However, there is some evidence suggesting that PFOA exposure may be associated with behavioral and physiological effects, areas of research that may warrant further analysis. Additionally, several single-dose studies indicate that neurodevelopmental endpoints may be sensitive indicators of PFOA toxicity.

Absolute and/or relative brain weights, as well as brain histopathology, were reported in studies using mice, rats, and monkeys; these studies generally reported null or inconsistent results across dose groups, generations, sexes, or studies (Yu et al., 2016; Butenhoff et al., 2012; Macon et al., 2011; Yahia et al., 2010; Butenhoff et al., 2004a; Perkins et al., 2004; Goldenthal et al., 1978). Statistically significant changes in brain weight were often not consistent across sexes or generations, were transient, were not dose-dependent, or occurred at relatively high doses compared with other health outcomes. For example, in a 2-year rat feeding study, Butenhoff et al. (2012) observed significantly increased absolute brain weights in males from the low-dose group (1.3 mg/kg/day) but not the high-dose group (14.2 mg/kg/day) or either female treatment groups. In a rat reproductive study, Butenhoff et al. (2004a) observed no change in absolute brain weight in P_0 males or females and no change in females from the F_1 generation but reported a significant decrease in absolute brain weight in the high-dose F_1 males (30 mg/kg/day) at PND 120. Similarly, Macon et al. (2011) reported a transient significant decrease in absolute brain weight in F_1 male mice exposed to 1 and 3 mg/kg/day during gestation at PND 63 (time points measured ranged from PND 7–84). There were no differences in absolute brain weight in females or in relative brain weight in either sex. However, sample sizes in control females were too low on PNDs 63 and 84 to conduct statistical analysis. Dam mice in the highest dose group reported by Yahia et al. (2010) in a gestational study (10 mg/kg/day) had significantly decreased absolute brain weight (approximately 7% decrease) and no statistical difference in relative brain weight. A 28-day study in male mice with doses up to 2.5 mg/kg/day (Yu et al., 2016) and a 13-week study with interim sacrifices at 4 and 7 weeks in male mice with doses up to 6.5 mg/kg/day (Perkins et al., 2004) also found no evidence of altered absolute or relative brain weights after PFOA exposure. One monkey study with a limited sample size (n = 2/sex/group) reported decreased absolute brain weight in females dosed with 10 mg/kg/day PFOA for 90 days (highest dose tested that did not induce mortality) (Goldenthal et al., 1978). There were no significant effects on brain weight in males from the same study. Despite several noted changes in brain weight, there were no reports of altered brain histopathology due to PFOA exposure in the available literature (NTP, 2020, 2019a; Li et al., 2017a; Butenhoff et al., 2012; Yahia et al., 2010; Butenhoff et al., 2004a). In a subchronic study in male C57BL/6J mice, Shi et al. (2020) observed increased neuronal apoptosis and cell shrinkage, though no quantitative data were provided.

Goulding et al. (2017) assessed behavioral effects in F_1 male offspring gestationally exposed to 0, 0.1, 0.3, or 1 mg/kg/day PFOA from GD 1–17. The authors conducted different behavioral assays across multiple periods of development through adulthood (~3 weeks–6 months of age). Significant effects were only observed in the highest dose group (1 mg/kg/day). Ambulatory activity in an open-field chamber, reported as the number of photocell breaks, was measured on PND 18–20. There was a significant increase in the number of photocell breaks in the 1 mg/kg/day dose group on PND 18, however, this response was not observed on PND 19 or PND 20. On PND 60, Goulding et al. (2017) reported no significant effects due to PFOA exposures in the auditory startle response, habituation, prepulse startle inhibition, and running wheel tests. The running wheel assay was repeated at PND 72 with similar results. On PND 168, mice were monitored for ambulatory activity following an acute injection of methamphetamine; the authors reported a significantly decreased number of photocell breaks in the 1 mg/kg/day group compared with controls. A few studies report clinical signs of toxicity that exhibit neurotoxicity including ataxia in potentially moribund animals (Butenhoff et al., 2012; Goldenthal et al., 1978).

Yu et al. (2016) analyzed tissue concentrations of four neurotransmitters in the brains of male mice exposed to 0, 0.5, or 2.5 mg/kg/day PFOA for 28 days. Concentrations of dopamine, serotonin, and norepinephrine were significantly altered in the 0.5 mg/kg/day dose group compared with controls but not the high-dose group; dopamine and serotonin were both increased while norepinephrine was decreased. Glutamate concentrations in the 2.5 mg/kg/day dose group were significantly decreased compared with controls. Guo et al. (2019) also reported a significant reduction in glutamate concentrations in male mice exposed to 10 mg/kg/day PFOA, but not to 0.4 or 2 mg/kg/day, for 28 days.

Several studies reported on additional behavioral and neurochemical effects. Onishchenko et al. (2011) and Sobolewski et al. (2014) observed behavioral effects including altered locomotor activity, exploratory behavior, circadian activity, and motor coordination in mouse offspring following gestational or perinatal exposure to single-dose levels of PFOA (0.3 and 0.1 mg/kg/day in the respective studies). Cheng et al. (2013) administered 10 ppm PFOA to pregnant rats from GD 1–PND 21 and similarly observed altered motor coordination and locomotor activity in male and female offspring. This study did not report drinking water consumption or body weights of the dams. Johansson et al. (2009; 2008) also observed behavioral (spontaneous behavior and locomotion) and neurochemical effects (altered cholinergic system responses and brain enzyme and protein levels) in adult mouse offspring after a single PFOA dose of either 0.58 or 8.7 mg/kg on PND 10.

C.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse nervous outcomes is discussed in Sections 3.2.4 and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 21 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 nervous effects. A summary of these studies is shown in Figure C-31. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to nervous effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	3	0	0	3
Cell Growth, Differentiation, Proliferation, Or Viability	2	0	5	6
Cell Signaling Or Signal Transduction	4	0	5	9
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	0	1	3
Hormone Function	1	0	2	3
Inflammation And Immune Response	0	1	0	1
Oxidative Stress	0	0	4	4
Xenobiotic Metabolism	0	0	1	1
Other	1	0	0	1
Not Applicable/Not Specified/Review Article	4	0	1	4
Grand Total	12	1	10	21

Figure C-31. Summary of Mechanistic Studies of PFOA and Nervous Effects

Interactive figure and additional study details available on HAWC.

C.4.4 Evidence Integration

There is *slight* evidence on an association between PFOA exposure and nervous effects in humans. The epidemiological studies reviewed since the 2016 Health Assessment provide mostly mixed results on the associations between PFOA and neurological outcomes. There were no new neurological studies identified that evaluated cerebral palsy. Outcomes investigated include those of depression, memory impairment, hearing impairment, ASD, and ID.

The recent epidemiological studies provide limited indication of adverse effects of PFOA on neurodevelopment, neuropsychological development (Niu et al., 2019; Goudarzi et al., 2016b), cognitive development (Oulhote et al., 2019; Harris et al., 2018), and executive function (Vuong et al., 2018b). Results for IQ were largely non-significant and inconsistent. There was no evidence of an association with depression; only two studies observed effects of PFOA on hearing (Li, 2020) and memory impairment (Gallo et al., 2013). Overall, results for neurodevelopmental, neuropsychological, cognitive, and executive function outcomes were somewhat mixed and limited in number of studies.

The recent epidemiological studies also provide limited indication of adverse effects of PFOA on behavioral problems, ADHD, ASD, and ID. There was suggestive evidence of an association between PFOA exposure and behavioral problems associated (Oulhote et al., 2019; Ghassabian et al., 2018; Oulhote et al., 2016); however, overall results were mixed. Of the multiple studies examining associations between PFOA and ADHD, only one (Lenters et al., 2019) observed associations with PFOA in a high-exposed population. No adverse associations of ID with PFOA were observed. Oulhote et al. (2016) observed a twofold increase in serum PFOA at age five was associated with significantly higher SDQ autism screening scores at age seven, but no associations between PFOA and autism screening scores were observed in other studies. However, many studies have methodological concerns, as PFOA exposures in cases and controls within the ADHD and ASD studies were often either similar to or had mean control exposures

greater than cases in many studies. A single category outcome for ASD may also not adequately encompass the heterogeneity in terms of developmental history, intelligence, comorbidity, and severity that might be important in accurately revealing associations.

The animal evidence for an association between PFOA exposure and neurological effects in animals is *slight*. In animal models, some changes in absolute brain weight were noted after PFOA exposure however, the changes in brain weight were not associated with histopathological effects. There is limited, but compelling evidence from several single-dose studies indicating neurodevelopmental consequences of PFOA exposure during perinatal periods, though these studies cannot be modeled for this assessment due to the exposure paradigm. In a multi-dose study, Goulding et al. (2017) assessed neurodevelopmental consequences of PFOA exposure, but the observed effects in neonates were transient and therefore, are difficult to interpret. This study also reported a suppression of ambulatory activity in mice from the high-dose group following an acute injection of methamphetamine on PND 168. The biological significance of the alterations in neurotransmitter levels observed in a separate study is unclear (Yu et al., 2016); however, these effects indicate a potential alteration of neural signaling and could be an additional outcome related to PFOA neurotoxicity or a potential toxicological mechanism underlying the observed behavioral changes.

C.4.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause nervous system effects in humans under relevant exposure circumstances (Table C-7). This conclusion is based primarily on effects on neurodevelopment, neuropsychological and cognitive development, executive function, and behavioral problem in studies in humans exposed to median PFOA ranging from 12 to 5.2 ng/mL, and on evidence from animal models showing alterations in neurodevelopment, neurobehavior, and neurotransmitter levels following exposure to doses as low as ≥0.3 mg/kg/day PFOA. There is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-7. Evidence Profile Table for PFOA Nervous System 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 C.4.1)					⊕⊙⊙ ***Evidence Suggests***
Neurodevelopment 1 *High* confidence study 4 *Medium* confidence studies 1 *Low* confidence study	Findings were mixed both across and within studies, often by sex. A *high* confidence study reported significant associations with development problems for both sexes, but with different skills. Two *medium* confidence studies reported significant associations with developmental effects, but results were inconsistent. Significant inverse associations were found only in 6-mo neonates in one study and only in girls in another study. Remaining studies did not report consistent associations.	• *High* and *medium* confidence studies	• *Low* confidence study • *Inconsistent direction* of effects within and across studies • *Small magnitude* of effects in significant associations	⊕⊙⊙ *Slight* Evidence for nervous system effects is based on *high* confidence studies reporting significant adverse findings, including for neurodevelopmental, behavioral, attention, autism, and visuospatial outcomes, which sometimes varied by sex and direction and magnitude of effect. Uncertainties remain due to inconsistent findings within studies and mixed findings across studies. Studies with mixed findings were primarily of *medium* or *low* confidence.	*Primary basis*: Human evidence indicated effects on neurodevelopment, neuropsychological and cognitive development, executive function, and behavioral problems. Animal evidence indicated alterations in neurodevelopment, neurobehavior, and neurotransmitter levels. There is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Cognitive Function 11 *Medium* confidence studies **Social-emotional and behavioral regulation** 1 *High* confidence study 4 *Medium* confidence studies 1 *Low* confidence study	Cognitive function findings were mixed both across and within studies, often by sex and timing of exposure measure. Of 11 studies examining children, studies observed significant positive associations with cognitive function measures such as reading,	• *Medium* confidence studies • *High* and *medium* confidence studies	• *Inconsistent direction* of effects within and across studies • *Low* confidence study • *Inconsistent direction* of effects across and within 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**	
	full-scale IQ, and verbal ability (4/11), while others reported significant inverse associations (2/11). Other non-significant results in these studies were mixed. The remaining studies observed inconsistent or no associations. Six studies examined social-emotional and behavioral effects in children, with mixed results. One *high* confidence study observed significant associations with behavioral and peer relationship problems at age seven alongside non-significant mixed associations for other behavioral measures. One *medium* study reported significant inverse associations with externalizing behaviors in boys at 18 mo. Another *medium* confidence study found significant positive associations with total SDQ scores, indicating increased behavioral problems with increased exposure. The remaining				

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**	
	studies reported non-significant, mixed associations.				
Depression 3 *Medium* confidence studies 1 *Low* confidence study	Two *medium* confidence studies reported results for depression in general population adults. An additional study of *medium* confidence reported results for depression among pregnant women exclusively. All three studies reported positive associations, though none reached significance. A *low* confidence study found an inverse relationship.	• *Medium* confidence studies	• *Low* confidence study • *Inconsistent direction* of effects across studies		
Executive function 3 *Medium* confidence studies	Two studies examined executive function impacts among children from the HOME Study. One study observed significant associations with increased odds of metacognition impairments, while the other observed no associations. In one *medium* confidence study of adults, exposure was associated with increased executive function.	• *Medium* confidence studies	• *Inconsistent direction* of effects across age groups and studies in same cohort • *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**	
Attention 1 *High* confidence study 7 *Medium* confidence studies 2 *Low* confidence studies	Studies examining attention-related effects, such as ADHD, inattention, and hyperactivity, occurred in children only. One *medium* confidence, and one *low* confidence study reported significant associations, though the observed effects were in opposite directions. The remaining studies reported no or non-significant associations.	• *High and medium* confidence studies • *Large magnitude* of effects	• *Low* confidence studies • *Inconsistent direction* of effects across studies		
Autism, autistic behaviors, and intellectual disability 1 *High* confidence study 5 *Medium* confidence studies	Six studies examined autism-related outcomes among children. One *high* confidence study observed significant positive associations between age 5 exposures and autism screening scores at age 7. One *medium* confidence study observed significant inverse associations with autism and with intellectual disability in the overall study population. The remaining *medium* confidence studies reported findings that were inconsistent.	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across studies, ages, and exposure windows in study with most significant association • *Small magnitude* of effect in significant associations		

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**	
Visuospatial performance 1 *High* confidence study 1 *Medium* confidence study	Two studies reported on visuospatial performance in children. One *high* confidence study observed significant inverse associations with visual-motor performance in mid-childhood but significant positive associations with visual-spatial and visual-motor performance in early childhood. The *medium* confidence study reported no significant associations in childhood.	• *High* and *medium* confidence studies • *Large magnitude* of effect	• *Inconsistent direction* of effects across studies and age groups • *Limited number* of studies examining outcome		
Memory impairment 4 *Medium* confidence studies	Two studies examined memory effects in children, with one *medium* confidence study reporting significant inverse associations with nonverbal working memory for the highest exposure category. Two studies examined memory impacts among adult populations. In one *medium* confidence study, a significant inverse association with memory impairment was reported. The other *medium* confidence study	• *Medium* confidence studies • *Large magnitude* of effect	• *Inconsistent direction* of effects across 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**	
	reported no significant associations.				
Hearing impairment 2 *Medium* confidence studies	Two *medium* confidence studies examined hearing impairment among adults. One study observed significant positive associations with hearing impairment for the highest exposure group, while the other reported inconsistent non-significant associations.	• *Medium* confidence studies • *Large magnitude* of effect	• *Inconsistent direction* of effects across studies • *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.4.2)					
Organ weights 1 *High* confidence study 3 *Medium* confidence studies	Significant effects for absolute brain weight were found only in developmental studies and only in males. One developmental study in mice reported transient reductions in absolute brain weight, while a developmental study in rats reported decreased absolute brain weight as well as decreased body weight. One chronic exposure study in rats found that absolute brain weight was increased in only the low-dose group, and one short-term study in mice found no effects.	• *High* and *medium* confidence studies • *Consistent direction* of some findings across studies	• *Incoherence* of findings in other neurological endpoints • *Confounding variables* such as decreases in body weights	⊕⊙⊙ *Slight* Changes in absolute brain weight, were noted after PFOA exposure; however, the changes in brain weight were not associated with histopathological effects. One study found transient neurobehavioral effects in neonates following developmental exposure and such findings are difficult to interpret. The same study also found neurobehavioral changes in adulthood. 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**	
Histopathology 3 *High* confidence studies 1 *Medium* confidence studies	No changes in brain histopathology were reported in rats (4/4).	• *High* and *medium* confidence studies • *Consistent direction* of effects across studies	• *Limited number* of studies examining outcomes	biological significance of the alterations in neurotransmitters levels in a separate study is unclear. However, these effects indicate a potential alteration of neural signaling and could be an additional outcome related to PFOA neurotoxicity or a potential toxicological mechanism underlying the observed behavioral changes.	
Neurobehavior 1 *Medium* confidence study	A developmental study in male mice observed a transient increase in locomotor activity level during the pre-weaning period and no changes in startle reactivity or prepulse inhibition (1/1).	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Neurotransmitters 2 *Medium* confidence studies	Two studies observed alterations of neurotransmitter concentrations in male mice following short-term PFOA exposure and observed decrease glutamate (2/2) and norepinephrine (1/1) and an increase in dopamine (1/1) and serotonin (1/1).	• *Medium* confidence studies • *Consistent direction* of effects across studies	• *Limited number* of studies examining specific outcomes • *Biological significance* of effects is unclear		

Notes: ADHD = attention deficit hyperactivity disorder; HOME = Health Outcomes and Measures of the Environment; IQ = intelligence quotient; mo = month; SDQ = Strengths and Difficulties Questionnaire.

C.5 Renal

EPA identified 23 epidemiological and 7 animal studies that investigated the association between PFOA and renal effects. Of the epidemiological studies, 1 was classified as *high* confidence, 2 as *medium* confidence, 19 as *low* confidence, and 1 was considered *uninformative* (Section C.5.1). Of the animal studies, 3 were classified as *high* confidence, and 4 were considered *medium* confidence (Section C.5.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.5.1 Human Evidence Study Quality Evaluation and Synthesis

C.5.1.1 Introduction

PFOA has the potential to affect the kidney's function of tubular resorption because of it uses tubular transporters for excretion and resorption (U.S. EPA, 2016c). Biomarkers of renal function include blood urea nitrogen (BUN), serum creatinine, estimated glomerular filtration rate (eGFR), and uric acid levels. eGFR is a marker of non-malignant renal disease.

The 2016 PFOA HESD (U.S. EPA, 2016c) concluded there was evidence of a suggestive association between PFOA and two renal outcomes (i.e., uric acid levels and eGFR) based on one occupational (Costa et al., 2009), two studies in high-exposed communities (Watkins et al., 2013; Steenland et al., 2010), and one general population study (Shankar et al., 2011). Kidney function was measured by eGFR, hyperuricemia, and uric acid levels. However, given the cross-sectional study designs, reverse causality as an explanation could not be ruled out. The report also concluded there was no probable link between PFOA exposure and kidney disease based on three occupational studies (Steenland et al., 2015; Raleigh et al., 2014; Steenland and Woskie, 2012).

For this updated review, 23 studies examined the association between PFOA and renal health outcomes. Five studies were in children and adolescents (Khalil et al., 2018; Qin et al., 2016; Kataria et al., 2015; Geiger et al., 2013), two in pregnant women (Nielsen et al., 2020; Gyllenhammar et al., 2018b), one study was in occupational workers (Rotander et al., 2015) and the remainder of the studies were in general population. Seventeen of the studies utilized a cross-sectional study design; the remaining studies included five cohort study designs (Nielsen et al., 2020; Blake et al., 2018; Conway et al., 2018; Gyllenhammar et al., 2018b; Dhingra et al., 2016b), and one controlled trial (Convertino et al., 2018) (Appendix D). All studies measured PFOA in blood components (i.e., plasma or serum). Two studies conducted in China investigated the same population from the Isomers of C8 Health Project (Wang et al., 2019b; Zeng et al., 2019c). Among those studying populations in the United States, five studies utilized data from the NHANES (Lee et al., 2020; Scinicariello et al., 2020b; Jain and Ducatman, 2019a, c; Kataria et al., 2015; Geiger et al., 2013). Outcomes evaluated in these studies included clinical conditions, such as chronic kidney disease (CKD) and gout, and biomarkers of renal function, including uric acid, eGFR, albumin, and creatinine.

C.5.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies examining kidney function and kidney disease. Since PFOA is removed from the blood by the kidney, cross-sectional analyses using serum PFOA as the exposure measure are problematic if individuals with compromised kidney function are included: PFOA concentrations could be increased in those individuals and an apparent association with GFR would be observed, even if one did not exist (Dhingra et al., 2017).

There are 23 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 renal effects. Study quality evaluations for these 23 studies are shown in Figure C-32.

Of the 23 studies identified since the 2016 assessment, one was classified as *high* confidence (Dhingra et al., 2016b), two as *medium* confidence (Gyllenhammar et al., 2018b; Dhingra et al., 2017)), 19 as *low* confidence, and one as *uninformative* (Seo et al., 2018). The main concerns with the *low* confidence studies included potential for residual confounding, selection bias, and reverse causality. Other concerns included small sample sizes (Nielsen et al., 2020; Khalil et al., 2018), selective reporting of significant results (Lee et al., 2020), and potential for selection bias (Rotander et al., 2015; Lin et al., 2013a). Additionally, *low* confidence studies utilizing cross-sectional analyses of kidney function with serum PFOA were impacted by the potential for reverse causation.

Seo et al. (2018) was considered *uninformative* due to use of bivariate statistical analyses, limiting the ability to interpret the results. Additionally, other potential sources of bias were identified, including a lack of information on participant recruitment and selection, unexplained discrepancies in sample sizes, and missing details on outcome assessment methods.

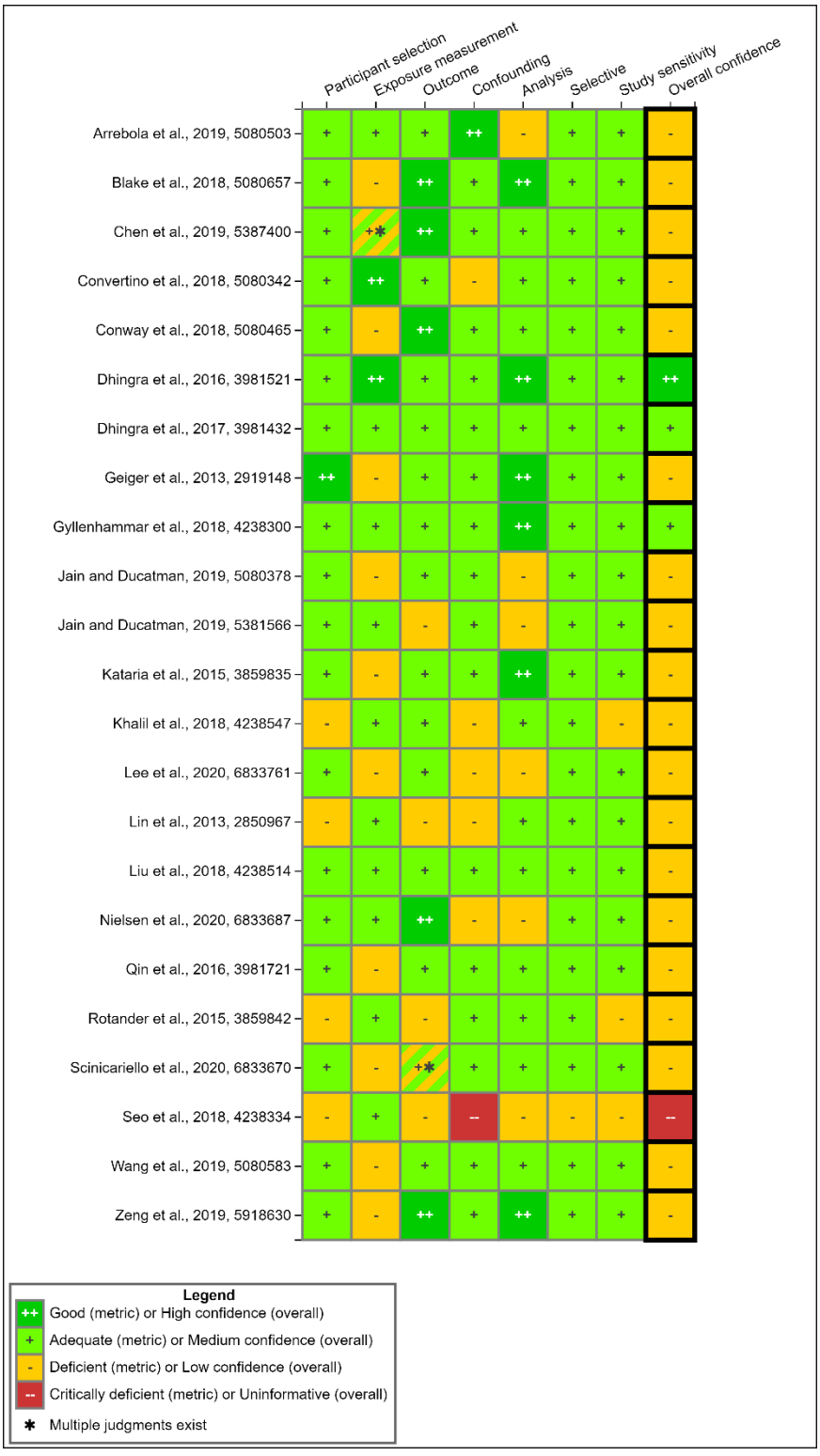


Figure C-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Renal Effects

Interactive figure and additional study details available on HAWC.

C.5.1.3 Findings From Children and Adolescents

Three *low* confidence studies examined uric acid among children and adolescents (Qin et al., 2016; Kataria et al., 2015; Geiger et al., 2013) with two also reporting on hyperuricemia (Qin et al., 2016; Geiger et al., 2013), defined as serum uric acid levels ≥6 mg/dL). Geiger et al. (2013) used NHANES data from 1999 to 2000 and 2003 to 2008 to assess the association between serum PFOA levels and hyperuricemia in children aged 12 to 18 years. A statistically significant positive association was observed between increasing quartiles of PFOA and hyperuricemia (p-trend = 0.0071), and serum uric acid (p-trend = 0.0001). An overlapping NHANES (2003–2010) study (Kataria et al., 2015) also observed a significant positive association for uric acid for the highest quartile of PFOA exposure (≥4.7 ng/mL) compared with the lowest (< 2.5 ng/mL). Qin et al. (2016) reported significant positive associations with uric acid and hyperuricemia in children aged 12 to 15 years from the GBCA in Taiwan. Positive associations were observed when the highest compared with the lowest PFOA quartiles. When stratified by sex, the associations were only evident among boys, including an increasing trend (p-trend = 0.033) (Qin et al., 2016).

One *low* confidence study (Kataria et al., 2015) reported on GFRs among children (12–19 years old) from NHANES (2003–2010). A negative association was reported between PFOA and eGFR, where the fourth quartile was associated with a statistically significant decrease in eGFR compared with the lowest exposure quartile, and the second and third quartiles showed a non-significant decrease.

Two *low* confidence studies investigated associations between PFOA and serum creatinine among children and adolescents (Khalil et al., 2018; Kataria et al., 2015). Kataria et al. (2015) reported a significant positive association with serum creatinine in the highest PFOA quartile when compared with the lowest quartile. Khalil et al. (2018) observed weak, non-significant negative association with serum creatinine in obese children (8–12 years).

C.5.1.4 Findings From the General Adult Population

Three studies examined CKD and no significant associations were observed (Wang et al., 2019b; Conway et al., 2018; Dhingra et al., 2016b). CKD was defined as an eGFR of < 60 mL/min/1.73 m^2. A *high* confidence C8 Health Project community study (Dhingra et al., 2016b) observed positive non-significant increases in the risk of CKD in both retrospective and prospective analyses, and among non-diabetic participants. In retrospective analyses, the magnitude of effect was diminished and inconsistent when modeling exposure using increasing lag periods (5-, 10-, and 20-year lag). In contrast, negative associations were observed in two *low* confidence studies (Wang et al., 2019b; Conway et al., 2018). Analyses of participants in the Isomers of C8 Health Project in China (Wang et al., 2019b) observed a significant negative association with odds of CKD. Analyses of diabetic individuals in the U.S.-based C8 Health Project (Conway et al., 2018) also showed significantly reduced odds, but this effect was not observed in non-diabetic participants. However, a concern for reverse causality makes interpretation of the results difficult in both *low* confidence studies.

Gout was examined in one *low* confidence study (Scinicariello et al., 2020b) on adults from NHANES (2009–2014) and a significant increased trend in risk of self-reported gout across PFOA quartiles was observed (p-value = 0.01). The observed effects were similar when stratifying by CKD status.

Seven *low* confidence general population studies (Scinicariello et al., 2020b; Arrebola et al., 2019; Chen et al., 2019a; Jain and Ducatman, 2019a; Zeng et al., 2019c; Seo et al., 2018; Lin et al., 2013a) and one *low* confidence occupational study (Rotander et al., 2015) examined uric acid levels, and three of these studies reported specifically on hyperuricemia (Scinicariello et al., 2020b; Arrebola et al., 2019; Zeng et al., 2019c). Significant findings were found in three studies, indicating a positive association with uric acid or increased odds of hyperuricemia, while non-significant positive associations were observed for uric acid in three general population confidence studies and one occupational study.

A *low* confidence NHANES (2009–2014) study (Scinicariello et al., 2020b) on adults reported a significant positive association between serum PFOA and serum uric acid in quartile analyses, and the trend was significant (p-trend = 0.0001). The association remained when restricted to participants without CKD, but the association was not consistent among those with CKD. Analyses of hyperuricemia were similar. A significant increasing trend in the odds of hyperuricemia was observed among the whole sample and those without CKD. Similarly, a positive association with serum uric acid was observed in a *low* confidence study on participants from the Isomers of C8 Health Project (Zeng et al., 2019c). In addition, a significant positive association was observed for hyperuricemia and total PFOA exposure (Zeng et al., 2019c). Results were similar among men and women in sex-stratified analyses. Utilizing NHANES data from 2007 to 2014, a *low* confidence study (Jain and Ducatman, 2019a) assessed the associations between serum PFOA and uric acid across gender and stages of GF. For males, serum PFOA and uric acid were positively associated ($p < 0.01$) at stage GF-1 and GF-2 and negatively associated ($p < 0.01$) at stage GF-3B/4. For females, all associations were positive but only reached significance for GF-1 and GF-3A. Two *low* confidence study (Chen et al., 2019a; Lin et al., 2013a) did not observe associations with plasma uric acid in Croatian adults aged 44–56 years, or in adolescents and young adults aged 12 to 30 years in the Young Taiwanese Cohort Study. A *low* confidence study (Arrebola et al., 2019) from the BIOAMBIENT.ES study observed a non-significant increase in risk of hyperuricemia.

One *low* confidence occupational study examined serum uric acid levels among firefighters with past exposure to AFFF (Rotander et al., 2015). Uric acid levels were elevated with increasing PFOA exposure in firefighters, but the result did not reach significance.

One *medium* and two *low* confidence studies in high-exposed populations examined eGFR, and two studies reported negative associations (Blake et al., 2018; Dhingra et al., 2017), while one reported a positive association (Wang et al., 2019b). Dhingra et al. (2017) reported a significant negative association with measured but not modeled PFOA and a negative trend in eGFR across measured serum PFOA quintiles in women from the Women from C8 Science Panel Project. The study used modeled PFOA as an approach to demonstrate that cross-sectional analyses using measured PFOA are affected by reverse causation (Dhingra et al., 2017). Blake et al. (2018) observed negative non-significant associations in participants of the Fernald Community Cohort (FCC) with high exposure to PFAS from their household water supplies. Wang et al. (2019b) observed positive associations in a high-exposed population from the Isomers of C8 Health Project.

The evidence on PFOA and renal effects among pregnant women was limited. Only two studies on pregnant women examined effects on eGFR (Nielsen et al., 2020; Gyllenhammar et al., 2018b). One *medium* confidence study (Gyllenhammar et al., 2018b) assessed the relationship

between maternal PFOA during pregnancy and maternal eGFR three weeks after delivery, calculated using both creatinine- and cystatin C-based estimates of GFR. A significant positive relationship between cystatin C-GFR and maternal PFOA was reported ($\beta = 0.004 \pm 0.002$, $p = 0.022$). Changes in kidney function during pregnancy were evaluated in a small group of pregnant women (n = 73) using creatinine-GFR and cystatin C-GFR in a *low* confidence study (Nielsen et al., 2020), but no significant effects were observed using partial Spearman rank correlations. While the *medium* confidence study in pregnant women reported a positive association between PFOA and eGFR (Gyllenhammar et al., 2018b), given the limited number of studies, there is not enough evidence to determine conclusive associations between PFOA renal function among pregnant women and an occupational group of firefighters.

Four *low* confidence studies examined albumin and creatinine as biomarkers for renal function (Lee et al., 2020; Chen et al., 2019a; Jain and Ducatman, 2019c; Convertino et al., 2018). The four studies provided differing conclusions. Jain and Ducatman (2019c) reported statistically significant positive with serum and urine creatinine, and serum albumin in NHANES (2005–2014) participants. Statistically significant negative associations were observed with urine albumin and urine albumin-creatinine ratios. Stratification by stages of GF was noted as better representing more severe stages of renal failure. For PFOA, stratification by stages of GF had inconsistent effects. However, Lee et al. (2020) observed a decreased risk of albuminuria (defined as urine albumin-to-creatinine ratio ≥30 mg/g) Chen et al. (2019a) did not observe significant associations with plasma creatinine. Convertino et al. (2018) did not observe any associations with serum creatinine during a phase 1 controlled trial assessing the chemotherapeutic potential of APFO.

One *low* confidence study (Liu et al., 2018b) examined serum proteins among NHANES (2013–2014) participants and reported a significant positive association using linear PFOA exposure levels. The result was similar for total PFOA but did not reach significance.

C.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and five studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and renal effects. Study quality evaluations for these seven studies are shown in Figure C-33.

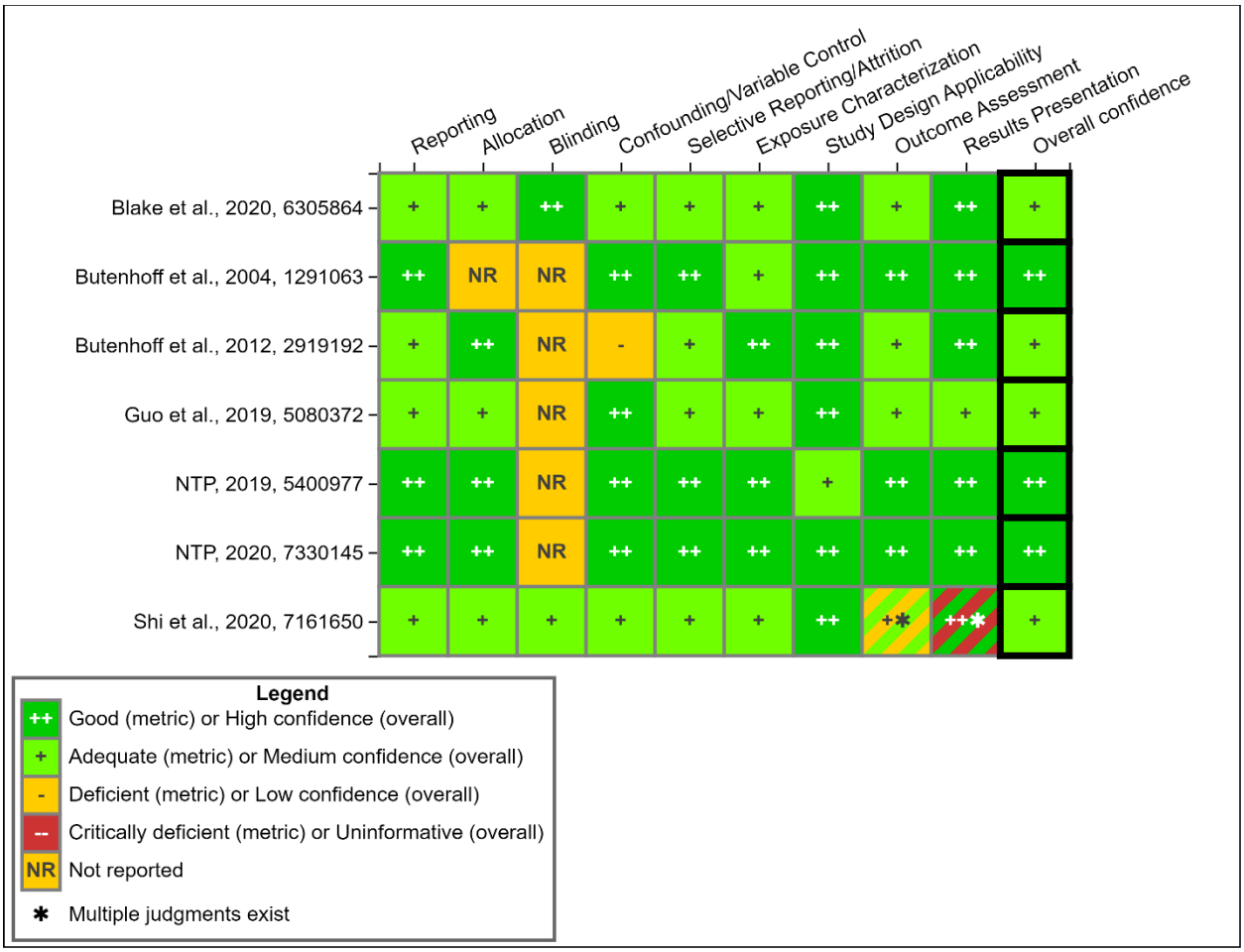


Figure C-33. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Renal Effects

Interactive figure and additional study details available on HAWC.

The available data suggest the renal system may be adversely affected by PFOA exposure, but the evidence primarily comes from studies conducted in rats. Two studies in mice (Blake et al., 2020; Shi et al., 2020) and one study in monkeys (Butenhoff et al., 2002) reported no effects on the renal system. In contrast, several short-term and chronic studies reported significant increases in absolute and/or relative kidney weights in rats (NTP, 2020, 2019a; Butenhoff et al., 2012; Cui et al., 2009; Butenhoff et al., 2004a) and/or alterations in serum biomarkers of renal function (NTP, 2020; Guo et al., 2019; NTP, 2019a; Cui et al., 2009). However, only two studies reported concurring histological changes in the kidney (NTP, 2020; Cui et al., 2009).

Effects on kidney weight were predominately observed in male rats rather than female rats, regardless of study design and exposure duration (Figure C-34 (absolute kidney weight), Figure C-35 (relative kidney weight in males), Figure C-36 (relative kidney weight in females)). This is true of both absolute and relative kidney weight metrics. However, across both sexes, several studies observed statistically significant decreases in absolute kidney weight at the highest doses tested (Figure C-34), which often corresponded to doses resulting in reduced body weight (see

Toxicity Assessment, (U.S. EPA, 2024b) and Section C.3.2). These changes in body weight may influence the interpretation of absolute and relative kidney weight changes.

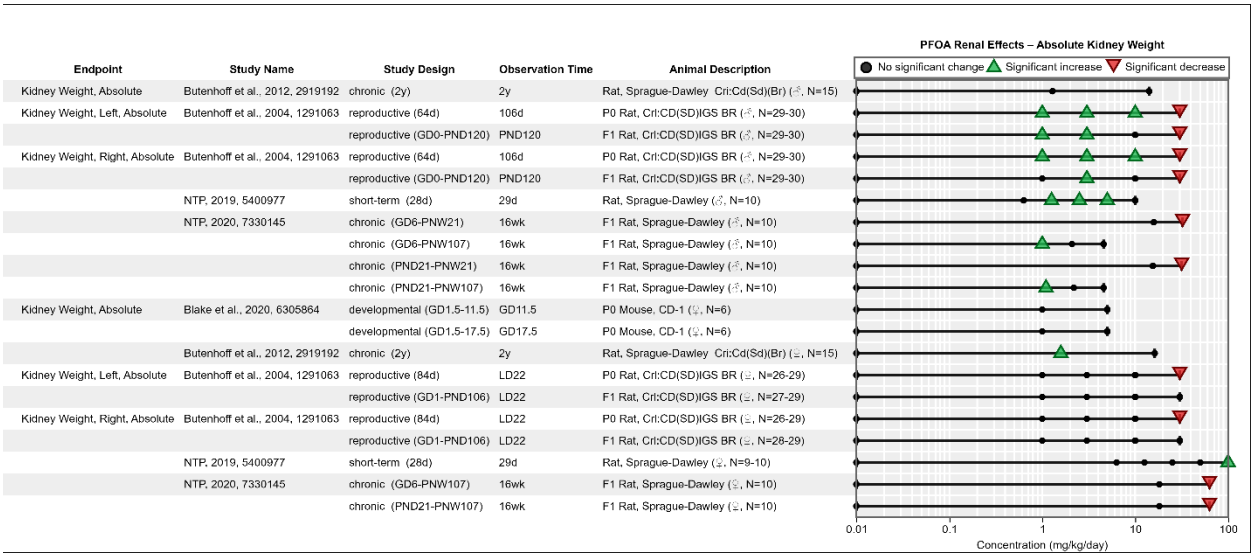


Figure C-34. Absolute Kidney Weights in Rodents Following Exposure to PFOA (Logarithmic Scale)

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; F_1 = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year.

NTP (2019a) observed dose-dependent increases in the absolute and relative kidney weights of male Sprague-Dawley rats treated with PFOA for 28 days. Absolute and relative kidney weights were increased in all treated groups (doses of 0.625–10 mg/kg/day), though the increase in absolute weight was only significant for the three middle dose groups (1.25, 2.5, and 5 mg/kg/day). The highest dose group (10 mg/kg/day) resulted in the largest increase in relative kidney weight of approximately 36% control weight. The lack of a clear dose-response trend in absolute kidney weights was likely related to decreased body weights observed at doses ≥2.5 mg/kg/day. Despite the increases observed in kidney weights, there were no significant histological changes observed in the kidneys of PFOA-treated rats (NTP, 2019a). Cui et al. (2009) similarly observed increased relative kidney weights in male rats administered 5 or 20 mg/kg/day for 28 days, though the increases were not dose-dependent (absolute weights were not reported); however, histological changes were observed in the kidneys of the high-dose group, including turbidness and tumefaction in the epithelia of the proximal convoluted tubule (reported qualitatively without incidence data).

A similar trend in kidney weight was observed for male rats in a two-generation reproduction study (Butenhoff et al., 2004a). Adult P_0 and F_1 males had significantly increased absolute kidney weights at 1, 3, and 10 mg/kg/day, but decreased kidney weights at the highest dose level of 30 mg/kg/day. Relative kidney weights were significantly increased in all treated males (increases of 16%–27% and 11%–19% change in P_0 and F_1 males, respectively). Kidney weights relative to brain weights were increased at 1, 3, and 10 mg/kg/day, but not 30 mg/kg/day. In the high-dose male group, absolute and relative kidney weight changes occurred in a pattern

typically associated with decrements in body weight. However, in the lower dose groups of males, significant increases in absolute kidney weight and relative to body and brain weights appear to be treatment-related and are consistent with the results reported for male rats in the 28-day study by NTP (2019a). Increased kidney weights observed following exposure to PFOA may be a response to the challenge of providing transporters for renal removal of the foreign molecule (U.S. EPA, 2016c). Increased kidney weight can be regarded as an adaptive response to the transport challenge. It is beneficial for the individual but adverse in the sense that it signifies the need to upregulate tubular transporters in the kidney to excrete the foreign material and a reflection of PFOA bioaccumulation in serum and tissues. Butenhoff et al. (2004a) did not report conducting kidney histopathology in this reproductive study.

Two chronic dietary studies in Sprague-Dawley rats evaluated effects on the renal system, but the results were not consistent across studies. Butenhoff et al. (2012) observed increased relative kidney weight in male rats administered 300 ppm in the diet (equivalent to 14.2 mg/kg/day) after 1 year of exposure, but no changes in absolute or relative kidney weight or histopathology were observed after 2 years of exposure. In contrast, a 2-year study by NTP (2020) observed altered kidney weights and increased incidences of nonneoplastic lesions in the kidneys of male rats exposed to postweaning dietary concentrations of 20, 40, 80, 150, or 300 ppm with or without perinatal exposure to 150 or 300 ppm (see Toxicity Assessment, (U.S. EPA, 2024b)). At the 16-week interim evaluation, absolute kidney weights were increased in males of the 0/20 and 300/20 ppm groups (perinatal/postweaning concentrations, equivalent to postweaning doses of 1.1, and 1.0 mg/kg/day, respectively) and decreased in males of the 0/300 and 300/300 ppm groups (31.7 and 32.1 mg/kg/day, respectively), but not significantly altered compared with controls in any of the intermediate dose groups. However, relative kidney weights were significantly increased in all treated groups (range of 21%–35% increases across all groups); body weights were also significantly reduced in all treatment groups (dose-dependent range of 9%–45% decreases across all groups). Substantially reduced body weights in treated males makes interpretation of kidney weight effects difficult.

Figure C-35. Percent Change in Relative Kidney Weights of Male Rats Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; F_1 = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year; CI = confidence interval.

Female rats were generally less sensitive to changes in kidney weights compared with male rats, with most differences occurring in the highest dose groups only (Figure C-35, Figure C-36). NTP (2019a) observed dose-dependent increases in absolute and relative kidney weights of female rats treated with PFOA for 28 days. Absolute kidney weight was only increased at the highest dose of 100 mg/kg/day (11% increase) while relative kidney weight was increased at 50 and 100 mg/kg/day (7% and 17% increases, respectively). Similar to males from this study, there were no significant histological changes observed in the kidneys of PFOA-treated rats (NTP, 2019a). In contrast, in a two-generation reproduction study (Butenhoff et al., 2004a), absolute and relative kidney weights of P_0 females were significantly decreased at 30 mg/kg/day (decreases of approximately 5%–8% change), and no effects were observed on kidney weight in F_1 females. There were no significant effects on the body weight of these animals at terminal sacrifice.

Butenhoff et al. (2012) observed an increase in absolute kidney weight (11% change) in female rats administered 30 but not 300 ppm PFOA in the diet for 2 years (equivalent to 1.6 and 16.1 mg/kg/day, respectively). In contrast, the authors reported a significant increase in relative kidney weights of female rats administered 300 but not 30 ppm (15% change). That dose group also experienced an approximately 12% decrease in body weight by the time of terminal sacrifice, but the change was not statistically significant. The authors reported no change in renal histopathology in female rats (Butenhoff et al., 2012). A second 2-year feeding study by NTP (2020) found alterations in absolute kidney weight and increased incidences of nonneoplastic lesions in the kidneys of female rats exposed to postweaning dietary concentrations of 300 or 1,000 ppm with or without perinatal exposure to 300 ppm (see Toxicity Assessment, (U.S. EPA, 2024b)). At the 16-week interim evaluation, absolute kidney weights were decreased in females of the 0/1,000 ppm and 300/1,000 ppm groups (equivalent to 63.4 and 63.5 mg/kg/day postweaning doses); however, relative kidney weights were unaltered in females. Body weights were significantly reduced in females exposed to 1,000 ppm postweaning (by 12%). Decreased absolute kidney weights observed in females exposed to 1,000 ppm were likely related to reduced body weights as there was no change in relative kidney weight.

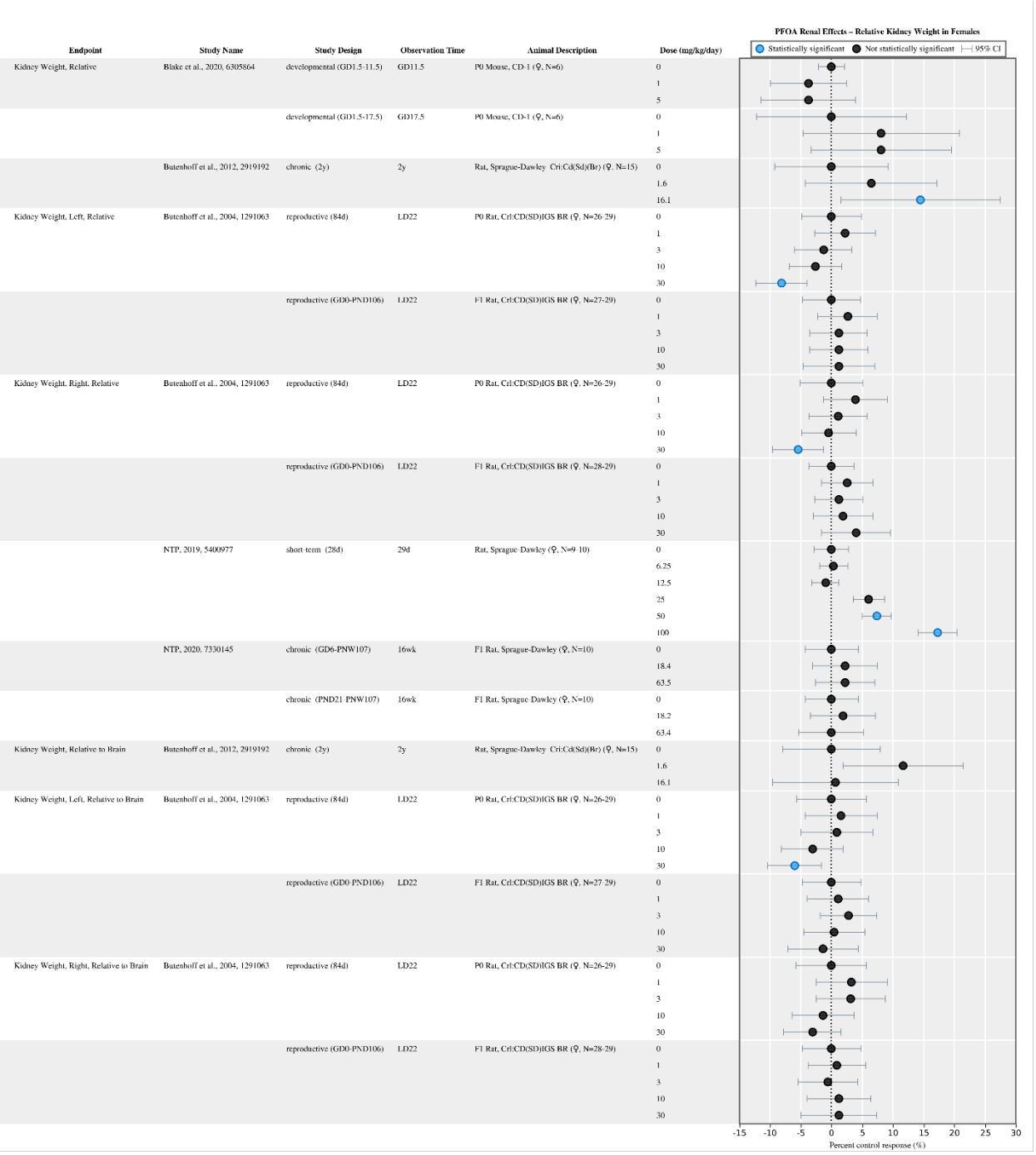


Figure C-36. Percent Change in Relative Kidney Weights of Female Rodents Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; F_1 = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year; CI = confidence interval.

Histopathological examination of male rats at the 16-week interim of a 2-year dietary study showed increased incidences of renal tubule mineralization in the 0/150, 0/300, and 300/300 ppm

groups compared with the 0/0 ppm control group (incidences of 40%, 50%, and 60%, respectively, compared with 0% incidence in the control group) (NTP, 2020). No other significant histological changes were observed in males, and the male groups were removed from that study shortly after the interim. However, examination of female rats revealed treatment-related increased incidences of renal tubule mineralization, hyperplasia of the urothelium that lines the renal papilla, and necrosis of the renal papilla (that was observed only after 2 years). As shown in Table C-8, these lesions were mainly found in the female groups with the highest postweaning exposure (1,000 ppm, equivalent to approximately 63 mg/kg/day).

Table C-8. Incidences of Nonneoplastic Lesions in the Kidneys of Female Sprague-Dawley Rats as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose		
	0 ppm	300 ppm	1,000 ppm
16 Weeks			
Renal Tubule, Mineralization			
0 ppm	2/10 (20%) (1.0)[a]	1/10 (10%) (1.0)	7/10* (70%) (1.0)
150 ppm	–	2/10 (20%) (1.0)	–
300 ppm	–	–	5/10 (50%) (1.2)
Renal Papilla Urothelium, Hyperplasia			
0 ppm	0/10 (0%)	0/10 (0%)	4/10* (40%) (1.3)
150 ppm	–	0/10 (0%)	–
300 ppm	–	–	4/10* (40%) (1.0)
Renal Papilla, Necrosis			
0 ppm	0/10 (0%)	0/10 (0%)	0/10 (0%)
150 ppm	–	0/10 (0%)	–
300 ppm	–	–	0/10 (0%)
107 Weeks			
Renal Tubule, Mineralization			
0 ppm	5/50 (10%) (1.2)	6/50 (12%) (1.3)	16/50** (32%) (1.0)
150 ppm	–	8/50 (16%) (1.0)	–
300 ppm	–	–	8/50 (16%) (1.5)
Renal Papilla Urothelium, Hyperplasia			
0 ppm	4/50 (8%) (1.0)	21/50** (42%) (1.0)	40/50** (80%) (1.9)
150 ppm	–	8/50 (16%) (1.0)	–
300 ppm	–	–	45/50** (90%) (1.8)
Renal Papilla, Necrosis			
0 ppm	0/50 (0%)	0/50 (0%)	12/50** (24%) (2.3)
150 ppm	–	0/50 (0%)	–
300 ppm	–	–	22/50** (44%) (2.1)

Notes:
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.
[a] Average severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

In a second similar study conducted by NTP in male rats only due to high mortality in the initial study, relative kidney weights of all groups exposed to postweaning dietary concentrations of 20,

40, or 80 ppm (equivalent to approximately 1, 2, or 4.6 mg/kg/day) for 16 weeks were significantly greater than the 0/0 ppm control group, but absolute kidney weights were significantly increased only in the groups exposed to 20 ppm postweaning (NTP, 2020). Body weights were significantly decreased in all treated groups (by 9%–21%), and that could explain why absolute kidney weights did not achieve statistical significance in the higher dose groups in these growing rats. These patterns in kidney weights are similar to those observed for male rats in the studies by NTP (2019a) and Butenhoff et al. (2004a). There were no significant histological changes in the kidneys for male rats found at the interim or 2-year terminal evaluations.

In contrast to results found in studies with rats, no treatment-related effects were reported for relative kidney weight in male mice administered PFOA for 5 weeks (Shi et al., 2020), kidney weight and histopathology in female mice administered PFOA during gestation (Blake et al., 2020), or kidney weight and histopathology in male monkeys administered PFOA for 6 months by oral capsule (Butenhoff et al., 2002). One short-term study in rats (NTP, 2019a) and three chronic studies in rats or monkeys also examined the urinary bladder for histopathology after exposure to PFOA, and no treatment-related effects were reported (NTP, 2020; Butenhoff et al., 2012; Butenhoff et al., 2002).

Several studies analyzed clinical chemistry and urinalysis endpoints related to renal toxicity, though there is uncertainty regarding adversity of the observed effects. In two separate studies, NTP observed increased concentrations of BUN in male and female rats following 28 days or 16 weeks of exposure (NTP, 2020, 2019a). However, without concomitant increases in blood creatinine concentrations, NTP concluded that the slight increases in urea nitrogen were likely due to a decrease in water intake (NTP, 2020, 2019a). In fact, creatinine concentrations were significantly decreased in male rats administered ≥0.625 mg/kg/day in the 28-day study, though NTP considered this change to be related to decreased food intake and body weight rather than a direct treatment effect (NTP, 2019a).

Butenhoff et al. (2012) also observed slight increases in BUN in male and female rats, but only at the 3- and 6-month evaluations of the 2-year study; creatinine was not measured. No significant differences were observed in serum BUN, serum creatinine, or urinary creatinine in female mice administered PFOA during gestation (Blake et al., 2020) or in male monkeys administered PFOA for 6 months (Butenhoff et al., 2002). However, a 28-day study in male mice found significant, dose-dependent decreases in BUN and increases in serum ammonia levels in all treated groups (0.4–10 mg/kg/day) compared with controls; the authors of this study suggest these changes are signs of urea cycle dysfunction caused by PFOA (Guo et al., 2019).

Two studies found that the activity of creatine kinase was decreased in male rats administered PFOA for 28 days or up to 2 years (NTP, 2019a; Butenhoff et al., 2012). NTP considered this effect to be treatment-related but not toxicologically relevant (NTP, 2019a). No effects on creatine kinase were observed in male or female rats at the 16-week interim evaluation of the NTP chronic dietary study (NTP, 2020).

No apparent treatment-related effects were observed on urinalysis endpoints (e.g., volume, pH, specific gravity, protein, blood) measured in male or female rats over the course of 2 years of treatment (Butenhoff et al., 2012) or in male monkeys over the course of 6 months of treatment (Butenhoff et al., 2002).

C.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse renal outcomes is discussed in Sections 3.1.1.4, 3.2.5, 3.3.4, and 3.4.3 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are four 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 renal effects. A summary of these studies is shown in Figure C-37. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to renal effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	1
Cell Signaling Or Signal Transduction	2	1	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	1	2
Grand Total	3	1	4

Figure C-37. Summary of Mechanistic Studies of PFOA and Renal Effects

Interactive figure and additional study details available on HAWC.

C.5.4 Evidence Integration

There is *slight* evidence for an association between PFOA exposure and renal effects in humans based on mixed evidence of decreased renal function. The 2016 PFOA HESD (U.S. EPA, 2016c) concluded there was evidence of an association between PFOA and two renal outcomes (i.e., uric acid levels and eGFR) based on one occupational study (Costa et al., 2009), two studies in higher exposed communities (Watkins et al., 2013; Steenland et al., 2010), and one general population study (Shankar et al., 2011). In this updated review, there was some evidence of associations with decreased kidney function, although reverse causality (i.e., increases in serum perfluoroalkyl levels could be due to a decrease in glomerular filtration and shared renal transporters for perfluoroalkyls and uric acid) cannot be ruled out. There were mixed results across the measures of renal function. A positive association was observed for CKD in a *high* confidence study in a C8 Health Project population including non-diabetics (Dhingra et al., 2016b); while two *low* confidence studies reported negative associations (Wang et al., 2019b; Conway et al., 2018). The results were also inconsistent when assessing eGFR, in three highly exposed population studies, with two reporting negative associations (Blake et al., 2018; Dhingra et al., 2017) and one positive association (Wang et al., 2019b). Regarding hyperuricemia and uric acid levels, results varied across gender and stages of GF. In children, there were mixed results for associations between PFOA and creatinine and uric acid. One *low* confidence study reported a statistically significant decrease in eGFR in adolescents across PFOA quartiles (Kataria et al., 2015).

The animal evidence for an association between PFOA exposure and renal toxicity is *slight* based on seven *high* or *medium* confidence animal studies that suggests the kidney can be a target of PFOA toxicity, although changes in kidney weight or histopathology have only been

observed in rats. Clinical chemistry and urinalysis endpoints do not provide strong evidence of damage to kidney structure or function; however, kidney weights, particularly in male rats, were significantly increased following short-term and chronic exposure. The observed increases in kidney weights may indicate an adaptive response that is adverse in the sense that it signifies the need to upregulate tubular transporters in the kidney to excrete the foreign material and is a reflection of PFOA bioaccumulation in serum and tissues. However, kidney weights appear to be heavily influenced by changes in body weight which impacts the ability to interpret and model these responses.

Studies in animals generally found no histological changes correlating with increased kidney weight. The NTP chronic study (NTP, 2020) in rats provides the most convincing evidence that the kidney can be damaged by exposure to PFOA, although the doses with effects observed were relatively high (approximately 18 and 63 mg/kg/day in females and 16 and 32 mg/kg/day in males). Renal lesions were mainly observed in treated females, except for increased tubule mineralization which was observed in both sexes. Cui et al. (2009) also observed kidney damage in male rats treated with 20 mg/kg/day PFOA for 28 days, but the incidences of specific lesions were not reported. The mechanisms of this kidney damage are unknown, but it may be related to direct cytotoxicity from the high concentration of PFOA in the urine (NTP, 2020).

C.5.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause renal effects in humans under relevant exposure circumstances (Table C-9). This conclusion is based primarily on effects on measures of kidney function observed in studies in humans exposed to median PFOA ranging from 3.5 to 11.9 ng/mL, and on evidence in rats showing increased kidney weights and renal lesions following exposure to doses as low as 1 mg/kg/day and 16 mg/kg/day PFOA, respectively. Although there is some evidence of negative effects of PFOA exposure on CKD, there is considerable uncertainty in the results due to inconsistency across studies, mixed findings, limited number of studies and potential for reverse causation.

Table C-9. Evidence Profile Table for PFOA Renal 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 C.5.1)					⊕⊙⊙ ***Evidence Suggests***
Uric acid 11 *Low* confidence studies	Studies in children observed significant increases in uric acid (3/3) and hyperuricemia (2/2) with increasing exposure to PFOA. In studies of adults, significant increases were observed in studies of the general population (3/7), while non-significant increases were reported in other general population studies (2/7) and an occupational study (1/1). Significant increases in the odds of hyperuricemia were also observed (2/7) in adults.	• *Consistent direction* of effect among children and adults	• *Low* confidence studies	⊕⊙⊙ *Slight* Several studies of *medium* and *low* confidence found evidence of decreased kidney function among children and adults, including increased uric acid and hyperuricemia and decreased eGFR. Overall, findings were inconsistent, with opposing directions of effect observed for some outcomes. Uncertainties remain due to the mixed results, limited studies evaluating albumin, gout, and proteins, and concerns about reverse causality in lower confidence studies.	*Primary basis*: Human evidence indicted effects on kidney function and animal evidence indicated increased kidney weight and renal lesions in rats. Although there is some evidence of negative effects of PFOA exposure on CKD, there is considerable uncertainty in the results due to inconsistency across studies, mixed findings, limited number of studies and potential for reverse causation. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Serum and urinary biomarkers 7 *Low* confidence studies	Increases in serum albumin were observed in adults (2/2), but urinary albumin was observed to be decreased (1/1). Significant increases in serum creatinine (1/1)	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining outcome • *Incoherence* of findings related to serum and urine albumin levels		

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 adults, along with increased urinary creatinine (1/1), leading to a decreased albumin-creatinine ratio. Results for urinary total protein and urea were not consistent (2/2). A limited number of studies evaluated effects in children, and one (1/2) observed increases in serum creatinine at the highest levels of exposure.				
Glomerular filtration rate 2 *Medium* confidence studies 4 *Low* confidence studies	Results for GFR were mixed. One study in children (1/1) reported a significant decrease in eGFR at the highest exposure level. In adults decreases in eGFR were observed in two studies (2/3), and a significant increase in eGFR was observed in one study (1/3). In studies of pregnant women, a positive association with GFR was observed (1/2).	• *Medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effect in studies of adults		
Chronic kidney disease 1 *High* confidence study 2 *Low* confidence studies	Three studies examined CKD in adults who were both diabetic and non-diabetic. The *high* confidence study reported non-significant increased	• *High* confidence study	• *Low* confidence studies • *Inconsistent direction* of effect across studies, which may be due to reverse		

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**	
	odds of CKD. The two *low* confidence studies found significant decreases in CKD (2/2), with one of those results reported for diabetic adults (1/3).		causality in *low* confidence studies • *Imprecision* of findings		
Gout 1 *Low* confidence study	Significantly increased odds of self-reported gout were observed in NHANES adults (1/1) at higher levels of exposure. The association remained in analyses stratified by CKD status.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining outcome • Potential outcome misclassification due to self-reported outcome		
Evidence From In Vivo Animal Studies (C.5.2)					
Kidney weight 3 *High* confidence studies 3 *Medium* confidence studies	Kidney weights were significantly changed following short-term and chronic exposure in several studies, particularly in male rats; however, concurrent decreases in body weight may have influenced results. No effects on absolute or relative kidney weight were reported in studies in mice (2/2). Absolute kidney weight in male rats was increased at lower doses and decreased at higher doses following PFOA	• *High* and *medium* confidence studies	• *Inconsistent direction* of results • Changes in body weight may limit ability to interpret these responses	⊕⊙⊙ *Slight* Evidence was based on 7 *high* and *medium* confidence studies. Kidney weights, particularly in male rats, were changed following short-term and chronic exposure. Most studies found no histological changes correlating with increased kidney weight, but one chronic study provides convincing evidence that the kidney can be damaged by	

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 (3/4). Absolute kidney weight in female rats was either increased (2/4) or decreased (2/4). Changes in relative kidney weight were also observed in rats. For male rats, only increases in relative kidney weight were observed (3/4). For female rats, increases (2/4) and decreases (1/4) were observed.			exposure to PFOA. Renal lesions were mainly observed in exposed females, except for increased tubule mineralization which was observed in both sexes. Clinical chemistry and urinalysis endpoints do not provide strong evidence of damage to kidney structure or function. Changes in clinical chemistry parameters such as increased serum BUN without further evidence of kidney dysfunction (e.g., increased serum creatinine) are not generally considered adverse and may be more reflective of changes in water consumption than effects on the kidney.	
Histopathology 2 *High* confidence studies 2 *Medium* confidence studies	Most studies found no histopathological changes in the kidneys of treated animals (3/4), including one developmental study in mice, one short-term study in rats, and one chronic study in rats. However, one *high* confidence chronic study found evidence of kidney damage in male and female rats following PFOA exposure. Increased hyperplasia and necrosis of the renal papilla were observed in female rats. Increased renal tubule mineralization was noted in both sexes.	• *High* and *medium* confidence studies	• 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**	
Serum biomarkers 2 *High* confidence studies 3 *Medium* confidence studies	Changes in serum BUN were observed in several studies (3/5); however, increases in BUN may be contributed to decreased water consumption. A decrease in serum creatinine was observed (1/3) but may be attributed to decreased food intake and body weight. Decreased serum creatine kinase (2/3) and increased serum ammonia (1/1) were also noted.	• *High* and *medium* confidence studies	• *Incoherence* of findings in serum biomarkers of renal function • Changes in water consumption, food intake, and body weight may limit ability to interpret these responses		
Urinalysis 2 *Medium* confidence studies	One study in rats measured several urinary endpoints at different timepoints over 2 years of exposure to PFOA and found no exposure-related changes. No changes in urinary creatinine were observed in mice exposed to PFOA during gestation.	• *Medium* confidence studies	• *Limited number* of studies examining outcome		

Notes: BUN = blood urea nitrogen; CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; GFR = glomerular filtration rate; NHANES = National Health and Nutrition Examination Survey.

C.6 Hematological

EPA identified eight epidemiological and three animal studies that investigated the association between PFOA and hematological effects. Of the epidemiological studies, three were classified as *medium* confidence, two as *low* confidence, and three were considered *uninformative* (Section C.6.1). Of the animal studies, one was classified as *high* confidence, and two were considered *medium* confidence (Section C.6.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.6.1 Human Evidence Study Quality Evaluation and Synthesis

C.6.1.1 Introduction

The mechanisms for PFOA effects on hematological parameters might include immune suppression, shifts in nutrients absorbed from the diet, or the influences related to other health outcomes such as cardiometabolic or kidney dysfunction (Abraham et al., 2020; Jain, 2020a; Chen et al., 2019a). PFOA has been implicated in endocrine disruption, which may affect vitamin D homeostasis (Etzel et al., 2019). It could also alter epigenetics via DNA methylation (van den Dungen et al., 2017). The effects of PFOA on hematological outcomes may differ by characteristics such as age, gender, race, and genetics.

Hematological health outcomes in humans were previously reviewed in the 2016 PFOA HESD for PFOA (U.S. EPA, 2016c). Six occupational studies and one general population study, published prior to 2010, provided hematology data. No statistically significant associations between PFOA exposure and hematology parameters were identified. The 2016 PFOA HESD did not specifically discuss or draw conclusions about these parameters independent of other health outcomes.

For this updated review, eight studies examined the association between PFOA and hematological health outcomes (Figure C-38). The specific hematological parameters investigated included hematology tests (calcium, erythrocytes, ferritin, fibrinogen, hematocrit, hemoglobin, iron), blood coagulation tests, Vitamin D levels and deficiency and anemia.

All studies assessed exposure to PFOA using biomarkers in blood. Samples were taken from pregnant women, children, adolescents, or adults. Most included studies were cross-sectional, meaning exposures and outcomes were evaluated during the same period. Four were from the United States, three from Europe, and one from Asia. Three studies used overlapping data from a large, ongoing survey in the United States, the NHANES (Jain, 2020a, b; Etzel et al., 2019). Etzel et al. (2019) (N = 7,040) used 2003–2010 NHANES data for adolescents and adults 12 and over (Etzel et al., 2019), and Jain (2020a) (N = 11,251) and Jain (2020b) (N = 10,644), used 2003–2016 NHANES data for adults 20 years and older (Jain, 2020a, b). Also in the United States, Khalil et al. (2018) used data on 48 obese children at 8–12 years old from a hospital lipid clinic in Dayton, Ohio. Abraham et al. (2020) included 101 healthy 1-year-old German children in the Berlin area, including 27 children living near a former copper smelting site. Jiang et al. (2014) recruited 141 pregnant women in Tianjin, China. Chen et al. (2019a) conducted a pilot study with 1,430 male and female adults from the island of Hvar, off the coast of Croatia.

Convertino et al. (2018) conducted a six-week trial with experimental exposure to APFO among late-stage cancer patients at two medical centers in Glasgow and Dundee, Scotland.

C.6.1.2 Study Quality

Several considerations were specific to evaluating the quality of studies on hematological parameters. Important considerations included the influence of diet, supplement or medication use, adiposity (due to lipid binding), disease status, and socioeconomic. In particular, the duration of breastfeeding is expected to be associated with both PFOA exposure and nutrition intake (Abraham et al., 2020). The blood matrix (whole blood vs. plasma or serum) could also affect the interpretation of results. 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., 2018b), current blood concentrations are expected to correlate well with past exposures.

There are eight 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 hematological effects. Study quality evaluations for these eight studies are shown in Figure C-38.

On the basis of the considerations mentioned, three studies were classified as *medium* confidence, two as *low* confidence, and three as *uninformative*. The *low* confidence had deficiencies in confounding and limited sample sizes. Convertino et al. (2018) did not control for confounding, although this concern is somewhat attenuated by the prospective trial study design wherein investigators manipulated the exposure levels. Khalil et al. (2018) was affected by a small sample size, and potential residual confounding attributable to differences in participants' socioeconomic status (SES). Three studies were rated as *uninformative* for hematological outcomes. For Jain (2020b), the use of PFOA as the dependent variable and health outcomes as the independent (predictive) variable rendered the study uninformative for hazard assessment (Jain, 2020b). Abraham et al. (2020) and Jiang et al. (2014) only performed unadjusted correlation analyses and therefore did not consider the influence of potential confounding factors.

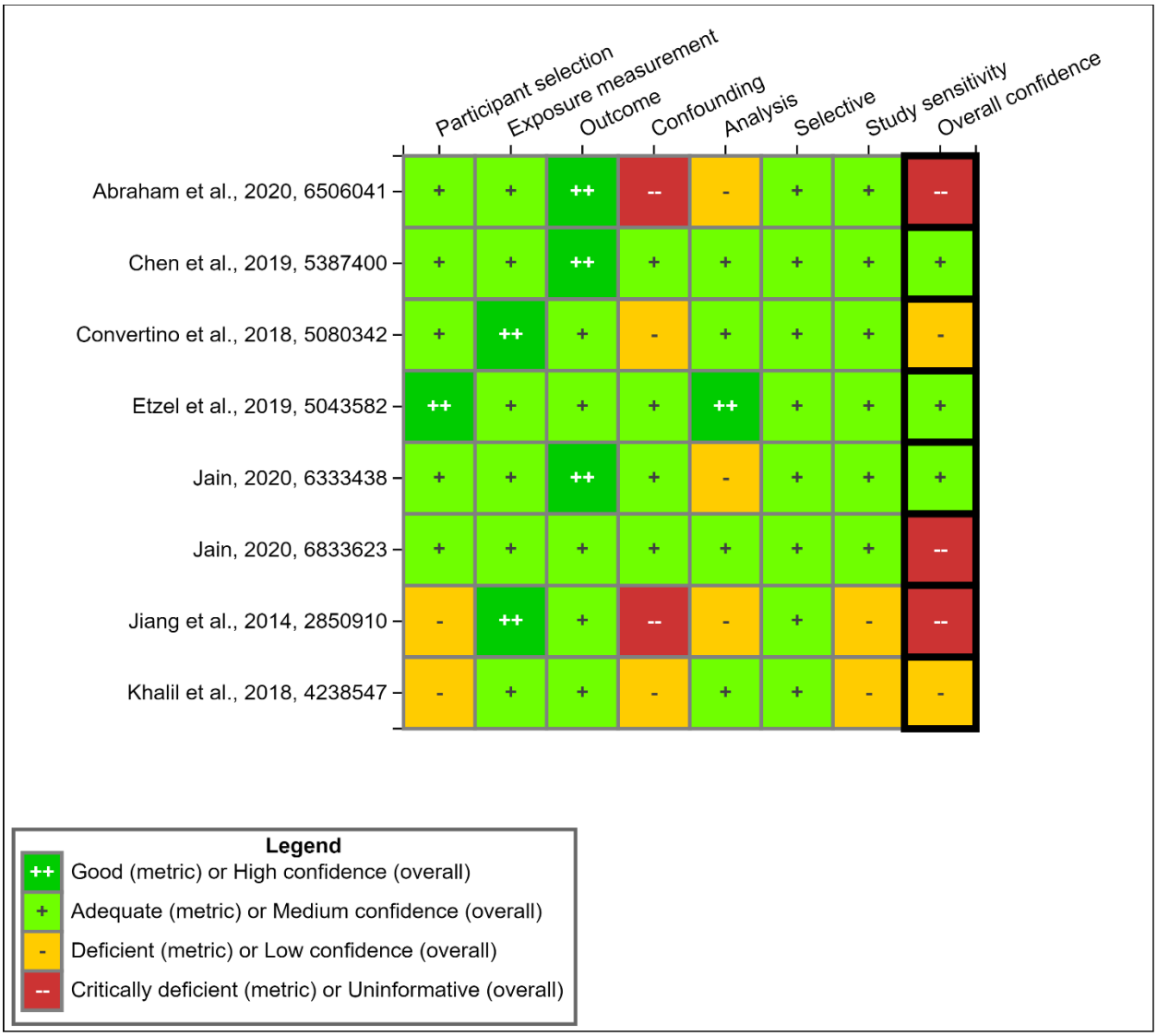


Figure C-38. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hematological Effects

Interactive figure and additional study details available on HAWC.

C.6.1.3 Findings

Two studies examined levels of 25-hydroxy vitamin D or vitamin D deficiency and observed no associations. In adolescents and adults from NHANES (2003–2010), Etzel et al. (2019) observed non-significant positive prevalence Ors for vitamin D deficiency and decreases in levels 25-hydroxy vitamin D pre doubling of PFOA. A *low* confidence study, Khalil et al. (2018) observed a non-significant positive association between PFOA exposure and 25-hydroxy vitamin D levels in 8–12-year-old United States children.

In adults from NHANES (2003–2016), Jain (2020a) observed small statistically significant increases in whole blood hemoglobin (WBHGB) levels (Appendix D). This was true for participants with or without anemia, and the magnitude of the association was larger among

those anemics. For example, associations (slopes) between PFOA and WBHGB for anemic females were more than 5 times higher as compared with non-anemic females (beta = 0.03413 vs. 0.00605). Anemia was defined as WBHGB concentrations < 12 g/dL for females or < 13 g/dL for males. Jain (2020a) also evaluated impact of deteriorating kidney function, by stratifying results by stages of GF. For anemic females, association between WBHGB and PFOA concentrations were uniformly positive across worsening stages of renal failure. For anemic males, association between WBHGB and PFOA concentrations were positive except at GF-3A ($45 \leq$ eGFR < 60 mL/min/1.73 m^2). Overall, the association between WBHGB and PFOA followed U-shaped distributions. Hemoglobin levels were also examined in pregnant women in Jiang et al. (2014). Significant positive correlations were observed between total PFOA and hemoglobin levels ($r = 0.192$, $p < 0.05$) and albumin ($r = 0.251$, $p < 0.01$), although these results did not consider the influence of confounding factors and should be interpreted with caution.

Chen et al. (2019a) observed non-significant decreases in serum calcium levels among Croatian adults.

Several markers of liver function blood clotting tests were examined in a phase 1 dose-calculation trial using APFO. In this *low* confidence study, Convertino et al. (2018), observed no clear differences in plotted probabilistic fibrinogen, prothrombin time (PPT), or activated partial thromboplastin time (aPPT) at various PFOA concentrations.

C.6.2 Animal Evidence Study Quality Evaluation and Synthesis

There is one study 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 hematological effects. Study quality evaluations for these three studies are shown in Figure C-39.

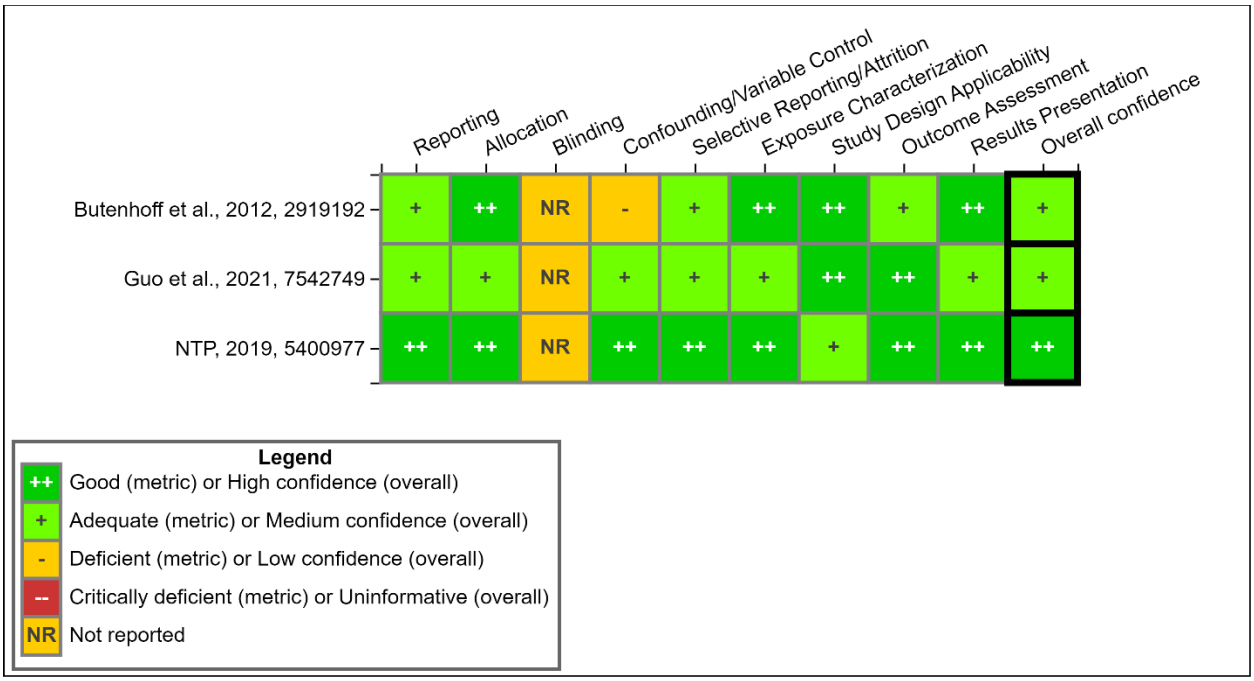


Figure C-39. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hematological Effects

Interactive figure and additional study details available on HAWC.

Hematological measures, along with other biomarkers or histopathological findings, may be informative for assessment of the health and function of blood-forming tissues such as the spleen and bone marrow. The focus of this section is clinical hematological endpoints including alterations in hemoglobin and hematocrit levels and changes in red blood cell production and structure. Five oral studies in rodents or monkeys with short-term to chronic exposure durations evaluated the effects of PFOA on the hematological system. Significant changes in some erythron parameters following PFOA exposure to rats at dose levels as low as 0.625 mg/kg/day (NTP, 2019a) and increases in aPPT and PPT in monkeys exposed to 30 mg/kg/day (Butenhoff et al., 2002) suggest the potential for the hematological system to be a target of PFOA toxicity.

In a 28-day study, significant decreases in erythrocyte count, hematocrit, and hemoglobin (≥1.25 mg/kg/day), reticulocytes (≥0.625 mg/kg/day), and mean cell volume (10 mg/kg/day) were observed in male Sprague Dawley rats (Figure C-40) (NTP, 2019a); however, the majority of these effects, except reticulocyte counts, were within 10% of control levels. Significant decreases in erythrocyte count (100 mg/kg/day), hematocrit (≥6.25 mg/kg/day), and hemoglobin (≥12.5 mg/kg/day) were observed in female rats from the same 28-day study, but the effects were also within 10% of control levels (Figure C-40) (NTP, 2019a). Loveless et al. (2008) administered PFOA to male Sprague-Dawley rats or male CD-1 mice at dose levels 0, 0.3, 1, 10, or 30 mg/kg/day for 29 days. In rats, hemoglobin and hematocrit were significantly decreased (91%–92% of control) at 10 and 30 mg/kg/day and a significant increase in reticulocytes (197% of control) was observed with 30 mg/kg/day. No other altered hematological effects were reported in rats or mice, though there was a slight increase in granulocytic bone marrow hyperplasia in mice dosed with 10 or 30 mg/kg/day.

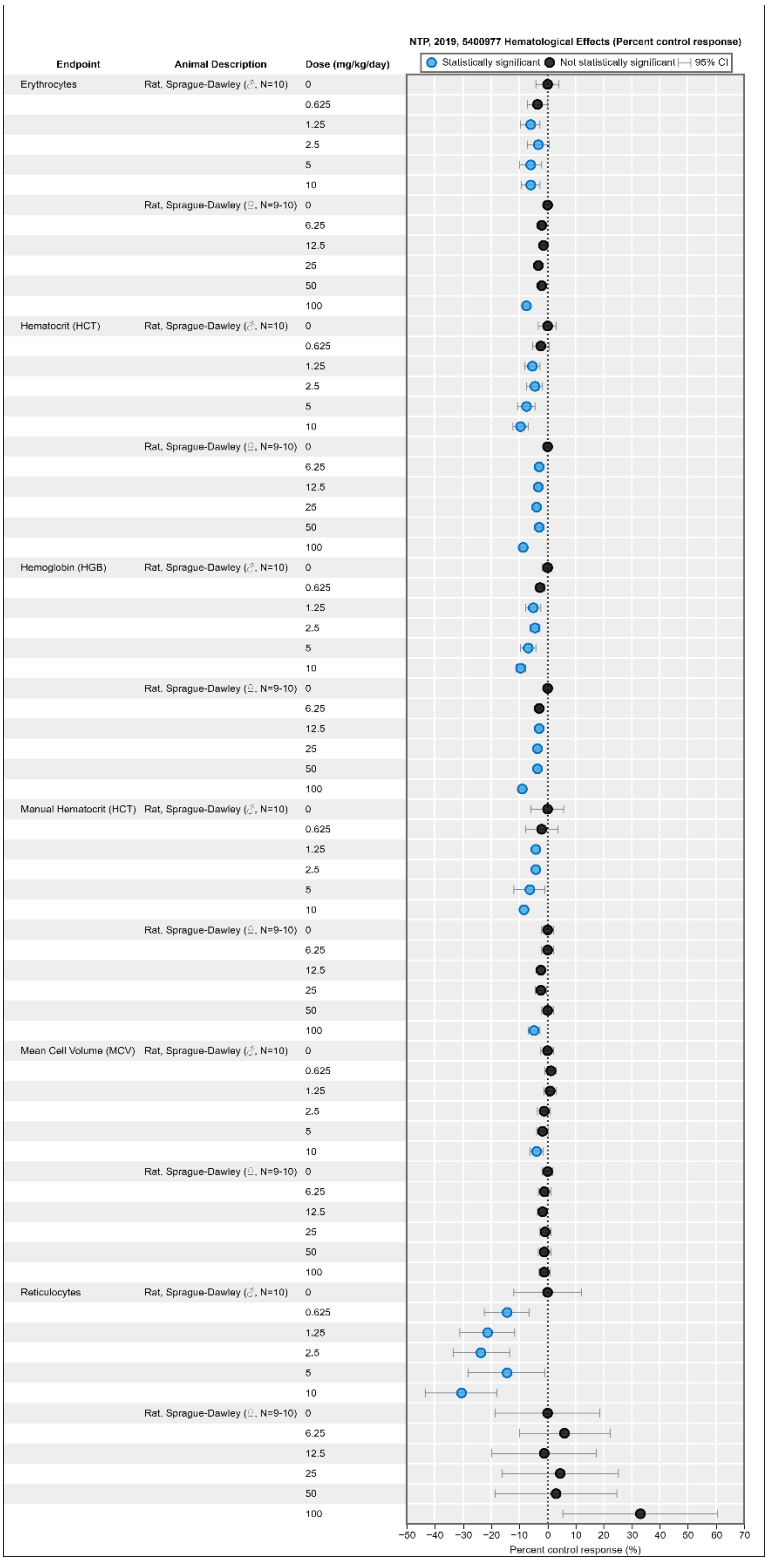


Figure C-40. Hematological Effects in Male and Female Sprague-Dawley Rats Dosed with PFOA for 28 Days as Reported by NTP (2019a)

Interactive figure and additional study details available on HAWC.
HCT = hematocrit; HGB = hemoglobin; MCV = mean cell volume; CI = confidence interval.

Dietary administration of 30 or 300 ppm PFOA (equivalent to 1.3 or 14.2 mg/kg/day in males and 1.6 or 16.1 mg/kg/day in females) to male and female Sprague-Dawley rats for 2 years produced mild or inconsistent effects on hematology (Butenhoff et al., 2012). The authors provided data on red blood cell counts, hemoglobin, and hematocrit at 3, 6, 12, 18, and 24 months, though only time points prior to 52 weeks are considered as clinical pathology testing in aging rodents may be affected by naturally occurring disease (Weingand et al., 1992). In males, Butenhoff et al. (2012) reported significant decreases in red blood cell counts in both dose groups at 6 months and in the 14.2 mg/kg/day group at 12 months. These decreases did not exceed 10% change from controls. Similarly, the authors reported significant decreases in hematocrit in both dose groups at 3 months and with 14.2 mg/kg/day at 12 months, but these changes also did not exceed 10% difference from controls. There was no observed effect on hemoglobin levels at any time point. In females, significant changes were often noted in the 1.6 mg/kg/day dose group but not the 16.1 mg/kg/day group. For example, minimal decreases in hemoglobin, hematocrit, and red blood cell counts were observed at 6 months in the 1.6 mg/kg/day group but not the high-dose group. Dose-dependent decreases in these three parameters were observed at the 12-month time point, though the magnitude of change did not exceed 10% difference from controls. Discussions on other parameters related to immune system function from this study are provided in (see Toxicity Assessment, (U.S. EPA, 2024b)).

In a 28-day study, significant decreases in serum levels of hemoglobin, bilirubin, platelets, and iron were observed in 6- to 8-week-old mice exposed to PFOA (0.4–10 mg/kg/day) via oral gavage (Guo et al., 2021b). Dose-dependent reductions in platelets were significantly reduced in animals by day 7 (≥2mg/kg/day) and all treatment groups by day 28. Reductions in hemoglobin were measured as early as day 7 in the highest dose tested (10 mg/kg/day), but by day 21 all exposure groups (≥0.4 mg/kg/day) experienced depletion. This decrease in hemoglobin also correlated to a dose-dependent reduction in serum iron content and significantly elevated bilirubin (10 mg/kg/day). Guo et al. (2021b) considered that reductions in hemoglobin, iron, and platelets and elevation of bilirubin are consistent with the pathophysiology of anemia.

In a 90-day study with rhesus monkeys, significant increases in aPPT and PPT were observed at 30 mg/kg/day at 1-month analyses (Goldenthal et al., 1978); at 3 months, the same effects were seen in the lone surviving monkey at 30 mg/kg/day (early mortality at the high-dose level of 100 mg/kg/day precluded hematological analyses). A 182-day oral (capsule) study in male cynomolgus monkeys reported no hematological findings at dose levels up to 20 mg/kg/day (Butenhoff et al., 2002).

C.6.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse hematological outcomes in the 2016 PFOA HESD (U.S. EPA, 2016c). There are four 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 hematological effects. A summary of these studies is shown in Figure C-41. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOA leads to hematological effects.

Mechanistic Pathway	Human	In Vitro	Grand Total
Atherogenesis And Clot Formation	1	1	2
Big Data, Non-Targeted Analysis	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	1	1
Oxidative Stress	0	1	1
Grand Total	1	3	4

Figure C-41. Summary of Mechanistic Studies of PFOA and Hematological Effects

Interactive figure and additional study details available on HAWC.

C.6.4 Evidence Integration

The evidence evaluating an association between PFOA exposure and hematological effects in humans is considered *indeterminate* based on limited number of studies and inconsistent and non-significant findings. Many of the outcomes were not studied in more than one study, making coherence hard to establish. Two studies that examined 25-hydroxy vitamin D levels reported mixed non-significant effects. There is evidence of an association between increased PFOA and slightly increased WBHGB, particularly among anemic adults from a large NHANES study (Jain, 2020a). Increases in hemoglobin and albumin may also affect pregnant women (Jiang et al., 2014). However, it is unclear whether the observed changes are clinically adverse.

The animal evidence for potential hematological effects is *indeterminate.* There is limited data on the hematological system being a target for PFOA in animal models, inconsistent results between sexes and species, and generally minimal effects observed (within 10% of the control). In the four studies that reported effects on red blood cells in rats (Guo et al., 2021b; NTP, 2019a; Butenhoff et al., 2012; Loveless et al., 2008), results were all within 10% of the controls except for the decrease in reticulocytes observed in male rats in NTP (2019a).

C.6.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOA exposure can cause hematological effects in humans under relevant exposure circumstances (Table C-10).

Table C-10. Evidence Profile Table for PFOA Hematological 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 C.6.1)					⊙⊙⊙ ***Inadequate Evidence***
25-hydroxy vitamin D 1 *Medium* confidence study 1 *Low* confidence study	Two studies examined changes in serum 25-hydroxy vitamin D. Results in both children and adults were inconsistent across exposure groups and largely imprecise.	• *Medium* confidence study	• *Inconsistent direction* of effects across studies • *Low* confidence study • *Imprecision* of findings • *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence for hematological effects is based on two studies reporting decreased 25-hydroxy vitamin D and one study reporting increased WBHGB. Considerable uncertainty due to limited number of studies and unexplained inconsistency across studies and endpoints.	*Primary basis*: Evidence in humans and animals were limited and largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Anemia and whole blood hemoglobin (WBHGB) 1 *Medium* confidence study	One study observed significant associations with increased WBGHB, particularly among anemic adults.	• *Medium* confidence study • *Consistent direction* of findings across subpopulations	• *Limited number* of studies examining outcome		
Serum electrolytes 1 *Medium* confidence study	One study observed a non-significant inverse association with serum calcium concentrations.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Liver function blood clotting 1 *Low* confidence study	Associations with concentrations of probabilistic fibrinogen, PPT, and aPTT were imprecise.	• No factors noted	• *Low* confidence study • *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.6.2)					
Complete blood count 1 *High* confidence study 2 *Medium* confidence studies	In a chronic and short-term exposure study, decreased hematocrit levels (2/2) were observed in male and female rats but this includes transient effects at only the 3-month timepoint in the chronic	• *High* and *medium* confidence studies • *Dose-dependent* response	• *Inconsistent direction* of effects across studies • *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited and inconsistent with direction of effect for hematological	

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**	
	study (1/1). One short-term study in rats reported a dose-response decrease in hematocrit in males but not females (1/1). Most studies found exposure associated decreases in hemoglobin (2/3) after 28 d in males (2/3) and females (1/2). RBC was decreased (2/2) in rats of both sexes at the highest dose in a short-term study (1/1), and at the 6-mo timepoints in a chronic study (1/1). One short-term study in rats found decreased mean cell volume in males only at the highest dose tested. One study in male mice found a dose-dependent decrease in platelets following a 28-day exposure to PFOA.			endpoints in animal models.	
Serum iron 1 *Medium* confidence study	One 28-day study in male mice observed a dose-dependent decrease in serum iron levels (1/1).	• *Medium* confidence study • Dose-dependent response	• *Limited number* of studies examining outcome		

Notes: aPTT = activated partial thromboplastin time; PPT = prothrombin time; RBC = red blood cell; WBHGB = whole blood hemoglobin.

C.7 Respiratory

EPA identified five epidemiological and four animal studies that investigated the association between PFOA and respiratory effects. Of the epidemiological studies, all five were classified as *medium* confidence (Section C.7.1). Of the animal studies, two were classified as *high* confidence, and two were considered *medium* confidence (Section C.7.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.7.1 Human Evidence Study Quality Evaluation and Synthesis

C.7.1.1 Introduction

Respiratory health can be ascertained by several measurements. The most informative are measurements of pulmonary function (e.g., lung volume and airflow measures determined by spirometry, as well as respiratory sounds, sputum analysis, and blood gas tension) or pulmonary structure (e.g., lung weight, histopathology, and chest radiography), while respiratory symptoms (shortness of breath, cough/presence of sputum, chest tightness), history of respiratory illnesses, and respiratory mortality have low specificity or sensitivity.

In the 2016 Health Assessment for PFOA (U.S. EPA, 2016c), no epidemiological evidence on pulmonary function was available; the C8 Science Panel concluded there was no probable link between PFOA exposure and respiratory health effects (e.g., chronic obstructive pulmonary disease (COPD)) (C8 Science Panel, 2012).

For this updated review, six new epidemiologic studies investigated the association between PFOA and respiratory effects: five studies targeting the general population reported on several lung function outcomes, and one occupational study examined COPD (Steenland et al., 2015) (Appendix D). All studies measured PFOA using biomarkers in blood. Three studies were mother-child cohort studies conducted in Europe (Agier et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018), one was a cross-sectional case-control study conducted in Taiwan (Qin et al., 2017); one was a cross-sectional study of adolescents and young adults residing near the WTC (Gaylord et al., 2019), and one was an occupational cohort study of workers and former workers at a chemical plant in West Virginia (Steenland et al., 2015). Five studies examined lung function measures in children and young adults, including forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio, forced expiratory flow at 25%–75% (FEF 25%–75%), peak expiratory flow rate (PEF) measured, lung volume and resistance at oscillation frequencies of 5 Hz or 20Hz, lung function at birth and severity of obstructive airways disease.

Studies that examined respiratory illnesses or symptoms reflecting immune system responses (e.g., asthma and allergies) and respiratory tract infections (e.g., cough) are discussed in the Toxicity Assessment (U.S. EPA, 2024b).

C.7.1.2 Study Quality

There are five 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 respiratory effects. Study quality evaluations for these five studies are shown in Figure C-42.

All five studies identified since the last assessment were classified as *medium* confidence. The *medium* confidence studies had minor deficiencies, including concerns that co-exposures in the WTC disaster could confound the results (Gaylord et al., 2019), reduced sensitivity because of low exposure levels and narrow ranges (Impinen et al., 2018), or concerns with potential bias in selection of non-asthmatic controls (Qin et al., 2017).

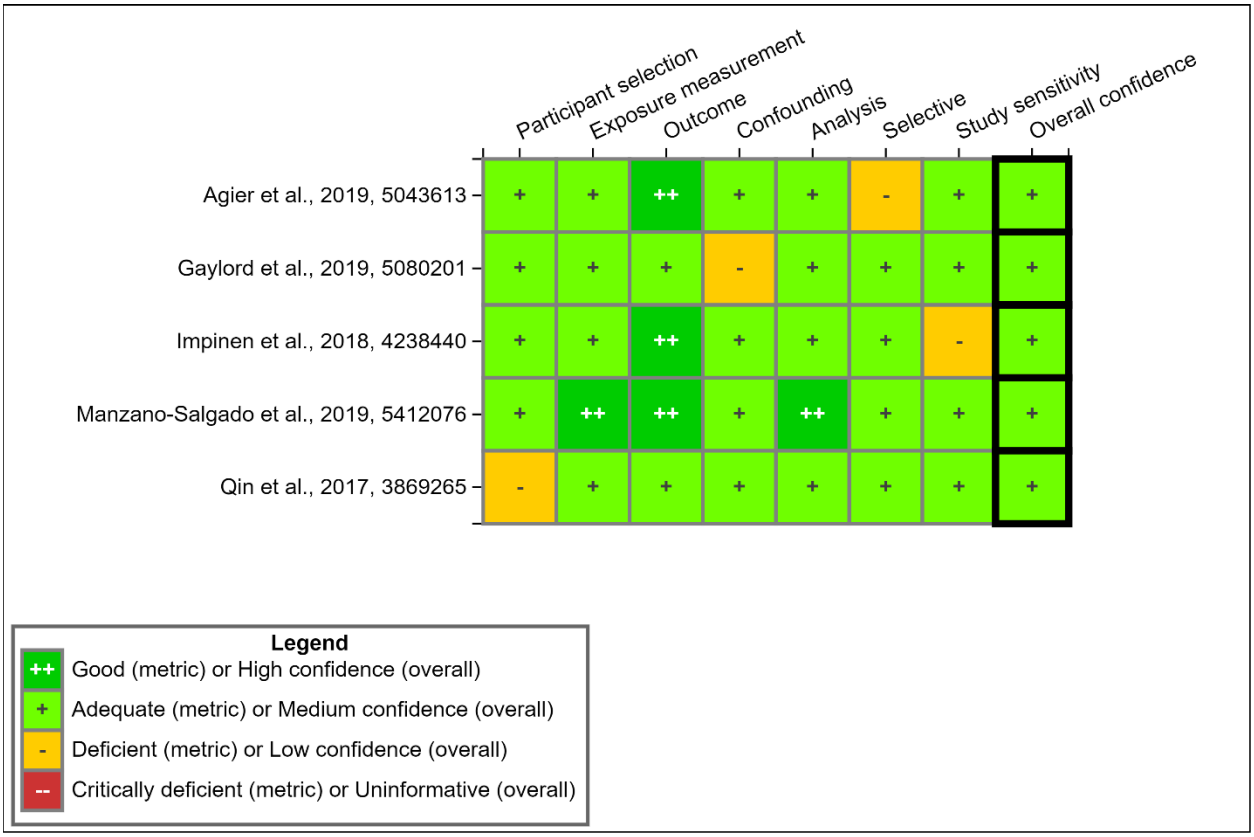


Figure C-42. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Respiratory Effects

Interactive figure and additional study details available on HAWC.

C.7.1.3 Findings From Children and Adolescents

Four studies examined respiratory health effects in children up to 15 years old (Agier et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018; Qin et al., 2017), and one examined adolescents and young adults ages 13–22 years (Gaylord et al., 2019) (Appendix D).

Of the four studies examining FEV1, all reported negative associations (i.e., decrease in FEV1 with higher PFOA levels). In children ages 6–12 years, Agier et al. (2019) reported statistically significant associations with prenatal exposure (beta per log2 increase PFOA = −1.4, 95% CI: −2.7, −0.1), but not for postnatal exposure. Qin et al. (2017) observed statistically significant associations for children ages 10–15 years with asthma (beta per ln increase PFOA = −0.10, 95%

CI: −0.19, −0.02), and in boys with asthma, but not in girls with asthma. There was also a significantly decreasing trend by quartiles of PFOA in children with asthma (p-trend = 0.002), but not observed in children without asthma. Negative non-significant associations were observed in two of the four studies (Gaylord et al., 2019; Manzano-Salgado et al., 2019).

For other lung function measures examined, there was limited evidence of associations. Manzano-Salgado et al. (2019) reported a statistically significant association between maternal PFOA concentrations and FVC at age 4 (beta = −0.17, 95% CI: −0.34, −0.01), but not for FVC at age 7 or for other measures of lung function, at either age 4 or age 7. Qin et al. (2017) observed statistically significant associations for FEF25%–75% (beta = −0.223, 95% CI: −0.4, −0.045) and a significant decreasing trend with quartiles of PFOA (p-value = 0.014) in children with asthma, but not for FVC or PEF or for any lung function measures in children without asthma. Impinen et al. (2018) reported a statistically significant association between prenatal PFOA exposure and severe obstructive airways disease at age 2 measured by the Oslo Severity Score (OSS), but only for the lowest severity category (OSS 1–5) (OR per log2 increase PFOA = 1.43, 95% CI: 1.03, 1.98). The study also reported a non-significant increase in odds of reduced lung function at birth, as measured by tidal flow volume. Other lung function measures (i.e., FVC, FVC/FEV1, lung resistance, total lung capacity, functional residual capacity, and residual volume) in adolescents and young adults residing near the WTC were all inversely associated with PFOA exposure, but none were significant (Gaylord et al., 2019).

C.7.1.4 Findings From the General Adult Population

One occupational cohort study (Steenland et al., 2015) assessed incidence of COPD and cumulative PFOA exposure in adult workers and former workers at a chemical plant in West Virginia. The study observed a non-significant increased risk of COPD in no-lag models, but no clear pattern of association in 10-year lag models.

C.7.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two 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 respiratory effects. Study quality evaluations for these four studies are shown in Figure C-43.

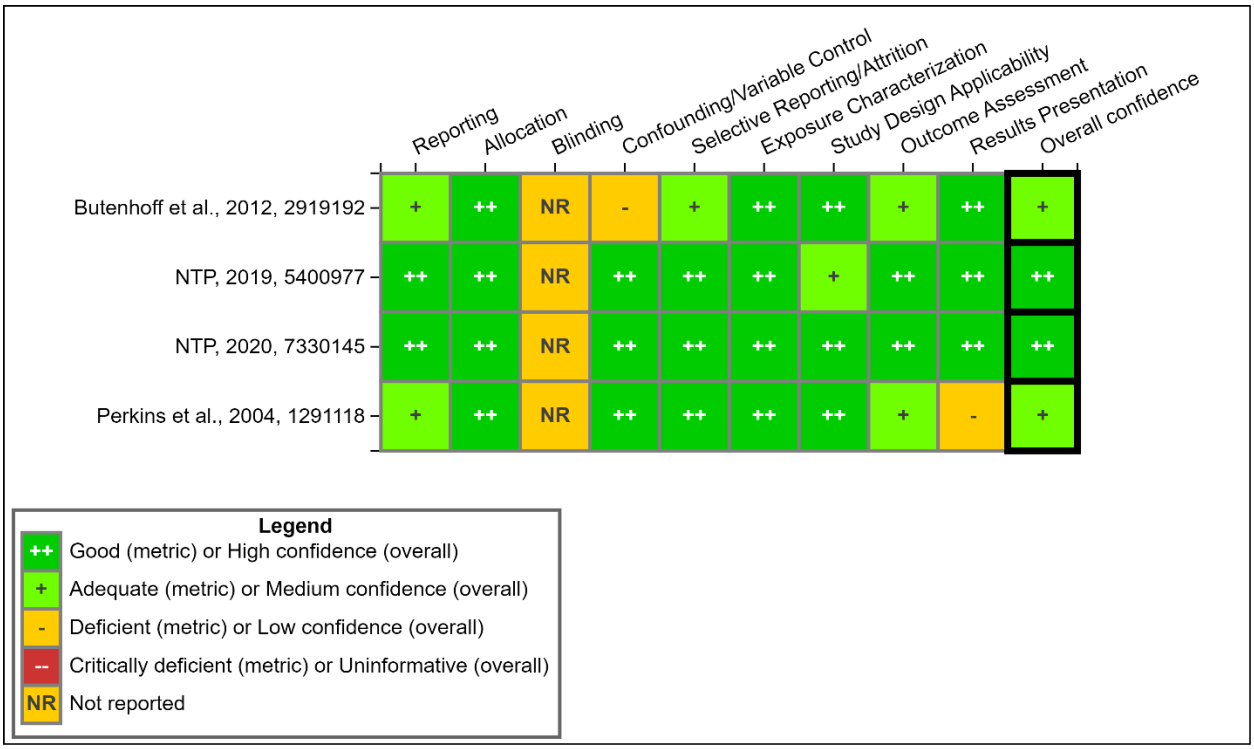


Figure C-43. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Respiratory Effects

Interactive figure and additional study details available on HAWC.

There is evidence suggesting oral PFOA exposure may adversely affect the nasal, olfactory, and pulmonary systems, though the database examining respiratory toxicity is generally limited. Adverse histopathological effects in the lung and nose were observed in short-term and chronic studies in adult rats (NTP, 2019a; Butenhoff et al., 2012; Cui et al., 2009). However, several other studies, including two chronic toxicity studies in rats and one developmental toxicity study in mice, did not report treatment-related alterations in the respiratory system of adults or neonates after treatment with PFOA (NTP, 2020; Yahia et al., 2010; Perkins et al., 2004).

In a 2-year rat feeding study, Butenhoff et al. (2012) observed significantly increased incidences of alveolar macrophages and pulmonary hemorrhage in males in the high-dose group (300 ppm, equivalent to 14.2 mg/kg/day) (Table C-11). However, the incidences of perivascular mononuclear infiltrate and interstitial pneumonia were decreased in both exposure groups. Incidence of perivascular mononuclear infiltrate was also reduced in females, though only in the low-dose group (1.6 mg/kg/day, 4% incidence compared with 26% in controls). There was also a significant increase in the incidence of lung vascular mineralization in females, though this was again observed only in the low-dose group (44%, 76%, and 52% incidence in the 0, 1.6, and 16.1 mg/kg/day groups, respectively). Altered lung histopathology in males was considered a co-critical effect for this study in derivation of candidate RfDs for PFOA (U.S. EPA, 2016c), though Butenhoff et al. (2012) questioned whether these effects were directly related to PFOA treatment. Two additional chronic dietary studies in rats found no treatment-related effects on lung weight or histopathology (NTP, 2020; Perkins et al., 2004). NTP (2020) reported significant

effects on lung weight in males and females that were considered secondary to decreased body weight and not direct toxicological effects of PFOA.

Table C-11. Incidences of Nonneoplastic Pulmonary Lesions in Male Rats as Reported by Butenhoff et al. (2012)

Pulmonary Lesion	Dose		
	0 ppm (0 mg/kg/day)	30 ppm (1.3 mg/kg/day)	300 ppm (14.2 mg/kg/day)
Alveolar Macrophages	10/49 (20%)	16/50 (32%)	31/49 (63%)*
Hemorrhage	10/49 (20%)	14/49 (29%)	22/50 (44%)*
Vascular Mineralization	43/49 (88%)	43/49 (88%)	47/50 (94%)
Perivascular Mononuclear Infiltrate	21/49 (43%)	3/49 (6%)*	7/50 (14%)*
Interstitial Pneumonia	16/49 (33%)	5/49 (10%)*	7/50 (14%)

Notes:
*Statistically significant at $p \leq 0.05$.

Cui et al. (2009) observed pulmonary congestion and focal or diffuse thickening of epithelial walls in the lungs of male rats gavaged with 5 or 20 mg/kg/day PFOA for 28 days (incidence data not provided). While NTP (2019a) did not report alterations in lung weight or histopathology after dosing for 28 days, there were several effects on the nasal cavity and olfactory system that were not suggestive of gavage-related reflux (Figure C-44). Chronic active inflammation of the nasal respiratory epithelium was observed in both males and females, though these effects did not exhibit a linear dose-response relationship. 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 (NTP, 2019a). Interestingly, these nasal and olfactory effects were observed across multiple PFAS (PFOA, perfluorohexanoic acid (PFHxA), PFNA, PFBS, PFHxS) in toxicity studies conducted by NTP (2019a, b), though not in the chronic PFOA feeding study (NTP, 2020). No other studies identified during this assessment reported examinations of nasal or olfactory systems in animal models.

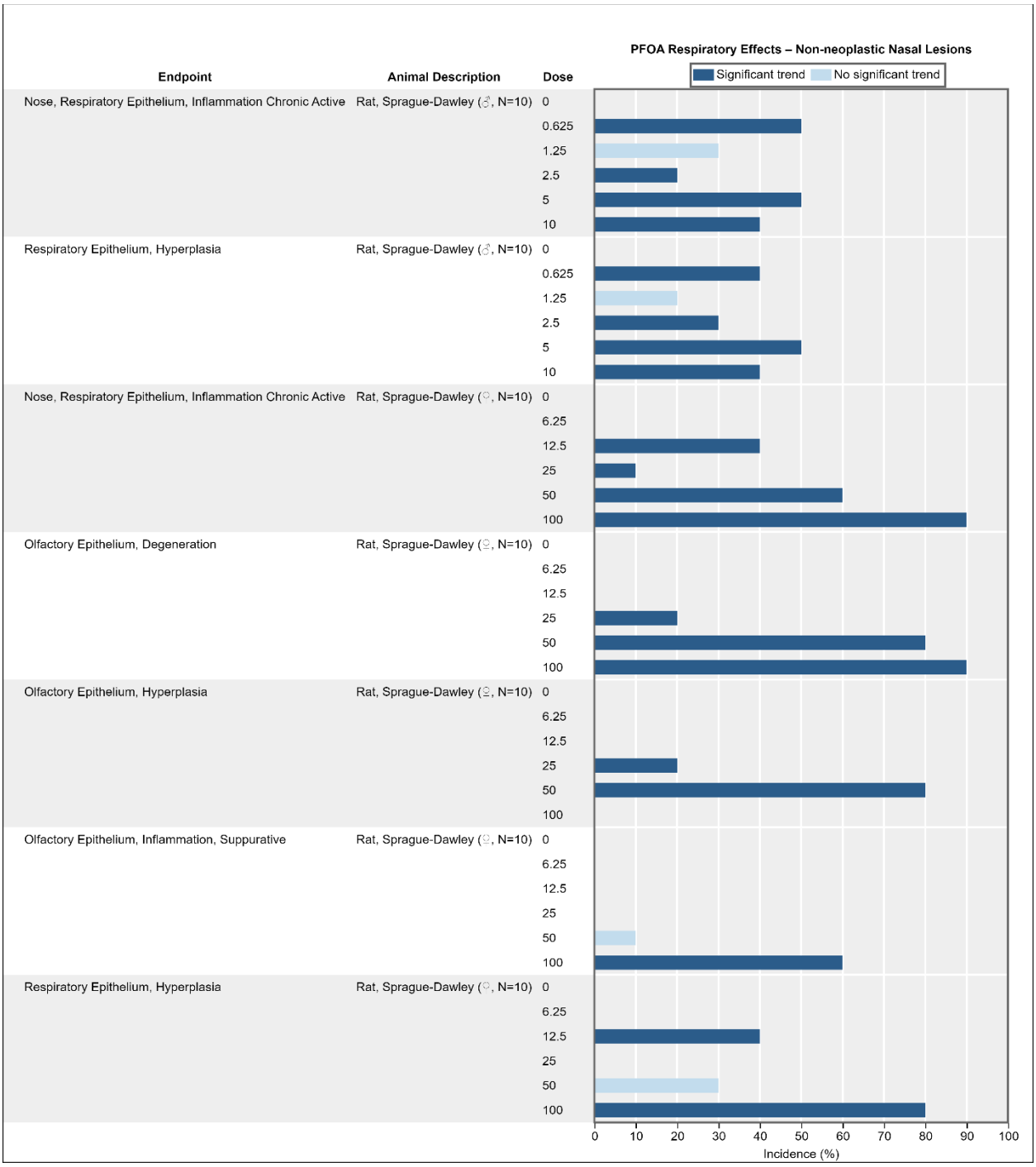


Figure C-44. Incidence of Nonneoplastic Nasal Lesions in Male and Female Sprague-Dawley Rats Following 28-day Oral Exposure to PFOA, as Reported by NTP (2019a)

Interactive figure and additional study details available on HAWC.
Statistical significance reached at $p \leq 0.05$.

There is one available study in mice that assessed potential pulmonary effects of PFOA exposure. In this developmental toxicity study, Yahia et al. (2010) saw no effect on the lungs of maternal or neonatal mice after up to 10 mg/kg/day PFOA treatment from GD 0–18.

C.7.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse respiratory outcomes is discussed in Section 3.3.4 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are three 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 respiratory effects. A summary of these studies is shown in Figure C-45. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to respiratory effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	1	2	3
Cell Signaling Or Signal Transduction	0	1	1
Inflammation And Immune Response	0	1	1
Oxidative Stress	0	1	1
Grand Total	1	2	3

Figure C-45. Summary of Mechanistic Studies of PFOA and Respiratory Effects

Interactive figure and additional study details available on HAWC.

C.7.4 Evidence Integration

The evidence of an association between PFOA exposure and respiratory effects in humans is *slight*, with an indication of decreased lung function among infants, children, and adolescents. However, the results are inconsistent and there are a small number of studies examining respiratory effects, particularly in adults. While there is some evidence of detrimental respiratory health effects, particularly in children with asthma, the available epidemiological evidence examining PFOA exposure and respiratory health is limited.

The animal evidence for an association between PFOA exposure and respiratory effects is *indeterminate*, based on inconsistencies in the available evidence in the *high* and *medium* confidence studies. While the increases in alveolar macrophages and hemorrhaging reported by Butenhoff et al. (2012) are suggestive of pulmonary damage, these results were not observed in two other chronic feeding studies in rats (NTP, 2020; Perkins et al., 2004). The authors of the study also call into question whether those effects were related to PFOA treatment (Butenhoff et al., 2012). NTP (2019a) provides data suggestive of nasal toxicity due to PFOA exposure, though the positive results in males do not follow a linear dose response and are difficult to interpret. The significant effects in females (i.e., olfactory epithelium degeneration and inflammation) occur at relatively high doses (50 mg/kg/day) compared with effects seen for other health outcomes. Therefore, it does not appear that respiratory effects are sensitive or replicable outcomes of PFOA toxicity.

C.7.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause respiratory effects in humans under relevant exposure circumstances (Table C-12). This conclusion is based on evidence of an association between PFOA and detrimental respiratory health effects, particularly in children with asthma, in a small number of epidemiologic studies with median exposure levels from 0.50 – 2.4 ng/mL; however, limited number of studies and issues with inconsistency across studies raise considerable uncertainty. Moreover, evidence in animals is sparse and largely uninterpretable regarding its relevance to humans.

Table C-12. Evidence Profile Table for PFOA Respiratory 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 C.7.1)					⊕⊙⊙ ***Evidence Suggests***
Lung function measures 5 *Medium* confidence studies	Studies in infants, children, and adolescents report significant decreases in FVC (1/5) and in FEV1 and FEF25%–75% among those with asthma (1/5). Studies in children observed significantly decreased FEV1 associated with prenatal and cross-sectional exposures (2/5). Other studies observed non-significant decreases in FEV1 (2/5).	• *Medium* confidence studies • *Consistent direction* of effect among infants, children, and adolescents	• No factors noted	⊕⊙⊙ *Slight* Several studies of *medium* confidence found evidence for decreases in lung function measures among infants, children, and adolescents, though other *medium* confidence studies did not observe significant effects. Few studies examined obstructive disease effects. Those studies that did were of lower confidence and showed imprecision of findings across exposure groups. Uncertainty remains about respiratory outcomes among adults in occupational settings and in the general population.	*Primary basis*: No evidence in animals and human evidence indicted detrimental respiratory health effects, particularly in children with asthma. However, limited number of studies and issues with imprecision across studies raise considerable uncertainty. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Obstructive disease 1 *Medium* confidence study 1 *Low* confidence study	One study in infants reported significantly increased odds of low severity obstructive airway disease. An occupational study of adult workers in a chemical plant observed no association with COPD.	• *Medium confidence study* •	• *Low* confidence study • *Imprecision* of observed effect across exposure groups in the occupational study • *Limited number* of studies examining outcome		
Evidence From In Vivo Animal Studies (Section C.7.2)					
Histopathology 2 *High* confidence studies 2 *Medium* confidence studies	Two studies evaluating chronic and short-term exposure to PFOA in male and female rats found increases in nonneoplastic lesions and inflammation in the lungs and nose	• *High* and *medium* confidence studies	• *Inconsistent direction* of results	⊙⊙⊙ *Indeterminate* Evidence was based on 4 *high* and *medium* confidence studies 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**	
	(2/4). Two additional chronic exposure studies in rats reported no changes in histopathological endpoints in the lungs (2/4).			provided inconsistent results. One study suggests alveolar macrophages and hemorrhaging increased, while two other chronic studies reported no change.	
Organ weight 2 *High* confidence studies 1 *Medium* confidence study	Studies evaluating rat lung weight found that short-term exposure to PFOA had no effect (2/3). One study found that lung weight increased in male and female rats after chronic PFOA exposure, however, this was attributed to decreased body weight and not considered a toxicological effect (1/3).	• *High* and *medium* confidence studies	• *Inconsistent direction* of results • Changes in body weight may limit ability to interpret these responses	Nasal toxicity reported in one study did not occur in a dose-dependent manner, while another required relatively high doses to occur. Lung weight was increased in one chronic exposure but occurred with decreased body weight.	

Notes: COPD = chronic obstructive pulmonary disease; FEF25%–75% = forced expiratory flow at 25%–75%; FEV1 = forced expiratory volume; FVC = forced vital capacity.

C.8 Musculoskeletal

EPA identified eight epidemiological and one animal studies that investigated the association between PFOA and musculoskeletal effects. Of the epidemiological studies, six were classified as *medium* confidence and two were considered *low* confidence (Section C.8.1). The animal study was considered *low* confidence (Section C.8.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.8.1 Human Evidence Study Quality Evaluation and Synthesis

C.8.1.1 Introduction

Musculoskeletal health outcomes include bone mineral density, risk of bone fractures, and risk of osteoarthritis. Osteoporosis (characterized by weak, brittle bone) and osteoarthritis disproportionately affect women, older individuals, and certain racial/ethnic groups (Khalil et al., 2016; Uhl et al., 2013).

The 2016 PFOA HESD (U.S. EPA, 2016c) did not previously evaluate musculoskeletal health outcomes in humans. The C8 Science Panel (C8 Science Panel, 2012) concluded there is no probable link between PFOA and osteoarthritis.

For this updated review, nine studies (nine publications) examined the association between PFOA exposure and musculoskeletal health outcomes. Different study designs were used; one was a cohort study (Jeddy et al., 2018), one used cross-sectional and prospective analyses (Hu et al., 2019), and the remainder were cross-sectional. All studies measured PFOA in blood components (i.e., blood, plasma, or serum), and one study (Di Nisio et al., 2019) measured PFOA in semen. Three studies (Khalil et al., 2016; Lin et al., 2014; Uhl et al., 2013) used data from participants in NHANES, but the study years and outcomes examined in these studies did not overlap. Other studies used data from various cohorts for cross-sectional analyses, including Project Viva (Cluett et al., 2019), the POUNDS Lost clinical trial (Hu et al., 2019), and the ALSPAC (Jeddy et al., 2018). The studies were conducted in different populations, including participants from England, Italy, and the United States. The specific outcomes investigated were osteoporosis; osteoarthritis; bone mineral density; bone area, thickness (e.g., endosteal and periosteal thickness), or circumference; bone mineral content (BMC); bone stiffness; ultrasound attenuation and speed of sound (indicators of bone quality); lean body mass; height; arm span; bone fracture; and plasma concentrations of β-C-telopeptides of type I collagen, a marker for bone turnover.

C.8.1.2 Study Quality

Musculoskeletal health outcomes include bone mineral density, risk of bone fractures, and risk of osteoarthritis. Osteoporosis (characterized by weak, brittle bone) and osteoarthritis disproportionately affect women, older individuals, and certain racial/ethnic groups (Khalil et al., 2016; Uhl et al., 2013).

The 2016 PFOA HESD (U.S. EPA, 2016c) did not previously evaluate musculoskeletal health outcomes in humans. The C8 Science Panel (C8 Science Panel, 2012) concluded there is no probable link between PFOA and osteoarthritis.

There are eight 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 musculoskeletal effects. Study quality evaluations for these eight studies are shown in Figure C-46.

Different study designs were used; one was a cohort study (Jeddy et al., 2018), one used cross-sectional and prospective analyses (Hu et al., 2019), and the remainder were cross-sectional. All studies measured PFOA in blood components (i.e., blood, plasma, or serum), and one study (Di Nisio et al., 2019) measured PFOA in semen. Three studies (Khalil et al., 2016; Lin et al., 2014; Uhl et al., 2013) used data from participants in NHANES, but the study years and outcomes examined in these studies did not overlap. Other studies used data from various cohorts for cross-sectional analyses, including Project Viva (Cluett et al., 2019), the POUNDS Lost clinical trial (Hu et al., 2019), and the ALSPAC (Jeddy et al., 2018). The studies were conducted in different populations, including participants from England, Italy, and the United States. The specific outcomes investigated were osteoporosis; osteoarthritis; bone mineral density; bone area, thickness (e.g., endosteal and periosteal thickness), or circumference; BMC; bone stiffness; ultrasound attenuation and speed of sound (indicators of bone quality); lean body mass; height; arm span; bone fracture; and plasma concentrations of β-C-telopeptides of type I collagen, a marker for bone turnover

Three cross-sectional or retrospective studies (Di Nisio et al., 2019; Khalil et al., 2018; Steenland et al., 2015) classified as *low* confidence had deficiencies in participant selection, confounding, outcome measurement, and study sensitivity. Participant selection was considered a deficiency mainly due to underreporting about participation rates and participant characteristics relative to non-participants (e.g., those who died before the retrospective study was conducted). Other deficiencies included potential for outcome misclassification when the musculoskeletal outcome (taking medication for osteoarthritis) was not validated using medical records (Steenland et al., 2015); potential for residual confounding by SES; small sample sizes and limited ranges of participant exposure to PFOA (Di Nisio et al., 2019; Khalil et al., 2018).

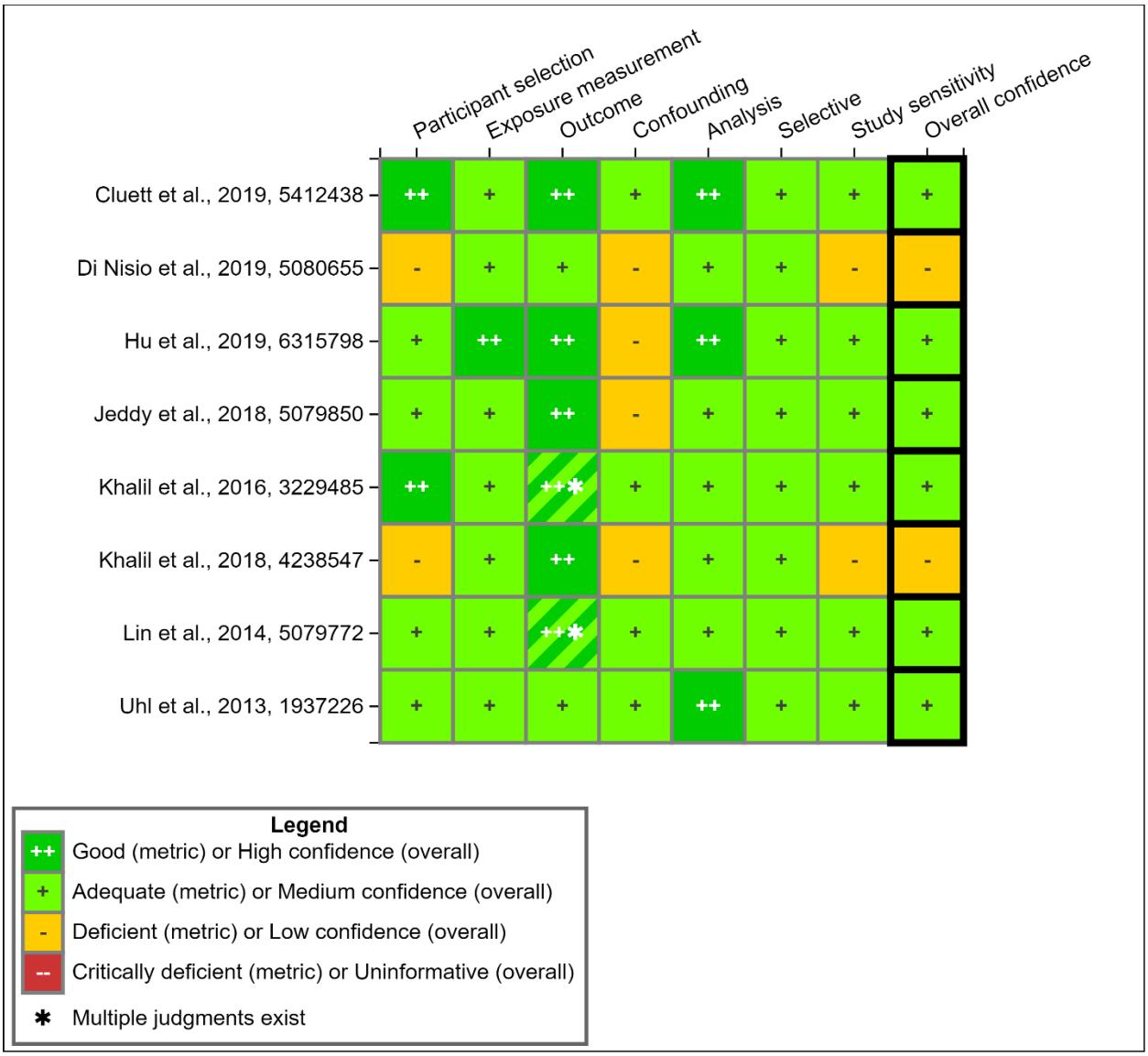


Figure C-46. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

C.8.1.3 Findings From Children and Adolescents

Three studies (Cluett et al., 2019; Jeddy et al., 2018; Khalil et al., 2018) examined musculoskeletal outcomes in children and adolescents, and two observed effects (Appendix D). While the *medium* confidence studies observed few statistically significant associations between PFOA and the musculoskeletal health outcomes examined, the associations supported a harmful, rather than beneficial, direction of effect. Cluett et al. (2019) observed a statistically significant inverse association with the areal bone mineral density (aBMD) z-score (a standardized measure of bone mineral amount relative to bone area) in children aged 6–10 years, with a greater magnitude of effect for females and was not significant for males. Inverse significant associations were also observed for BMC z-score. Jeddy et al. (2018) observed a statistically

significant inverse association between prenatal PFOA exposure and height in 17-year-old girls. A statistically significant inverse association was also observed with whole-body bone area, but this was no longer significant after adjusting for participant height.

A *low* confidence study in 8–12-year-old children from a hospital lipids clinic in Dayton, Ohio, (Khalil et al., 2018) observed non-significant inverse associations with bone stiffness index, broadband ultrasound attenuation, or speed of sound.

None of the studies identified in this updated review examined musculoskeletal outcomes in pregnant women and infants.

C.8.1.4 Findings From the General Adult Population

Five studies (Di Nisio et al., 2019; Hu et al., 2019; Khalil et al., 2016; Lin et al., 2014; Uhl et al., 2013) examined musculoskeletal outcomes in adults in the general population and three observed effects (Appendix D).

The four *medium* confidence studies observed a small number of statistically significant associations, but a consistently harmful direction of effect. The same outcomes were not examined by multiple studies. Khalil et al. (2016) observed higher odds of osteoporosis in women aged 12–80 years from NHANES (2009–2010). Uhl et al. (2013) observed statistically significantly increased odds of osteoarthritis in women aged 20–84 years in NHANES cycles (2003–2008). This was most apparent among younger premenopausal women aged 20–49, who may have differing susceptibility to endocrine disruption. An overlapping NHANES study (Lin et al., 2014) observed no statistically significant associations with history of bone fractures in women aged 20 and older. In adults aged 30–70 years from the POUNDS Lost study, Hu et al. (2019) observed small but statistically significant inverse associations with bone mineral density (or 2-year change in bone mineral density) in five of six sites examined: the spine, total hip, femoral neck, hip trochanter, and hip intertrochanteric area.

A low confidence study in young men (18–24 years) from the Padova area of northeastern Italy (Di Nisio et al., 2019) did not find evidence of associations between PFOA exposure and arm span.

C.8.1.5 Findings From Occupational Studies

One *low* confidence study of occupational exposure (Steenland et al., 2015) reported limited, conflicting evidence related to osteoarthritis in predominantly male workers: participants with elevated PFOA exposure had lower odds of self-reported osteoarthritis after a 10-year time lag, but this finding was not supported across exposure quartiles.

C.8.2 Animal Evidence Study Quality Evaluation and Synthesis

There is one study from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (Bulka et al., 2021 3603279) that investigated the association between PFOA and musculoskeletal effects. Study quality evaluations for this one study is shown in Figure C-47.

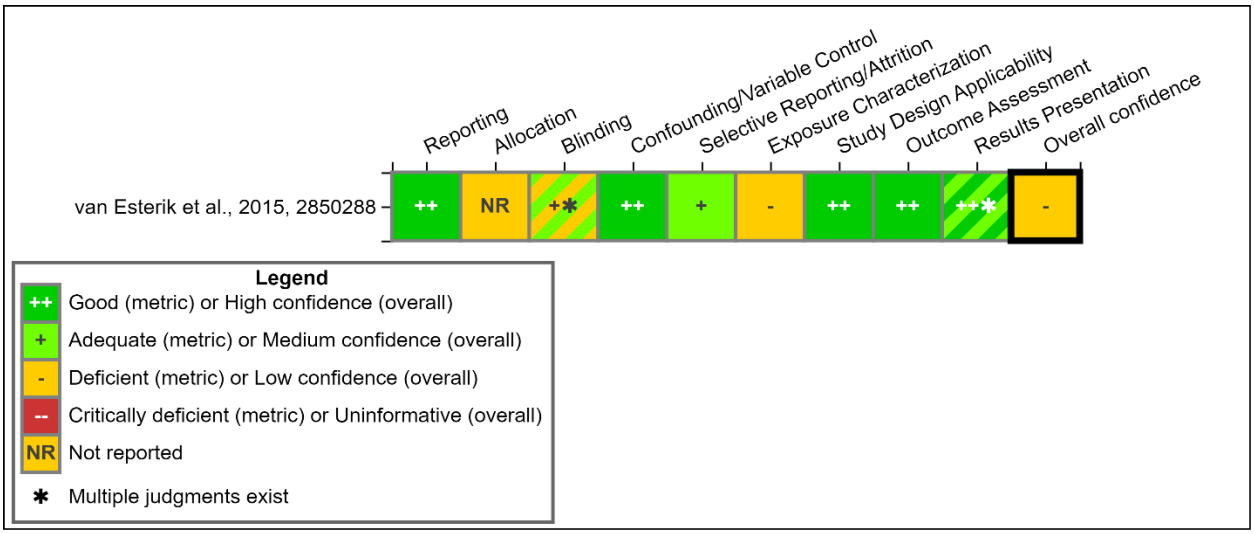


Figure C-47. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

Limited data are available on the effect of PFOA on the musculoskeletal system other than developmental skeletal defects resulting from gestational exposure that are discussed in Section 3.4.4.2 of the Toxicity Assessment (U.S. EPA, 2024b). EPA did not identify any publications that reported musculoskeletal effects outside of those associated with developmental toxicity from the 2016 PFOA HESD (Bulka et al., 2021 3603279) or the recent literature searches that were PECO relevant and determined to be *medium* or *high* confidence rating during study quality evaluation.

C.8.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse musculoskeletal outcomes in 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 musculoskeletal effects. A summary of these studies is shown in Figure C-48. Additional mechanistic synthesis will not be conducted since evidence suggests but is not sufficient to infer that PFOA leads to musculoskeletal effects.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	0	7	7
Cell Signaling Or Signal Transduction	1	3	4
Extracellular Matrix Or Molecules	1	1	2
Oxidative Stress	0	2	2
Grand Total	2	7	8

Figure C-48. Summary of Mechanistic Studies of PFOA and Musculoskeletal Effects

Interactive figure and additional study details available on HAWC.

C.8.4 Evidence Integration

There is *slight* evidence of an association between PFOA exposure and musculoskeletal effects in humans based on observed effects on bone mineral density and bone health in several *medium* confidence studies. Additionally, there is limited evidence of negative effects of PFOA on skeletal size (height and arm span) and connective tissue disorders (osteoarthritis). No epidemiological studies examined the relationship between PFOA and muscular disorders. No musculoskeletal health outcome epidemiology studies were previously reviewed in the 2016 PFOA HESD (U.S. EPA, 2016c).

Although relatively few studies have investigated musculoskeletal health outcomes related to PFOA exposure, some shared conclusions can be drawn. The observed associations in epidemiological studies were primarily between increased PFOA exposure and decreased bone mineral density (consistently among various skeletal sites), bone mineral density relative to bone area, height in adolescence, osteoporosis, and osteoarthritis. These issues with bone density may correspond with the reports of reduced ossification and skeletal deformities in developmental animal models with gestational PFOA exposure (see Toxicity Assessment, (U.S. EPA, 2024b)). Rarer outcomes, such as fracture, were not observed to be associated with PFOA exposure. In general, links to musculoskeletal disease were more commonly observed among older women. Some outcomes, such as osteoporosis and osteoarthritis, may be more relevant to examine in females, due to greater prevalence and potentially greater susceptibility to endocrine-disrupting chemicals. Study limitations led to reduced confidence in most studies; common issues included cross-sectional design or potential for residual confounding.

The animal evidence for an association between PFOA exposure and effects in the musculoskeletal system is considered *indeterminate* based on lack of information in animal models. There is one *low* confidence study where there was some change in bone length.

C.8.4.1 Evidence Integration Judgment

Overall, ***evidence suggests*** that PFOA exposure has the potential to cause musculoskeletal effects in humans under relevant exposure circumstances (Table C-13). This conclusion is based primarily on effects on bone mineral density and bone health observed in studies in humans exposed to median PFOA ranging from 0.99 to 5.4 ng/mL. Although there is some evidence of

negative effects of PFOA exposure on skeletal size (height and arm span) and connective tissue disorders (osteoarthritis, especially in older women), there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies.

Table C-13. Evidence Profile Table for PFOA Musculoskeletal 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 C.8.1)					⊕⊙⊙ ***Evidence Suggests***
Bone parameters 5 *Medium* confidence studies 1 *Low* confidence study	Decreases in bone mineral content (BMC) were observed in two studies (2/6) on children and adults. Reductions in bone mineral density (BMD) were also observed in children and adults (4/6), including site specific BMD measures. However, there was some inconsistency in direction of effect when stratified by sex. Decreases in other measures of bone health, such as the stiffness index, bone area, and broadband ultrasound attenuation, were observed in children.	• *Medium* confidence studies	• *Imprecision* of findings across studies, including for bone area association, due to wide confidence intervals and measures of BMD • *Inconsistent direction* of effect based on sex • *Low* confidence study	⊕⊙⊙ *Slight* Evidence for musculoskeletal effects is based on studies reporting reductions in bone health, bone density, lean body mass, and increased odds of osteoporosis. Uncertainties remain due to inconsistent or imprecise results, and limited evidence for fractures, size measures, and odds of osteoarthritis or osteoporosis.	*Primary basis*: *No evidence* in animals and human evidence indicated effects on bone mineral density and bone health. Although there is some evidence of negative effects of PFOA exposure on skeletal size (height and arm span) and connective tissue disorders (osteoarthritis, especially in older women), there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Fractures 1 *Medium* confidence study	Study authors reported no significant association with incidence of bone fractures.	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome		
Size measures 1 *Medium* confidence study 1 *Low* confidence study	Studies among children found significantly decreased height (1/2), but results for arm span were not precise in a study of high school students in a	• *Medium* confidence study	• *Imprecision* of findings • *Limited number* of studies examining outcome • *Low* confidence study		

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-exposure community (1/2).				
Lean body mass 1 *Medium* confidence study	Study authors reported no significant association among adolescent females.	• *Medium* confidence study	• *Limited number* of studies examining outcome		
Osteoarthritis 1 *Medium* confidence study 1 *Low* confidence study	Findings for osteoarthritis were mixed. Significantly increased odds of osteoarthritis were observed among females ages 20–84 in both continuous and categorical analyses, among the highest exposure group of females ages 20–49, and among all adults ages 20–49 (1/2). The risk of osteoarthritis was decreased in an occupational study, but findings were not precise.	• *Medium* confidence study	• *Imprecision* of findings • *Inconsistent direction* of effect based on study population • *Limited number* of studies examining outcome • *Low* confidence study		
Osteoporosis 1 *Medium* confidence study	Significant increases for the odds of osteoporosis were observed in a study of females 12–80 yr of age.	• No factors noted	• *Imprecision* of findings from categorical analyses • *Limited number* of studies examining outcome		

Notes: BMC = bone mineral content; BMD = bone mineral density; yr = years.

C.9 Gastrointestinal

EPA identified four epidemiological and three animal studies that investigated the association between PFOA and gastrointestinal effects. Of the epidemiological studies, one was classified as *medium* confidence and three were considered *low* confidence (Section C.9.1). Of the animal studies, one was classified as *high* confidence, and two were considered *medium* confidence (Section C.9.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.9.1 Human Evidence Study Quality Evaluation and Synthesis

C.9.1.1 Introduction

PFOA exposure may affect gastrointestinal health by altering molecular processes (such as those involved in inflammation), gut mucosa integrity (by acting as surfactants) and intestinal permeability, gut microbiota, and/or systemic susceptibility to infection (Xu et al., 2020d; Steenland et al., 2018b). Gastrointestinal outcomes only assessed in the context of immune system health, including ulcerative colitis and Crohn's disease, are discussed in the Toxicity Assessment (U.S. EPA, 2024b). However, some research suggests an overall immunosuppressive effect of PFOA could reduce the efficiency of routine childhood immunizations (Dalsager et al., 2016) which might include that for rotavirus, a common childhood cause of diarrhea and vomiting. In addition, inflammatory bowel disease (IBD), or the chronic inflammation of the gastrointestinal tract in response to environmental triggers, can be considered an immune dysregulation response occurring in genetically susceptible individuals (Hammer et al., 2019).

For this updated review, four studies examined the association between PFOA and gastrointestinal health outcomes (Timmermann et al., 2020; Xu et al., 2020d; Hammer et al., 2019; Dalsager et al., 2016). PFOA was measured in serum or blood, and the outcomes measured included diarrhea and vomiting, and IBD biomarkers zonulin and calprotectin. Dalsager et al. (2016) measured PFOA in pregnant women in Denmark and collected self-reported health outcomes for their children (≤ 4 years). Hammer et al. (2019) examined a subset of the general population in the Faroe Islands enrolled in the Children's Health and the Environment in the Faroes (CHEF) study. Xu et al. (2020d) examined child and adult residents of Ronneby, Sweden, exposed to PFAS in drinking water, as well as unexposed individuals from a nearby town. Timmermann et al. (2020) examined a subset of 4–18-month-old children from a randomized controlled trial of early measles vaccination, conducted in Guinea-Bissau in West Africa from 2012 to 2015.

C.9.1.2 Study Quality

Several considerations were specific to evaluating the quality of the studies of gastrointestinal symptoms. For example, fever or a stool test might help to confirm that diarrhea and vomiting are attributable to infection, as opposed to a chronic underlying condition or other chemical or dietary irritant. Medical diagnoses are preferred to self-reported symptoms, although knowledge of gastrointestinal disorders has developed substantially over recent decades and diagnostic

indicators continue to rapidly evolve. Causal factors in developing gastrointestinal conditions have likewise shifted over time, such as changes in emerging contaminants, hygiene, the gut microbiome, activity and stress levels, and dietary trends. These underlying trends may affect cohort studies with extended recruitment or follow-up periods. Reverse causation is possible if gastrointestinal conditions lead to increased intake of PFOA from food packaging or preparation methods, increased PFOA absorption through the gastrointestinal tract, or reduced fecal excretion. Measuring PFOA and gastrointestinal outcomes 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., 2018b), current blood concentrations are expected to correlate well with past exposures.

There are four 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 gastrointestinal effects. Study quality evaluations for these four studies are shown in Figure C-49.

Consistent with the considerations mentioned, one study was considered *medium* confidence (Timmermann et al., 2020) and three as *low* confidence (Xu et al., 2020d; Hammer et al., 2019; Dalsager et al., 2016). The *medium* confidence study (Timmermann et al., 2020) relied on retrospective reporting of gastrointestinal outcomes, which is subject to recall bias, and did not detail the interview question used. Study sensitivity was also limited by small case numbers and relatively low PFOA exposure levels. However, the concerns were considered relatively minor and likely to minimally impact interpretation of the results.

Concerns in the *low* confidence studies included potential for selection bias, including using unclear recruitment methods and, a convenience sample (Xu et al., 2020d). Another concern was potential for outcome misclassification or underreporting due to inconsistent participation and adherence to the parent reporting mechanism (Dalsager et al., 2016). Another common reason for *low* confidence was a serious risk for residual confounding by SES (Hammer et al., 2019). Exposure misclassification was also a concern in Xu et al. (2020d), due to use of residential history as a proxy. Deficiencies in multiple domains contributed to an overall *low* confidence rating.

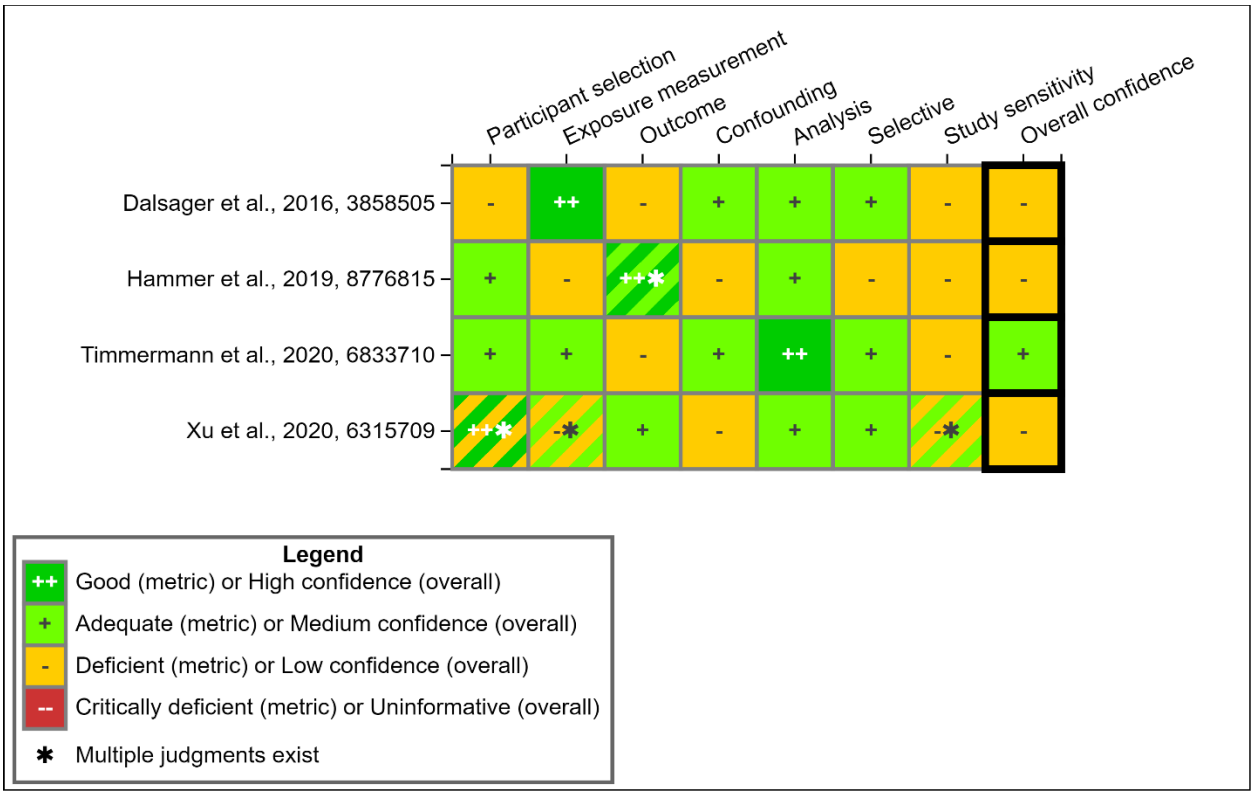


Figure C-49. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

C.9.1.3 Findings

Results for the studies that examined gastrointestinal outcomes are presented in (Appendix D). Both studies examining diarrhea observed non-significant increased association with PFOA. Timmermann et al. (2020) observed increased odds of diarrhea in very young children (up to 9 months old) in Guinea-Bissau. Dalsager et al. (2016) observed non-significant increased odds and incidence of diarrhea, decreased incidence of vomiting, and inconsistent non-significant odds of vomiting across exposure tertiles in 1–4-year-old children in Denmark.

Both studies examining IBD observed no associations with PFOA. Hammer et al. (2019) observed a non-significant decrease in incidence of IBD in Faroese children and adults. Xu et al. (2020d) observed non-significant decreases in levels of IBD biomarkers calprotectin or zonulin in children and adults from Sweden.

C.9.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two studies from the most recent literature search conducted in 2020 and one key study from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and gastrointestinal effects. Study quality evaluations for these three studies are shown in Figure C-50.

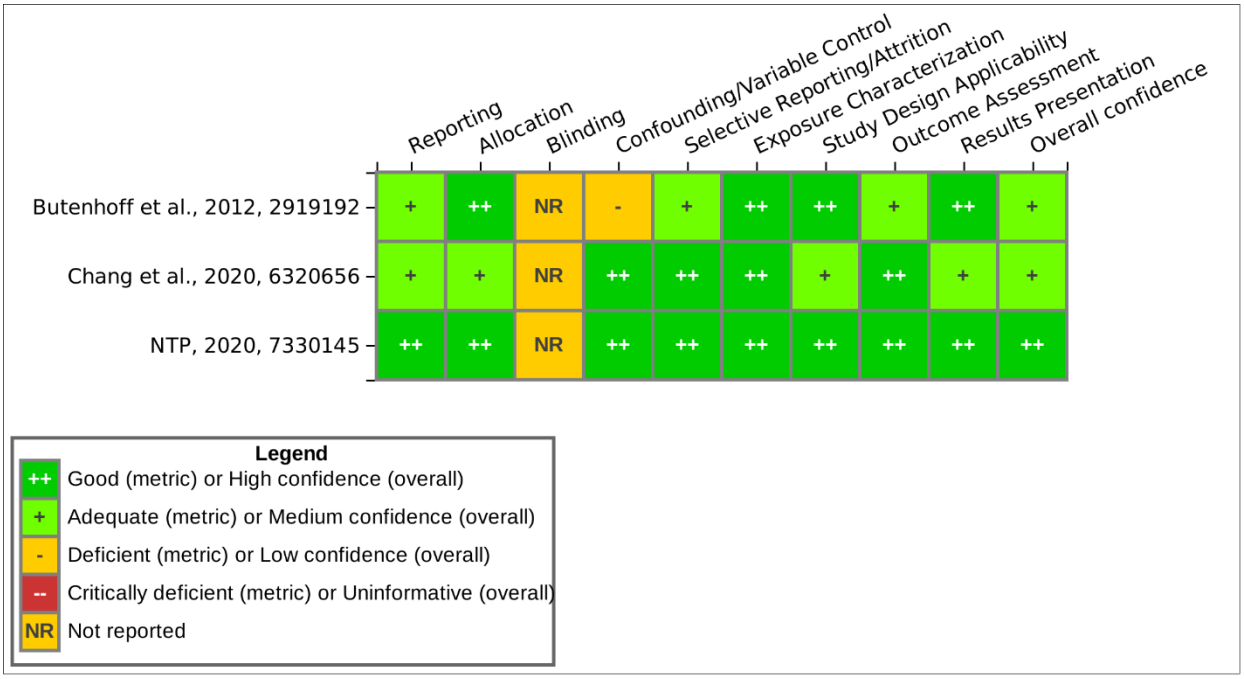


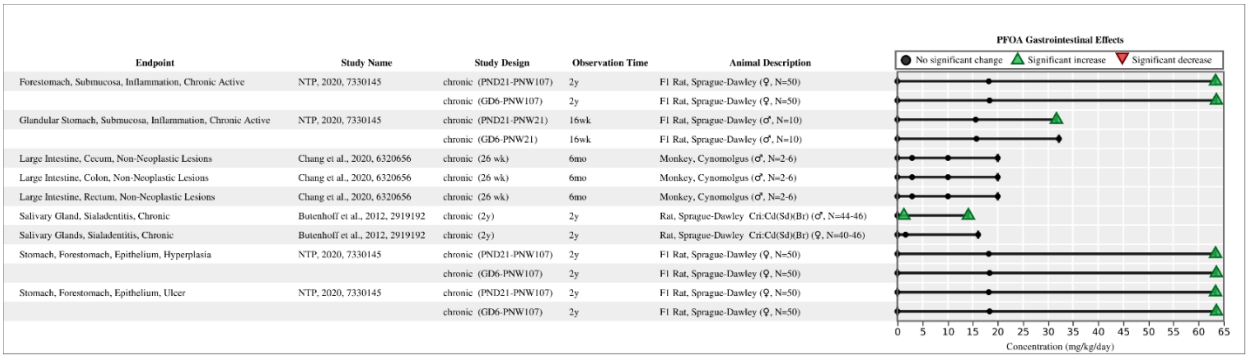
Figure C-50. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

The only information available to assess the gastrointestinal tract is histopathological evaluations (Figure C-51). In many cases, this was evaluated in the control and high-dose groups only. Chronic studies in rats suggest that oral exposure to PFOA may increase the incidence of nonneoplastic lesions in the gastrointestinal tract (Chang et al., 2020; NTP, 2020). However, shorter durations may not elicit the response as noted in a study where no histopathological findings were observed in the duodenum, jejunum, or ileum of the small intestine or the cecum, colon, or rectum of the large intestine of rats after 28 days. Likewise, no adverse effects were seen in the forestomach and glandular stomach or salivary gland (NTP, 2019a).

NTP (2020) used a matrix-type exposure paradigm whereby pregnant rats were administered PFOA 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 0/0–300/1000 ppm in females (see Toxicity Assessment, (U.S. EPA, 2024b) for further study design details). At the 16-week interim evaluation, incidences of chronic active inflammation of the glandular stomach submucosa were increased in all male treated groups compared with the control; however, statistical significance was only achieved in the 0/300 ppm group. No significant differences were noted in groups with and without perinatal exposure and no effects were seen in females at interim sacrifice. At the 2-year evaluation, females of the 0/1000 and 300/1000 ppm groups exhibited increased incidences of ulcer, epithelial hyperplasia, and chronic active inflammation of the submucosa of the forestomach when compared with controls. In addition, a single case of squamous cell papilloma was noted in both exposure groups (NTP, 2020).

In a dietary study, male and female rats fed 30 or 300 ppm PFOA for 2 years exhibited no stomach abnormalities during histopathological examination. In the salivary glands of male rats, significant increases in chronic sialadentitis were noted at 30 ppm (27%) and 300 ppm (30%). However, study authors reported this as being associated with antemortem viral infection. This effect was not observed in females (Butenhoff et al., 2012).



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Forestomach, Submucosa, Inflammation, Chronic Active	NTP, 2020, 7330145	chronic (PND21-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)
		chronic (GD6-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)
Glandular Stomach, Submucosa, Inflammation, Chronic Active	NTP, 2020, 7330145	chronic (PND21-PNW21)	16wk	F1 Rat, Sprague-Dawley (♂, N=10)
		chronic (GD6-PNW21)	16wk	F1 Rat, Sprague-Dawley (♂, N=10)
Large Intestine, Cecum, Non-Neoplastic Lesions	Chang et al., 2020, 6320656	chronic (26 wk)	6mo	Monkey, Cynomolgus (♂, N=2-6)
Large Intestine, Colon, Non-Neoplastic Lesions	Chang et al., 2020, 6320656	chronic (26 wk)	6mo	Monkey, Cynomolgus (♂, N=2-6)
Large Intestine, Rectum, Non-Neoplastic Lesions	Chang et al., 2020, 6320656	chronic (26 wk)	6mo	Monkey, Cynomolgus (♂, N=2-6)
Salivary Gland, Sialadentitis, Chronic	Butenhoff et al., 2012, 2919192	chronic (2y)	2y	Rat, Sprague-Dawley Crl:Cd(Sd)(Br) (♂, N=44-46)
Salivary Glands, Sialadentitis, Chronic	Butenhoff et al., 2012, 2919192	chronic (2y)	2y	Rat, Sprague-Dawley Crl:Cd(Sd)(Br) (♀, N=40-46)
Stomach, Forestomach, Epithelium, Hyperplasia	NTP, 2020, 7330145	chronic (PND21-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)
		chronic (GD6-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)
Stomach, Forestomach, Epithelium, Ulcer	NTP, 2020, 7330145	chronic (PND21-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)
		chronic (GD6-PNW107)	2y	F1 Rat, Sprague-Dawley (♀, N=50)

Figure C-51. Gastrointestinal Effects in Rodents and Nonhuman Primates Following Exposure to PFOA (Logarithmic Scale)

Interactive figure and additional study details available on HAWC.
GD = gestation day; F_1 = first generation; PND = postnatal day; PNW = postnatal week; mo = month; wk = week; y = year.

Archived colon tissues from the previously mentioned 2-year dietary study in rats conducted by Butenhoff et al. (2012) were subjected to pathology review by Chang et al. (2020). Minimal neutrophilic infiltration was observed in 8/39 males and 4/34 females treated with PFOA compared with 0/36 and 2/33 male and female control animals, respectively Mild subacute inflammation was noted in 1/39 treated male rats with no incidences occurring in treated females or control animals. These incidences were not significant when compared with controls. In addition, signs of overt inflammation, including infiltration of inflammatory leukocytes and tissue destruction and/or reaction were not observed. Therefore, these incidences were considered part of the normal mucosal immune system. Minimal to mild nematodiasis was observed in 6/50 male controls, 2/50 female controls, and 1/50 treated females. Study authors stated that it unknown whether PFOA contributed to the presence of the parasite in the treated group and noted that at the time of the original study, use of parasite-free animals was not common practice (Chang et al., 2020).

In the same study, Chang et al. (2020) examined archived cecum, colon, and rectum tissues of male cynomolgus monkeys administered gelatin capsules containing 0 (n = 6), 3 (n = 4), 10 (n = 6), or 30/20 (n = 2) mg/kg/day of PFOA for six months. Animals in the highest dose group received 30 mg/kg/day for the first 12 days; however, due to systemic toxicity, treatment halted and was resumed on day 22 at the reduced dose of 20 mg/kg/day. Isolated incidences of mild, brown pigment were noted in the cecum and colon and minimal eosinophil infiltrate was noted in the colon. These findings were not statistically significant and were considered to be normal background histomorphology. Isolated incidences of granulomatous lesions consistent with *Oesophagostomum* spp. Were observed but were considered common in the intestinal tract of non-human primates at the time the study was conducted (Chang et al., 2020).

NTP conducted a 28-day study in which 10 or 100 mg/kg/day of PFOA were orally administered to male or female rats, respectively. No histopathological findings were noted in the duodenum, jejunum, or ileum of the small intestine or the cecum, colon, or rectum of the large intestine. Likewise, no adverse effects were seen in the forestomach and glandular stomach or salivary gland (NTP, 2019a).

C.9.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse gastrointestinal outcomes in the 2016 PFOA HESD (U.S. EPA, 2016c). There are five 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 gastrointestinal effects. A summary of these studies is shown in Figure C-52. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOA leads to gastrointestinal effects.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	1	2
Cell Signaling Or Signal Transduction	1	0	0	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	0	1	1
Inflammation And Immune Response	0	0	1	1
Other	0	1	1	2
Grand Total	1	1	3	5

Figure C-52. Summary of Mechanistic Studies of PFOA and Gastrointestinal Effects

Interactive figure and additional study details available on HAWC.

C.9.4 Evidence Integration

The evidence evaluating an association between PFOA and gastrointestinal health effects in humans is *indeterminate* based on a paucity of research and the quality of the available studies. In the 2016 PFOA HESD (U.S. EPA, 2016c), gastrointestinal outcomes from epidemiological studies were only assessed in the context of immune system health, with limited evidence of associations with gastroenteritis. The available research has not demonstrated conclusive effects of PFOA exposure and gastrointestinal health effects, including vomiting, or diarrhea.

The animal evidence for an association between PFOA exposure and gastrointestinal tract effects is *indeterminate* based on limited data in animal models. The only significant nonneoplastic lesions observed were noted in the stomachs of male rats treated at 0/300 ppm and female rats treated at high doses (0/1000 ppm and 300/1000 ppm) in a 2-year feeding study (NTP, 2020). Additionally, lack of significant effects in rat colon and cynomolgus monkey cecum, colon, and rectum indicated no signs of ulcerative colitis (Chang et al., 2020).

C.9.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOA exposure can cause gastrointestinal effects in humans under relevant exposure circumstances (Table C-14).

Table C-14. Evidence Profile Table for PFOA Gastrointestinal 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 C.9.1)					⊙⊙⊙ ***Inadequate Evidence***
Diarrhea and vomiting 1 *Medium* confidence study 1 *Low* confidence study	Two studies examining diarrhea observed non-significant increased associations with PFOA in young children. One study also observed decreased incidence of vomiting, but odds of vomiting across exposure tertiles in children ages 1–4 yr were non-significant and inconsistent. No studies were conducted in adults.	• *Medium* confidence study	• *Low* confidence study • *Inconsistent directions* of effects across exposure levels and endpoints • *Limited number* of studies examining outcome • *Imprecision* of findings • Potential outcome misclassification or underreporting due to inconsistent parental participation	⊙⊙⊙ *Indeterminate* Evidence for gastrointestinal effects is based on two studies reporting increases in diarrhea and vomiting and two other studies reporting decreases in IBD. Considerable uncertainty due to limited number of studies and unexplained inconsistency across exposure levels and endpoints.	*Primary basis*: Evidence in humans and animals are largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Inflammatory bowel disease 2 *Low* confidence studies	Both studies examining IBD observed no associations with PFOA. Non-significant decreases in IBD incidence or IBD biomarkers were observed in association with PFOA.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining outcome • *Imprecision* of findings • Potential for residual confounding by socioeconomic status and decreased study sensitivity		
Evidence From In Vivo Animal Studies (Section C.9.2)					
Histopathology 1 *High* confidence study 2 *Medium* confidence studies	One chronic exposure study found evidence of increased incidence of nonneoplastic lesions including ulcer, epithelial hyperplasia, and/or inflammation in male and	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Inconsistent direction* of effects across animal models	⊙⊙⊙ *Indeterminate* Evidence was limited to three studies that demonstrated unexplained inconsistency across	

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**	
	female rats. Two chronic exposure studies found no evidence of nonneoplastic lesions within the gastrointestinal tract in both sexes in rats or in male monkeys.			animal models regarding gastrointestinal toxicity.	

Notes: IBD = inflammatory bowel disease; yr = years.

C.10 Dental

EPA identified two epidemiological studies that investigated the association between PFOA and dental effects. No animal studies were identified. The two epidemiological studies were both classified as *medium* confidence (Section C.10.1). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.10.1 Human Evidence Study Quality Evaluation and Synthesis

C.10.1.1 Introduction

PFOA exposure could potentially adversely affect both dentin and bone mineralization, skeletal formation, thyroid hormones that stimulate tooth maturation and enamel sufficiency, and immune responses to cariogenic bacteria (Puttige Ramesh et al., 2019). At a molecular level, PFAS such as PFOA may influence tooth growth and development via activation of peroxisome proliferator-activated receptor alpha, initiation of oxidative stress, altering gene expression in the vascular endothelial growth factor signaling pathway for gastric cells, hemoprotein binding, estrogen disruption, or disruption of carbonic anhydrase (needed for enamel development) (Wiener and Waters, 2019).

For this updated review, two studies examined the association between PFOA exposure and dental caries in children and adolescents (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). Dental caries was defined as presence of decay or a restoration on any tooth surface or the loss of a tooth following tooth decay, excluding third molars (Puttige Ramesh et al., 2019). Trained dentists performed visual and tactile exams using appropriate tools, but X-rays were not taken. No other dental health outcomes were evaluated.

The two cross-sectional studies used data from the NHANES: Puttige Ramesh et al. (2019) assessed data from 2,869 12–19-year-old adolescents included in the 1999–2012 NHANES and Wiener and Waters (2019) examined data from 639 children ages 3–11 years in the 2013–2014 NHANES cycle. Therefore, no participant overlap is expected between these studies. Exposure to PFOA was assessed via biomarkers in blood.

C.10.1.2 Study Quality

Important considerations specific to evaluating the quality of studies on dental outcomes relate to the difficulty of characterizing risk factors for dental caries, such as diet and oral hygiene practices. Self-reported frequency of brushing, fluoridated product use, and dental visits may be useful indicators. Fluoride levels in local public drinking water supplies are also thought to influence development of dental caries and tap water consumption habits differ among households and individuals (Wiener and Waters, 2019). Measuring PFOA and dental outcomes 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., 2018b), current blood concentrations are expected to correlate well with past exposures.

There are two 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 dental effects. Study quality evaluations for these two studies are shown in Figure C-53.

On the basis of the considerations mentioned, the two included studies were considered *medium* confidence, wherein limitations were not expected to severely affect results interpretation. Limitations included cross-sectional study design, which introduces some concern about whether the exposure preceded the outcome or vice versa (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). Puttige Ramesh et al. (2019) was primarily limited by participant selection, since NHANES data necessarily excluded participants who were unable or unwilling to submit to a dental examination. This could have resulted in selection bias against individuals with the most severe tooth decay. Dental examinations were performed on all NHANES participants aged 2+ who did not have orofacial pain, specific medical conditions, physical limitations, inability to comply, or were uncooperative.

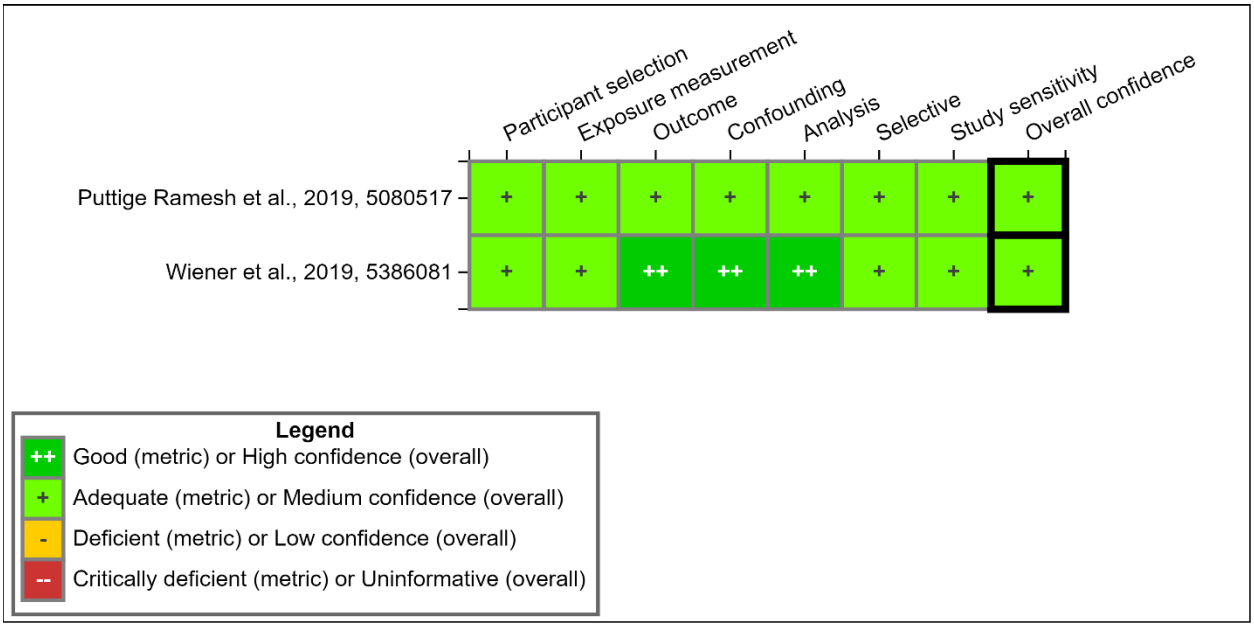


Figure C-53. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Dental Effects

Interactive figure and additional study details available on HAWC.

C.10.1.3 Findings

Results for the studies that examined dental outcomes are presented in Appendix D. Both studies observed non-significantly increased odds of dental caries with increased PFOA exposure children and adolescents (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). Puttige Ramesh et al. (2019) also observed increased odds of dental caries in the third highest quartile of exposures, but decreased odds in the second and highest quartiles compared with the lowest. Analyses did not account for age, but considered gender, race, education level of parent/guardian, family-poverty-to-income ratio, blood lead level, and serum cotinine level (an indicator of exposure to smoking). Wiener and Waters (2019) adjusted the analysis for age, sex, race/ethnicity, ratio of family-income-to-poverty guidelines, tooth brushing frequency, fluoride

in water, percentage of sugar in the diet, and dental visits. No studies of dental health outcomes were available for pregnant women, adults, or occupational workers.

C.10.2 Animal Evidence Study Quality Evaluation and Synthesis

In the available literature, there is no reported biological consequence of PFOA exposure on dental outcomes in animals.

C.10.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse dental outcomes in the 2016 PFOA HESD (U.S. EPA, 2016c). There are no 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 dental effects. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOA may cause dental effects.

C.10.4 Evidence Integration

The evidence evaluating an association between PFOA exposure and dental effects in humans is *indeterminate* based on the limited number of available studies and imprecision of observed results. Dental outcomes were not previously reviewed in the 2016 PFOA HESD (U.S. EPA, 2016c). The present epidemiological review identified only two dental studies in humans in which prevalence of dental caries was evaluated. Both studies observed non-significantly increased odds of dental caries (Puttige Ramesh et al., 2019; Wiener and Waters, 2019). These studies have exposure levels typical in the general population, large sample sizes and low risk of bias.

The animal evidence for an association between PFOA exposure and dental effects is *indeterminate* because there are no available studies in animal models that examine dental effects due to PFOA exposure.

C.10.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOA exposure can cause dental effects in humans under relevant exposure circumstances (Table C-15).

Table C-15. Evidence profile table for PFOA Dental 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 C.10.1)					⊙⊙⊙ ***Inadequate Evidence***
Dental caries 2 *Medium* confidence studies	Two studies observed non-significant increase in odds of dental caries. No significant associations observed in studies of children from NHANES.	• *Medium* confidence studies	• *Limited number* of studies examining outcome • *Imprecision* of findings	⊙⊙⊙ *Indeterminate* Evidence was limited to two studies that reported non-significant positive associations with dental caries in children and adolescents, but results are imprecise. Uncertainties remain regarding effects in adults in the general population.	*Primary basis:* No evidence in animals and evidence in humans is largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.

Notes: NHANES = National Health and Nutrition Examination Survey.

C.11 Ocular

EPA identified one epidemiological and two animal studies that investigated the association between PFOA and ocular effects. The one epidemiological study was classified as *medium* confidence (Section C.11.1). Of the animal studies, two were classified as *high* confidence (Section C.11.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.11.1 Human Evidence Study Quality Evaluation and Synthesis

C.11.1.1 Introduction

For this updated review, there is one epidemiological study that investigated the association between PFOA and ocular effects (Zeeshan et al., 2020).

This cross-sectional study was conducted in Shenyang, China part of the "Isomers of C8 Health Project in China," focused on a high-exposed population, including adults aged 20 years and older, who were randomly selected using multistage, stratified cluster sampling. Median total PFOA serum concentrations among the 1,202 study participants were 6.06 ng/mL (Q1 = 3.97 ng/mL, Q3 = 9.12 ng/mL). Participants were subject to a complete ophthalmic examination which included ocular history, visual acuity, and anterior and posterior segment examinations. Several ocular conditions, reflecting effects on different segments of the eyes, were assessed, including visual impairment (VI), vitreous disorder, synechia, macular disorder, corneal pannus, anterior chamber depth (ACD)-shallow, retinal disorder, lens opacity, and conjunctival disorder.

C.11.1.2 Study Quality

There is one study 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 ocular effects. Study quality evaluation for this one study is shown in Figure C-54.

Zeeshan et al. (2020) was classified as *medium* confidence. The main limitation of this study is the cross-sectional design, which does not allow for establishing temporality. Participants' serum samples were collected at study enrollment only and the utilization of a single exposure measurement may not adequately represent exposure variability; additionally, it is unclear whether exposure occurred at an etiologically relevant time period to reflect changes in ocular function.

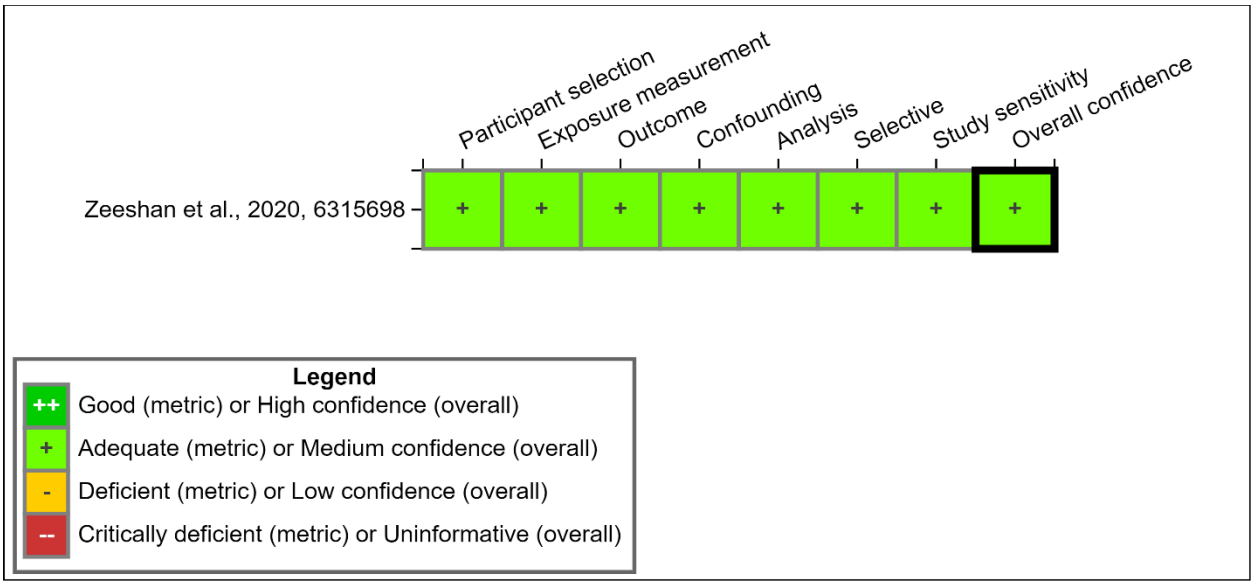


Figure C-54. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Ocular Effects

Interactive figure and additional study details available on HAWC.

C.11.1.3 Findings

Zeeshan et al. (2020) examined the effects of exposure to PFOA in adults aged 22–96 years, who had lived for at least 5 years in in Shenyang, China (Appendix D). Ocular outcomes examined included VI, vitreous disorder, synechia, macular disorder, corneal pannus, and ACD, and combined eye disease (aggregating all 9 ocular conditions examined). A positive statistically significant association between VI and total serum PFOA was observed (OR: 1.80; 95% CI: 1.37, 2.37). When stratified by age, the association between combined eye disease and total serum PFOA was statistically significant for participants aged ≤ 65 years (OR: 1.25; 95% CI: 1.01, 1.56) but not for the older participants (OR: 1.19; 95% CI: 0.71, 1.98). No other associations were observed.

C.11.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two 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 ocular effects. Study quality evaluations for these two studies are shown in Figure C-55.

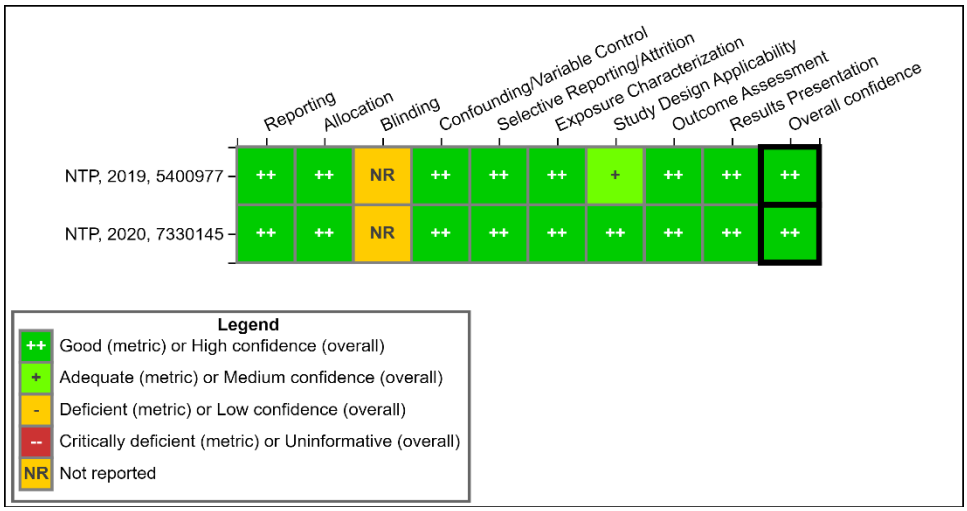


Figure C-55. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Ocular Effects

Interactive figure and additional study details available on HAWC.

Eye irritation studies in rabbits suggest that PFOA acts as an ocular irritant (Gabriel, 1976); however, no adverse lesions were noted in eye tissues during histopathological examination in repeated-dose oral toxicity studies in rats. In a 28-day oral toxicity study where only control and high-dose groups were evaluated, no histopathological findings were noted in eyes of male rats treated with 10 mg/kg/day or female rats treated with 100 mg/kg/day (NTP, 2019a). In a chronic exposure study, male and female Sprague-Dawley rats were fed diets containing PFOA for approximately 2 years (see Toxicity Assessment, (U.S. EPA, 2024b) further study design details). Observation of gross abnormalities and histopathological examination of eye tissues were conducted in pups at 16 weeks and 2 years with no treatment-related abnormalities noted (NTP, 2020).

C.11.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse ocular outcomes in the 2016 PFOA HESD (U.S. EPA, 2016c). There is one study 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 ocular effects. A summary of these studies is shown in Figure C-56. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOA leads to ocular effects.

Mechanistic Pathway	In Vitro	Grand Total
Atherogenesis And Clot Formation	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	1
Cell Signaling Or Signal Transduction	1	1
Inflammation And Immune Response	1	1
Grand Total	1	1

Figure C-56. Summary of Mechanistic Studies of PFOA and Ocular Effects

Interactive figure and additional study details available on HAWC.

C.11.4 Evidence Integration

The evidence evaluating an association between PFOA exposure and ocular effects in humans is considered *indeterminate* based on a limited number of studies. In the 2016 Health Assessment for PFOA (U.S. EPA, 2016c), no epidemiological evidence of an association between PFOA exposure and ocular health effects was observed. One recent epidemiological study reported an association between PFOA exposure and visual impairment and combined eye disease in humans. However, since only one study was available for review and given its cross-sectional design, existing epidemiological evidence does not allow for a definitive conclusion regarding potential detrimental ocular health effects due to exposure to PFOA.

The animal evidence for an association between PFOA and ocular effects is *indeterminate* due to the limited evidence available in animal models. In two available studies in animal models that assess ocular toxicity, there were no observed ocular effects with short-term or chronic PFOA exposure in male or female rats.

C.11.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOA exposure can cause ocular effects in humans under relevant exposure circumstances (Table C-16)

Table C-16. Evidence Profile Table for PFOA Ocular 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 C.11.1)					⊙⊙⊙ **Inadequate Evidence**
Eye disease 1 *Medium* confidence study	The only study examining eye disease was a cross-sectional study that observed significant positive associations between visual impairment and serum PFOA. The association was also significant for combined eye disease, but only in participants aged ≤65 yr.	• *Medium* confidence study	• *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited to one study reporting increases in visual impairment in all ages and increases in combined eye disease in participants aged ≤65 yr.	*Primary basis:* Evidence in humans is limited and evidence in animals is largely non-significant. *Human relevance, cross-stream coherence, and other inferences:* No specific factors are noted.
Evidence From In Vivo Animal Studies (Section C.11.2)					
Histopathology 2 *High* confidence studies	No changes in ocular histopathology were reported in one 28-day and one chronic study in male and female rats.	• *High* confidence studies	• *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited to two studies reporting no findings of ocular toxicity.	

Notes: yr = years.

C.12 Dermal

EPA identified one epidemiological and two animal studies that investigated the association between PFOA and dermal effects. The one epidemiological study was classified as *medium* confidence (Section C.12.1). Of the animal studies, two were classified as *high* confidence (Section C.12.2). Studies may have *mixed* confidence ratings depending on the endpoint evaluated. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.12.1 Human Evidence Study Quality Evaluation and Synthesis

C.12.1.1 Introduction

For this updated review, one study examined the association between age at the occurrence of acne and PFOA exposure. In the Puberty Cohort, a large sub-cohort of the DNBC in Denmark, Ernst et al. (2019) examined the association between prenatal PFOA exposure and pubertal development in. Mother-child pairs were recruited for the DNBC from 1996 to 2002, and eligibility for the Puberty Cohort was determined in 2012. PFAS levels in maternal blood, largely collected during the first trimester of pregnancy, were used to assess prenatal exposure, and age at the occurrence of acne was self-reported by children via bi-annual questionnaire starting in 2012 or at 11 years of age.

C.12.1.2 Study Quality

There is one study 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 dermal effects. Study quality evaluation for this one study is shown in Figure C-57.

Ernst et al. (2019) was considered a *medium* confidence study, with no major concerns with the overall quality of the study and any identified concerns were not likely to impact the results. Self-reporting was used to assess the occurrence of acne, a study limitation that could introduce minor bias to the outcome assessment. Additionally, some children were sampled for the Puberty Cohort after the onset of puberty, thus their self-reported outcome information has increased risk of inaccurate recall. However, this was not expected to substantially impact the accuracy of the outcome measures.

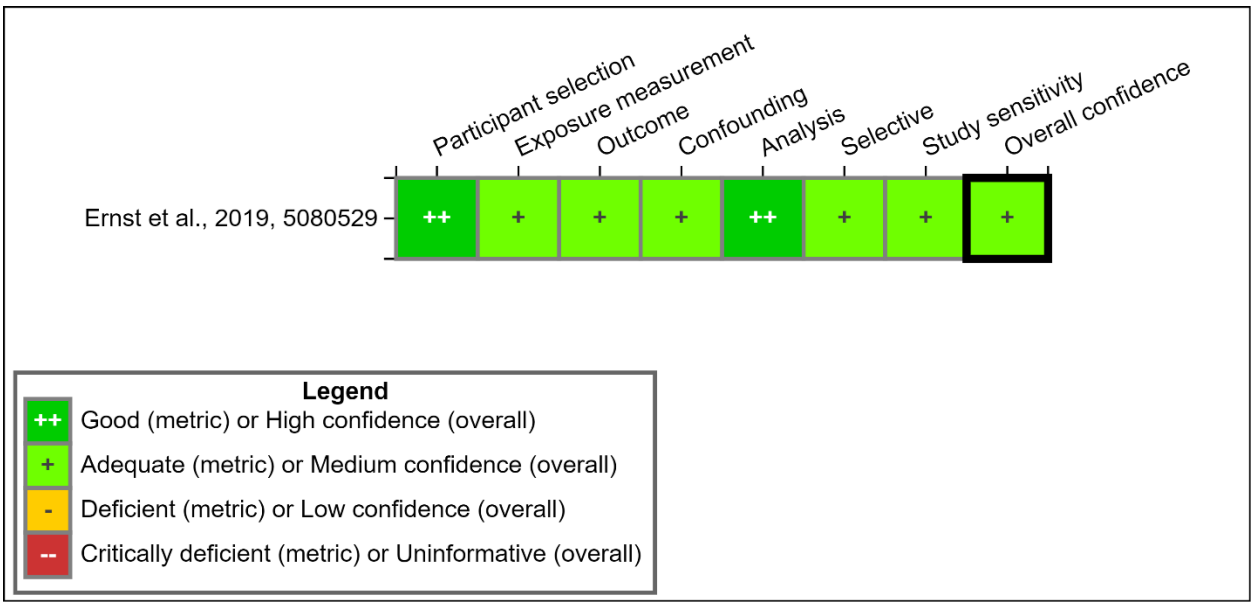


Figure C-57. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Dermal Effects

Interactive figure and additional study details available on HAWC.

C.12.1.3 Findings

Results for the studies that examined dermal outcomes are presented in Appendix D. Ernst et al. (2019) observed negative associations between prenatal PFOA exposure and age at the occurrence of acne. Significant negative associations were observed for girls per doubling of PFOA (β: −5.16; 95% CI: −8.50, −1.82), and in the highest tertile of PFOA exposure compared with the lowest (β: −6.09; 95% CI: −12.10, −1.70) (Ernst et al., 2019). Associations in boys were negative and non-significant.

C.12.2 Animal Evidence Study Quality Evaluation and Synthesis

There are two 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 dermal effects. Study quality evaluations for these two studies are shown in Figure C-58.

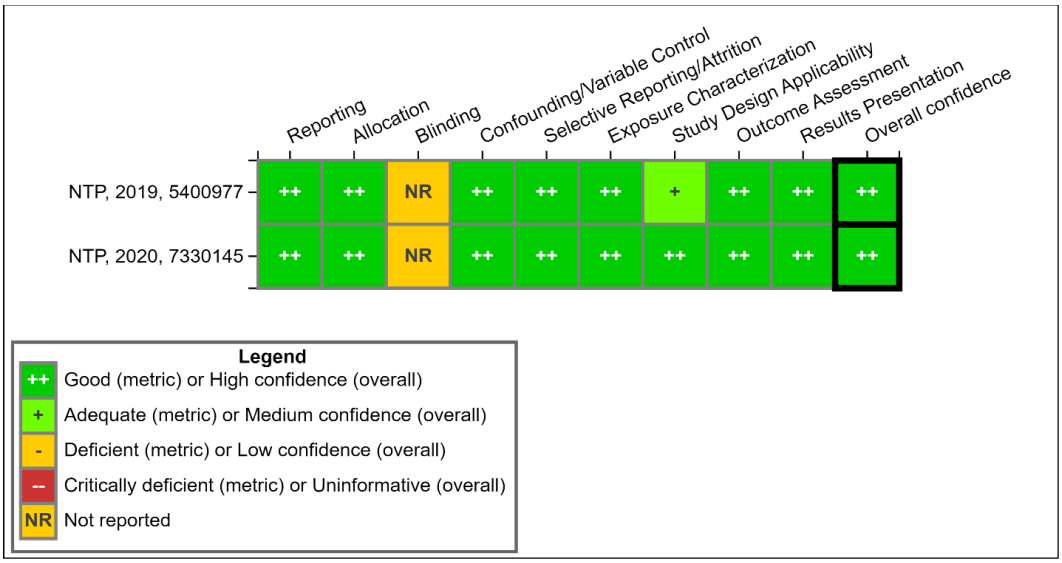


Figure C-58. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Dermal Effects

Interactive figure and additional study details available on HAWC.

There is no evidence in the literature that oral PFOA exposure results in dermal toxicity in animal models. An NTP (2019a) study explored histopathology of the skin following 28 days of oral gavage of up to 10 mg/kg/day PFOA in male and up to 100 mg/kg/day PFOA in female Sprague-Dawley rats. They observed no lesions of dermal tissue. Similarly, in a subsequent report, NTP (2020) reported no lesions in dermal tissue in male or female Sprague-Dawley rats that received PFOA via feed for 2 years (see Toxicity Assessment, (U.S. EPA, 2024b)).

C.12.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse dermal outcomes in the 2016 PFOA HESD (U.S. EPA, 2016c). There are two 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 dermal effects. A summary of these studies is shown in Figure C-59. Additional mechanistic synthesis will not be conducted since evidence is inadequate to infer that PFOA may cause dermal effects.

Mechanistic Pathway	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	2	2
Extracellular Matrix Or Molecules	1	1
Inflammation And Immune Response	1	1
Oxidative Stress	2	2
Grand Total	2	2

Figure C-59. Summary of Mechanistic Studies of PFOA and Dermal Effects

Interactive figure and additional study details available on HAWC.

C.12.4 Evidence Integration

The evidence evaluating an association between PFOA exposure and dermal effects in humans is *indeterminate* based on the limited number of studies available. The 2016 PFOA HESD (U.S. EPA, 2016c) did not report on the association between oral PFOA exposure and dermal effects. In this updated review, one epidemiological study examined the association between PFOA exposure and dermal effects during puberty and observed an inverse association with age at the occurrence of acne, which was significant only in girls, suggesting earlier occurrences of acne with increasing PFOA exposure.

The animal evidence for an association between PFOA exposure and dermal effects is *indeterminate*. There are two *high* confidence studies that evaluated the skin as part of the histopathological evaluation that observed no dermal lesions. There are no reported biological consequences of oral PFOA exposure on dermal tissue in animal models.

C.12.4.1 Evidence Integration Judgment

Overall, there is ***inadequate evidence*** to assess whether PFOA exposure can cause dermal effects in humans under relevant exposure circumstances (Table C-17).

Table C-17. Evidence Profile Table for PFOA Dermal 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 C.12.1)					⊙⊙⊙ ***Inadequate Evidence*** *Primary basis*: Evidence in humans and animals are largely non-significant. *Human relevance, cross-stream coherence, and other inferences*: No specific factors are noted.
Acne 1 *Medium* confidence study	One study found a significant inverse association with age of acne onset in adolescents, but this was significant **only** in girls.	• *Medium* confidence study	• *Limited number* of studies examining outcome • *Inconsistent directions* of effects across sexes	⊙⊙⊙ *Indeterminate* Evidence was limited to one study reporting associations that vary in significance by sex.	
Evidence From In Vivo Animal Studies (Section C.12.2)					
Histopathology 2 *High* confidence studies	No changes in dermal histopathology were reported in one 28-day and one chronic study in male and female rats.	• *High* confidence studies	• *Limited number* of studies examining outcome	⊙⊙⊙ *Indeterminate* Evidence was limited to two studies reporting no findings of dermal toxicity.	

Appendix D. Detailed Information from Epidemiology Studies

D.1 Developmental

Table D-1. Associations Between PFOA Exposure and Developmental Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Ashley-Martin et al. (2017) *High*	Canada, 2008–2011	Cohort	Pregnant women (enrolled if <14 wk gestation, ≥18 yr of age) and their infants at recruitment and from MIREC N = 1,509	Maternal blood Early pregnancy 1.7 (1.2–2.4)	BW (z-score): adequate, inadequate, and excess weight gain	Regression coefficient per log10-unit increase in PFOA	BW: Females: −89.51 (–263.4, 84.38) Males: –35.51 (–198.99, 127.97) BW z-score: –0.1 (–0.34, 0.13) Adequate weight gain: –0.36 (–0.85, 0.11) Excess weight gain: –0.08 (–0.44, 0.27) Inadequate weight gain: –0.08 (–0.78, 0.63)
	MIREC = Maternal-Infant Research on Environmental Chemicals (MIREC) **Outcome:** Weight gain adequacy based on Institute of Medicine (IOM) guidelines **Confounding:** Maternal age, pre-pregnancy BMI, parity, household income, smoking, each PFAS.[c]						
Bach et al. (2016) *High*	Denmark, 2008–2013	Cohort	Pregnant women and their infants from the Aarhus Birth Cohort N = 1,507	Maternal serum Early pregnancy 2.0 (1.5–2.6)	BL (cm), BW (g, z-score), gestational length (weeks), HC (cm), PTB	Regression coefficient per IQR increase in PFOA and by quartiles OR per 0.1-unit increase in PFOA, per IQR increase, and by quartiles	BL: 0.1 (–0.1, 0.2) Q2: 0 (–0.4, 0.4) Q3: 0 (−0.4, 0.4) Q4: 0.1 (−0.3, 0.4) BW (g): 7 (–10, 23) Q2: 3 (–54, 59) Q3: 15 (–42, 72) Q4: 9 (–47, 64)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BW (z-score): 0.02 (–0.02, 0.06) Q2: 0.009 (–0.13, 0.14) Q3: 0.04 (–0.09, 0.17) Q4: 0.02 (–0.1, 0.16) Gestational length: 0.1 (0, 0.2) Q2: 0 (–0.3, 0.2) Q3: 0.1 (–0.2, 0.3) Q4: 0.1 (–0.2, 0.4) HC: 0.1 (0, 0.2) Q2: 0 (–0.2, 0.3) Q3: 0.1 (–0.2, 0.4) Q4: 0.1 (–0.1, 0.4)
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI and educational level, GA.						
Bell et al. (2018) *High*	United States, 2008–2010	Cross-sectional	Singleton and twin infants born in from Upstate KIDS N = 2,071 singletons; 1,040 twins	Blood Later pregnancy Singletons: 1.10 (0.69–1.63) Twins: 1.01 (0.69–1.53)	BL (cm), BW (g), GA (weeks), HC (cm), ponderal index	Regression coefficient per log(PFOA+1) unit increase	BL S: 0.02 (–0.13, 0.17) T: 0.21 (–0.11, 0.52) BW S: –11.55 (–35.72, 12.62) T: 18.48 (–17.18, 54.13) GA S: 0.01 (–0.07, 0.08) T: –0.01 (–0.12, 0.11) HC S: 0.04 (–0.17, 0.26) T: 0.12 (–0.22, 0.46) Ponderal index S: –0.01 (–0.03, 0.01)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T: –0.01 (–0.04, 0.02)
	Results: S = Singletons; T = Twins **Comparison:** Logarithm base not specified. **Confounding:** Maternal age, maternal BMI, maternal education, infertility treatment, parity.						
Bjerregaard-Olesen et al. (2019) *High*	Denmark, 2011–2013	Cohort	Pregnant women and their children from FETOTOX N = 671	Maternal serum Early pregnancy IQR = 0.92	BL (cm), BW (g), HC (cm)	Regression coefficient per IQR increase in PFOA	BL 1.68(–0.1, 0.2) Females: –0.2 (–0.5, 0) Males: 0.2 (0, 0.3), Interaction p-value = 0.008 BW 18 (–9, 45) Females: –23 (–78, 31) Males: 31 (6, 56) HC 1.68(0, 0.2) Females: –0.1 (–0.3, 0.1) Males: 0.2 (0.1, 0.3), Interaction p-value = 0.004
	Confounding: Age at delivery, pre-pregnancy BMI, educational level, smoking, alcohol intake, GA at birth.						
Buck Louis et al. (2018) *High*	United States, 2009–2013	Cohort	Pregnant women (age range 18–40 yr) with singleton pregnancies from the NICHD Fetal Growth Studies N = 2,106	Maternal blood Early pregnancy 1.985 (1.297–3.001)	BL (cm), BW (g), GA at delivery (weeks), HC (cm), umbilical circumference (cm), upper arm length (cm), upper thigh length (cm)	Regression coefficient per SD increase in log-PFOA	BL: –0.23 (–0.35, –0.1) BW: –5.9 (–28.75, 16.94) GA: 0.01 (–0.08, 0.1) HC: –0.04 (–0.12, 0.03) Umbilical circumference: –0.06 (–0.19, 0.07) Upper arm length: –0.02 (–0.07, 0.03) Upper thigh length: –0.19 (–0.26, –0.12)
	NICHD = National Institute of Child Health and Human Development. **Comparison:** Logarithm base not specified. **Confounding:** Maternal age, education, pre-pregnant body mass index, serum cotinine, infant sex, chemical-maternal race/ethnic interaction, mode of delivery.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Chu et al. (2020) *High*	China, 2013	Cohort	Pregnant women (aged 18–45 yr) and infants from Guangzhou Birth Cohort Study N = 372	Maternal serum Later pregnancy 1.538 (0.957–2.635) Girls: 1.497 (0.920–2.642) Boys: 1.558 (0.988–2.628)	BW (g), GA (weeks), LBW, PTB	Regression coefficient (BW, GA) or OR (LBW, PTB) per ln-unit increase in PFOA or by quartiles	BW −73.64 (−126.39, −20.88) Girls: −56.04 (−129.32, 17.24) Boys: −71.8 (−148.61, 5.00) p-value for interaction by sex = 0.958 GA −0.21 (−0.44, 0.02) Girls: −0.53 (−0.83, −0.23) Boys: 0.17 (−0.16, 0.51) p-value for interaction by sex = 0.002 LBW 1.16 (0.52, 2.58) Q2: 0.61 (0.14, 2.69) Q3: 0.27 (0.05, 1.42) Q4: 1.00 (0.23, 4.35) p-trend = 0.007 PTB 1.49 (0.94, 2.36) Q2: 0.71 (0.23, 2.14) Q3: 1.60 (0.60, 4.23) Q4: 1.84 (0.72, 4.71) p-trend = 0.273
	Outcome: LBW defined as BW < 2500 g **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, maternal occupation, maternal education, family income, parity for all outcomes; GA for BW and LBW; child sex for BW and GA.						
Costa et al. (2019) *High*	Spain, 2003–2008	Cohort	Pregnant women and their children from INMA study	Maternal plasma 2.35 (1.6–3.30)	AC, FL, BPD, estimated fetal weight at 12 wk, 20 wk, and 34 wk	Percent change per twofold increase in PFOA	AC 12 wk: 0.8 (–2.4, 4.0) Girls: 2.9 (–1.7, 7.2) Boys: –1.5 (–6.0, 2.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 1,230 (Girls = 597, Boys = 633)				20 wk: –0.5 (–3.7, 2.8) Girls: 2.7 (–1.9, 6.9) Boys: –3.1 (–7.5, 1.2) 34 wk: (1.1 (–2.1, 4.3) Girls: 1.2 (–3.2, 5.4) Boys: 1.1 (–3.3, 5.4) FL 12 wk: 1.9 (–1.4, 5.2) Girls: 4.2 (–0.5, 8.3) Boys: –0.6 (–5.0, 3.8) 20 wk: –1.4 (–4.6, 1.9) Girls: 0.2 (–4.3, 4.6) Boys: –3.0 (–7.5, 1.3) 34 wk: –0.2 (–3.5, 3.1) Girls: –1.8 (–6.3, 2.7) Boys: 1.2 (–3.4, 5.5) BPD 12 wk: –0.5 (–5.6, 4.5) Girls: 3.9 (–0.7, 8.2) Boys: –4.7 (–11.1, 1.8) 20 wk: 0.0 (–3.2, 3.3) Girls: 2.9 (–1.5, 7.3) Boys: –2.6 (–7.1, 1.8) 34 wk: 1.9 (–1.3, 5.1) Girls: 1.6 (–2.9, 6.0) Boys: 2.2 (–2.4, 6.6) Estimated Fetal Weight 12 wk: 1.2 (–2.1, 4.4) Girls: 3.3 (–1.4, 7.5) Boys: –1.2 (–5.7, 3.2) 20 wk: –0.8 (–4.0, 2.4) Girls: 2.0 (–2.5, 6.4) Boys: –3.5 (–8.0, 0.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							34 wk: 1.3 (–1.9, 4.5) Girls: 0.7 (–3.8, 5.0) Boys: 2.1 (–2.4, 6.4)
	INMA = Infancia y Medio Ambiente (Environment and Childhood) Project **Confounding:** Cohort, parity, maternal age, country of birth, smoking at week 12, maternal pre-pregnancy BMI, studies, season of last menstrual period.						
Darrow et al. (2013) *High*	United States Cohort 2005–2011	Pregnant women from the C8HP exposed through drinking water, Ages ≥19 LBW, all births N = 1,629 LBW, first prospective birth N = 783 BW, all births N = 1,470 BW, first prospective birth N = 710 PTB, all births N = 1,628 PTB, first prospective birth N = 783	Maternal serum at enrollment 14.3 (8.0–29.8)	LBW, BW (g), PTB	OR (LBW, PTB), regression coefficient (BW) per ln-unit increase in PFOA, per IQR increase in PFOA, or by quintiles	LBW All births Per ln-unit increase: 0.94 (0.75, 1.17) Per IQR increase: 0.95 (0.85, 1.06) Q2: 0.94 (0.45, 1.98) Q3: 0.99 (0.48, 2.05) Q4: 1.25 (0.63, 2.46) Q5: 0.92 (0.44, 1.95) First prospective birth Per ln-unit increase: 1.07 (0.78, 1.47) Per IQR increase: 0.99 (0.87, 1.12) Q2: 0.82 (0.23, 2.85) Q3: 1.03 (0.35, 3.06) Q4: 1.86 (0.67, 5.14) Q5: 1.06 (0.32, 3.54) BW All births Per ln-unit increase: −8 (−28, 12) Per IQR increase: −5 (−13, 2) Q2: 35 (−33, 105) Q3: −9 (−79, 61) Q4: 4 (−65, 72) Q5: 0 (−68, 69)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-trend = 0.701 First prospective birth Per ln-unit increase: 5 (−22, 33) Per IQR increase: 1 (−10, 11) Q2: 135 (34, 276) Q3: 26 (−71, 124) Q4: 56 (−37, 149) Q5: 74 (−20, 169) p-trend = 0.622 PTB All births Per ln-unit increase: 0.93 (0.78, 1.1) Per IQR increase: 0.95 (0.88, 1.04) Q2: 1.56 (0.88, 2.76) Q3: 1.19 (0.66, 2.14) Q4: 1.21 (0.67, 2.19) Q5: 1.01 (0.55, 1.86) p-trend = 0.629 First prospective birth Per ln-unit increase: 1.09 (0.86, 1.37) Per IQR increase: 1.01 (0.92, 1.1) Q2: 1.11 (0.42, 2.89) Q3: 1.30 (0.51, 3.27) Q4: 1.49 (0.62, 3.61) Q5: 1.32 (0.53, 3.32) p-trend = 0.409

C8HP = C8 Health Project
Outcome: PTB defined as births occurring before 37 wk gestation. LBW defined as those weighing less than 2,500 g.
Results: Lowest quintile used as reference.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, educational level, smoking status, parity, BMI, self-reported diabetes, time between conception and serum management (year strata). Additional confounding for BW: indicator variables for gestational week.						
Eick et al. (2020) *High*	United States 2014–2018	Cohort	Second trimester pregnant women from the CIOB cohort BW (g) N = 461 GA, BW (z-score), PTB N = 506	Maternal serum from the second trimester 0.76 (0.46–1.12)	BW (g, z-score), GA (weeks), PTB	Regression coefficient by tertile PTB: OR by tertile	BW (g) T2: 62.93 (−42.94, 168.8) T3: 86.07 (−36.31, 208.45) BW (z-score) T2: 0.13 (−0.10, 0.35) T3: 0.12 (−0.14, 0.37) GA T2: −0.29 (−0.74, 0.17) T3: −0.10 (−0.63, 0.43) PTB T2: 1.79 (0.75, 4.28) T3: 2.37 (0.88, 6.38)
	CIOB = Chemicals in our Bodies. **Outcome:** PTB defined as birth at <37 wk gestation. **Results:** Lowest tertile used as reference. **Confounding:** Maternal age, maternal race/ethnicity, pre-pregnancy BMI, maternal education, smoking status, parity, and food insecurity.						
Gardener et al. (2021) *High*	United States Recruitment: 2009	Cohort	Pregnant women in third trimester (ages 18–49) and children at birth from the Vanguard Pilot Study of the NCS GA at birth N = 433 BW N = 403	Maternal serum from primarily third trimester 1.4 (0.9–2.0)	GA at birth (weeks), BW (z-score), GA <37 wk	GA at birth and BW: Mean by quartile GA <37 wk and BW: OR by quartile	GA at birth Mean Q1: 38.94 (38.60, 39.27) Q2: 38.53 (38.19, 38.88) Q3: 38.67 (38.35, 38.98) Q4: 38.85 (38.49, 39.20) p-trend = 0.79 BW Mean Q1: −1.35 (−4.69, 2.02) Q2: 0.41 (−3.00, 3.86) Q3: 0.75 (−2.38, 3.91) Q4: 1.95 (−1.5, 5.41) p-trend = 0.20

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							OR Q2: 1.2 (0.56, 2.59) Q3: 0.84 (0.40, 1.80) Q4: 0.91 (0.41, 2.02) p-trend = 0.62 GA <37 wk OR Q2: 3.17 (0.94, 10.7) Q3: 3.14 (0.95, 10.31) Q4: 1.38 (0.32, 5.97) p-trend = 0.53
	NCS = National Children's Study. **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, education, race/ethnicity, pre-pregnancy BMI, prenatal smoking, parity, GA at serum collection.						
Govarts et al. (2016) *High*	Belgium, 2008–2009	Cohort	Mother-newborn pairs from FLEHS II N = 248	Cord blood 1.52 µL (1.10–2.10 µL)	BW (g)	Regression coefficient per IQR increase in PFOA	–34.5 (–129.02, 60.02)
	FLEHS II = Flemish Environmental and Health Study II **Confounding:** GA, child's sex, smoking of the mother during pregnancy, parity, maternal pre-pregnancy BMI.						
Huo et al. (2020b) *High*	China, 2013–2016	Cohort	Mothers (aged ≥20 yr) and their children from the Shanghai Birth Cohort N = 2,849	Maternal blood Later pregnancy 11.85 (9.20–15.26)	GA (weeks), PTB (indicated, non-spontaneous, spontaneous, and overall)	Regression coefficient (GA) and OR (PTB) per ln-unit increase in PFOA and per tertile	GA: 0 (–0.14, 0.13) T1: 0.11 (–0.31, 0.54) T2: –0.69 (–1.75, 0.37) T3: 0.03 (–0.29, 0.35) OR T2: 0.11 (–0.03, 0.24) OR T3: –0.01, –0.15, 0.12) PTB, overall: 0.92 (0.61, 1.33) Females: 0.82 (0.44, 1.55) Males: 1.02 (0.59, 1.78) PTB, indicated: 1.71 (0.8, 3.67)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T2: 0.96 (0.44, 2.11) T3: 1.02 (0.47, 2.22) PTB, non-spontaneous: Females: 2.64 (0.83, 8.39) Males: 1.23 (0.44, 3.39) PTB, spontaneous: 0.73 (0.45, 1.19) T2: 0.71 (0.43, 1.17) T3: 0.76 (0.46, 1.22) Females: 0.54 (0.26, 1.13) Males: 0.95 (0.49, 1.81)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, parity, parental education levels, pregnancy complicated with chronic disease, infant sex, GA at blood drawing.						
Lauritzen et al. (2017) *High*	Norway and Sweden, 1986–1988	Cohort	Mother-infant pairs from NICHD SGA N = 424 (265 from Norway, 159 from Sweden (78 girls, 81 boys))	Maternal serum Later pregnancy Norway: 1.62 (Range = 0.31–7.97) Sweden: 2.33 (Range = 0.60–6.70)	BL (cm), BW (g), GA (weeks), HC (cm), SGA	Regression coefficient or OR (SGA) per ln-unit increase in PFOA	BL −0.49 (−0.99, 0.02); p-value = 0.06 NO: −0.1 (−0.7, 0.4); p-value = 0.656 SE: −1.3 (−2.3, −0.3); p-value = 0.01 SE-girls: −0.8 (−2.4, 0.8); p-value = 0.34 SE-boys: −1.6 (−2.9, −0.4) BW −81.7 (−202, 39.2); p-value = 0.185 NO: 37 (−99, 174); p-value = 0.59 SE: −359 (−596, −122), p-value = 0.003 SE-girls: −156 (−541, 228); p-value = 0.419

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							SE-boys: −526 (−828, −222); p-value = 0.001 GA −0.20 (−0.34, 0.14); p-value = 0.255 NO: −0.2 (−0.6, 0.2); p-value = 0.431 SE: −0.3 (−0.9, 0.3); p-value = 0.318 SE-girls: −0.1 (−1.1, 0.9); p-value = 0.802 SE-boys: −0.4 (−1.2, 0.5); p-value = 0.365 HC −0.02 (−0.32, 0.27) NO: 0.2 (−0.2, 0.5); p-value = 0.354 SE: −0.4 (−1.0, 0.1); p-value = 0.115 SE-girls: −0.1 (−1.0, 0.7); p-value = 0.728 SE-boys: −0.6 (−1.3, 0.1); p-value = 0.103 SGA 1.21 (0.69, 2.11) NO: 0.66 (0.33, 1.33); p-value = 0.246 SE: 5.25 (1.68, 16.4); p-value = 0.004 SE-girls: 4.73 (0.79, 28.3); p-value = 0.089 SE-boys: 6.55 (1.14, 37.45); p-value = 0.035

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	NICHD SGA = The US National Institute of Child Health and Human Development (NICHD) Scandinavian Successive SGA Births Study. **Outcome:** SGA defined as BW below the 10th percentile for GA, sex, and parity. **Results:** NO = Norway; SE = Sweden **Confounding:** Maternal age, height, pre-pregnancy BMI, education, parity, smoking status at conception, interpregnancy interval, offspring sex.						
Lind et al. (2017a) *High*	Denmark 2010–2012	Cohort	Infants prenatally exposed to PFAS from the Odense Child Cohort N = 212 girls, 299 boys	Maternal serum Early pregnancy 1.7 (1.1–2.3)	BW (g), HC (cm), gestational length (days)	Regression coefficient per ln-unit increase in PFOA or by quartiles	BW Males: −5 (−92, 82) p-trend by quartiles = 0.88 Females: 6 (−90, 102) p-trend by quartiles = 0.88 HC Males: (−0.3, 0.3) p-trend by quartiles = 0.80 Females: 0.1 (−0.3, 4) p-trend by quartile = 0.72 Gestational length Males Continuous: −0.7 (−2.9, 1.5) Q2: 1.0 (−2.4, 4.4) Q3: 2.7 (−0.9, 6.3) Q4: −0.9 (−4.6, 2.7) p-trend by quartiles = 0.63 Females Continuous: −1.5 (−4.3, 1.3) Q2: 1.1 (−2.7, 4.9) Q3: −2.7 (−6.3, 1.2) Q4: −3.6 (−8.0, 0.8) p-trend by quartiles = 0.04 BW and HC:Quartile analysis did not show any

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, weight-for-age z-score, pre-pregnancy BMI, parity, smoking.						
Luo et al. (2021) *High* for BW *Medium* for birth length, ponderal index	China, 2017–2019	Cohort	Mother-newborn pairs N = 224	Maternal blood within three days of delivery 3.51 (2.23–4.80)	BW (g), BL (cm), ponderal index (kg/m^3)	Regression coefficient per ln-unit increase in PFOA	BW: –62.37 (–149.08, 24.35) BL: 0.08 (–0.36, 0.52) Ponderal index: –0.61 (–1.15, –0.06), p-value < 0.05
	Confounding: Maternal age, pre-pregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, GA, and newborn sex.						
Manzano-Salgado et al. (2017a) *High*	Spain, 2003–2008	Cohort	Mother (aged ≥16 yr)-child pairs from INMA N = 1,202	Maternal plasma Early pregnancy Mean = 2.35 (SD = 1.25)	I Regression I coefficient and (OR per doubling c of PFOA and per r quartiles) , I V (g) , C A (v e e k s)	BL: −0.01 (–0.15, 0.14) Q2: 0.01 (–0.28, 0.29) Q3: –0.06 (–0.36, 0.24) Q4: –0.03 (–0.34, 0.28) Females: 0.04 (–0.16, 0.24) Males: 0.01 (–0.18, 0.21) BW: –9.33 (–38.81, 20.16) Q2: –29.6 (–92.82, 33.63) Q3: –32.99 (–97.08, 31.09) Q4: –32.77 (–97.65, 32.11) Females: 13.81 (–26.67, 54.3) Males: –24.75 (–66.71, 17.22) GA: –0.05 (–0.16, 0.07) Q2: –0.05 (–0.29, 0.2) Q3: 0.03 (–0.23, 0.28) Q4: –0.12 (–0.37, 0.17) Females: –0.08 (–0.24, 0.08) Males: –0.04 (–0.2, 0.13) HC: –0.07 (–0.17, 0.03)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					, I C (c r) , I I V , I I V a t t e r r , I T I , S G A		Q2: –0.01 (–0.22, 0.19) Q3: 0.04 (–0.17, 0.25) Q4: –0.16 (–0.38, 0.06) Females: 0.03 (–0.1, 0.17) Males: –0.13 (–0.27, 0) LBW: 0.9 (0.63, 1.29) Females: 0.76 (0.48, 1.21) Males: 1.12 (0.64, 1.99) LBW at term: 0.85 (0.53, 1.34) Females: 0.62 (0.36, 1.06) Males: 1.67 (0.72, 3.86), interaction p-value = 0.05 PTB: 0.92 (0.72, 1.19) Females: 1.19 (0.62, 2.31) Males: 0.74 (0.43, 1.25) SGA: 0.92 (0.72, 1.19) Females: 0.72 (0.5, 1.04) Males: 1.18 (0.82, 1.69), interaction p-value = 0.08
	INMA = Infancia y Medio Ambiente (Environment and Childhood Project) **Outcome:** SGA defined as newborns weighing below the 10th percentile for GA and sex according to national references. **Results:** Lowest quartile used as reference. **Confounding:** Maternal age, parity, pre-pregnancy BMI, fish intake during pregnancy, type of delivery.						
Sagiv et al. (2018) *High*	United States, 1999–2002	Cohort	Pregnant women and infants from Project Viva	Maternal blood Early pregnancy 5.8 (IQR = 3.8)	BW-for-GA (z-score), gestational	Regression coefficient per	BW-for-GA: –0.02 (–0.08, 0.03) Q2: –0.04 (–0.17, 0.09)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 1,644		length (weeks), PTB	IQR increase and by quartiles	Q3: –0.12 (–0.25, 0.02) Q4: –0.07 (–0.21, 0.07)
						PTB: OR per IQR increase and by quartiles	Gestational length: –0.05 (–0.16, 0.06) Q2: 0.05 (–0.22, 0.32) Q3: 0 (–0.28, 0.28) Q4: –0.04 (–0.33, 0.24) PTB: 1 (0.9, 1.3) Q2: 1.1 (0.6, 2) Q3: 1.1 (0.6, 1.9) Q4: 1.2 (0.7, 2.2) BW-for-GA and gestational length: no statistically significant associations by sex
	Outcome: PTB was defined as <37 wk **Results:** Lowest quartile used as reference. **Confounding:** Maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, paternal education, household income, child's sex, GA at blood draw.						
Shoaff et al. (2018) *High*	United States, 2003–2006; follow-up 4 wk to 2 yr from recruitment	Cohort	Pregnant women (aged ≥18 yr) and their children at birth, 4 wk and 2 yr from the HOME study N = 345	Maternal blood Later pregnancy 5.5 (3.8–7.7)	Regression coefficient by tertile (per doubling in PFOA) Rapid weight gain: Relative risk by tertile	BW T2: 0.18 (–0.06, 0.42) T3: –0.15 (–0.4, 0.1) Length-for-age T2: 0.19 (–0.2, 0.5) T3: –0.32 (–0.72, 0.07) Weight gain T2: 1.08 (0.78, 1.5) T3: 0.8 (0.56, 1.15) Weight-for-age T2: –0.02 (–0.34, 0.29)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					r g t h - f c r - a g e (z - s c c r e) , r a p i d v e i g h t g a i		T3: –0.46 (–0.78, –0.14), p-trend < 0.01 Weight-for-length T2: –0.31 (–0.56, –0.06) T3: –0.34 (–0.59, –0.08), p-trend = 0.02 BW, length-for-age, and weight gain: no statistically significant trends

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					r, weight-for-age (z-score), weight-for		

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
					-length (z-score)		
	HOME = Health Outcomes and Measures of the Environment. **Outcome:** Rapid weight gain defined as increase in weight z-score > 0.67 SDs any time between age 4 wk and 2 yr. **Results:** Lowest tertile used as reference **Confounding:** Maternal age at delivery, race, marital status, insurance, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable and fish consumption during pregnancy, prenatal vitamin use.						
Starling et al. (2017) *High*	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 yr) and infants from Healthy Start at birth N = 628	Maternal serum 1.1 (0.7–1.6)	Adiposity (% fat mass), BW (g)	Regression coefficient per ln-unit increase PFOA and by tertiles	Adiposity: –0.43 (–0.91, 0.04) T2: –0.34 (–1.06, 0.38) T3: –0.97 (–1.74, –0.2) BW: –51.4 (–97.2, –5.7) T2: –15.9 (–84.9, 53.2) T3: –92.4 (–166.2, –18.5)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, race/ethnicity, education, gestational weight gain, smoking during pregnancy, gravidity, GA at blood draw, infant sex, and GA at birth.						
Starling et al. (2019) *High*	United States, 2009–2014	Cohort	Pregnant women (aged ≥16 yr) and infants from	Maternal serum 1.0 (0.7–1.6)	Adiposity (%), weight-for-age z-score (WAZ),	Regression coefficient per ln-unit increase	Adiposity: 0.76 (–0.03, 1.55) T2: 1.4 (0.18, 2.62)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			Healthy Start assessed up to 5 mo N = 415 (202 girls, 213 boys)		weight-for-length z-score (WLZ), WAZ and WLZ growth from birth to 5 mo, rapid growth in WAZ or WLZ	in PFOA and by tertiles Rapid growth: OR per ln-unit increase in PFOA	T3: 1.16 (–0.18, 2.49) Females: 0.27 (–0.85, 1.4) T2: 1.71 (–0.06, 3.48) T3: 0.03 (–1.77, 1.83) Males: 1.53 (0.35, 2.71) T2: 1.2 (–0.56, 2.97) T3: 2.81 (0.79, 4.84) p-value for sex interaction = 0.07 WAZ: 0.01 (–0.14, 0.15) T2: 0.17 (–0.05, 0.39) T3: 0.08 (–0.16, 0.32) Females: –0.14 (–0.34, 0.06) T2: 0.01 (–0.3, 0.33) T3: –0.18 (–0.51, 0.14) Males: 0.17 (–0.05, 0.39) T2: 0.31 (–0.01, 0.63) T3: 0.38 (0.01, 0.75) No statistically significant interaction by sex WLZ: 0.01 (–0.16, 0.18) T2: 0.1 (–0.16, 0.35) T3: 0.07 (–0.21, 0.35) Females: –0.11 (–0.34, 0.12) T2: –0.01 (–0.38, 0.35) T3: –0.17 (–0.55, 0.2) Males: 0.14 (–0.11, 0.39) T2: 0.17 (–0.21, 0.55) T3: 0.33 (–0.1, 0.76) WAZ, growth from birth: 0.07 (–0.08, 0.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							WAZ, rapid growth: 1.25 (0.77, 2.04) WLZ, growth from birth: 0.09 (–0.10, 0.27) WLZ, rapid growth: 1.43 (0.92, 2.22)
	Outcome: Rapid growth defined as change in WAZ or WLZ >0.67 between birth and 5 mo **Confounding:** Maternal age, race/ethnicity, pre-pregnancy BMI, any previous pregnancies, any smoking during pregnancy, education, gestational weight gain z-score, infant sex, exclusive breastfeeding to follow-up visit, infant age (days) at follow-up.						
Tanner et al. (2020) *High*	Sweden, Recruitment: 2007–2010; followed up to 5.5 yr	Cohort	Mother-infant pairs from SELMA study N = 1,334	Maternal serum GM = 1.6 (Range = 0.2–21.1)	Age of infant PGV (months), infant growth slope (log10), infant PGV (log10), infant spurt duration (log10), infant weight plateau (kg)	Regression coefficient per log10-unit increase in PFOA	Age of infant PGV: 0.58 (0.17, 0.99), p-value = 0.01 Growth slope: –0.06 (–0.11, –0.01), p-value = 0.02 PGV: –0.02 (–0.05, 0.02) Spurt duration: 0.06 (0.01, 0.11), p-value = 0.02 Weight plateau: 0.81 (0.21, 1.41), p-value = 0.01
	SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy **Outcome:** PGV = peak growth velocity **Confounding:** Sex, PTB, mother's age, weight, parity, and smoking.						
Valvi et al. (2017) *High*	Faroe Islands 1997–2000	Cross-sectional	Pregnant women and their children N = 604 (288 girls, 316 boys)	Maternal serum Later pregnancy 3.31 (2.54–3.99)	HC (cm), body length (cm), BW (g)	Regression coefficient per doubling of PFOA	HC 1 (−0.22, 0.23) Girls: 0.10 (−0.23, 0.44) Boys: −0.05, (−0.36, 0.26) p-value for sex interaction = 0.90 Body length 0.03 (−0.29, 0.35) Girls: −0.01 (−0.48, 0.46) Boys: 0.02 (−0.42, 0.47)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-value for sex interaction = 0.64 BW −11 (−88, 67) Girls: 58 (−48, 164) Boys: −71 (−184, 42) p-value for sex interaction = 0.04
	Confounding: Maternal age at delivery, education, parity, pre-pregnancy BMI, smoking during pregnancy, child sex.						
Wang et al. (2016) *High*	Taiwan Recruitment 20002001, assessment up to age 11	Cohort	Children from Taiwan Maternal and Infant Cohort Study, assessed at ages 2, 5, 8, and 11 yr N = 106 girls, 117 boys	Maternal serum Later pregnancy Girls: 2.34 (1.57–3.43) Boys: 2.37 (1.35–3.47)	HC (cm), BL (cm), BW (kg), SGA, height z-score at each age, average childhood height z-score, weight z-score, average childhood weight z-score	Regression coefficient per ln-unit increase in PFOA or by quartiles SGA: OR per ln-unit increase in PFOA	HC Girls: 0.11 (−0.26, 0.47) Boys: 0.06 (−0.24, 0.36) BL Girls: −0.32 (−0.92, 0.28) Boys: 0.31 (−0.22, 0.84) BW Girls: −0.08 (−0.18, 0.01) Boys: 0.04 (−0.05, 0.12) SGA Girls: 1.48 (0.63, 3.48) Boys: 0.63 (0.32, 1.13) Girls' analysis by quartiles: no statistically significant associations Height and weight z-scores by age: NR, no significant interactions for either sex (p-value > 0.10)
	Outcome: SGA defined as BW below the 10th percentile for GA by sex using 1998–2002 Taiwan nationwide singleton BW charts. **Results:** Lowest quartile used as reference.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Family annual income, maternal age at delivery, maternal education, maternal previous live children, maternal pre-pregnancy BMI.						
Whitworth et al. (2012) *High*	Norway 2003–2004	Cohort	Pregnant women and their children from MoBa PTB, LGA, SGA N = 901 BW N = 849	Maternal plasma Around 17 wk of gestation 2.2 (1.7–3.0)	PTB, BW (z-score), LGA, SGA	Regression coefficient and OR per unit increase in PFOA, or by quartile	PTB Q2: 0.3 (0.1, 1.3) Q3: 0.7 (0.2, 2.4) Q4: 0.1 (0.03, 0.6) p-trend = 0.02 LGA Q2: 0.9 (0.5, 1.7) Q3: 1.0 (0.5, 1.9) Q4: 0.6 (0.3, 1.4) p-trend = 0.33 SGA Q2: 0.8 (0.3, 2.3) Q3: 1.3 (0.5, 3.2) Q4: 1.0 (0.3, 2.8) p-trend = 0.92 BW Per unit increase: −0.03 (−0.10, 0.04) Q2: −0.06 (−0.28, 0.16) Q3: −0.08 (−0.32, 0.16) Q4: −0.21 (−0.45, 0.04) p-trend = 0.10
	MoBa = Norwegian Mother and Child Cohort Study. **Results:** Lowest quartile used as reference. **Outcome:** PTB defined as GA <37 wk. SGA defined as gender- and gestation age-specific BW less than the 10th percentile. LGA defined as gender- and GA-specific BW greater than the 90th percentile. **Confounding:** Maternal age, pre-pregnancy BMI, parity. Additional confounding for BW: Weight gain at 17 wk.						
Wikström et al. (2020) *High*	Sweden 2007–2010	Cohort	Infants exposed prenatally to PFAS from the SELMA study	Maternal serum Early pregnancy 1.61 (1.11–2.30)	BW (g), BW-SDS, SGA	Regression coefficient (BW, BW-SDS) or OR (SGA) per ln-	BW Per increase: −68 (−112, −24) Q2: 27 (−35, 89)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 1533 (732 girls, 801 boys)			unit increase in PFOA or by quartiles	Q3: −41 (−106, 23) Q4: −90 (−159, −91) Girls Per increase: −86 (−145, −26) Q2: 30 (−55, 115) Q3: −36 (−124, 52) Q4: −136 (−231, −40) Boys Per increase: −49 (−113, 15) Q2: 26 (−66, 116) Q3: −44 (−139, 50) Q4: −47 (−147, 54) BW-SDS Per increase: −0.152 (−0.251, −0.052) Q2: 0.065 (0.076, 0.206) Q3: −0.088 (−0.235, 0.058) Q4: −0.204 (−0.362, −0.047) Girls Per increase: −0.191 (−0.325, −0.057) Q2: 0.065 (−0.124, 0.255) Q3: −0.088 (−0.285, 0.109) Q4: −0.299 (−0.513, −0.085) Boys Per increase: −0.111 (−0.258, 0.036) Q2: 0.065 (−0.144, 0.274) Q3: −0.086 (−0.302), 0.131) Q4: −0.117 (−0.348, 0.114)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							SGA Per increase: 1.43 (1.03, 1.99) Q2: 0.77 (0.45, 1.32) Q3: 0.96 (0.57, 1.61) Q4: 1.44 (0.86, 2.40) Girls Per increase: 1.96 (1.18, 3.28) Q2: 1.00 (0.40, 2.51) Q3: 1.64 (0.71, 3.83) Q4: 2.33 (1.00, 5.43) Boys Per increase: 1.16 (0.75, 1.78) Q2: 0.67 (0.34, 1.31) Q3: 0.66 (0.33, 1.29) Q4: 1.04 (0.54, 2.01)
	SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy. **Outcomes:** SGA defined as BW below the 10th percentile for GA and sex. **Results:** Lowest quartile used as reference. **Confounding:** Sex, GA, maternal weight, parity, cotinine levels.						
Wikström et al. (2021) *High*	Sweden 2007–2010	Nested case-control	Pregnant women from the SELMA study N = 1,527	Serum First trimester Case: 2.00 (1.44–2.76) Control: 1.64 (1.13, 2.32)	Miscarriage	OR per doubling in PFOA, or by quartile	Per doubling: 1.48 (1.09, 2.01); p-value < 0.05 Q2: 1.69 (0.8, 3.56) Q3: 2.02 (0.95, 4.29) Q4: 2.66 (1.26, 5.65)
	SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy. **Results:** Lowest quartile used as reference. **Confounding**: Parity, age, and cotinine (tobacco smoke) exposure.						
Xiao et al. (2019) *High*	Denmark 1994–1995	Cohort	Pregnant women and their children N = 171	Maternal blood Later pregnancy GM = 2.37 µg/g (range: 0.8–6.9 µg/g)	BL, BW, and cranial circumference (z-scores)	Regression coefficient per log2-unit	BL z-score −0.14 (−0.40, 0.13) Girls: −0.02 (−0.37, 0.32) Boys: −0.27 (−0.65, 0.10)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
						increase in PFOA	BW z-score −0.29 (−0.56, −0.01) Girls: −0.20 (−0.57, 0.16) Boys: −0.39 (−0.79, −0.01) Cranial circumference z-score −0.17 (−0.48, 0.15) Girls: −0.30 (−0.74, 0.13) Boys: −0.03 (−0.46, 0.15)
	Confounding: Child sex, parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury.						
Yao et al. (2021) *High*	China 2010–2013	Cross-sectional	Parents and their children at birth from LWBC N = 369	Maternal and paternal serum within three days of birth Maternal: 42.83 (Range = 1.16–602.79) Paternal: 103.38 (Range = 1.24–2,077.93)	BW (g)	Regression coefficient per ln-unit increase in PFOA	BW by maternal exposure Model A: −25.2 (−75.29, 24.89) BW by paternal exposure Model A: –5.67 (−54.05, 42.72)
	LWBC = Laizhou Wan Birth Cohort. **Confounding**: All models adjusted for characteristics of parent with measured exposure: age, education, BMI (before pregnancy for maternal exposure). Maternal exposure models additionally adjusted for parity. "Adjusted" models additionally adjusted for other parent's exposure and characteristics.						
Yeung et al. (2019) *High*	United States Recruitment 2008–2010, assessment up to age 3	Cohort	Children aged 0–3 from Upstate KIDS study N = 1,954 singletons (S) (930 girls, 1,024	Blood 1.1 (0.7–1.6)	BMI, BMI z-score, length (cm), length z-score, obesity, weight (g), weight z-score, rapid weight gain,	Regression coefficient or OR (rapid weight gain, obesity) per log-SD increase in	BMI S: −0.11 (−0.17, −0.05); p-value < 0.05 S-girls: −0.18 (−0.27, −0.09); p-value < 0.05 S-boys: −0.05 (−0.12, 0.03) T: 0.04 (−0.06, 0.14)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			boys) and 902 twins (T)		weight-for-length (WFL) z-score	PFOA or by quartiles	BMI z-score S: −0.08 (−0.12, −0.04); p-value < 0.05 Q2: −0.189 (−0.30, −0.07); p-value < 0.05 Q3: −0.22 (−0.33, −0.10); p-value < 0.05 Q4: −0.24 (−0.35, −0.12); p-value < 0.05 S-girls: −0.13 (−0.19, −0.07); p-value < 0.05 Q2: −0.16 (−0.32, 0.01) Q3: −0.23 (−0.39, −0.06); p-value < 0.05 Q4: −0.33 (−0.50, −0.16); p-value < 0.05 S-boys: −0.04 (−0.09, 0.02) Q2: −0.21 (−0.37, −0.05); p-value < 0.05 Q3: −0.20 (−0.37, −0.03); p-value < 0.05 Q4: −0.16 (−0.32, 0.01) T: 0.05 (−0.03, 0.12) Q2: 0.23 (0.03, 0.42); p-value < 0.05 Q3: 0.21 (0.01, 0.40); p-value < 0.05 Q4: 0.19 (−0.02, 0.39) Length S: 0.13 (0.02, 0.25); p-value < 0.05 S-girls: 0.19 (0.01, 0.37) S-boys: 0.09 (−0.06, 0.25) T: 0.16 (−0.03, 0.34)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Length z-score S: 0.05 (0.001, 0.11); p-value < 0.05 S-girls: 0.07 (−0.004, 0.15) S-boys: 0.04 (−0.03, 0.11) T: 0.07 (−0.01, 0.15) Weight S: −12.57 (−49.47, 24.33) S-girls: −30.22 (−84.05, 23.60) S-boys: 6.60 (−44.69, 57.89) T: 94.04 (33.82, 154.26); p-value < 0.05 Weight z-score S: −0.03 (−0.07, 0.01) S-girls: −0.05 (−0.11, 0.01) S-boys: −0.01 (−0.06, 0.05) T: 0.09 (0.03, 0.16); p-value < 0.05 WFL z-score S: −0.08 (−0.12, −0.04); p-value < 0.05 S-girls: −0.13 (−0.19, −0.06); p-value < 0.05 S-boys: −0.04 (−0.0p, 0.02) T: 0.04 (−0.04, 0.12) Rapid weight gain, obesity: not statistically significant for all children
	Outcome: Rapid weight gain defined as the child's weight gain SD above 0.5 for 4 or 9 mo or about 0.67 for 12 mo.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as reference. **Confounding:** Child's age at measurement, age squared, age cubed, sex-age interactions, maternal age, pre-pregnancy BMI category, maternal education, maternal race, private insurance, infertility treatment.						
Andersen et al. (2010) *Medium*	Denmark, 1996–2002	Cohort	Pregnant women and their children followed up at birth, 5 mo, and 12 mo from DNBC N at birth = 1114 (552 boys, 562 girls)	Maternal plasma First and second trimesters 5.21 (0.5–21.9)	BW (z-score, g); weight at 5 and 12 mo (z-score, g); height at 5 and 12 mo (z-score, cm); BMI at 5 and 12 mo (kg/m^2, z-score)	Regression coefficient per unit increase in PFOA	BW z-score: −0.024 (−0.046, −0.002); p-value < 0.05 g: −12.8 (−24.5, −1.2); p-value < 0.05 Boys z-score: −0.018 (−0.051, 0.015) g: −9.5 (−26.6, 7.6) Girls z-score: −0.03 (−0.058, 0.001) g: −15.2 (−31.1, 0.7) Weight 5 mo follow-up z-score: −0.009 (−0.031, 0.012) g: −9.4 (−28.6, 9.9) Boys z-score: −0.032 (−0.063, −0.001); p-value < 0.05 g: −30.2 (−59.3, −1.1); p-value < 0.05 Girls z-score: 0.009 (−0.020, 0.038) g: 7.9 −17.7, 33.4) 12 mo follow-up z-score: −0.015 (−0.038, 0.007) g: −19.0 (−44.9, 6.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Boys z-score: −0.036 (−0.069, −0.003); p-value < 0.05 g: –43.1 (−82.9, −3.3); p-value < 0.05 Girls z-score: 0.002 (−0.029, 0.034) g: 2.5 (−30.9, 36.0) Height 5 mo follow-up z-score: 0.017 (−0.007, 0.040) cm: 0.044 (−0.017, 0.105) Boys z-score: 0.0015 (−0.020, 0.050) cm: 0.039 (−0.050, 0.127) Girls z-score: 0.018 (−0.014, 0.049) cm: 0.047 (−0.038, 0.132) 12 mo follow-up z-score: 0.016 (−0.009, 0.042) cm: 0.049 (−0.026, 0.124) Boys z-score: 0.011 (−0.027, 0.048) cm: 0.032 (−0.079, 0.143) Girls z-score: 0.021 (−0.013, 0.056) cm: 0.064 (−0.039, 0.166)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BMI 5 mo follow-up z-score: −0.015 (−0.040, 0.010) kg/m^2: −0.025 (−0.067, 0.017) Boys z-score: −0.04 (−0.078, −0.003); p-value < 0.05 kg/m^2: −0.067 (−0.129, −0.004); p-value < 0.05 Girls z-score: 0.007 (−0.027, 0.041) kg/m^2: 0.012 (−0.045, 0.069) 12 mo follow-up z-score: −0.025 (−0.052, 0.002) kg/m^2: −0.042 (−0.086, 0.002) Boys z-score: −0.046 (−0.086, −0.006); p-value < 0.05 kg/m^2: −0.078 (−0.0144, −0.011); p-value < 0.05 Girls z-score: −0.006 (−0.043, 0.030) kg/m^2: −0.01 (−0.068, 0.048)

DNBC = Danish National Birth Cohort.
Results: "Models for weight at 5 or 12 mo included BW, models for length at 5 or 12 mo included birth length, and models for body mass index at 5 or 12 mo included birth body mass index."; adjusted models were used for all results.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, parity, pre-pregnancy body mass index, smoking, socioeconomic status, GA at blood drawing, breastfeeding. Additional confounding for BMI and 5 and 12 mo: birth BMI. Additional confounding height at 5 and 12 mo: birth height. Additional confounding for weight at 5 and 12 mo: BW.						
Apelberg et al. (2007) *Medium*	United States 2004–2005	Cross-sectional	Pregnant women and their newborns from Baltimore THREE Study, N = 293	Cord blood at birth 1.6 (1.2–2.1)	BW (g), HC (cm), BL (cm), ponderal index (g/cm^3 * 100), GA (days)	Regression coefficient per ln-unit increase in PFOA, regression coefficient per IQR increase in PFOA	BW Per ln-unit increase: −104 (−213, 5) Per IQR increase: −58 (−119, 3) HC Per ln-unit increase: −0.41(−0.76, −0.07), p-value < 0.05 Per IQR increase: −0.23 (−0.42, −0.04), p-value < 0.05 BL Per ln-unit increase: −0.10 (−0.64, 0.44) Per IQR increase: −0.06 (−0.36, 0.24) Ponderal index Per ln-unit increase: −0.07 (−0.138, −0.001), p-value < 0.05 Per IQR increase: −0.039 (−0.077, −0.001), p-value < 0.05 GA Per ln-unit increase: 1.1 (−1.2, 3.4) Per IQR increase: 0.9 (−1.1, 2.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: GA, maternal age, BMI, race, parity, smoking, baby sex, height, net weight gain, diabetes, hypertension. Additional confounding for head circumference: delivery mode.						
Fei et al. (2008a) *Medium*	Denmark Recruitment 1996–2002, Assessment 6–18 mo later	Cohort	Pregnant women and their children at 6 and 18 mo from the DNBC N = 1,400	Maternal plasma first trimester	Apgar score <10	OR for Q4 vs. Q1	1.14 (0.57, 2.25)
	DNBC = Danish National Birth Cohort. **Confounding:** Maternal age, maternal occupation and educational status, pregnancy body mass index (BMI), smoking and alcohol consumption during pregnancy, gestational weeks at blood drawing, child's sex.						
Fei et al. (2008b) *Medium*	Denmark 1996–2002	Cohort	Pregnant women and their newborns from the DNBC Placental weight N = 1,337 Birth length N = 1,376 Head circumference N = 1,347 Abdominal circumference N = 1,325	Maternal plasma between 4–14 wk gestation 5.21 (3.91–6.97)	Placental weight (g), BL (cm), HC (cm), abdominal circumference (cm)	Regression coefficient per unit increase in PFOA, or by quartile	Placental weight Per unit increase: −2.06 (−5.39, 1.28) Q2: −11.4 (−34, 11.2) Q3: −13.6 (−36.8, 9.7) Q4: −21.3 (−46.1, 3.4) Birth length Per unit increase: −0.069 (−0.113, −0.024) Q2: −0.21 (−0.51, −0.09) Q3: −0.04 (−0.35, 0.27) Q4: −0.49 (−0.81, −0.16) Head circumference Per unit increase: −0.03 (−0.064, 0.004) Q2: −0.09 (−0.32, 0.14) Q3: −0.23 (−0.47, 0.01) Q4: −0.14 (−0.39, 0.12) Abdominal circumference −0.059 (−0.106, −0.012) Q2: −0.07 (−0.38, 0.25) Q3: −0.16 (−0.49, 0.16)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: −0.29 (−0.63, 0.06)
	DNBC = Danish National Birth Cohort. **Results:** Lowest quartile used as reference group. **Confounding:** GA, quadratic GA, infant sex, maternal age, socio-occupational status, parity, cigarette smoking, pre-pregnancy body mass index, gestational week at blood drawing.						
Stein et al. (2009) *Medium*	United States Cohort 2005–2006		Pregnant women and their infants from the C8HP Birth defects N = 1,505 PTB N = 1,571 LBW N = 1,589	Maternal serum within 5 yr after pregnancy 21.2 (10.3–49.8)	Birth defects, PTB, LBW	OR per IQR increase in PFOA	Birth defects 1.68(0.8, 1.6) PTB 0.8 (0.8, 1.1) LBW 0.7 (0.5, 1.0)
	C8HP = C8 Health Project. **Population:** Includes "women who lived in the same contaminated water district from the approximate start of the pregnancy through the time of enrollment… to ensure that the PFOA level measured at C8 Health Project enrollment would reflect the level at the time of pregnancy." **Outcome:** PTB defined as birth at <37 wk gestation; LBW defined as <5.5 pounds at birth. **Confounding:** Maternal age, parity, education level at interview, smoking status at interview, PFOS levels.						
Savitz et al. (2012a) *Medium*	United States Cohort 1990–2005		Pregnant women from the C8HP N = 11,737	Modeled 1990–1994 6.0 (4.5–27.6) 1995–1999 10.7 (5.1–50.4) 2000–2005 15.9 (5.9–56.2)	PTB, term LBW, birth defects	OR per 100 ng/mL increase in estimated PFOA, OR by quintile, OR per IQR increase in estimated PFOA	PTB Per 100 ng/mL: 0.97 (0.93, 1.02) Q3: 1.0 (0.9, 1.2) Q4: 1.0 (0.8, 1.1) Q5: 1.0 (0.8, 1.1) Per IQR: 0.96 (0.89, 1.05) Term LBW Per 100 ng/mL: 0.96 (0.79, 1.16) Q3: 1.2 (0.8, 1.9) Q4: 1.2 (0.7, 1.9) Q5: 0.8 (0.4, 1.4)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Per IQR: 0.89 (0.66, 1.2) Birth defect Per 100 ng/mL: 0.97 (0.9, 1.06) Q3: 1.0 (0.7, 1.3) Q4: 1.1 (0.8, 1.4) Q5: 1.0 (0.8, 1.3) Per IQR: 1.0 (0.86, 1.16)
	C8HP = C8 Health Project. **Outcome:** PTB defined as birth 3 or more weeks before the due date; LBW defined as <5.5 pounds at birth. **Results:** Lowest two quintiles used as reference. Quintile ranges defined as follows: <40th percentile = 3.9–<6.8; 60th percentile = 16.6; 80th percentile = 63.1. **Confounding:** Exposure year, maternal age, parity, education level at interview, smoking status at interview.						
Arbuckle et al. (2020) *Medium*	Canada, 2008–2011	Cohort	Pregnant women (age range = 17–42 yr) and their infants from MIREC N = 205	Maternal blood 1.70 (1.10–2.50)	Anoclitoris distance (ACD, mm), anofourchette distance (AFD, mm), anopenile distance (APD, mm), anoscrotal distance (ASD, mm)	Regression coefficient per ln-unit increase in PFOA and by quartiles	ACD: 0.78 (–0.25, 1.82) Q2: 0.88 (–0.79, 2.54) Q3: 0.48 (–1.22, 2.17) Q4: 1.06 (–0.65, 2.76) AFD: 0.06 (–1.2, 1.32) Q2: –0.69 (–2.66, 1.28) Q3: 0.73 (–1.27, 2.74) Q4: –0.56 (–2.6, 1.48) APD: 0.1 (–0.94, 1.14) Q2: –0.76 (–2.65, 1.12) Q3: –0.02 (–1.91, 1.88) Q4: –0.51 (–2.5, 1.48) ASD: 1.36 (0.3, 2.41) Q2: 0.23 (–1.67, 2.13) Q3: –0.43 (–2.34, 1.47) Q4: 1.77 (–0.23, 3.77)
	MIREC = Maternal-Infant Research on Environmental Chemicals. **Results:** Lowest quartile used as reference. **Confounding:** Household income, education, active smoking status, GA, weight-for-length z-score, and recruitment site.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Chang et al. (2022) *Medium*	United States 2014–2018	Cohort	Mother-infant pairs from the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study N = 370	Maternal serum, Early pregnancy, 0.71 (0.45–1.07)	BW (g), SGA	BW: Regression coefficient per doubling in PFOA and by quartiles SGA: Odds ratio per doubling in PFOA and by quartiles	BW Per doubling: –14 (–49, 21) Q2: –126 (–241, –10) p < 0.05 Q3: –44 (–162, 73) Q4: –107 (–227, 13) p-trend = 0.23 SGA Per doubling: 1.20 (0.97, 1.49) Q2: 2.22 (1.10, 4.50) p < 0.05 Q3: 2.44 (1.21, 4.92) p < 0.05 Q4: 2.23 (1.10, 4.54) p < 0.05 p-trend = 0.06
	Outcome: SGA defined as a BW below the 10th percentile for GA. **Confounding:** maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex (BW only).						
Chen et al. (2012) *Medium*	Taiwan, 2004–2005	Cross-sectional	Mother-infant pairs from TBPS N = 429	Cord blood at birth GM (SD) = 1.84 (2.23)	BW (g), BL (cm), GA (weeks), HC (cm), ponderal index (g/cm^3), PTB, LBW, SGA	BW, BL, GA, HC, ponderal index: Regression coefficient per ln-unit increase in PFOA PTB, LBW, SGA: OR per ln-unit increase in PFOA	BW: –19.2 (–63.5, 23.1) BL: –0.003 (–0.21, 0.21) GA: 0.06 (–0.14, 0.26) Head circumference: –0.05 (–0.22, 0.17) Ponderal index: –0.01 (–0.04, 0.02) PTB: 0.64 (0.4, 1.02) LBW: 0.53 (0.18, 1.55) SGA: 1.24 (0.75, 2.05)
	TBPS = Taiwan Birth Panel Study. **Outcome:** PTB defined as GA <37 wk. LBW defined as a BW <2,500 g. SGA defined as a BW below the 10th percentile for GA.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, pre-pregnancy body mass index, education level, log (Ln)-transformed cord blood cotinine levels, type of delivery, parity and infant sex.						
Chen et al. (2017b) *Medium*	Taiwan, 2004–2005	Cohort	Mother-infant pairs from the Taiwan Birth Panel Study (TBPS) N = 429	Cord blood	BMI (z-score, kg/m^2), height (z-score, cm), weight (z-score, kg)	Regression coefficient per ln increase in PFOA	At Birth BMI: –0.09 (–0.2, 0.02) Females: 0.02 (–0.13, 0.17) Males: –0.2 (–0.36, –0.04) Height: –0.04 (–0.16, 0.08) Females: –0.007 (–0.18, 0.17) Males: –0.05 (–0.22, 0.12) Weight: –0.07 (–0.18, 0.03) Females: 0.02 (–0.14, 0.17) Males: –0.15 (–0.3, –0.006)
	Population: Infants were followed up at 4, 6, 13, 24, 60, 84, and 108 mo. **Results:** Regression coefficients reported at birth; BMI, height, and weight (overall and stratified by infant sex) at follow-up points were not statistically significant. **Confounding:** Maternal age, pre-pregnancy BMI, education level, ln-cord blood cotinine, infant sex, PTB, postnatal ETS exposure, breastfeeding.						
Chen et al. (2021) *Medium*	China Recruitment: 2013–2015	Cohort	Mother-child pairs from the SBC, Ages ≥20, N = 214 (95 male children, 119 female children)	Maternal plasma from the first trimester 15.2 (11.08–20.88)	BW (g), BL (cm), HC (cm)	Regression coefficient per ln-unit increase in PFOA	BW 33.7 (−83.9, 151.3) BL −0.27 (−0.61, 0.07) Males −0.21 (−0.73, 0.32) Females −0.21 (−0.74, 0.33) HC −45.9 (−113.9, 22.0)
	SBC = Shanghai Birth Cohort. **Confounding:** Maternal age, BMI, educational level, occupation, income, fetal sex, parity, GA, smoking, and alcohol.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Darrow et al. (2014) *Medium*	United States, Recruitment: 2005–2006; Follow-up: 2008–2011	Cohort	Pregnant women with known PFAS exposure (ages ≥20 yr) from C8HP N = 1,438 (first pregnancy = 1,129)	Serum collected before pregnancy 15.6 (9.0–31.9)	Primary analysis miscarriage, first pregnancy miscarriage	OR per ln-unit increase in PFOA and by quintiles	Primary Analysis: 1.01 (0.88, 1.16) Q2: 0.84 (0.53, 1.32) Q3:1.08 (0.69, 1.69) Q4: 1.08 (0.69, 1.68) Q5: 1.00 (0.63, 1.58) First Pregnancy: 1.04 (0.89, 1.21) Q2: 1.03 (0.62, 1.71) Q3: 1.27 (0.78, 2.08) Q4: 1.34 (0.81, 2.20) Q5: 1.07 (0.64, 1.77)
	C8HP = C8 Health Project. **Outcome:** Primary analysis includes more than one pregnancy for some women (304 miscarriages). First pregnancy is restricted to the first pregnancy conceived per woman after serum measurement (213 miscarriages). **Results:** Lowest quintile used as reference. **Confounding:** Maternal age, educational level, smoking status, BMI, self-reported diabetes, time between conception, and serum measurement.						
de Cock et al. (2014a) *Medium*	The Netherlands Recruitment: 2011–2013 Follow-up at 1, 2, 4, 6, 9, and 11 mo after birth	Cohort	Mother-child pairs N = 89	Cord blood 870 ng/L (Range = 300–2,700 ng/L)	BMI (kg/m^2), HC (cm), height (cm), weight (kg)	Regression coefficient for quartiles of PFOA	BMI, HC, height, and weight: no statistically significant associations
	Confounding: BW, GA, maternal height.						
de Cock et al. (2016) *Medium*	The Netherlands, 2011–2013	Cross-sectional	Mother-infant pairs N = 64	Cord blood 870 ng/L (Range = 200–2,700 ng/L)	BW (g)	Regression coefficient by tertiles	T2: 24.6 (–270.12, 319.33) T3: 191.3 (–137.17, 519.73) Females T2: 238.1 (–183.42, 659.57) T3: –10.8 (–487.87, 466.34) Males

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T2: –184.8 (–623.06, 253.41) T3: 168.4 (–239.18, 575.92) No statistically significant associations or trends by tertiles
	Results: Lowest tertile used as reference. **Confounding:** GA, maternal BMI, maternal height, maternal age at birth, and parity, paternal BMI, paternal height, education, fish intake.						
Govarts et al. (2018) *Medium*	Belgium, the Netherlands, Norway, and Slovakia 2002–2012	Cohort	Mother-child pairs from FLEHS I and II, HUMIS, LINC, and PCB Cohort N = 662	Cord blood 550 ng/L (299–1,200 ng/L)	SGA	OR per IQR increase of PFOA	1.637 (0.971, 2.761)
	FLEHS = Flemish Environmental and Health Study; HUMIS = Human Milk Study; LINC = Linking EDCs in Maternal Nutrition to Child Health. **Outcome:** SGA defined as newborns weighing below the 10th percentile for the norms defined by GA, country, and infant's sex. **Confounding:** Maternal education, maternal age at delivery, maternal height, maternal pre-pregnancy BMI, smoking during pregnancy, parity, child's sex.						
Gyllenhammar et al. (2018b) *Medium*	Sweden, 1996–2011 and follow-up at 5 yr of age	Cohort and cross-sectional	Mother-infant pairs of singleton births from POPUP study N = 381	Maternal serum Later pregnancy 2.3 (1.6–3.0)	BL (SD scores), BW (SD scores), gestational length (days), HC (SD scores), length (SD scores), weight (SD scores)	Regression coefficient per IQR increase in maternal PFOA	BL: 0.0014 (–0.1435, 0.1478) BW: –0.0579 (–0.1852, 0.0695) Gestational length: –0.2201 (–1.5028, 1.055) HC: –0.0219 (–0.1648, 0.121)
	POPUP = Persistent Organic Pollutants in Uppsala Primiparas. **Confounding:** Sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, smoking during pregnancy, total fish consumption.						
Hamm et al. (2010) *Medium*	Canada Recruitment: 2005–2006 Follow-up at	Cohort	Pregnant women (≥18 yr of age) and their singleton children	Maternal serum collected at 15–16 wk gestation	BW (g, z-score), length of gestation (weeks), SGA, PTB	BW, GA: Regression coefficient per ln-unit or per	BW: −37.4 (−86.0, 11.2) T2: 20.54100.51, 141.57) T3: 14.80 (107.29, 136.89)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	delivery: 2006–2007		delivered at or after 22 wk gestation N = 252	GM (SD) = 1.3 (2.9)		unit increase in PFOA and by tertiles SGA, PTB: Relative risk by tertiles	BW (g per unit): 12.4 (−32.8, 8.0) BW (z-score): −0.078 (−0.19, 0.032) T2: 0.055 (0.22, 0.33) T3: 0.031 (0.25, 0.31) GA: −0.06 (−0.28, 0.15) T2: 0.012 (0.54, 0.52) T3: 0.086 (0.62, 0.45) SGA: T2: 0.55 (0.16, 1.83) T3: 0.99 (0.25, 3.92) PTB: T2: 0.88 (0.28, 2.78) T3: 1.31 (0.38, 4.45)
	Outcome: SGA defined as BW <10th percentile for GA and infant gender; PTB defined as delivery at 22–36 wk. **Results:** Lowest tertile used as reference. **Confounding:** Maternal age, maternal race, gravida, maternal weight, height, smoking. Additional confounding for BW: Infant gender, GA at birth. Additional confounding for PTB: Infant gender.						
Hjermitslev et al. (2019) *Medium*	Greenland, Recruitment: 2010–2011, 2013–2015	Cohort	Pregnant women (≥18 yr of age) and their children from ACCEPT N = 256	Maternal serum Early pregnancy, later pregnancy 1.06 (Range = 0.10–7.26)	BW (g), GA at birth (weeks), HC (cm), PTB	Regression coefficient and OR per ln-unit increase in PFOA	BW: –119 (–202, –36.6), p-value = 0.005 Females: –161 (–283, –40.1), p-value = 0.01 Males: –81.2 (–194, 31.2) GA: 0.45 (0.17, 0.74), p-value = 0.002 Female: 0.48, p-value = 0.019 Male: 0.42, p-value = 0.043 HC: –0.14 (–0.42, 0.14)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Females: –0.51 (–0.88, 0.15) Males: 0.22 (–0.56, 0.12) PTB OR: –0.146, p-value = 0.011
	ACCEPT = Adapting to Climate Change, Environmental Pollution and Dietary Transition. **Confounding:** Maternal age, plasma cotinine, alcohol consumption during pregnancy, pre-pregnancy BMI, GA at birth.						
Jensen et al. (2020a) *Medium*	Denmark, 2010–2012 and follow-up at 18 mo of age	Cohort	Pregnant women and infants at 3 and 18 mo of age from Odense Child Cohort N = 593	Maternal serum 1.62 (0.67–4.03)	Ponderal index standard deviation score (SDS)	Regression coefficient per unit increase in PFOA	0.07 (0.01, 0.13), p-value = 0.02
	Outcome: Ponderal index (kg/m^3) was calculated as weight (kg) divided by the length cubed (m^3). **Results:** PFOA pooled 3 and 18 mo. **Confounding:** Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI^2, education, smoking, sex, visit, adiposity marker at birth.						
Kashino et al. (2020) *Medium*	Japan, 2003–2009	Cohort	Mother-infant pairs from the Hokkaido Study on Environment and Children's Health N = 1,949	Plasma Later pregnancy 2.0 (1.3–3.3)	Birth HC (cm), BL (cm), BW (g)	Regression coefficient per log10-unit increase in PFOA	HC: 0.053 (–0.189, 0.295) Females: 0.039 (–0.32, 0.398) Males: 0.099 (–0.228, 0.425) Length: –0.032 (–0.309, 0.246) Females: –0.013 (–0.4, 0.373) Males: –0.041 (–0.442, 0.36) BW: –18.7 (–69.8, 32.4) Females: –1.8 (–75.1, 71.5) Males: –29.5 (–101.3, 42.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							HC, BL, and BW: no statistically significant associations overall or stratified by sex
	Confounding: GA, maternal age, pre-pregnancy BMI, parity, infant sex, maternal educational level, plasma cotinine concentration during pregnancy.						
Kobayashi et al. (2017) *Medium*	Japan, 2002–2005	Cross-sectional	Pregnant women at 22–35 wk gestation and infants from Hokkaido Study on Environment and Children's Health N = 177	Maternal serum Later pregnancy 1.4 (0.9–2.1)	BL (cm), BW (g)	Regression coefficient per ln-unit increase in PFOA	Length: 0.01 (–0.37, 0.4) BW: –494. (–130.4, 31.6) Length and BW: no statistically significant associations
	Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, GA, infant sex, maternal blood sampling period.						
Kobayashi et al. (2022) *Medium*	Japan Recruitment: 2002–2005	Cohort	Mother-child pairs from the Sapporo Cohort of the Hokkaido Birth Cohort N = 372 (198 female children, 174 male children)	Maternal blood in the third trimester 1.3 (0.8–1.8) Females 1.2 (0.8–1.7) Males 1.4 (0.9–1.8)	BL (cm), BW (g)	Regression coefficient per log10-unit increase in PFOA	BL –0.408 (–1.112, 0.307), p-value = 0.262 Females: –0.608 (–1.538, 0.302), p-value = 0.187 Males: –0.077 (–1.253, 1.099), p-value = 0.897 BW –107.1 (–232.5, 18.4), p-value = 0.094 Females: –183 (–361.9, –4.1), p-value = 0.045 Males: –55.8 (–235.4, 123.8), p-value = 0.540
	Confounding: Maternal age (continuous), pre-pregnancy BMI (continuous), maternal smoking in the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), parity (primiparous/multiparous), educational level, annual household income, cesarean section (yes/no), maternal blood sampling period, GA (continuous), and infant sex.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Kwon et al. (2016) *Medium*	Korea, 2006–2010	Cohort	Pregnant women and infants from EBGRC N = 268	Cord blood 0.91 (0.68–1.15)	BW (g)	Regression coefficient per log-unit increase in PFOA	–77.93 (–153.56, –2.3), p-value = 0.04
	EBGRC = Ewha Birth & Growth Retrospective Cohort. **Comparison:** Logarithm base not specified. **Confounding:** Mother's age, pre-pregnancy BMI, past history of alcohol consumption and child's GA, gender, parity.						
Lenters et al. (2016) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cohort	Pregnant women and singleton infants from INUENDO N = 1,250	Maternal serum Later pregnancy GM = 1.421 (2-SD ln-PFOA = 1.175)	BW at term (g)	Regression coefficient per 2-SD increase in ln-PFOA	–68.94 (–134.25, –3.63), p-value = 0.039
	INUENDO = Biopersistent Organochlorines in Diet and Human Fertility. **Confounding:** Study population, maternal age, pre-pregnancy BMI, parity.						
Liew et al. (2020) *Medium*	Denmark, 1996–2002	Case-control	Females from the Danish National Birth Cohort, N = 438	Plasma, Cases: 3.96 (3.02, 5.22) Controls: 3.56 (2.76, 4.66)	Miscarriage	OR per doubling of PFOA and by quartiles	1.4 (1, 1.9) Q2: 1 (0.5, 1.8) Q3: 1.4 (0.8, 2.6) Q4: 2.2 (1.2, 3.9) p-value for trend <0.01
	Results: Lowest quartile used as the reference group. **Confounding:** Maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, parity.						
Louis et al. (2016) *Medium*	United States, 2005–2009	Cohort	Females from the LIFE study, Ages ≤24, 24–29, 30–34, ≥35, N = 344	Serum, Pregnant women: 3.3 (2.2, 4.9) Infertile females: 3.2 (2.5, 4.3)	Pregnancy loss	HR per log-unit increase in PFOA	0.93 (0.75, 1.16)
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI, prior pregnancy loss conditional on previous pregnancy, any alcohol consumption during pregnancy, any cigarette smoking during pregnancy.						
Liu et al. (2020c) *Medium*	China, 2009–2013	Nested case-control	Pregnant women and infants N = 519	Maternal blood 0.79 (0.51–1.17)	PTB (spontaneous)	OR per log10-unit increase in PFOA and by quartiles	1.08 (0.41, 1.6), p-value = 0.538 Q2: 1.22 (0.68, 2.16) Q3: 0.87 (0.48, 1.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Q4: 1.02 (0.55, 1.88) No statistically significant association by quartiles
	Population: Cases, n = 144; controls, n = 375. **Exposure Level**: Cases: 0.74 (0.51–1.17); controls: 0.80 (0.51–1.18). **Results**: Lowest quartile used as reference. **Confounding:** Sampling time, maternal age, pre-pregnancy BMI, occupation, parity, gravidity, spontaneous abortion history, child gender, folic acid use, passive smoking, fasting status, medication use.						
Maisonet et al. (2012) *Medium*	Great Britain Recruitment: 1991–1992, Followed up until 20 mo of age	Cohort	Pregnant women and their singleton girls assessed at birth and 2, 9, and 20 mo from ALSPAC BW N = 422 BL N = 356 GA N = 444 Ponderal index N = 360 Weight at 20 mo N = 320 (106 upper tertile of BW, 107 middle tertile of BW, 107 lower tertile of BW)	Maternal serum during pregnancy (median 15 wk) 3.7 (Range = 1.0–16.4)	BW (g), BL (cm), GA (weeks), ponderal index (g/cm^3), weight at 20 mo (g)	Regression coefficient by tertiles	BW: T2: –56.81 (–153.05, 39.43) T3: –133.45 (–237.37, –29.54) p-trend = 0.0120 BL: T2: 0.14 (–0.34, 0.61) T3: –0.44 (–0.96, 0.08) p-trend = 0.0978 GA: T2: –0.25 (–0.61, 0.12) T3: –0.34 (–0.73, 0.05) p-trend = 0.0833 Ponderal Index: T2: –0.06 (–0.12, 0.01) T3: 0.02 (–0.05, 0.09) p-trend = 0.5920 Weight at 20 mo: T2: –184.21 (–465.9, 97.48) T3: 128.4 (–180.94, 437.74) p-trend = 0.4147 Upper tertile of BW: T2: 15.13 (–573.62, 603.87) T3: –27.39 (–785.4, 730.61)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							p-trend = 0.9430 Middle tertile of BW: T2: –121.55 (–708.11, 465.01) T3: 169.83 (–497.87, 837.54) p-trend = 0.6149 Lower tertile of BW: T2: –21.13 (–827.99, 785.72) T3: 248.27 (–570.54, 1,067.08) p-trend = 0.5488
	ALSPAC = Avon Longitudinal Study of Parents and Children. **Results:** Lowest tertile used as reference. **Confounding**: BW: maternal smoking during pregnancy, maternal pre-pregnancy BMI, previous live births, and GA; BL additionally adjusted for maternal education. GA: GA when maternal serum sample was obtained. Ponderal index: maternal pre-pregnancy BMI, previous live births, and GA when maternal serum sample was obtained. Weight at 20 mo (all tertiles): height at 20 mo, BW, maternal education, maternal age at delivery, and previous live birth; intratertile analyses adjusted for maternal education, maternal age at delivery, previous live birth, and BW.						
Manzano-Salgado et al. (2017b) *Medium*	Spain, 2003–2008	Cohort	Mother (aged ≥16 yr)-child pairs from INMA assessed at birth and 6 mo N = 1,154 (568 girls, 586 boys)	Maternal blood GM = 2.32 (1.63–3.31)	Rapid growth, weight gain (z-score)	Relative risk and regression coefficient per log2-unit increase in PFOA	Rapid growth: 0.99 (0.86, 1.14) Weight gain z-score: 0.04 (–0.04, 0.12) Females: –0.03 (–0.14, 0.08) Males: 0.13 (0.01, 0.26) p-value for sex interaction = 0.28
	INMA = Infancia y Medio Ambiente (Environment and Childhood Project). **Outcome:** Rapid growth defined as a z-score >0.67 standard deviation for weight gain from birth until 6 mo. **Confounding:** Maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI), age and sex of child.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Meng et al. (2018) *Medium*	Denmark, 1996–2002	Cohort	Pregnant women and their infants from DNBC N = 3,507	Maternal serum Early pregnancy, Later pregnancy 4.6 (3.3–6.0)	BW (g), GA (days), low LBW, PTB	BW and GA: Regression coefficient per doubling of PFOA and by quartiles LBW and PTB: OR per doubling of PFOA and by quartiles	BW: –35.6 (–66.3, –5) Q2: –20.4 (–70, 29.2) Q3: –25.9 (–77.7, 25.9) Q4: –117 (–172.3, –61.6) Females: –25 (–71.4, 21.5) Males: –41.5 (–82.1, –0.9) GA: –0.4 (–1, 0.3) Q2: –1.4 (–2.4, –0.3) Q3: –1.2 (–2.2, –0.1) Q4: –1.7 (–2.9, –0.6) Females: –0.1 (–1.1, 0.9) Males: –0.6 (–1.4, 0.3) LBW: 1 (0.7, 1.5) Q2: 1.5 (0.8, 3.1) Q3: 1.2 (0.5, 2.5) Q4: 1.5 (0.7, 3.3) PTB: 1.1 (0.8, 1.5) Q2: 3.2 (1.8, 5.6) Q3: 1.7 (0.9, 3.2) Q4: 1.9 (1, 3.6) BW and GA: no statistically significant associations by sex
	DNBC = Danish National Birth Cohort. **Results:** Lowest quartile used as reference. **Confounding:** Infant sex, infant birth year, gestational week of blood draw, maternal age, parity, socio-occupational status, pre-pregnancy body mass index, smoking during pregnancy, alcohol intake during pregnancy, study sample.						
Ou et al. (2021) *Medium*	China, 2014–2018	Nested case-control	Pregnant women and their children with (cases) and without (controls) CHD	Maternal blood and cord blood at delivery Maternal blood	Septal defects, conotruncal defects, and total CHD	OR for >75th percentile vs. <75th percentile PFOA	Maternal PFOA Septal defects: 0.54 (0.18, 1.62) Conotruncal defects: 0.28 (0.07, 1.10)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
			N = 316	Cases: 1.524 (1.275–1.914) Controls: 1.491 (1.178–2.230) Cord blood Cases: 1.083 (0.778–1.379) Control: 1.169 (0.895–1.397)			Total CHD: 0.64 (0.34, 1.21) Cord PFOA Septal defects: 0.58 (0.16, 2.10) Conotruncal defects: 1.66 (0.12, 22.1) Total CHD: 0.66 (0.23, 1.88)
	CHD = congenital heart defects. **Outcome**: Total congenital heart defects included septal defects and conotruncal defects, as well as individual congenital heart defect subtypes with a large number of cases. **Confounding**: Maternal age, parity, infant sex.						
Robledo et al. (2015) *Medium*	United States, 2005–2009	Cohort	Couples and their children from the LIFE study N = 234	Serum Early pregnancy Girls: GM = 3.16 (95% CI: 2.92, 3.42) Boys: GM = 5.00 (95% CI: 4.70, 5.32)	BW (g), HC (cm), BL (cm), ponderal index (g/cm^3)	Regression coefficient for mean change per 1-SD increase in ln(maternal PFOA) or in ln(paternal PFOA)	Maternal PFOA Girls: BW: –61.64 (–159.15, 35.87) HC: –0.18 (–0.59, 0.23) BL: –0.17 (–0.74, 0.40) Ponderal Index: –0.02 (–0.09, 0.04) Boys: BW: 4.78 (–85.44, 95.01) HC: 0.18 (–0.25, 0.60) BL: –0.24 (–0.77, 0.29) Ponderal Index: 0.04 (–0.02, 0.10) Paternal PFOA Girls: BW: 19.82 (–69.37, 109.02) HC: –0.03 (–0.42, 0.36) BL: –0.27 (–0.79, 0.25) Ponderal Index: 0.06 (0.00, 0.12)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Boys: BW: –11.04 (–112.32, 90.23) HC: –0.04 (–0.52, 0.43) BL: –0.26 (–0.86, 0.34) Ponderal Index: 0.03 (–0.04, 0.10)
	LIFE = Longitudinal Investigation of Fertility and the Environment. **Confounding:** Maternal and paternal serum lipids, serum cotinine, BMI, maternal age, difference in paternal age, infant gender, individual and partner sum of remaining chemical concentrations in each chemical's respective class.						
Savitz et al. (2012b) *Medium*	United States, 1990–2004	Nested case-control	Pregnant women and their infants, Study II linked to C8HP data Study I: N = 3,695 Study II: N = 4,547	Modeled Study I: 7.7 (4.9–17.2) Study II: 13.4 (5.6–61.2)	PTB, stillbirth, term SGA, term LBW, BW (g)	PTB, stillbirth, LBW, low SGA: OR per 100-unit increase in PFOA, or by quartiles, or per IQR increase in ln-PFOA BW: Adjusted mean difference per 100-unit increase in PFOA, or by quartiles, or per IQR increase in ln-PFOA	Study I: PTB: 1.02 (0.94, 1.1) Q2: 1.0 (0.8, 1.1) Q3: 1.0 (0.9, 1.2) Q4: 1.0 (0.9, 1.2) Per IQR: 1.02 (0.96, 1.08) Stillbirth: 1.2 (0.86, 1.68) Q2: 0.9 (0.4, 2.0) Q3: 1.0 (0.5, 1.7) Q4: 0.8 (0.5, 1.5) Per IQR: 1.0 (0.76, 1.32) Term SGA: 0.86 (0.67, 1.11) Q2: 1.0 (0.7, 1.4) Q3: 1.0 (0.7, 1.5) Q4: 0.8 (0.6, 1.2) Per IQR: 0.91 (0.78, 1.06) Term LBW: 1.0 (0.86, 1.15) Q2: 0.9 (0.7, 1.2) Q3: 1.0 (0.8, 1.3) Q4: 1.0 (0.8, 1.3) Per IQR: 1.02 (0.92, 1.13)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							BW: –14.8 (–43.28, 13.68) Q2: 22.8 (–32.9, 78.5) Q3: 2.3 (–50.3, 54.8) Q4: –9.5 (–58.4, 39.4) Per IQR: –10.72 (–32.26, 10.82) Study II: PTB: 1.09 (1.0, 1.18) Q2: 1.2 (0.9, 1.5) Q3: 0.8 (0.6, 1.1) Q4: 1.2 (0.9, 1.6) Per IQR: 1.09 (0.91, 1.32) Term SGA: 1.07 (0.98, 1.17) Q2: 1.0 (0.7, 1.4) Q3: 1.1 (0.8, 1.6) Q4: 1.3 (0.9, 1.7) Per IQR: 1.18 (0.97, 1.43) Term LBW: 1.0 (0.82, 1.21) Q2: 0.9 (0.5, 1.7) Q3: 1.6 (1.0. 2.8) Q4: 0.9 (0.5, 1.7) Per IQR: 1.04 (0.75, 1.44) BW: –9.14 (–20.3, 2.02) Q2: –3.8 (–40.4, 32.8) Q3: –25.4 (–63.7, 12.9) Q4: –33.3 (–73.1, 6.5) Per IQR: –21.89 (–45.91, 2.13)

C8HP = C8 Health Project.
Outcome: PTB defined as birth at <37 wk gestation. Term SGA is defined as BW <10th percentile by GA and sex. LBW defined as BW <2,500 g. Stillbirths are only reported for Study I.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Results: Lowest quartile used as reference. **Confounding**: Maternal age, education, parity, smoking status, exposure year, state of residence. Additional confounding for term LBW and BW: GA.						
Vesterholm et al. (2014) *Medium*	Denmark and Finland Recruitment 1997–2002, follow-up 3 mo after birth	Nested case-control	Boys with (107 cases) or without (108 controls) cryptorchidism N = 215	Cord blood 2.6 (5th–95th percentile: 1.4–4.4)	Cryptorchidism	OR per ln-unit increase in PFOA or by tertiles	Continuous: 0.51 (0.21, 1.2) T2: 0.58 (0.28, 1.22) T3: 0.46 (0.20, 1.02) p-trend = 0.06
	Outcome: Cryptorchidism defined as by Scorer (1964). **Exposure Level:** Denmark cases: 2.4 (5th–95th percentile: 1.4–4.4); controls: 2.70 (5th–95th percentile: 1.4, 4.0); Finland cases: 1.9 (5th–95th percentile: 1.0–3.9); controls: 2.3 (5th–95th percentile: 1.2–4.8). **Results:** Lowest tertile used as reference. **Confounding:** BW, GA, parity.						
Wang et al. (2019a) *Medium*	China 2013	Cross-sectional	Pregnant women and their children at birth N = 340 (171 girls, 169 boys)	Cord blood Later pregnancy 1.99 (1.22–3.11)	BL (cm), BW (g), BW z-score, HC cm), ponderal index (g/cm^3)	Regression coefficient per log10-unit increase in PFOA	BL 0.09 (−0.39, 0.58); p-value = 0.702 Girls: −0.13 (−0.86, 0.59); p-value = 0.715 Boys: 0.06 (−0.59, 0.72); p-value = 0.855 p-value for interaction by sex = 0.913 BW −33.42 (−149.6, 82.77); p-value = 0.573 Girls: −84.07 (−260.42, 92.28); p-value = 0.35 Boys: −21.24 (−171.66, 129.17); p-value = 0.782 p-value for interaction by sex = 0.959 BW z-score

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							−0.09 (−0.41, 0.23); p-value = 0.589 HC −0.37 (−0.70, −0.04); p-value = 0.028 Girls: −0.57 (−1. 07, −0.08); p-value = 0.023 Boys: −0.35 (−7.89, −0.96); p-value = 0.124 p-value for interaction by sex = 0.992 Ponderal index −0.05 (−0.10, 0.01); p-value = 0.103 Girls: −0.05 (−0.13, 0.03); p-value = 0.23 Boys: −0.03 (−0.10, 0.04); p-value = 0.401 p-value for interaction by sex = 0.980
	Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, GA, parity, pre-pregnant maternal body mass index, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain.						
Woods et al. (2017) *Medium*	United States, Recruitment: 2003–2006; outcome assessed at birth	Cohort	Pregnant women and their children at birth from the HOME study N = 272	Maternal serum Later pregnancy 5.4 (3.8–8.1)	BW (g)	Regression coefficient per log10-unit increase maternal PFOA	−13.1 (−53.2, 27.0)
	HOME = Health Outcomes and Measures of Environment. **Confounding:** Maternal race, age at delivery, infant sex, maternal education, tobacco exposure, household annual income, employment, maternal insurance status, marital status, prenatal vitamin use, maternal BMI, GA.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Yang et al. (2022In Press) *Medium*	China 2018–2019	Nested case-control	Infants from the KBCS, N = 768 (384 term births, 384 PTBs)	Cord blood at birth Term births 0.455 (0.221–0.785) PTBs 0.289 (0.167–0.562)	PTB, GA (weeks)	OR (PTB) and regression coefficient (GA) per IQR increase in PFOA	PTB 1.03 (0.89, 1.2), p-value = 0.71 GA Term births −0.38 (−1.33, 0.57), p-value = 0.44 PTBs −1.04 (−3.72, 1.63), p-value = 0.44
	KBCS = Kashgar Birth Cohort Study. **Outcome:** PTBs defined as live born infants with GA at delivery 28–36 wk. **Confounding:** Maternal age, maternal ethnicity, maternal BMI, household income, maternal education level, maternal tobacco smoking during pregnancy, maternal alcohol consumption during pregnancy, parity, living near factory, periconceptional folic acid intake, gestational diabetes, gestational hypertension, infant's sex.						
Callan et al. (2016) *Low*	Australia, 2008–2011	Cross-sectional	Mother-infant pairs enrolled in AMETS, Ages 19–44, N = 98	Maternal blood 0.86 (0.21–3.1)	BW (g), BL (cm), Proportion of optimal BW (POBW), HC (cm), ponderal index ($g/cm^3 \times 100$), proportion of optimal BL (POBL), proportion of optimal HC (POHC)	Regression coefficient per ln-unit increase in PFOA	BW −48 (−203, 108) BL 0.06 (−0.7, 0.81) POBW 0.83 (−3.6, 5.3) HC −0.4 (−0.96, 0.16) Ponderal Index −0.06 (−0.16, 0.05) POBL 0.42 (−1, 1.9) POHC −0.66 (−2.3, 1)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	AMETS = Australian Maternal Exposure to Toxic Substances. **Confounding:** For BW, BL, HC, and ponderal index: GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy, sex of infant. For POHC: Weight gain during pregnancy, annual household income. For POBL: Weight gain during pregnancy, maternal age, annual household income.						
Cao et al. (2018) *Low*	China, 2013–2015	Cohort	Infants from Zhoukou City, China, N = 337 (183 males, 154 females) Postnatal weight, postnatal length, postnatal head circumference N = 282 (157 males, 125 females)	Cord blood 1.25 (0.87–1.82)	BW (g), BL (cm), ponderal index (g/cm^3), postnatal weight (g), postnatal length (cm), postnatal HC, birth defects	Regression coefficient and OR by tertiles	BW T2: −42.3 (−165.6, 81) T3: −26.3 (−149.1, 96.4) Males T2: −121.7 (−293.3, 49.8) T3: −15.4 (−181.9, 151.2) Females T2: 41.3 (−135.1, 217.7) T3: −65.3 (−247.1, 116.6) BL T2: −0.21 (−0.56, 0.14) T3: −0.45 (−0.79, −0.1) Males T2: −0.22 (−0.68, 0.23) T3: −0.36 (−0.8, 0.09) Females T2: −0.16 (−0.68, 0.37) T3: −0.58 (−1.12, −0.04) Ponderal Index T2: −0.01 (−0.09, 0.09) T3: 0.06 (−0.03, 0.15) Males T2: −0.07 (−0.21, 0.08) T3: 0.06 (−0.08, 0.2) Females T2: 0.07 (−0.04, 0.17) T3: 0.05 (−0.07, 0.16) Postnatal Weight

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							T2: −429.2 (−858.4, −0.121) T3: −114.9 (−562, 332.1) Males T2: −661.1 (−1,193.8, −128.4) T3: −284.6 (−830.9, 261.7) Females T2: −103.3 (−825.5, 618.8) T3: 8.1 (−757.5, 773.6) Postnatal Length T2: −0.47 (−2.3, 1.37) T3: 1.37 (−0.5, 3.28) Males T2: −1.95 (−4.3, 0.4) T3: 0.58 (−1.82, 2.99) Females T2: 1.4 (−1.51, 4.31) T3: 2.13 (−0.95, 5.21) Postnatal HC T2: 0.12 (−0.8, 1.03) T3: −0.04 (−0.09, 0.92) Males T2: 0.2 (−0.99, 1.4) T3: 0.72 (−0.51, 1.94) Females T2: −0.23 (−1.65, 1.19) T3: −1.46 (−2.96, 0.05) Birth Defects T2 OR: 0.87 (0.38, 1.96) T3 OR: 1.24 (0.57, 2.61)

Comparison: Tertiles were defined as follows: T2 = 0.99–1.59 vs. <0.99. T3 = >1.59 vs. <0.99. T2 OR = 0.99–1.59 vs. <0.99. T3 OR = >1.52 vs. <0.74.
Results: Lowest tertile used as reference.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Maternal age, household income, parity, infant's gender. Additional confounding for BW, birth defects, ponderal index: smoking of father, drinking of father. Additional confounding for BW, birth defects, ponderal index, postnatal weight, postnatal length, POHC: maternal education. Additional confounding for postnatal weight, postnatal length, and POHC: infant's age.						
Espindola Santos et al. (2021) *Low*	Brazil Recruitment: 2017	Cross-sectional	Mother-child pairs of women enrolled in the PIPA project BW: N = 63 BL, weight-for-length N = 56 HC N = 53	Cord blood from newborns 0.44 (0.21–1.02)	BW, BL, weight-for-length, and HC (z-scores)	Regression coefficient per log10-unit increase in PFOA	BW: 0.38 (–0.18, 0.93) BL: 0.26 (–0.21, 0.73) Weight-for-length: 0.50 (–0.17, 1.16) HC: 0.62 (–0.096, 1.269)
	PIPA = Rio Birth Cohort Study. **Population**: Mothers were recruited between 28th–32nd weeks of gestation and were over 16 yr of age. **Exposure**: Year of assessment not reported. **Confounding**: Education, income, race, pre-gestational BMI, smoking active and passive, alcohol consumption, GA, primiparity, age (continuous), and fish consumption.						
Gross et al. (2020) *Low*	United States 2012–2014	Nested case-control	Healthy and overweight 18-mo-old Hispanic children from StEP, N = 98	Newborn blood Mean (SD) = 0.376 (0.249)	BW (z-score), overweight	Regression coefficient (BW) and OR (overweight) for PFOA >mean level vs. PFOA ≤ mean level	BW (z-score) −0.26 (−0.63, 0.11) Overweight 0.91 (0.36, 2.29)
	StEP = Starting Early Program **Outcome:** Overweight defined as 18-mo weight-for-length z-score ≥85th percentile. **Confounding:** Maternal age, maternal education, maternal depressive symptoms, pre-pregnancy BMI, GA, parity, and intervention status.						
Nolan et al. (2010)	United States 2003–2005	Cross-sectional	Mother-child pairs	Drinking Water	Congenital anomalies	OR by LHWA exposure level	Congenital abnormalities LHWA vs. No LHWA

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
Low			N = 1,548	LHWA 5.7 (Range = 1.7–17.1) Non-LHWA 0.0049 (Range = 0.0–0.017)			1.1 (0.34, 3.3) LHWA vs. partial LHWA 1.68(0.4, 3.1)
	LHWA = Little Hocking Water Association (water service area with high PFOA). **Population:** No LHWA was defined as residing in ZIP codes served by Marietta and Warren Water Service. Partial LHWA was defined as ZIP codes served in part by the LHWA and in part by Belpre Water. **Confounding:** Maternal age, PTB, parity, sec, race, maternal education, diabetic status, alcohol, and tobacco use.						
Wu et al. (2012) *Low*	China, 2007	Cross-sectional	Pregnant women residing in e-waste recycling (Guiyu) and non-e-waste recycling (Chaonan) areas, N = 167 (108 Guiyu, 59 Chaonan) Still births N = 146 LBW N = 150 Premature delivery N = 146	Maternal serum Guiyi: 16.95 (5.5–58.5) Chaonan: 8.70 (4.4–30.0)	BW (g), Apgar score (5-minute), BL (cm), GA (weeks), ponderal index ($g/cm^3 \times 100$), premature delivery, still birth, term LBW	Apgar score, BL, BW, GA, ponderal index: regression coefficient per log10-unit increase in PFOA Premature delivery, still birth, term LBW: comparison of mean log10 unit PFOA concentrations	BW −267.3 (−573.27, −37.18), p-value < 0.05 Apgar score −1.37 (−2.42, −0.32), p-value < 0.05 BL −1.91 (−3.31, −0.52), p-value < 0.01 GA −2.28 (−3.96, −0.61), p-value < 0.01 Ponderal index 0.095 (−0.2, 0.389) Premature delivery No: Mean = 1.1 (SD = 0.22) Yes: Mean = 1.34 (SD = 0.18) p-value = 0.003

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Sample Timing, Levels[a]	Outcome	Comparison	Results[b]
							Still birth No: Mean = 1.11 (SD = 0.22) Yes: Mean = 1.42 (SD = 0.14) p-value < 0.001 Term LBW Low: Mean = 1.10 (SD = 0.22) Normal: Mean = 1.25 (SD = 0.24) p-value = 0.025
	Comparison: Logarithm base not specified. **Confounding:** Apgar score, BL, BW, GA, ponderal index: Maternal age, educational level, smoking, husband smoking, catching cold during pregnancy, parity, premature delivery history, spontaneous abortion history. Additional confounding for Apgar score, BL, BW, ponderal index: baby sex, GA.						

Notes: AC = abdominal circumference; ACCEPT = Adapting to Climate Change, Environmental Pollution and Dietary Transition; ALSPAC = Avon Longitudinal Study of Parents and Children; AMETS = Australian Maternal Exposure to Toxic Substances; BL = birth length; BMI = body mass index; BPD = biparietal diameter; BW = birth weight; C8HP = C8 Health Project; CI = confidence interval; CIOB = Chemicals in our Bodies; DNBC = Danish National Birth Cohort; EBGRC = Ewha Birth & Growth Retrospective Cohort; FL = femur length; FLEHS = Flemish Environmental and Health Study; HUMIS = Human Milk Study; LINC = Linking EDCs in Maternal Nutrition to Child Health GA = gestational age; GM = geometric mean; HC = head circumference; HOME = Health Outcomes and Measures of Environment; INMA = Infancia y Medio Ambiente (Environment and Childhood Project); INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; IOM = Institute of Medicine; IQR = interquartile range; KBCS = Kashgar Birth Cohort Study; LBW = low birth weight; LHWA = Little Hocking Water Association; LIFE = Longitudinal Investigation of Fertility and the Environment; LWBC = Laizhou Wan Birth Cohort ; MIREC = Maternal-Infant Research on Environmental Chemicals; MoBa = Norwegian Mother and Child Cohort Study; NCS = National Children's Study; NICHD = National Institute of Child Health and Human Development; NICHD SGA = The US National Institute of Child Health and Human Development (NICHD) Scandinavian Successive SGA Births Study; OR = odds ratio; PIPA = Rio Birth Cohort Study; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; PTB = preterm birth; SBC = Shanghai Birth Cohort; SD = standard deviation; SE = standard error; SGA = small-for-gestational-age; SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy; StEP = Starting Early Program; TBPS = Taiwan Birth Panel Study.
T2 = tertile 2; T3 = tertile 3; mo = months; wk = weeks; yr = years.
[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.2 Reproductive

D.2.1 Male

Table D-2. Associations Between PFOA Exposure and Male Reproductive Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Jensen et al. (2020b) *High*	Denmark 2010–2012	Cohort	Infants from Odense Child Cohort N = 208 boys	Maternal serum 1.64	Levels of FSH (IU/L), testosterone (nmol/L), LH (IU/L), testosterone/LH ratio, DHEAS (nmol/L), DHEA (nmol/L), Androstenedione (nmol/L), 17-OHP (nmol/L)	Regression coefficient (testosterone), or percent change (%) per doubling of PFOA	FSH: 10% (−0.4, 21.4); p-value = 0.06 Testosterone, LH, testosterone/LH, DHEAS, DHEA, androstenedione, 17-OHP: no statistically significant associations
	Confounding: Age of the child at examination time, maternal parity.[c]						
Lind et al. (2017a) *High*	Denmark 2010–2012	Cohort	Infants from Odense child cohort N = 649 (296 boys)	Maternal serum Total cohort: 1.7	Penile width (mm), Anogenital distance (AGD) (mm); penile (AGDap), scrotal (AGDas)	Regression coefficient per ln-unit increase in PFOA or by quartiles	AGDap Continuous: 0.1 (−1.1, 1.3) p-trend by quartiles = 0.71 AGDas Continuous: −0.3 (−1.6, 1.0) p-trend by quartiles = 0.58 Penile width: no statistically significant associations; p-trend by quartiles = 0.86
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, weight-for-age z-score, pre-pregnancy BMI, parity, smoking.						
Itoh et al. (2016) *Medium*	Japan 2002–2005	Cohort	Infants from Sapporo Cohort	Maternal serum 1.60	In cord blood, log10-transformed levels of	Regression coefficient per	Inhibin B

			of the Hokkaido study N = 83 boys		E2 (ng/mL), FSH (mIU/mL), Inhibin B (pg/mL), insulin-like 3 (ng/mL), LH (mIU/mL), progesterone (ng/mL), prolactin (ng/mL), SHBG (not log10-transformed, nmol/L), testosterone (pg/mL) Testosterone/E2 ratio, testosterone/SHBG ratio	log10-unit increase in PFOA, least squares mean (LSM) by quartiles	0.197 (0.009, 0.384); p-value = 0.04 Q1: 36.9 (29.1, 46.7) Q2: 44.3 (36.0, 55.3) Q3: 48.5 (39.0, 60.7) Q4: 50.3 (39.2, 64.2) E2, FSH, insulin-like 3, LH, progesterone, prolactin, SHBG, testosterone, testosterone/E2, testosterone/SHBG: No statistically significant associations
	Confounding: Age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement, gestational age at birth.						
Lopez-Espinosa et al. (2016) *Medium*	United States 2005–2006	Cross-Sectional	Male children ages 6–9 yr N = 1,169	Serum 34.8	Total testosterone (ln-ng/dL)	Percent difference between 75th and 25th percentile of ln-unit PFOA or by quartiles	Total testosterone: −4.9 (−8.7, −0.8) Q2: −3.2 (−10.6, 4.7) Q3: −10.4 (−17.6, −2.6) Q4: −10 (−17, −2.4) p-trend = 0.030
	Results: Results by quartile used lowest quartile as reference. **Confounding:** Age, month, time of sampling.						
Goudarzi et al. (2017a) *Medium*	Japan 2002–2005	Cohort	Children from the Hokkaido Study N = 185 (81 males)	Serum Total cohort: 1.40	Levels (log10-ng/mL) of DHEA, androstenedione	Regression coefficient per log10-unit increase in PFOA or by quartiles	DHEA: −0.312 (−0.642, −0.043); p-value = 0.025 Androstenedione: −0.23 (−0.49, 0.038); p-value = 0.093
	Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period.						
Liu et al. (2020b) *Medium*	China 2013–2014	Cross-sectional	Neonates N = 374 (183 males)	Serum Total cohort: 1.65	Cord blood levels (ng/mL) of 17-OHP, progesterone	Percent change per interquartile ratio increase in PFOA	17-OHP: 7.82 (−0.22, 16.51); p-value = 0.57 Progesterone: 9.45 (3.23, 16.05); p-value < 0.01
	Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education status, passive smoking during pregnancy, parity, gestational weeks, sample collecting time.						
Ernst et al. (2019)	Denmark 1999–2017	Cohort	Children from the Puberty Cohort of	Maternal blood Sample 1: 5.1	Age (months) at axillary hair	Regression coefficient per	No statistically significant associations

Medium			the Danish National Birth Cohort N = 565 boys	Sample 2: 4.3	attainment, voice break, first ejaculation, Tanner stages 2–5 for genital development or pubic hair growth; combined sex-specific puberty indicator	log2-unit increase in first trimester maternal serum PFOA Puberty indicator: mean difference in age at puberty by tertiles	
	Confounding: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy body mass index, daily number of cigarettes smoked in first trimester.						
Tian et al. (2019b) *Medium*	China 2012–2013	Cohort	Male infants at birth, 6 mo, and 12 mo N = 500	Maternal plasma 20.13	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln-unit increase in maternal PFOA or by quartiles	AGDap Birth: 0.28 (−0.62, 1.18); p-value = 0.533 6 mo.: −1.82 (−4.25, 0.62); p-value = 0.147 Q2: −3.57 (−6.73, −0.41); p-value < 0.05 Q3: −1.44 (−4.70, 1.81) Q4: −3.05 (−6.19, 0.10) 12 mo.: −1.55 (−4.76, 1.66); p-value = 0.342 AGDas Birth: −0.16 (−0.92, 0.61); p-value = 0.686 6 mo.: −2.17 (−4.58, 0.24); p-value = 0.079 Q2: −3.36 (−6.51, −0.21); p-value < 0.05 Q3: −2.39 (−5.62, 0.84) Q4: −2.58 (−5.71, 0.54) 12 mo.: 1.12 (−1.56, 3.79); p-value = 0.411
	Results: Lowest quartile used as reference. **Confounding:** Maternal age at delivery, gestational age, maternal education, parity, pre-pregnancy BMI, infant age at physical examination, and infant body size (birth weight at birth; WLZ at 6 and 12 mo of age).						

Arbuckle et al. (2020) *Medium*	Canada 2008–2011	Cohort	Newborns from the MIREC cohort N = 205 boys	Maternal plasma 1.7	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln-unit increase in maternal PFOA, or by quartiles	AGDap Per ln increase: 0.1 (−0.94, 1.14) Q2: −0.76 (−2.65, 1.12) Q3: −0.02 (−1.91, 1.88) Q4: −0.51 (−2.50, 1.48) p-value for trend = 0.807 AGDas Per ln increase: 1.36 (0.30, 2.41); p-value < 0.05 Q2: 0.23 (−1.67, 2.13) Q3: −0.43 (−2.34, 1.47) Q4: 1.77 (−0.23, 3.77) p-value for trend = 0.148
	Results: Lowest quartile used as reference. **Confounding:** AGDap: recruitment site, education, active smoking status, gestational age; AGDas: household income, active smoking status, gestational age.						
Di Nisio et al. (2019) *Low*	Italy 2017–2018	Cross-sectional	Male high school students N = 100 (50 unexposed controls, 50 exposed)	Serum Unexposed controls: 4.70 Exposed: 7.35 Semen Unexposed controls: 0.1 Exposed: 0.24	AGD (cm), crown-to-pubis distance (cm), pubis-to-floor distance (cm), crown-to-pubis/pubis-to-floor ratio, penis circumference (cm), penis length (cm), testicular volume (mL), normal morphology (%), semen pH, immotile sperm (%), nonprogressive motility (%), progressive motility (%), total sperm count (10^6), semen volume (mL), sperm concentration (10^6/mL), viability (%), FSH (U/L), testosterone (nmol/L)	Mann-Whitney test (Exposed *vs.* Unexposed controls)	AGD Controls: 4.50 (4.0, 5.2) Exposed: 4.00 (3.5, 5.0) Adjusted p-value for comparison of medians = 0.114 Pubis-to-floor distance Controls: 97.0 (93.0, 101.1) Exposed: 95.0 (90.3, 99.0) Adjusted p-value for comparison of medians = 0.320 Penis circumference Controls: 10.10 (9.9, 11.0) Exposed: 9.50 (9.0, 10.0) Adjusted p-value for comparison of medians < 0.001 Penis length Controls: 10.0 (9.0, 11.0) Exposed: 9.00 (8.0, 10.0) Adjusted p-value for comparison of medians < 0.001

							Testicular volume Controls: 16.13 (14.8, 19.0) Exposed: 14.00 (12.6, 16.0) Adjusted p-value for comparison of medians < 0.001 Normal morphology Controls: 7.0 (4.0, 12.0) Exposed: 4.0 (2.0, 6.0) Adjusted p-value for comparison of medians < 0.001 Semen pH Controls: 7.60 (7.5, 7.7) Exposed: 7.70 (7.6, 7.7) Adjusted p-value for comparison of medians = 0.042 Testosterone Controls: 18.98 (12.9, 17.9) Exposed: 18.98 (16.3, 21.8) Adjusted p-value for comparison of medians < 0.001 Crown-to-pubis, Crown-to-pubis/pubis-to-floor, sperm motility, sperm count, semen volume, sperm concentration, viability, FSH: No statistically significant associations after adjusting for comparison of medians
	Results: Values for each outcome are reported as median (25th–75th percentile). **Confounding:** Age.						
General Population							
Cui et al. (2020) *Medium*	China 2015–2016	Cross-sectional	Chinese adult men N = 651	Serum 8.57	Serum levels (ln-transformed) of E2 (pmol/L), FSH	Percent change per ln-unit increase in serum or semen	Free testosterone Serum PFOA: −2.7 (−4.83, −0.53); p-value = 0.015

				Semen 0.23	(IU/L), LH (IU/L), SHBG (nmol/L), free testosterone, total testosterone (nmol/L); free androgen index, total testosterone/LH ratio	PFOA or by quartiles	p-trend by quartiles = 0.036 Semen PFOA: −4.42 (−7.12, −1.55); p-value = 0.003 p-trend by quartiles = 0.001 Total testosterone Serum PFOA: −3.1 (−5.32, −0.84); p-value = 0.008 p-trend by quartiles = 0.012 Semen PFOA: −5.56 (−8.4, −2.62); p-value < 0.000 p-trend by quartiles < 0.001 E2, semen PFOA: −5.49 (−10.6, −0.17); p-value = 0.044 p-trend by quartiles = 0.031 Total testosterone/LH, semen PFOA: −4.83 (−9.12, −0.35); p-value = 0.035 p-trend by quartiles = 0.018 No other statistically significant associations or trends by quartile
	Results: Lowest quartile used as reference. **Confounding:** Age, BMI, smoking status, blood sampling time, fasting status.						
Petersen et al. (2018) *Medium*	Denmark 2007–2009	Cross-sectional	Faroese men born between 1981 and 1984 N = 263	Serum 2.8	Levels (log-transformed) of E2 (nmol/L), FSH (IU/L), free testosterone (pmol/L), inhibin B (pg/mL), LH (IU/L), SHBG (nmol/L), testosterone (nmol/L) Ratios of free testosterone/E2, free testosterone/LH, Inhibin B/FSH,	Regression coefficient per log-unit increase PFOA	Free testosterone: −0.28 (−0.56, 0.002) Free testosterone/E2: −0.12 (−0.21, 0.02)[d]; p-value = 0.02 No other statistically significant associations

					testosterone/E2, testosterone/LH Normal morphology (%), motile sperm (logit-%), total sperm count ($(10^6)^{1/3}$) semen volume ($mL^{1/3}$), sperm concentration ($((10^6/mL)^{1/3}$)		
	Outcome: Logarithm base not specified. **Comparison:** Logarithm base not specified. **Confounding:** Age, BMI groups, current smoking, time of sampling.						
Kvist et al. (2012) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cross-sectional	Male partners of pregnant women from INUENDO N = 359	Serum Mean Greenland: 4.84 Poland: 5.19 Ukraine: 1.91	Y:X-chromosome ratio of sperm	Linear regression adjusted r^2	0.013; p-value = 0.05
	Confounding: Age, abstinence time, alcohol intake, CB-153.						
Leter et al. (2014) *Medium*	Greenland, Poland, and Ukraine 2002–2004	Cross-sectional	Male partners of pregnant women from INUENDO N = 262	Serum Mean = 4.0	Sperm DNA methylation level (% 5-mC) at LINE-1, Alu, or Sat-alpha; global DNA methylation level (FCM DGML channel no.)	Regression coefficient per ln-unit increase PFOA	LINE-1: 1.1 (−0.3, 2.5) Ukraine: 2.6 (0.3, 5.0); p-value < 0.05 Greenland: −1.7 (−4.2, 0.7) Poland: 1.7 (−1.4, 4.8) Alu, Sat-alpha, or global methylation levels: No statistically significant associations
	Confounding: Site, age (ln-transformed), smoking status.						
Pan et al. (2019) *Medium*	China 2015–2016	Cross-sectional	Adult men in Nanjing N = 664	Serum 8.567 Semen 0.229	Sperm normal morphology (%), count ($(10^6)^{1/3}$), concentration ($((10^6/mL)^{1/3}$), progressive motility (%), curvilinear velocity (VCL)	Regression coefficient per ln-unit increase PFOA in serum or in semen, or by quartiles	No statistically significant associations by serum PFOA levels; following results are by semen PFOA Sperm count 0.247 (0.061, 0.432) p-value = 0.05

		(μm/s); straight-line velocity (VSL) (μm/s), DNA fragmentation index (DFI) (ln-%), high DNA stainability (HDS) (ln-%); semen volume (ln-mL)	Q2: 0.37 (0.02, 0.71) Q3: −0.08 (−0.43, 0.27) Q4: 0.42 (0.06, 0.78) p-trend = 0.2 Sperm concentration 0.193 (0.075, 0.311) p-value = 0.02 Q2: 0.3 (0.08, 0.52) Q3: 0.06 (−0.16, 0.28) Q4: 0.36 (0.13, 0.59) p-trend = 0.2 Progressive motility −2.377 (−3.94, −0.815) p-value = 0.03 Q2: 0.31 (−2.65, 3.27) Q3: −1.49 (−4.48, 1.50) Q4: −4.26 (7.30, 1.22) p-trend = 0.02 Sperm VCL −1.155 (−2.064, −0.245) p-value = 0.06 Q2: −1.65 (−3.38, 0.07) Q3: −1.61, (−3.35, 0.12) Q4: −2.64 (−4.41, −0.87) p-trend = 0.08 Sperm VSL −0.92 (−1.676, −0.165) p-value = 0.08 Q2: −1.68 (−3.11, −0.24) Q3: −0.87 (−2.32, 0.57) Q4: −2.13 (−3.60, −0.66) p-trend = 0.1 Sperm DFI 0.136 (0.064, 0.209) p-value = 0.01

							Q2: 0.05 (−0.09, 0.19) Q3: 0.14 (0, 0.28) Q4: 0.21 (0.07, 0.35) p-trend = 0.03 Sperm morphology, sperm HDS, semen volume: no statistically significant associations or trends
	Results: Lowest quartile used as reference. **Confounding:** Age, BMI, BMI^2, smoking, alcohol intake, abstinence time.						

Notes: 17-OHP = 17-hydroxyprogesterone; AGD = anogenital distance; AGDap = anopenile distance; AGDas = anoscrotal distance; BMI = body mass index; DHEA = dehydroepiandrosterone; DFI = DNA fragmentation index; DNA = deoxyribonucleic acid; E2 = estradiol; FSH = follicle stimulating hormone; HDS = high DNA stainability; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; LH = luteinizing hormone; LSM = least squares mean; MIREC = Maternal-Infant Research on Environmental Chemicals; mo = months; PFOA = perfluorooctanoic acid; SHBG = sex hormone-binding globulin; VCL = curvilinear velocity; VSL = straight-line velocity; wk = weeks; yr = years.

[a] Exposure levels reported as median in ng/mL unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

[d] Values are reproduced as reported in publication.

D.2.2 Female

Table D-3. Associations Between PFOA Exposure and Female Reproductive Health Effects in Female Children and Adolescents

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Jensen et al. (2020b) *High*	Denmark, 2010–2012	Cohort	Female infants from the Odense Child Cohort, Age 4 mo, N = 165	Maternal serum, 1.70 (5th–95th percentile = 0.67, 3.70)	Levels of 17-OHP (nM), DHEA (nM), FSH (IU/L), LH (IU/L)	Percent change per doubling in PFOA	17-OHP 1 (−7.9, 15.2) DHEA −4.7 (−15.5, 7.4) FSH 3.8 (−6.4, 15) LH 13.3 (−4.8, 34.9)
	Confounding: Age of the child at examination time, maternal parity.[c]						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Lind et al. (2017a) *High*	Denmark, 2010–2012	Cohort	Infants from Odense child cohort N = 649 (353 girls)	Maternal serum Total cohort: 1.7	Anogenital distance (AGD) (mm); clitoral (AGDac), fourchette (AGDaf)	Regression coefficient per ln-unit increase in PFOA or by quartiles	AGDac Continuous: −0.5 (−1.8, 0.8) p-trend by quartiles = 0.71 AGDaf Continuous: 0.1 (−0.9, 1.1) p-trend by quartiles = 0.94 Quartile analysis did not show any statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Age at examination, weight-for-age z-score, pre-pregnancy BMI, parity, smoking.						
Yao et al. (2019) *High*	China, 2010–2013	Cross-sectional	Pregnant women (aged > 18 yr) and female infants, N = 171	Cord blood, 34.67 (20.48, 57.84)	Levels of estradiol (log10-pg/mL), testosterone (log10-ng/mL), testosterone-to-estradiol ratio	Regression coefficient per log10-unit increase in PFOA	Estradiol 0.03 (−0.01, 0.07) Testosterone 0.07 (−0.03, 0.17) Testosterone-to-estradiol ratio 0.04 (−0.05, 0.13)
	Confounding: Maternal age, pre-pregnancy BMI, parity, mode of delivery, passive smoking during pregnancy, gestational age, household income level among female infants separately.						
Ernst et al. (2019) *Medium*	Denmark, 1999–2017	Cohort	Female adolescents from the Danish National Birth Cohort, N = 555	Maternal blood, Sample 1: 4.8 (10th–90th percentile = 2.7, 8.2)	Breast development, pubic hair development, age at attainment of axillary hair (months), age at menarche	Regression coefficient per log10-unit increase in PFOA	Breast development −1.37 (−6.14, 3.4) Pubic hair development 3.05 (−0.94, 7.04) Axillary hair −1.49 (−4.56, 1.58) Menarche −1.09 (−3.25, 1.07)
	Exposure Levels: For Sample 2, median = 4.1 (10th–90th percentile = 2.3, 6.4). Samples 1 and 2 combined for analysis. **Outcome**: Age in months at Tanner stage 5 used to measure breast development and pubic hair development. **Confounding**: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy body mass index, daily number of cigarettes smoked in first trimester.						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Donley et al. (2019) *Medium*	United Kingdom, Recruitment 1991–1992, outcome assessed at adolescence	Case-control	Mothers and their daughters from ALSPAC, N = 446	Maternal serum, 3.7 (2.8, 4.8)	AMH (log10-ng/mL)	Regression coefficient per unit increase in PFOA	Complete AMH data 0.05 (0.01, 0.09) Multiple imputation model 0.04 (−0.01, 0.09)
	Results: N for complete data = 173; N for imputation model = 446. **Confounding**: Maternal age at delivery, pre-pregnancy BMI, maternal education.						
Goudarzi et al. (2017a) *Medium*	Japan, 2002–2005	Cohort	Pregnant women and their infants from the Hokkaido Study on the Environment and Children's Health, N = 104	Maternal serum, 1.40 (<LOD–5.30)	Levels of androstenedione (log10-ng/mL), DHEA (log10-ng/mL)	Regression coefficient per log10-unit increase in PFOA	Androstenedione −0.17 (−0.46, 0.07) DHEA −0.10 (−0.27, 0.11)
	Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period.						
Itoh et al. (2016) *Medium*	Japan, 2002–2005	Cohort	Female infants from the Sapporo Cohort of the Hokkaido Study, N = 106	Maternal serum, 1.35 (0.80, 2.00)	Levels of estradiol (log_{10}- ng/mL), progesterone (log10-ng/mL), prolactin (log10-ng/mL), SHBG (nmol/L), testosterone (log10-pg/mL)	Regression coefficient per log10 increase in PFOA	Estradiol –0.04 (– 0.19, 0.11) Progesterone 0.04 (–0.22, 0.29) Prolactin –0.16 (–0.36, 0.05) SHBG –0.12 (–0.29, 0.05) Testosterone –0.03 (–0.27, 0.20)
	Confounding: Age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement.						
Liu et al. (2020b) *Medium*	China, 2013–2014	Cross-sectional	Female neonates, N = 191	Cord blood, 1.65 (1.31, 2.11)	Levels of progesterone (ng/mL), 17-OHP (ng/mL)	Percent change per IQR-unit increase in PFOA	Progesterone –0.03 (–5.64, 5.9) 17-OHP 0.69 (–5.98, 7.84)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education status, passive smoking during pregnancy, parity, gestational weeks, sample collecting time.						
Lopez-Espinosa et al. (2016) *Medium*	United States, 2005–2006	Cross-sectional	Females from the C8 Health Project, Ages 6−9, N = 1,123	Serum, 30.1 (13.5, 74.0)	Levels of estradiol (ln-pg/mL)	Percent difference by quartiles of PFOA	Q2: 12.6 (3, 23.1) Q3: 6.2 (–3, 16.4) Q4: 8.1 (–1.2, 18.4)
	Results: Lowest quartile used as the reference group. **Confounding**: Age, month of sampling.						
Maisonet et al. (2015a) *Medium*	United Kingdom, 1991–1992	Cohort	Female adolescents from ALSPAC, Age 15, N = 72	Maternal serum, 3.6 (2.7, 4.7)	Levels of serum total testosterone (nmol/L), SHBG (nmol/L)	Regression coefficient by tertiles of PFOA	Testosterone T2: 0.15 (–0.02, 0.32) T3: 0.24 (0.05, 0.43) SHBG T2: 0.32 (–15.97, 16.61) T3: 5.02 (–13.07, 23.11)
	Results: Lowest tertile used as the reference group. **Confounding**: Maternal education, maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, time of day daughter's blood sample was obtained, daughter's age at menarche, daughter's BMI at 15 years. SHBG concentration included in testosterone model.						
Tsai et al. (2015) *Medium*	Taiwan, 2006–2008	Cross-sectional	Female adolescents, Ages 12–17, N = 95	Serum, GM = 2.74 (GSD = 2.95)	Levels of serum FSH (ln-mIU/mL), serum SHBG (ln-nmol/L)	Means by quartile of PFOA	FSH Q1: 1.47 (SE = 0.2) Q2: 1.38 (SE = 0.21) Q3: 1.23 (SE = 0.25) Q4: 1.35 (SE = 0.29) SHBG Q1: 3.5 (SE = 0.24), p-value < 0.05 Q2: 3.5 (SE = 0.25), p-value < 0.05 Q3: 3.45 (SE = 0.29), p-value < 0.05 Q4: 2.96 (SE = 0.34), p-value < 0.05
	Confounding: Age, gender, BMI, high-fat diet.						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Wang et al. (2019a) *Medium*	China, 2013	Cross-sectional	Pregnant women and their children, N = 171	Cord blood, 1.99 (1.22–3.11)	Levels of estrone (log10-ng/mL), b-estradiol (log10-ng/mL), estriol (log10-ng/mL)	Regression coefficient per ln-unit increase in PFOA	Estrone 0.07 (–0.07, 0.21) b-estradiol 0.14 (–0.04, 0.32) Estriol 0.29 (0.02, 0.56), p-value = 0.034
	Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, gestational age, parity, pre-pregnant maternal body mass index, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain.						

Notes: 17-OHP = 17-hydroxyprogesterone; ALSPAC = Avon Longitudinal Study of Parents and Children; AMH = anti-Müllerian hormone; BMI = body mass index; DHEA = dehydroepiandrosterone; DNBC = Danish National Birth Cohort; FSH = follicle stimulating hormone; LH = luteinizing hormone; mo = months; GM = geometric mean; GSD = geometric standard deviation; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; SD = standard deviation; SE = standard error; SHBG = sex hormone binding globulin; T1 = tertile one; T2 = tertile two; T3 = tertile 3; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

Table D-4. Associations Between PFOA Exposure and Female Reproductive Health Effects in Pregnant Women

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Huo et al. (2020a) *High*	China, 2013–2016	Cohort	Females from the Shanghai Birth Cohort Study, Ages > 20, N = 3,220	Plasma, 11.85 (9.18, 15.29)	Gestational hypertension, hypertensive disorders of pregnancy, preeclampsia	OR per ln-unit increase in PFOA	Gestational hypertension 1.37 (0.76, 2.48) Hypertensive disorders 1.09 (0.72, 1.66) Preeclampsia 0.89 (0.5, 1.57)
	Confounding: Maternal age, pre-pregnancy BMI, parity, parental educational levels, gestational age of blood drawn, fetal sex.[c]						
Mitro et al. (2020) *High*	United States, 1999–2005	Cohort	Females from Project Viva, N = 812	Plasma, 5.6 (4.0, 7.6)	Levels of SHBG (nmol/L)	Percent difference per log2-unit increase in PFOA	–1.5 (–9.3, 7) Women under 35 yr during pregnancy –0.9 (–11.4, 10.8)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Women over 35 yr during pregnancy –1.8 (–13.7, 11.6)
	Confounding: Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity.						
Borghese et al. (2020) *Medium*	Canada, 2008–2011	Cohort	Females from the MIREC study, Ages > 18, N = 1,739	Plasma, GM = = 1.65 (95% CI: 1.61, 1.70)	Gestational hypertension, preeclampsia, DBP (mmHg), SBP (mmHg)	OR (GH, PE) or regression coefficient (DBP, SBP) per log2-unit increase in PFOA	Gestational hypertension 1.06 (0.84, 1.35) Preeclampsia 1.36 (0.9, 2.08) DBP 0.64 (0.24, 1.05), p-value = = = 0.002 SBP 0.82 (0.23, 1.42), p-value = 0.006
	Confounding: Maternal age**,** education**,** smoking status**,** pre-pregnancy BMI**,** parity.						
Huang et al. (2019) *Medium*	China, 2011–2012	Cross-sectional	Females from mother-infant pairs, N = 687	Cord blood plasma, 6.98 (4.95, 9.54)	Gestational hypertension, hypertensive disorders of pregnancy, preeclampsia	OR per increase in standardized PFOA	Gestational hypertension 0.95 (0.61, 1.48) Hypertensive disorders of pregnancy 1.02 (0.73, 1.44) Preeclampsia 1.12 (0.68, 1.84)
	Results: Standardized PFOA calculated by subtracting PFOA concentration from mean PFOA concentration and dividing by the SD. **Confounding:** Age, pre-pregnancy BMI, parity, education level.						
Lyngsø et al. (2014) *Medium*	Greenland, 2002–2004	Cross-sectional	Pregnant women from the INUENDO cohort, N = 1,623	Serum, 1.5 (10th–90th percentile = 0.7, 3.1)	Menstrual cycle length (long), irregularity	OR per log-unit increase in PFOA and by tertile	Length 1.5 (1.0, 2.1) T2: 1.4 (0.8, 2.3) T3: 1.8 (1.0, 3.3) Irregularity 1.68(0.8, 1.9) T2: 1.3 (0.8, 2.3) T3: 1.3 (0.7, 2.3)
	Results: Lowest tertile used as the reference group. **Comparison:** Logarithm base not specified. **Confounding**: Age at menarche, age at pregnancy, parity, pre-pregnancy BMI, smoking, country.						

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Romano et al. (2016) *Medium*	United States, 2003–2006	Cohort	Females from the HOME study, Ages > 18, N = 336	Serum, 5.5 (3.8, 7.7)	Breastfeeding termination at 3 mo and at 6 mo	RR by quartiles of PFOA	Breastfeeding termination At 3 mo Q2: 1.32 (0.92, 1.88) Q3: 1.63 (1.16, 2.28) Q4: 1.77 (1.23, 2.54) p-value = 0.003 At 6 mo Q2: 1.25 (0.96, 1.62) Q3: 1.38 (1.06, 1.79) Q4: 1.41 (1.06, 1.87) p-value for trend = 0.038
	Results: Lowest quartile used as the reference group. **Confounding:** Maternal age at delivery, household income, total weeks of prior breastfeeding, gestational week at blood draw, marital status, race, parity, maternal serum cotinine during pregnancy, alcohol use.						
Rylander et al. (2020) *Medium*	Sweden, 1989	Case-control	Females with or without pre-eclampsia, Ages 15–44, N = 876	Serum, Primiparous cases: 2.82 (Minimum, Maximum = 0.55, 10.9)	Preeclampsia	OR by quartiles of PFOA	Q2: 0.94 (0.56, 1.57) Q3: 1.42 (0.87, 2.31) Q4: 1.13 (0.68, 1.87)
	Exposure Levels: [Multiparous cases] Median = 1.96 ng/mL (Minimum, Maximum = 0.42, 6.93 ng/mL); [Primiparous controls] Median = 2.83 ng/mL (Minimum, Maximum = 0.39, 9.38 ng/mL); [Multiparous controls] Median = 1.81 ng/mL (Minimum, Maximum = 0.40, 9.34 ng/mL). **Confounding:** Maternal age, BMI in early pregnancy, maternal smoking in early pregnancy, parity.						
Starling et al. (2014a) *Medium*	Norway, 2003–2007	Nested case-control	Females from MoBa, Ages 16–44, N = 976	Plasma, 2.78 (2.14, 3.57)	Preeclampsia onset	HR per ln-unit increase in PFOA	0.89 (0.65, 1.22)
	Confounding: Maternal age, pre-pregnancy BMI, education completed, smoking during pregnancy.						
Timmermann et al. (2017b) *Medium*	Denmark, 1997–2000, 2007–2009	Cohort	Pregnant and postpartum females, N = 987	Serum, 2.40 (1.45, 3.59)	Total breastfeeding duration (months), exclusive breastfeeding	Regression coefficient per doubling of PFOA	Total breastfeeding duration –1.3 (–1.9, –0.7) Exclusive breastfeeding duration –0.5 (–0.7, –0.3)

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
					duration (months)		
	Confounding: Cohort, maternal age, pre-pregnancy BMI, pregnancy alcohol intake, pregnancy smoking, education, employment, parity.						
Wikström et al. (2019) *Medium*	Sweden, 2007–2010	Cohort	Females from the SELMA study, Ages 28–35, N = 1,773	Serum, 1.61 (1.12, 2.31)	Preeclampsia	OR per log2-unit increase in PFOA	PE All women: 1.31 (0.93, 1.87) Nulliparous women: 1.38 (0.90, 2.21)
	Population: N for nulliparous women = 812. **Confounding:** Parity, women's age, body weight, smoke exposure.						

Notes: BMI = body mass index; CI = confidence interval; DBP = diastolic blood pressure; GM = geometric mean; GSD = geometric standard deviation; HOME = Health Outcomes and Measures of the Environment; HR = hazard ratio; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; LIFE = Longitudinal Investigation of Fertility and the Environment Study; MIREC = Maternal-Infant Research on Environmental Chemicals; mo = months; MoBa = Norwegian Mother and Child Cohort Study; OR = odds ratio; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; RR = relative risk ratio; SBP = systolic blood pressure; SD = standard deviation; SE = standard error; SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study; SHBG = sex hormone binding globulin.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

Table D-5. Associations Between PFOA Exposure and Female Reproductive Health Effects in Nonpregnant Adult Women

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Ding et al. (2020) *High*	United States, 1999–2017	Cohort	Pre-menopausal women from the Study of Women's Health Across the Nation, Ages 42–52, N = 1,120	Serum, 4.0 (2.8, 5.7)	Natural menopause	HR per doubling of PFOA and by tertiles	1.11 (0.99, 1.24) T2: 1.12 (0.9, 1.4) T3: 1.31 (1.04, 1.65) p-value for trend = 0.01
	Results: Lowest tertile used as the reference group. **Confounding**: Education, parity, BMI at baseline, physical activity, smoking status, prior hormone use at baseline.[c]						

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Crawford et al. (2017) *Medium*	United States, 2008–2009	Cohort	Females from the Time to Conceive Study, Ages 30−44, N = 99	Serum, 2.79 (2.48, 3.16)	Cycle-specific time to pregnancy, day-specific time to pregnancy; levels of AMH (ln-ng/mL)	Times to pregnancy: FR per ln-unit increase in PFOA AMH: Regression coefficient per ln-unit increase in PFOA	Cycle-specific time to pregnancy 1.15 (0.66, 2.01) Day-specific time to pregnancy 0.96 (0.31, 1.94) AMH –0.56 (p-value = 0.75)
	Confounding: Age, mean cycle length (for cycle-specific outcome).						
Dhingra et al. (2017) *Medium*	United States, 2005–2006, 2008–2011	Cohort	Females from the C8 Science Panel, Age > 40, N = 9,192	Serum, measured and modeled Measured: Mean = 69.2 µg/mL (SD = 195.6) Modeled: Mean = 81.8 µg/mL (SD = 175.0)	Natural menopause	OR per ln-unit increase in PFOA, or by quintiles, or by deciles	Measured 1.09 (1.002, 1.18), p-value = 0.04 Quintile 2: 1.68 (1.21, 2.35), p-value = 0.002 Quintile 3: 1.45 (1.04, 2.02), p-value = 0.03 Quintile 4: 1.39 (1, 1.93), p-value = 0.05 Quintile 5: 1.58 (1.14, 2.19), p-value = 0.006 Modeled 0.98 (0.7, 1.37) Quintile 2: 0.98 (0.7, 1.37) Quintile 3: 1.05 (0.75, 1.45) Quintile 4: 0.78 (0.56, 1.08) Quintile 5: 0.92 (0.65, 1.3) Dose response by deciles: increased up to the 4th decile and then, except for a drop

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							at the 5th decile, remained approximately level thereafter
	Results: Lowest quintile used as the reference group. **Confounding**: Age, parous/nulliparous status, smoking status, education, BMI, birth year.						
Kim et al. (2020b) *Medium*	Australia, 2006–2011	Cross-sectional	Females undergoing fertility treatment, Ages 23–42, N = 97	Follicular fluid Mean = 2.4 (Minimum-Maximum = 0.3, 14.5)	Fertilization rate	Regression coefficient per unit increase in PFOA	0.71 (–2.22, 3.63)
	Confounding: Age.						
Lum et al. (2017) *Medium*	United States, 2005–2009	Cohort	Females from the LIFE study, Ages 18–40, N = 483	Serum Women with ≤24-day cycle: 3.1 (2.5, 4.0) Women with 25 to 31-day cycle: 3.5 (2.3, 5.0) Women with ≥32-day cycle: 3.1 (2.0, 4.7)	Day-specific probability of pregnancy, menstrual cycle length	Regression coefficient by tertiles of PFOA	All women: Day-specific probability of pregnancy T2: 1 (0.7, 1.5) T3: 0.7 (0.5, 1.5) Menstrual cycle length T2: 0.98 (0.95, 1.01) T3: 0.98 (0.96, 1)
	Results: Lowest tertile used as the reference group. **Exposure Levels:** Presented for females with 25–31-day cycles. The study also present exposure levels for females with 24-day cycles or shorter and females with cycles longer than 31 d. **Results:** Lowest tertile used as the reference group. **Confounding**: For menstrual cycle length: female age, BMI, active smoking at enrollment; For day-specific probability of pregnancy: couple intercourse pattern, female menstrual cycle length, age, BMI, active smoking at enrollment.						
Tsai et al. (2015) *Medium*	Taiwan, 2006–2008	Cross-sectional	Females, Ages 18–30, N = 265	Serum, GM = 2.74 (GSD = 2.95)	Levels of FSH in serum (ln-mIU/mL), SHBG in serum (ln-nmol/L)	Means by quartile of PFOA	FSH Q1: 1.69(SE = 0.24) Q2: 1.65 (SE = 0.24) Q3: 1.64 (SE = 0.25) Q4: 1.79 (SE = 0.26) SHBG

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
							Q1: 3.83 (SE = 0.21) Q2: 3.86 (SE = 0.2) Q3: 3.81 (SE = 0.22) Q4: 3.78 (SE = 0.23)
	Confounding: Age, BMI, high-fat diet.						
Wang et al. (2017) *Medium*	China, 2014–2015	Case-control	Females of reproductive age, N = 335	Plasma, Cases: 14.67 (7.32, 23.73)	Endometriosis-related infertility	OR by tertiles of PFOA	T2: 0.89 (0.5, 1.59) T3: 1.05 (0.58, 1.91)
	Population: [Cases] Females with endometriosis; [Controls] Females from couples seeking treatment for male infertility **Exposure Levels:** [Control] Median = 12.09 (25th–75th percentile = 7.33, 22.59) **Results:** Lowest tertile used as the reference group. **Confounding**: Age, BMI, household income, and education.						

Notes: 17-OHP = 17-hydroxyprogesterone; ALSPAC = Avon Longitudinal Study of Parents and Children; AMH = anti-Müllerian hormone; BMI = body mass index; DHEA = dehydroepiandrosterone; DNBC = Danish National Birth Cohort; FR = fecundability ratio; FSH = follicle stimulating hormone; HR = hazard ratio; LH = luteinizing hormone; GM = geometric mean; GSD = geometric standard deviation; OR = odds ratio; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; SD = standard deviation; SE = standard error; SHBG = sex hormone binding globulin; T1 = tertile one; T2 = tertile two; T3 = tertile 3.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

D.3 Hepatic

Table D-6. Associations Between PFOA Exposure and Hepatic Effects in Epidemiology Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Adults			
Omoike et al. (2020) *Medium*	United States 2005–2012	Cross-sectional	Adults from NHANES, Age ≥20, N = 6,652	Serum 3.20 (20th–80th percentile = 1.82–5.50)	Levels of iron in serum, bilirubin, and albumin	Percent change per one percent increase in PFOA	Iron concentration in serum 0.10 (0.07, 0.12), p-value < 0.05 Bilirubin 0.06 (0.04, 0.08), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Albumin 0.03 (0.03, 0.04), p-value < 0.05
	Confounding: Age, sex, race, education, poverty-income ratio, serum cotinine, BMI.						
Jain (2019) *Medium*	United States 2003–2014	Cross-sectional	Adults from NHANES Age > 20, N = 108–3,562	Serum	Levels of ALT (log10-IU/L), AST (log10-IU/L)	Regression coefficient per log10-unit increase in PFOA	ALT Non-obese, GF-1: 0.009 GF-2: 0.047, p-value = 0.02 GF-3A: 0.001 GF-3B/4: −0.001 Obese, GF-1: 0.077, p-value < 0.01 GF-2: 0.035 GF-3A: 0.057 GF-3B/4: 0.164, p-value < 0.01 AST Non-obese, GF-1: 0.014 GF-2: 0.028 GF-3A: 0.002 GF-3B/4: 0.055, p-value = 0.03 Obese, GF-1: 0.039, p-value < 0.01 GF-2: 0.029 GF-3A : 0.036, p-value = 0.03 GF-3B/4: 0.050, p-value < 0.01
	Confounding: Gender, race/ethnicity, smoking status, age, log10(BMI), diabetes status, hypertension status, fasting time, poverty-income ratio, survey year, alcohol consumption.[c]						
Liu et al. (2018a) *Medium*	United States, 2004–2007	Controlled trial	Overweight and Obese patients from the POUNDS Lost, Age 30–70 study, N = 150	Plasma Males 5.2 (3.9–8.6) Females 4.1 (2.8–5.6)	Hepatic fat mass	Partial Spearman correlation coefficient among baseline PFOA (ng/ml)	Hepatic fat mass: 0.12

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
						and hepatic fat mass	
	Confounding: age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups.						
Liu et al. (2018b)	United States, 2013–2014	Cross-sectional	Adults from NHANES, Age > 18, N = 1871	Serum GM = 1.86 (SE = 1.02)	Levels of albumin (g/dL)	Regression coefficient per ln-unit increase in PFOA	Albumin 0.09, SE = 0.02, p-value < 0.005
	Confounding: age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, antihyperglycemic, and antihyperlipidemic agents).						
Salihovic et al. (2018) *Medium*	Sweden 2001–2014	Cohort	Elderly adults in Sweden, Age 70 N = 1.002 Age 75 N = 817 Age 80 N = 603	Plasma Age 70 3.31 (2.52–4.39) Age 75 3.81 (2.71–5.41) Age 80 2.53 (1.82–3.61)	Levels of ALT (µkat/L)	Regression coefficient per ln-unit increase in PFOA	0.04 (0.03, 0.06), p-value < 0.0016
	Confounding: Sex, LDL and HDL, serum triglycerides, BMI, fasting glucose levels, statins use, and smoking.						
Nian et al. (2019) *Medium*	China 2015–2016	Cross-sectional	Adults in high exposure area in China, Ages 22–96, N = 1,605	Serum 6.19 (4.08–9.31)	Levels of ALT (ln-U/L), AST (ln-U/L)	Percent change per 2.71-fold increase in PFOA	ALT 7.4 (3.9, 11.0) AST 2.9 (0.7, 5.2)
	Confounding: Age, sex, career, income, education, drink, smoke, giblet, seafood consumption, exercise, BMI.						
Yamaguchi et al. (2013) *Medium*	Japan 2008–2010	Cross-sectional	Participants from the "Survey on the Accumulation of Dioxins and Other Chemical Compounds" project from urban, agricultural and fishing areas, Ages 15–76,	Blood 2.1 (1.5–3.3)	Levels of GGT (IU/L), AST (IU/L), ALT (IU/L)	Spearman rank correlation	GGT 0.06, p-value = 0.120 AST 0.13, p-value = 0.002 ALT 0.09, p-value = 0.040

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 590				
	Confounding: Age, sex, BMI, regional block, smoking habits, frequency of alcohol intake.						
Gallo et al. (2012) *Medium*	United States 2005–2006	Cross-sectional	Adults from the C8 Health Project, Ages ≥18 yr, N = 46,452	Serum 28.0 (13.5–70.8)	Levels of GGT (ln-IU/L), ALT (ln-IU/L), Direct bilirubin (ln-mg/dL), ALT (IU/L, elevated)	GGT, ALT, direct bilirubin: Regression coefficient per ln-unit increase in PFOA Elevated ALT: OR per ln-unit increase in PFOA, or by deciles	GGT 0.015 (0.01, 0.019), p-value < 0.001 ALT 0.022 (0.018, 0.025), p-value < 0.001 ALT, elevated (OR): Decile 2: 1.09 (0.94, 1.26) Decile 3: 1.19 (1.03, 1.37) Decile 4: 1.26 (1.09, 1.45) Decile 5: 1.40 (1.22, 1.62) Decile 6: 1.39 (1.21, 1.60) Decile 7: 1.31 (1.14, 1.52) Decile 8: 1.42 (1.23, 1.64) Decile 9: 1.40 (1.21, 1.62) Decile 10: 1.54 (1.33, 1.78) p-trend < 0.001 Per ln-unit increase: 1.1 (1.07, 1.13), p-value < 0.001 Direct bilirubin: No statistically significant associations
	Results: Lowest decile used as the reference group. **Confounding:** Age, sex, alcohol consumption, socioeconomic status, fasting status, month of blood sample collection, smoking status, BMI, physical activity, insulin resistance. Additional confounding for GGT, ALT, and direct bilirubin regression analyses: Race. Additional confounding for OR analyses: increased serum iron.						
Lin et al. (2010) *Medium*	United States 1999–2000, 2003–2004	Cross-sectional	Adults from NHANES, Ages ≥18 yr, Total N = 2,216, Men	Serum Total: 4.20 (2.90–5.95) Mean (SE): Men: 5.05 (1.03)	Levels of ALT (U/L), GGT (log-U/L), bilirubin (μM)	Regression coefficient per log-unit increase in PFOA	ALT Total: Separate analysis: 1.86 (SE = 0.62), p-value = 0.005 Composite analysis: 2.19 (SE = 0.79), p-value = 0.009

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 1,063, Women N = 1,134, Ages 18–39 N = 944, Ages 40–59 N = 534, Ages ≥60 N = 719	Women: 4.06 (1.04) Ages 18–39: 4.48 (1.03) Ages 40–59: 4.71 (1.04) Ages ≥60: 4.22 (1.04)			Men: 1.55 (SE = 0.84), p-value = 0.076 Women: 1.87 (SE = 1.13), p-value = 0.109 Ages 18–39: 1.02 (SE = 0.84), p-value = 0.234 Ages 40–59: 1.83 (SE = 1.84), p-value = 0.329 Ages ≥60: 1.93 (SE = 1.10), p-value = 0.089 GGT Total: Separate analysis: 0.08 (SE = 0.03), p-value = 0.019 Composite analysis: 0.15 (SE = 0.04), p-value = 0.001 Men: (SE = 0.03), p-value = 0.766 Women: 0.09 (SE = 0.05), p-value = 0.087 Ages 18–39: 0.06 (SE = 0.04), p-value = 0.078 Ages 40–59: 0.04 (SE = 0.08), p-value = 0.641 Ages ≥60: 0.06 (SE = 0.04), p-value = 0.146 Bilirubin, total Separate analysis: −0.09 (SE = 0.20), p-value = 0.645 Composite analysis: −0.20 (SE = 0.22), p-value = 0.378

Population: Stratified population counts do not include 19 individuals who were excluded due to their iron saturation being above 50%.
Comparison: Logarithm base not specified.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Age, gender, race/ethnicity, smoking status, drinking status, education level, BMI, HOMA-IR, metabolic syndrome, iron saturation status. Additional confounding for bilirubin, GGT and ALT composite analyses: PFHxS exposure, PFNA exposure, PFOS exposure.						
Costa et al. (2009) *Medium*	Italy 2007	Cross-sectional	Current and former male employees of an Italian chemical production plant, Comparison of means analysis N = 68, Exposed vs Unexposed analysis N = 141, Continuous regression analysis N = 56	Serum Production workers (2007): 3.89 µg/mL (2.18–18.66 µg/mL)	Levels of AST (U/L), ALT (U/L), GGT (U/L), ALP (U/L), Albumin (%)	Comparison of mean outcome (Exposed vs unexposed workers) Regression coefficient (exposed workers vs all workers) Regression coefficient per unit increase in PFOA	No significant differences in comparison of mean hepatic outcomes ALT Exposed vs Unexposed: −5.18 (−13.7, 3.32) Continuous: 0.116 (0.054, 0.177), p-value < 0.01 ALP Exposed vs Unexposed: −0.78 (−8.51, 6.95) Continuous: 0.057 (0.007, 0.107), p-value < 0.05 AST Exposed vs. Unexposed: 1.35 (−2.72, 5.41) Continuous: 0.038 (−0.003, 0.08) GGT Exposed vs. Unexposed: 0.32 (−17.5, 18.1) Continuous: 0.177 (0.076, 0.278), p-value < 0.01 Albumin Exposed vs. Unexposed: −0.73 (−3.44, 1.97) Continuous: −0.009 (−0.017, 0.001)
	Confounding: Age, job seniority, body mass index, smoking and alcohol consumption. Additional confounding for continuous regression analyses: year of observation.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Sakr et al. (2007a) *Medium*	United States 2004	Cross-sectional	Active employees at a Washington Works site where APFO is used, AST analysis N = 1,016, ALT analysis N = 1,018, GGT and bilirubin analysis N = 1,019, AST analysis, workers not on lipid-lowering medications N = 838, ALT and GGT analysis, workers not on lipid-lowering medications N = 840	Serum Mean (SD) = 0.428 (0.86) ppm	Levels of AST (ln-IU/L), ALT (ln-IU/L), GGT (ln-IU/L), Bilirubin (ln-IU/L)	Regression coefficient per unit increase in PFOA	AST 0.012 (SE = 0.012), p-value = 0.317 ALT 0.023 (SE = 0.015), p-value = 0.124 GGT 0.048 (SE = 0.020), p-value = 0.016 Bilirubin 0.008 (SE = 0.014), p-value = 0.59 AST, workers not on lipid-lowering medication 0.023 (SE = 0.013), p-value = 0.079 ALT, workers not on lipid-lowering medication 0.031 (SE = 0.017), p-value = 0.071 GGT, workers not on lipid-lowering medication 0.05 (SE = 0.023), p-value = 0.03 Bilirubin, workers not on lipid-lowering medication 0.008 (SE = 0.017), p-value = 0.637
	Confounding: Age, gender, BMI.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Sakr et al. (2007b) *Medium*	United States 1979–2007	Cohort	Fluoropolymer manufacturing site workers, N = 454	Serum Mean (SD) = 1.13 (2.1) ppm	Levels of total bilirubin (mg/dL), GGT (IU/L), AP (IU/L), AST (IU/L), ALT (IU/L)	Regression coefficient per unit increase in PFOA	Bilirubin, total −0.008 (−0.0139, −0.0021) GGT 1.24 (−1.09, 3.57) AP −0.21 (−0.60, 0.18) AST 0.35 (0.10, 0.60) ALT −0.54 (−0.46, 1.54)
	Confounding: Age, BMI, gender, decade of hire. Additional confounding for total bilirubin regression analysis: age squared.						
Olsen et al. (2001) *Medium*	United States, Belgium 1994–2000	Cohort	Male 3M fluorochemical plant workers in Antwerp, Belgium and Decatur, Alabama N = 175	Serum Antwerp (2000) Mean (SD): 1.43 ppm (1.21) Decatur (2000): 1.83 ppm (1.53)	Levels of ALT (ln-IU/L), ALP (ln-IU/L), AST (ln-IU/L), GGT (ln-IU/L)	Regression coefficient per unit increase in PFOA	ALT 0.015 (SE = 0.02), p-value = 0.46 PFOA × Years of observation interaction p-value = 0.19 AST 0.027 (SE = 0.015), p-value = 0.06 PFOA × Years of observation interaction p-value = 0.41 ALP 0.005 (SE = 0.012), p-value = 0.69 PFOA × Years of observation interaction p-value = 0.62 GGT –0.009 (SE = 0.025), p-value = 0.72 PFOA × Years of observation interaction p-value = 0.29

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Years of observation, PFOA × Years of observation, age, BMI, drinks/day, cigarettes/day, location, entry period, baseline years worked, triglycerides.						
Olsen et al. (2000) *Medium*	United States 1993–1997	Cross-sectional	Male workers involved in ammonium perfluorooctanoate production, N = 265	Serum 1993: 1.1 (Range = 0.0–80.0) ppm 1995: 1.2 (Range = 0.0–114.1) ppm 1997: 1.3 (Range = 0.1–81.3) ppm	Levels of ALT (IU/L), Cholecystokinin (pg/mL)	Regression coefficient per unit increase in PFOA	ALT 1993: 0.89 (SE = 2.88), p-value = 0.76 1995: 0.81 (SE = 2.62), p-value = 0.75 1997: 2.77 (SE = 1.27), p-value = 0.03 Cholecystokinin −0.008 (SE = 0.004), p-value = 0.07
	Confounding: Age, alcohol use, cigarette use. Additional confounding for cholecystokinin regression analysis: Body mass index (BMI).						
Olsen et al. (2012) *Low*	United States 2008–2010	Cohort	3M Fluorochemical plant employees and contractors, N = 179	Serum Mean change from baseline, Employees: −218.3; Contractors: 32.1	Levels of ALT (IU/L), AST (IU/L)	Regression coefficient per unit increase in PFOA	ALT −0.0097 (SD = 0.005), p-value = 0.00495 AST −0.0032 (SD = 0.003)
	Confounding: Sex, age at baseline, BMI at baseline, alcohol consumption at baseline.						
Wang et al. (2012) *Low*	China 2010–2011	Cross-sectional	Male fluorochemical plant workers and nearby residents, N = 55–132	Serum Residents: 284.34 (Range = 10.20–2,436.91); Workers: 1,635.96 (Range = 84.98–7,737.13)	Levels of ALT (ln-IU/L), AST (ln-IU/L)	Regression coefficient per ln-unit increase in PFOA	ALT Residents: −0.1 (−0.19, 0.00), p-value = 0.05 Workers: 0.04 (−0.06, 0.15) AST Residents: −0.04 (−0.10, 0.02) Workers: −0.12 (−0.22, −0.02), p-value = 0.02
	Confounding: None.						
Darrow et al. (2016) *Medium*	United States 2005–2006	Cohort and Cross-sectional	Adults from the C8 Health Project, Ages ≥18 yr, N = 30,723	Modeled cumulative PFOA,	Levels of ALT (IU/L), Liver (enlarged,	Regression coefficient per ln-unit increase in	ALT Modeled, 0.012 (0.008, 0.016) Quintile 2: 0.023 (0.006, 0.040) Quintile 3: 0.035 (0.018, 0.052)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				20th–80th percentile: 191–3,998yr-ng/mL; Estimated in serum 16.5 (range = 2.6–3,559)	fatty, or cirrhosis), Liver disease (any)	PFOA or by quintiles Liver (enlarged, fatty, or cirrhosis) and disease (any): HR per 1-ln y-ng/mL increase in PFOA or by quintiles	Quintile 4: 0.039 (0.022, 0.056) Quintile 5: 0.058 (0.040, 0.076) p-trend < 0.0001 Estimated, 0.012 (0.009, 0.016) Quintile 2: 0.001 (−0.016, 0.018) Quintile 3: 0.023 (0.007, 0.040) Quintile 4: 0.036 (0.019, 0.053) Quintile 5: 0.048 (0.031, 0.066) p-trend < 0.001 Liver (enlarged, fatty, or cirrhosis) No lag, 0.97 (0.91, 1.04) Quintile 2: 0.90 (0.65, 1.25) Quintile 3: 0.83 (0.60, 1.15) Quintile 4: 0.75 (0.54, 1.03) Quintile 5: 0.83 (0.60, 1.16) 10-yr lag, 1.00 (0.94, 1.07) Quintile 2: 1.04 (0.72, 1.50) Quintile 3: 0.91 (0.64, 1.31) Quintile 4: 0.84 (0.59, 1.21) Quintile 5: 0.87 (0.61, 1.25) Liver disease (any) No lag, 0.97 (0.92, 1.03) Quintile 2: 1.19 (0.88, 1.59) Quintile 3: 1.08 (0.81, 1.45) Quintile 4: 1.04 (0.78, 1.40) Quintile 5: 0.95 (0.70, 1.27) 10-yr lag, 0.98 (0.93, 1.04) Quintile 2: 1.15 (0.81, 1.63) Quintile 3: 1.08 (0.76, 1.54) Quintile 4: 0.90 (0.63, 1.28) Quintile 5: 0.99 (0.70, 1.42)
	Results: Regression coefficient for modeled continuous PFOA is per ln y-ng/mL increase. Lowest quintile used as the reference group.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Age, sex, BMI, alcohol consumption, regular exercise, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, race.						
	Adults – Other Hepatic Outcomes						
Girardi and Merler (2019) *Low*	Italy 1960–2018	Cohort	Male workers at a PFAS production plant N = 462	Serum T2: GM = 13,051 ng/m L-years T3: GM = 81,934 ng/m L-years	Liver cancer or cirrhosis mortality Liver cirrhosis mortality	SMR by tertiles Mortality risk ratio by tertiles	Liver cancer or cirrhosis mortality SMRs: T1: 0.44 (0.06, 3.15) T2: 2.76 (1.15, 6.63) T3: 2.86 (1.36. 6.00) RRs: T1: 1.17 (0.15, 9.42) T2: 7.26 (2.37, 22.3) T3: 6.68 (2.41, 18.5) Liver cirrhosis mortality SMRs: T2: 2.76 (0.89, 8.56) T3: 2.63 (0.85, 8.14) RRs: T2: 6.59 (1.57, 27.7) T3: 5.04 (1.19, 21.3)
	Results: Workers at nearby non-chemical factory used as reference. Tertile 1 used as the reference group. **Confounding**: For mortality risk ratio: age at risk, calendar period.						
Rantakokko et al. (2015) *Medium*	Finland 2005–2011	Cross-sectional	Morbidly obese adults undergoing bariatric surgery, N = 160	Serum 2.56 (5th–95th percentile: 1.04–4.66)	Lobular inflammation	OR per log10 unit increase in PFOA by level of lobular inflammation	<2 foci vs. none: 0.71 (0.10, 5.18) 2–4 foci vs. none: 0.02 (<0.01, 0.66), p-value = 0.027
	Results: No foci used as the reference group. Foci measured per 200x field. **Confounding**: Age, sex, BMI, serum lipids, fasting insulin.						
	Children and Adolescents						
Gleason et al. (2015) *Medium*	United States 2007–2010	Cross-sectional	Adolescents from NHANES, Ages ≥12, N = 4,333	Serum 3.7 (2.5–5.2)	Levels of ALT (ln-U/L), AST (ln-U/L), GGT (ln-U/L), ALP (ln-U/L);	Regression coefficient per ln-unit increase in PFOA	ALT 0.038 (0.014, 0.062), p-value < 0.001 ALT, elevated, OR: Q2: 1.43 (1.11, 1.86)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
					elevated ALT, GGT, or AST	Elevated ALT, GGT, or AST: OR by quartile	Q3: 1.56 (1.15, 2.12) Q4: 1.52 (1.18, 1.96) p-trend = 0.07 GGT 0.058 (0.021, 0.096), p-value < 0.01 GGT elevated, OR: Q2: 1.10 (0.80, 1.53) Q3: 1.12 (0.80, 1.53) Q4: 1.36 (1.00, 1.82) p-trend = 0.042 AST 0.025 (0.007, 0.043), p-value < 0.01 AST elevated, OR Q2: 1.32 (1.03, 1.67) Q3: 1.27 (0.98, 1.66) Q4: 1.40 (1.07, 1.83) p-trend = 0.058 ALP –0.003 (–0.023, 0.016)
	Outcome: Elevated clinical biomarkers defined based on the 75th percentile value in the 2007–2010 NHANES. **Results:** Lowest quartile used as reference group. **Confounding:** Age, gender, race/ethnicity; and BMI group, poverty, smoking, alcohol consumption "if statistically significant associated with both the exposure and outcome in univariate analysis."						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children, Ages 8–12, N = 48	Serum 0.99 (IQR = 0.45)	Levels of ALT (u/L), AST (u/L)	Regression coefficient per unit increase in PFOA	ALT 1.64 (−8.68, 12.00) AST 0.14 (−4.73, 5.00)
	Confounding: Age, sex, race.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Attanasio (2019) *Medium*	United States 2013–2016	Cross-sectional	Adolescents from NHANES, Ages 12–19, N = 305–354	Serum Boys: GM = 1.5 (SE = 0.06) Girls: GM = 1.22 (SE = 0.06)	Levels of ALT (ln-IU/L), AST (ln-IU/L)	Regression coefficient per ln-unit increase in PFOA or by quartiles	ALT Boys, −0.07 (−0.13, −0.01) Q2: 0.02 (−0.16, 0.19) Q3: −0.01 (−0.13, 0.10) Q4: −0.11 (−0.21, −0.01), p-value = 0.03 p-trend = 0.09 Girls, 0.09 (0.02, 0.17) Q2: 0.09 (0.01, 0.18) Q3: 0.16 (0.05, 0.28) Q4: 0.17 (0.05, 0.28) p-trend = 0.02 AST Boys, −0.06 (−0.12, 0) Q2: −0.01 (−0.14, 0.12) Q3: 0.00 (−0.08, 0.08) Q4: −0.05 (−0.15, 0.04) Girls, 0.06 (0.00, 0.13) Q2: 0.04 (−0.02, 0.11) Q3: 0.10 (0.01, 0.19) Q4: 0.11 (0.01, 0.20)
	Results: Lowest quartile used as reference group. **Confounding**: Age, race/ethnicity, body weight status, education, poverty-income ratio, exposure to smoking.						
Mora et al. (2018) *Medium*	United States 1999–2010	Cohort	Children from Project Viva, N = 508–630	Plasma Prenatal: 5.4 (3.9–7.6); Mid-childhood: 4.3 (3.0–6.0)	Levels of ALT (U/L)	Regression coefficient per IQR increase in PFOA	Prenatal exposure: −0.5 (−1.3, 0.2) Mid-childhood exposure: −0.7 (−1.4, 0)
	Confounding: For prenatal exposure maternal education, prenatal smoking, gestational age at blood draw, and child's sex, race/ethnicity, age at lipids/ALT measurements; For mid-childhood exposure maternal education, prenatal smoking, and child's sex, race/ethnicity, age at lipids/ALT measurements.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Children and Adolescents – Other Hepatic Outcomes							
Jin et al. (2020) *Medium*	United States 2007–2015	Cross-sectional	Children and adolescents diagnosed with nonalcoholic fatty liver disease, Ages 7–19, N = 74	Plasma 3.42 (2.5–4.1)	Ballooning, Grade of steatosis, Liver fibrosis, Lobular inflammation, Nonalcoholic steatohepatitis, Portal inflammation	OR per IQR increase in PFOA	Ballooning Few balloon cells: 0.99 (0.52, 1.86) Many cells/prominent ballooning: 0.42 (0.07, 2.60) Grade of steatosis 34%–66% steatosis: 1.41 (0.61, 3.23) >66% steatosis: 1.21 (0.60, 2.47) Liver fibrosis Mild (Stage 1): 1.68 (0.75, 3.73) Significant (Stages 2–4): 0.97 (0.33, 2.82) Lobular inflammation <2 foci: 0.90 (0.45, 1.81) 2–4 foci: 1.32 (0.52, 3.39) Nonalcoholic steatohepatitis 1.21 (0.67, 2.18) Portal inflammation Mild: 1.26 (0.65, 2.43) Moderate-to-severe: 0.65 (0.18, 2.39)
	Results: For ballooning, none was used as the reference group. For grade of steatosis < 5%–33% was used as the reference group. For liver fibrosis, none was used as the reference group. For lobular inflammation, no foci used as the reference group. Foci measured per 200x field. For portal inflammation, none was used as the reference group. **Confounding**: Age, sex, ethnicity, BMI z-score.						

Notes: ALP = alkaline phosphatase; ALT = alanine aminotransferase; APFO = ammonium perfluorooctanoate; AST = aspartate aminotransferase; BMI = body mass index; GF = glomerular filtration; GGT = γ-glutamyltransferase; GM = geometric mean; HOMA-IR = homeostasis model assessment of insulin resistance; HR = hazard ratio; IQR = interquartile range; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POUNDS = Preventing Overweight Using Novel Dietary Strategies; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; rr = risk ratio; SD = standard deviation; SE = standard error; SMR = standardized mortality ratio; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.
[c] Confounding indicates factors the models presented adjusted for.

D.4 Immune

Table D-7. Associations Between PFOA Exposure and Vaccine Response in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Children			
Grandjean et al. (2012) *Medium*	Faroe Islands, Denmark Recruitment 1997–2000, Follow-up through 2008	Cohort	Children followed from birth to age 7 Birth and infancy: N = 587 Prebooster (mean age 5.0) examination: N = 532 Postbooster (mean age 5.2) examination: N = 456 Age 7 (mean age 7.5) examination: N = 464	Maternal serum (prenatal) Geometric mean = 3.20 (2.56, 4.01) Child serum (5 yr) Geometric mean = 4.06 (3.33–4.96)	Antibody concentrations (log-IU/mL) for tetanus and diphtheria	Percent change per doubling in age 5 and maternal serum PFOA	Child serum Anti-diphtheria, prebooster, age 5 −6.8 (−28.3, 21.0) Anti-diphtheria, postbooster, age 5 −6.1 (−23.6, 15.5) Anti-diphtheria, age 7 −25.2 (−42.9, −2.0) Anti-diphtheria, age 7 adjusted for age 5 Ab −23.4 (−39.3, −3.4) Maternal serum Anti-diphtheria, prebooster, age 5 −16.2 (−34.2, 6.7) Anti-diphtheria, postbooster, age 5 −6.2 (−22.4, 13.3) Anti-diphtheria, age 7 −22.8 (−39.4, −1.7) Anti-diphtheria, age 7 adjusted for age 5 Ab −16.8 (−32.9, 3.3) Child serum Anti-tetanus, prebooster, age 5 −13.3 (−31.6, 9.9) Anti-tetanus, postbooster, age 5 −9.7 (−30.7, 17.7)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Anti-tetanus, age 7 −35.8 (−51.9, 14.2) Anti-tetanus, age 7 adjusted for age 5 Ab −28.2 (−42.7, −10.1) Maternal serum Anti-tetanus, prebooster, age 5 −10.5 (−28.2, 11.7) Anti-tetanus, postbooster, age 5 14.5 (−10.4, 46.4) Anti-tetanus, age 7 7.4 (−17.1, 39.0) Anti-tetanus, age 7 adjusted for age 5 Ab 12.3 (−8.6, 38.1)
	Confounding: Age, sex. Additional confounding for postbooster analyses: time since vaccination, booster type. Additional confounding for year 7 analyses: booster type. Additional confounding for year 7 analyses adjusted for age 5 Ab: booster type, child's specific antibody concentration at age 5 years.						
Granum et al. (2013) *Medium*	Norway 1999–2008	Cohort	Mother-infant pairs from MoBa at 3-yr follow-up N = 56	Maternal serum with three days of delivery 1.1 (0.8–1.4)	Levels (OD) of rubella anti-vaccine antibodies	Regression coefficient per unit increase in PFOA	Rubella antibody –0.4 (–0.64, –17) p-value = 0.001
	Confounding: Maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up.						
Mogensen et al. (2015a) *Medium*	Faroe Islands, Denmark 2002–2007	Cohort	Children aged 5–7 yr N = 443 (7 yr)	Serum 4.4 (3.5–5.7)	Antibody concentrations (log2-IU/mL) for diphtheria or tetanus	Percent change per doubling of PFOA	Anti-diphtheria, age 7 −25.4 (−40.9, −5.8) Anti-tetanus, age 7 −20.5 (−38.2, 2.1)
	Confounding: Age, sex, booster type.[c]						
Stein et al. (2016) *Medium*	United States,	Cross-sectional	Children aged 12-19 years, NHANES	Serum	Antibody concentrations for measles,	Percent change per doubling serum PFOA	Measles antibody All –0.1 (–13.8, 15.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	1999–2000, 2003–2004, 2005–2006		N = 1,190 (All) N = 1,152 (Seropositive)	GM = 4.13 (95% CI: 3.76, 4.53)	mumps, and rubella		Seropositive –3.4 (–16.7, 11.9) Mumps antibody All –6.0 (–12.4, 0.9) Seropositive –6.6 (–11.7, –1.5) Rubella antibody All –2.5 (–9.1, 5.3) Seropositive –8.9 (–14.6, –2.9)
	Confounding: Age, sex, race/ethnicity, survey year.						
Grandjean et al. (2017a) *Medium*	Faroe Islands, Denmark Enrollment: 1997–2000	Cohort and cross-sectional	Children followed up at 7 yr and 13 yr N = 505 (13 yr) N = 427 (7 yr)	Serum 13 yr: 2.0 (1.6–2.5) 7 yr: 4.4 (3.5–5.7)	Levels of diphtheria antibody (log2-IU/mL), tetanus antibody (log2-IU/mL)	Percent change per doubling of PFOA	Diphtheria antibody Age 7: −4.1 (−25.4, 23.3) p-value = 0.742 Age 13: −17.5 (−35.6, 5.8) p-value = 0.129 Tetanus antibody Age 7: 9.4 (−24.7, 58.9) p-value = 0.637 Age 13: 3.3 (−27.3, 46.9) p-value = 0.856
	Confounding: Sex, age at antibody assessment, booster type at age 5.						
Grandjean et al. (2017b) *Medium*	Faroe Islands, Denmark 1997–2000 and 2007–2009 (year of birth)	Cohort and Cross-sectional	Infants 2 wk after expected term date, followed up at 18 mo and 5 yr	Serum 18 mo: median = 2.8 (2.0–4.5) 5 yr:	Age 5 levels of tetanus antibody (IU/mL), diphtheria antibody (IU/mL)	Percent change per doubling of PFOA	2007–2009 cohort Tetanus antibody Birth: −22.25 (−35.25, −6.63) p-value = 0.007 18 mo: −16.31 (−29.04, −1.31) p-value = 0.034 5 yr: −25.26 (−42.63, −2.64) p-value = 0.031

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			All: N = 490, 18 mo: N = 275, 5 yr: N = 349	median = 2.2 (1.8–2.8)			Diphtheria antibody: Birth: −18.93 (−33.16, −1.66) p-value = 0.033 18 mo: 4.19 (−11.76, 23.02) p-value = 0.63 5 yr: 18.31 (−10.72, 56.78) p-value = 0.24 Combined cohort Tetanus antibody: Birth: −17.59 (−28.38, −5.17) p-value = 0.007 18 mo: −16.47 (−28.84, −1.96) p-value = 0.028 5 yr: −18.75 (−31.79, −3.21) p-value = 0.020 Diphtheria antibody: Birth: −17.82 (−29.11, −4.74) p-value = 0.009 18 mo: 5.44 (−10.28, 23.92) p-value = 0.52 5 yr: 3.38 (−14.16, 24.50) p-value = 0.73
	Confounding: Age, sex.						
Abraham et al. (2020) *Medium*	Berlin, Germany Enrollment: 1997–1999	Cross-sectional	Children, 1 yr old All: N = 101, formula fed: N = 21, breastfed: N = 80	Plasma Formula fed: mean = 3.8 ± 1.1 (range = 1.6–6.4) Breastfed:	Levels of Hib antibody, tetanus antibody IgG, tetanus antibody IgG1, diphtheria antibody	Spearman correlation coefficient	Hib antibody: −0.32 p-value < 0.05 Tetanus antibody IgG: −0.25 p-value < 0.05 Tetanus antibody IgG: −0.22 p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				mean = 16.8 ± 6 .6 (range = 2.6–36.7)			Diphtheria antibody: −0.23 p-value < 0.05
	Confounding: Time since last vaccination.						
Timmermann et al. (2020) *Medium*	Guinea-Bissau 2012–2015	Cohort	Infants enrolled at 4–7 mo old (inclusion), followed up at 9 mo and 2 yr Inclusion: N = 236 9-mo Unvaccinated controls: N = 100 Intervention: N = 134 2-yr Unvaccinated controls: N = 102 Intervention: N = 92	Maternal blood 0.68 (0.53–0.92)	Measles antibody concentration (mIU/mL)	Percent difference per doubling of PFOA	Inclusion (no measles vaccination): −12 (−28, 7) 9-mo visit Control (no measles vaccination): −11 (−36, 22) Intervention (1 measles vaccination): 7 (−15, 35) 2-yr visit Control (1 measles vaccination): −9 (−30, 18) Intervention (2 measles vaccinations): 12 (−11, 40)
	Confounding: Weight and age at inclusion, maternal education, breastfeeding without solids, maternal measles antibody concentration, sex, and time from vaccination to blood sampling.						
Timmerman et al. (2021) *Medium*	Greenland Recruitment: 1999–2005, Examination: 2012–2015	Cohort and cross-sectional	Vaccinated children ages 7–12 yr and their mothers at pregnancy Maternal serum N = 57	Maternal serum from pregnancy 2.28 (1.89–2.88) Child serum 2.13 (1.68–2.54)	Levels (IU/mL) of diphtheria and tetanus antibody	Percent difference per unit increase in PFOA OR per log10-unit increase in PFOA	Diphtheria antibody Child serum Percent difference: −22 (−48, 16) OR: 1.41 (0.91, 2.19) Maternal serum Percent difference: 44 (−15, 145) Tetanus antibody

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Child serum N = 169				Child serum Percent difference: −8 (−30, 21) Maternal serum Percent difference: −7 (−44, 56)
	Confounding: Area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq). Additional confounding for percent difference analyses: duration of being breastfed (<6 mo, 12 mo, >1 yr). Additional confounding for child serum analyses: time since vaccine booster (only children with known vaccination date were included).						
Zeng et al. (2019b) *Low*	China 2013	Cohort	Infants from Guangzhou Birth Cohort Study at birth and 3 mo Birth N = 194 (91 girls, 103 boys) 3-mo N = 180 (89 girls, 91 boys)	Cord blood 1.22 (0.86–1.74)	HFMD antibody titers (CA16 or EV71) in serum of cord blood or at 3 mo	Percent change or OR (below clinical protection) per doubling of PFOA	CA16 Cord blood: −16.3 (−25.3, 6.1) Girls: −8.7 (−22.6, 7.6) Boys: −22.0 (−33.1, −8.9) p months: −6.9 (−13.2, 0) Girls: −3.2 (−11.2, 5.5) Boys: −11.1 (−20.7, −0.3) CA16 below clinical protection Cord blood: 1.56 (1.13, 2.14); p-value = 0.007 Girls: 1.16 (0.72, 1.87) Boys: 1.95 (1.16, 3.27) p-value for interaction by sex = 0.181 q months: 1.73 (1.08, 2.75); p-value = 0.022 Girls: 1.31 (0.71, 2.44) Boys: 2.49 (1.23, 5.04) p-value for interaction by sex = 0.263 EV71 Cord blood: −18.7 (−28.6, −7.4) Girls: −14.6 (−30.4, 4.6) Boys: −20.6 (−32.5, −6.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							r months: −7.2 (−13.2, −0.8) Girls: −4.9 (−13.7, 4.8) Boys: −8.2 (−16.2, 0.5) EV71 below clinical protection Cord blood: 1.49 (1.09, 2.05); p-value = 0.014 Girls: 1.27 (0.84, 1.93) Boys: 1.76 (1.07, 2.87) p-value for interaction by sex = 0.282 s months: 2.11 (1.27, 3.48); p-value = 0.004 Girls: 1.52 (0.81, 2.85) Boys: 2.90 (1.34, 6.29) p-value for interaction by sex = 0.202
	Outcome: Clinical protection threshold defined as titers ≥1:8 in modified cytopathogenic effect assay. **Confounding:** Sex, age, parental education, parental occupation, family income, parity, and birth weight.						
Adults and Adolescents							
Looker et al. (2014) *Medium*	United States Baseline: 2005–2006, Follow-up: 2010	Cohort	Adults near water districts of Ohio and West Virginia with contaminated drinking water N = 403	Serum GM (95% CI) = 33.74 (29.78–38.22)	Influenza antibodies (titer ratio and titer rise, log10-transformed): A/H1N1, A/H3N2, type B; influenza A/H3N2 seroconversion	Regression coefficient per log10-unit increase in PFOA, or by quartiles Influenza A/H3N2 seroconversion: OR per log10-unit increase in	Influenza type B titer rise Per log10-unit: –0.2 (–0.13, 0.09), p-value = 0.73 Q2: –0.03 (–0.19, 0.13), p-value = 0.69 Q3: –0.02 (–0.19, 0.15), p-value = 0.82 Q4: –0.07 (–0.24, 0.10), p-value = 0.42 Influenza type B titer ratio Per log10-unit: –0.02 (–0.11, 0.08), p-value = 0.73

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						PFOA, or by quartiles	Q2: 0.05 (–0.09, 0.19), p-value = 0.53 Q3: 0.07 (–0.07, 0.22), p-value = 0.32 Q4: –0.03 (–0.17, 0.12), p-value = 0.71 Influenza A/H3N2 titer rise Per log10-unit: –0.01 (–0.17, 0.14), p-value = 0.86 Q2: –0.28 (–0.51, –0.06), p-value = 0.02 Q3: –0.37 (–0.60, –0.13), p-value = 0.002 Q4: –0.12 (–0.36, 0.13), p-value = 0.35 Influenza A/H3N2 titer ratio Per log10-unit: –0.12 (–0.25, 0.02), p-value = 0.09 Q2: –0.10 (–0.30, 0.10), p-value = 0.31 Q3: –0.07 (–0.28, 0.14), p-value = 0.49 Q4: –0.22 (–0.43, –0.01), p-value = 0.04 Influenza A/H1N1 titer rise Per log10-unit: –0.30 (–0.14, 0.09), p-value = 0.63 Q2: –0.09 (–0.27, 0.08), p-value = 0.31 Q3: –0.10 (–0.28, –0.09), p-value = 0.30 Q4: –0.12 (–0.30, 0.06), p-value = 0.19

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Influenza A/ H1N1 titer ratio Per log10-unit: 0.07 (–0.06, 0.21), p-value = 0.30 Q2: –0.08 (–0.29, 0.12), p-value = 0.43 Q3: –0.04 (–0.25, 0.18), p-value = 0.72 Q4: 0.07 (–0.14, 0.29, p-value = 0.51 Influenza A/H3N2 seroconversion not statistically significant
	Results: Lowest quartile used as reference group. **Confounding:** Age (cubic spline), gender, mobility, and history of previous influenza vaccination.						
Pilkerton et al. (2018) *Medium* for youth *Low* for adult	United States 1999–2000	Cross-sectional	Adults and adolescents 12 yr and older Youths: N = 1,012 Adults: N = 542 women, 613 men	Serum Women: mean = 4.3, SE = 0.2 Men: mean = 6.0 SE = 0.3	Rubella IgA titers (log-IU)	Regression coefficient by quartiles or per quartile increase in PFOA	Adolescents: Per quartile increase: No associations. F-value = 0.34, p-value = 0.80 Adults: Per quartile increase: F-value = 6.60, p-value = 0.002 Women Q2: −0.25 (−0.52, 0.02) p-value = 0.064 Q3: −0.15 (−0.9, 0.6) p-value = 0.686 Q4: −0.17 (−0.97, 0.64) p-value = 0.677 Men Q2: −0.14 (−0.43, 0.15) p-value = 0.339 Q3: −0.55 (−0.81, −0.28) p-value = 0.0002

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: –0.45 (−0.84, −0.05) p-value = 0.028
	Outcome: Logarithm base not reported. **Results:** Lowest quartile used as reference group. **Confounding:** Women: age, ethnicity, BMI, educational level, number of live births; men: age, ethnicity, BMI, educational level.						
Bulka et al. (2021) *Medium*	United States 1999–2000, 2003–2016	Cross-sectional	NHANES adolescents and adults aged 12–49 yr 12–19 yr: N = 3,189 20–49 yr: N = 5,589	Serum 12–19 yr: GM (SE) = 2.54 (0.06) 20–49 yr: GM (SE) = 2.68 (0.03)	Persistent infections of cytomegalovirus, Epstein-Barr virus, hepatitis C, hepatitis E, herpes simplex virus 1, herpes simplex virus 2, Toxoplasma gondii, and Toxocara species; pathogen burden	Persistent infections: Prevalence ratio per doubling in PFOA Pathogen burden: Relative difference per log2-unit increase in PFOA	Cytomegalovirus 12–19 yr: 0.87 (0.70, 1.08), p-value = 0.24 20–49 yr: 0.98 (0.91, 1.05), p-value = 0.57 Epstein-Barr virus 12–19 yr: 0.99 (0.94, 1.05), p-value = 0.83 Hepatitis C virus 20–49 yr: 0.89 (0.62, 1.29), p-value = 0.54 Hepatitis E virus 20–49 yr: 1.01 (0.78, 1.31), p-value = 0.92 Herpes simplex virus 1 12–19 yr: 1.02 (0.93, 1.11), p-value = 0.75 20–49 yr: 1.03 (1.01, 1.06), p-value = 0.01 Herpes simplex virus 2 20–49 yr: 1.11 (1.05, 1.17), p-value < 0.01 Toxoplasma gondii

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							12–19 yr: 0.99 (0.68, 1.42), p-value = 0.94 20–49 yr: 1.03 (0.89, 1.18), p-value = 0.70 Toxocara species 12–19 yr: 1.21 (0.56, 2.65), p-value = 0.63 20–49 yr: 1.23 (1.00, 1.51), p-value = 0.08 Pathogen burden 12–19 yr: 1.36 (1.27, 1.45) 20–49 yr:1.09 (1.06, 1.12)
	Outcome: Pathogen burden defined as the sum of pathogens for which an individual was seropositive (including any pathogens with a seroprevalence < 1.0%). **Confounding:** Age, race/ethnicity, sex, ratio of family income to the federal poverty threshold, educational attainment, serum cotinine concentrations, and BMI.						
Lopez-Espinosa et al. (2021) *Medium*	United States 2005–2006, 2010	Cohort and cross-sectional	Adults from C8HP 2005–2006: N = 42,782 2010: N = 526	Serum 2005–2006: 26.9 (13.2–69.2) 2010: 35.7 (15.0–93.7)	Levels (ln-cells/µL or percentage of white blood cells/lymphocytes) of white blood cells, neutrophils, monocytes, eosinophils, lymphocytes, CD3+ T cells, CD3+CD4+ T-helper cells, CD3+CD4+CD8+ double positive T cells,	Levels: Relative difference per 1-ln IQR increase in PFOA Percentages: Difference per 1-ln IQR increase in PFOA	White blood cells, total 2005–2006: −0.27 (−0.62, 0.08) 2010: 0.84 (−2.20, 3.97) Likelihood ratio test p-value < 0.001 for the comparison between the two time points

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					CD3+CD8+ T-cytotoxic cells, CD3-CD16+CD56+ natural killer cells, CD3-CD19+ B cells; CD4+/CD8+ ratio		
	Outcome: All cell types reported as cell counts; eosinophils, lymphocytes, monocytes, and neutrophils additionally reported as percentage of white blood cells; CD3+ T cells, CD3+CD4+ T-helper cells, CD3+CD4+CD8+ double positive T cells, CD3+CD8+ T-cytotoxic cells, CD3-CD16+CD56+ natural killer cells, and CD3-CD19+ B cells additionally reported as percentage of lymphocytes. **Confounding:** Gender, age, smoking, month of sampling, alcohol intake, and educational level.						
Shih et al. (2021) *Medium*	Faroe Islands, Denmark Recruitment: 1986–1987, Follow-up through 2015	Cohort and cross-sectional	Faroe Island residents at birth, 7, 14, 22, and 28 yr N = 399	Cord blood at birth 1.11 (IQR = 0.62) Serum 7 yr: 5.11 (IQR = 2.45) 14 yr: 4.98 (IQR = 2.11) 22 yr: 2.96 (IQR = 1.69) 28 yr: 1.28 (IQR = 0.90)	Levels (IU/mL) of hepatitis A antibody, hepatitis B antibody, diphtheria antibody, tetanus antibody; Hepatitis A antibody signal-to-cutoff ratio	Percent change per doubling of PFOA	Hepatitis Type B Cord blood: −4.34 (−30.69, 32.02) 7-yr serum: −9.39 (−43.4, 45.04) 14-yr serum: −7.4 (−47.65, 63.81) 22-yr serum: −21.24 (−42.2, 7.34) 28-yr serum: −16.77 (−35.47, 7.35) Hepatitis Type A Cord blood: 0.05 (−0.36, 0.46) 7-yr serum: 0.1 (−0.52, 0.72) 14-yr serum: −0.71 (−1.52, 0.09) 22-yr serum: −0.06 (−0.48, 0.35) 28-yr serum: −0.24 (−0.59, 0.1) Diphtheria Cord blood: 28.14 (−0.38, 64.82) 7-yr serum: −4.89 (−37.24, 44.11) 14-yr serum: −11.6 (−47.55, 48.97) 22-yr serum: 9.8 (−12.62, 37.96) 28-yr serum: 23.56 (3.65, 47.29) Tetanus

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Cord blood: −2.57 (−20.38, 19.22) 7-yr serum: 4.68 (−23.9, 43.99) 14-yr serum: −0.77 (−36.35, 54.7) 22-yr serum: −0.39 (−17.12, 19.72) 28-yr serum: 3.1 (−10.42, 18.66)
	Confounding: Sex.						
Zeng et al. (2020) *Low*	China 2015–2016	Cross-sectional	Adults from the Isomers of C8 Health Project N = 605	Serum 5.12 (3.82–8.11)	Hepatitis B surface antibody (HbsAb) (log-mIU/mL) or surface antigen (HbsAg) (mIU-mL); HbsAb seronegative (<10 mIU/mL)	Regression coefficient or OR (HbsAb seronegative) per log10-unit increase in PFOA	HbsAb concentration −0.38 (−0.79, 0.02); p-value = 0.061 HbsAb seronegative 1.89 (1.23, 2.90); p-value = 0.004 HbsAg concentration 0.41 (−0.42, 1.25); p-value = 0.33
	Confounding: Age, gender, BMI, career, income, alcohol drinking, smoking, regular exercise; education for HbsAb concentration alone.						
Zhang et al. (2023c) *Medium*	United States 2003–2004, 2009–2010	Cross-sectional	Children and adolescents aged 12–19 from NHANES N = 819	Serum Mean 3.33 (2.50–4.70)	Levels of rubella antibody, mumps antibody, measles antibody	Percent change per 2.7-fold increase in serum PFOA	Rubella −4.36 (−11.53, 3.40) Mumps −11.05 (−18.56, −2.85) p-value < 0.05 Measles −1.94 (−14.64, 12.64)
	Confounding: Age, sex, race, income-poverty ratio, BMI, serum cotinine concentrations, survey cycle, and dietary intake of milk.						

Notes: Ab = antibody; BMI = body mass index; C8HP = C8 Health Project; CI = confidence interval; GM = geometric mean; HAI = hemagglutinin inhibition; HFMD = hand, foot, and mouth disease; ICH = immunohistochemistry; IQR = interquartile range; mo = month/s; MoBa = Norwegian Mother and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; OD = optical density; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SE = standard error; T2 = tertile 2; T3 = tertile 3; wk = week/s; yr = year/s.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-8. Associations Between PFOA Exposure and Infectious Disease in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Children			
Fei et al. (2010) *Medium*	Denmark, Recruitment: 1996–2003; Follow-up: 2008	Cross-sectional and cohort	Mother-infant pairs with follow-up to 11 yr (DNBC) N = 1,400	Maternal plasma Mean (range) = 5.6 (<LLOQ–41.5); LLOQ = 1.0 ng/mL	Infectious disease hospitalizations	IRR by quartiles or per quartile increase in PFOA	Girls Q2: 1.20 (0.76, 1.89) Q3: 1.63 (1.03, 2.58) Q4: 1.74 (1.06, 2.87) Per quartile increase: 1.21 (1.04, 1.42) Boys Q2: 0.58 (0.40, 0.83) Q3: 0.53 (0.36, 0.76) Q4: 0.57 (0.38, 0.86) Per quartile increase: 0.83 (0.73, 0.95) All children Q2: 0.71 (0.53, 0.94) Q3: 0.77 (0.59, 1.03) Q4: 0.84 (0.62, 1.13) Per quartile increase: 0.96 (0.87, 1.06) Results stratified by age not statistically significant
	Results: Lowest quartile used as reference group **Confounding:** Parity, maternal age, pre-pregnancy BMI, breastfeeding, smoking during pregnancy, socio-occupational status, home density, child's age, sibling age difference, gestational age at blood drawing, birth year, and birth season.						
Gourdazi et al. (2017b) *Medium*	Hokkaido, Japan 2003–2009	Cohort	Children, early pregnancy followed up at 4 yr N = 1,558 (793 boys, 765 girls)	Maternal blood 2.01 (1.31–3.35)	Infectious diseases, total (including Otitis media, Pneumonia, RS virus, Varicella)	OR by quartiles	Girls Q2: 1.45 (0.92, 2.3) Q3: 1.37 (0.87, 2.19) Q4: 1.37 (0.86, 2.21) p-value for trend = 0.242 Boys Q2: 1.02 (0.67, 1.56) Q3: 1.34 (0.87, 2.11)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: 0.952 (0.61, 1.49) p-value for trend = 0.854 All Q2: 1.17 (0.87, 1.6) Q3: 1.32 (0.97, 1.82) Q4: 1.11 (0.81, 1.54) p-value for trend = 0.393
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, number of elder siblings, child sex, breastfeeding period, and smoking during pregnancy.[c]						
Manzano-Salgado et al. (2019) *Medium*	Spain, 2003–2008	Cohort	Children aged 1.5, 4, or 7 yr Age 1.5: N = 1,188 Age 4: N = 1,184 Age 7: N = 1,068	Maternal blood 2.35 (1.63–3.30)	LRTI	OR or RR per log2-unit increase in PFOA	OR 1.5 yr: 0.92 (0.79, 1.07) 4 yr: 1.11 (0.94, 1.31) 7 yr: 0.69 (0.47, 1.01) RR, 1.5–7 yr All: 0.96 (0.85, 1.08) Boys: 0.97 (0.82, 1.14) Girls: 0.99 (0.83, 1.18)
	Confounding: OR assessment: Age-at-follow-up of the child; RR assessment: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth.						
Ait Bamai et al. (2020) *Medium*	Hokkaido, Japan Enrollment: 2003–2012	Cohort	Children, early pregnancy followed up at 7 yr N = 2,689	Maternal blood 1.94 (1.30–2.95)	Chicken pox, RSV, otitis media, pneumonia, wheeze, eczema	OR or RR per ln-unit increase in PFOA	Pneumonia: OR: 1.17 (1.01, 1.37); p-value = 0.043 Otitis media: OR: 1.06 (0.92, 1.22); p-value = 0.45 Chicken pox: OR: 0.94 (0.81, 1.09); p-value = 0.381 RSV: OR: 0.96 (0.8, 1.17); p-value = 0.694

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Wheeze: RR: 0.92 (0.84, 1.01); p-value = 0.089 Eczema: RR: 0.85 (0.77, 0.94); p-value = 0.001
	Confounding: Sex, maternal age, parity, maternal smoking during pregnancy, BMI pre-pregnancy, annual household income during pregnancy, duration nursing, and presence of siblings.						
Grandjean et al. (2020) *Medium*	Denmark 2020	Cross-sectional	Adults, ages 30–70 yr, with known SARS-CoV-2 infection N = 323	Plasma 0.77 (0.43–1.18)	Covid-19 severity	OR per unit increase in PFOA	Covid-19 severity 0.83 (0.57, 1.20) Covid-19 severity (hospitalization vs. no hospitalization) 1.11 (0.37, 3.32) Covid-19 severity (intensive care unit and/or deceased vs. hospitalization) 0.90 (0.29, 2.80)
	Confounding: Age, sex, kidney disease, other chronic disease, national origin, place of testing, and days between blood sampling and diagnosis.						
Huang et al. (2020) *Medium*	China Recruitment: 2011–2013, Follow-up at 5 yr	Cohort	Children ages 1–5 yr N = 344 (182 boys, 162 girls)	Cord blood 6.68 (4.82–9.13)	Respiratory tract infections (total and recurrent)	Recurrent respiratory tract infections: OR for >75th percentile vs. ≤75th percentile PFOA	Total respiratory tract infections 0.37 (–3.63, 4.38), p-value = 0.854 Recurrent respiratory tract infections 0.90 (0.49, 1.64), p-value = 0.73 Results stratified by age and sex not statistically significant
	Confounding: Infant sex, maternal age, maternal education level, birth weight.						
Dalsager et al. (2021) *Medium*	Denmark Recruitment: 2010–2012, Follow-up until 2015	Cohort	Pregnant women and their children from the OCC, followed up to 4 yr	Maternal serum 1.68 (0.27–12.5)	Hospitalization from infection (any infection, upper respiratory tract, lower	Hazard ratio per log2-unit increase in PFOA	Any infection 1.13 (0.97, 1.29) Upper respiratory infection 1.18 (0.93, 1.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 1,472		respiratory tract, gastrointestinal, other)		Lower respiratory infection 1.27 (1.01, 1.59) Gastrointestinal infection 0.55 (0.32, 0.95) Other infection 1.12 (0.93, 1.35) Results stratified by sex not statistically significant
	Confounding: Maternal age, parity, maternal educational level, child sex, child age.						
Ji et al. (2021) *Medium*	China 2020	Case-control	Adults N = 160	Urine Controls: 24.8 (16.9–36.3) ng/g creatinine Cases: 39.6 (27.5–48.9) ng/g creatinine	COVID-19 infection	OR per log2-SD change in PFOA	COVID-19 2.73 (1.71, 4.55)
	Confounding: Age, gender, body mass index, diabetes, cardiovascular diseases, and urine albumin-to-creatinine ratio.						
Wang et al. (2022b) *Medium*	China Recruitment: 2010–2013, Follow-up after 1 yr	Cohort	Pregnant women and their children at 1 yr from LWBC N = 235	Maternal serum at delivery 45.82 (28.72–77.34)	Common cold, bronchitis/pneumonia, diarrhea	OR per log10-unit increase in PFOA IRR per log10-unit increase in PFOA	Common cold OR: 1.36 (0.60, 3.09), p-value = 0.469 IRR: 1.18 (0.85, 1.63), p-value = 0.329 Bronchitis/pneumonia OR: 1.14 (0.37, 3.54), p-value = 0.822 IRR: 0.68 (0.30, 1.53), p-value = 0.350 Diarrhea

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							OR: 4.99 (1.86, 13.39), p-value = 0.001 IRR: 1.97 (1.32, 2.94), p-value = 0.001
	Confounding: Maternal age, pre-pregnancy BMI, smoking during pregnancy, maternal education level, and parity.						
Dalsager et al. (2016) *Low*	Odense, Denmark 2010–2012	Cohort	Children, pregnancy followed up at 1–4 yr N = 346	Maternal serum 1.68 (range = 0.32–10.12)	Fever, cough, nasal discharge, diarrhea, vomiting	OR (of proportion of days with symptoms) by tertiles	Fever T2: 1.55 (0.90, 2.95) T3: 1.97 (1.07, 3.62); p-value < 0.05 Cough T2: 0.72 (0.42, 1.24) T3: 1.01 (0.42, 1.24) Nasal discharge T2: 1.19 (0.70, 2.04) T3: 1.37 (0.75, 2.51) Diarrhea T2: 1.10 (0.64, 1.89) T3: 0.94 (0.51, 1.74) Vomiting T2: 1.05 (0.62, 1.78) T3: 0.95 (0.52, 1.72)
	Results: Lowest tertile used as reference group. **Confounding:** Maternal age, maternal educational level, parity, and child age.						
Impinen et al. (2018) *Low*	Oslo, Norway Recruited 1992–1993, followed up for 10 yr	Cohort, Nested case-control	Infants followed up at 2 and 10 yr of age N = 641	Cord blood 1.6 (1.2–2.1)	Common cold episodes from 0 to 2 yr, LRTI episodes from 0 to 10 yr	Regression coefficient per log2-unit increase in PFOA	Common cold 0–2 yr −0.04 (−0.08, 0.01) p-value = 0.089 LRTI 0–10 yr 0.28 (0.22, 0.35) p-value < 0.0001
	Confounding: Child sex.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Impinen et al. (2019) *Low*	Oslo, Norway, Enrollment: 1999–2008	Cohort	Pregnant women and their infants followed up at 3 and 7 yr 0–3 yr: N = 1,207 6–7 yr: N = 921	Maternal blood 2.54 (1.86–3.30)	Common cold, bronchitis/pneumonia, throat infection with strep, pseudocroup, ear infection, diarrhea/gastric flu, urinary tract infection	OR per 1-IQR increase in PFOA	Common cold, 0–3 yr: 0.96 (0.94, 0.99); p-value < 0.05 Bronchitis/pneumonia 0–3 yr: 1.27 (1.12, 1.43); p-value < 0.05 6–7 yr: 0.75 (0.45, 1.23) Throat infection with strep, 0–3 yr: 1.14 (0.96, 1.35) Other throat infections, 0–3 yr: 0.91 (0.80, 1.04) Pseudocroup, 0–3 yr: 1.22 (1.07, 1.38); p-value < 0.05 Ear infection 0–3 yr: 1.00 (0.92, 1.08) 6–7 yr: 1.12 (0.88, 1.41) Diarrhea/gastric flu 0–3 yr: 1.00 (0.94, 1.06) 6–7 yr: 1.48 (1.31, 1.67); p-value < 0.05 Urinary tract infection 0–3 yr: 0.78 (0.69, 0.88); p-value < 0.05 6–7 yr: 0.66 (0.43, 1.01)
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy.						
Kvalem et al. (2020) *Low*	Norway Enrollment: 1992–1993	Cohort and cross-sectional	Children, 10 yr N = 378 (193 boys, 185 girls)	Serum All: 4.36 (IQR: 1.77)	Common cold, LRTI	Colds: OR (reference: 1–2 colds)	Colds, 10–16 yr 3–5 colds All: 1.23 (0.33, 4.58) p-value = 0.76

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up: 2002–2009		Children, 10–16 yr N = 375 (191 boys, 184 girls) Children, 16 yr N = 375 (191 boys, 184 girls)	Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)		LRTI: RR per IQR-unit increase in PFOA	Boys: 1.41 (0.29, 6.89) p-value = 0.67 Girls: 1.32 (0.19, 9.21) p-value = 0.78 > 5 colds: All: 1.29 (0.36, 4.64) p-value = 0.7 Boys: 1.38 (0.29, 6.54) p-value = 0.69 Girls: 1.67 (0.26, 1.09) p-value = 0.59 LRTI 10–16 yr All: 1.1 (1.02, 1.19) p-value = 0.01 Boys: 1.11 (0.97, 1.26) p-value = 0.12 Girls: 1.49 (1.15, 1.92) p-value = 0.002 16 yr All: 1.14 (0.81, 1.59) p-value = 0.45 Boys: 1 (0.64, 1.59) p-value = 0.99 Girls: 1.61 (0.72, 3.58) p-value = 0.25
	Confounding: Puberty status at 16 yr, mother's education, physical activity level at 16 yr.						
				Occupational			
Costa et al. (2009) *Medium*	Italy 2007	Cross-sectional	Current and former male employees of an Italian chemical production plant,	Serum Production workers (2007): 3.89 µg/mL (2.18–18.66 µg/mL)	Concentration of WBC (× 10^9/L)	Comparison of mean outcome (Exposed vs. unexposed workers)	No significant difference in comparison of mean WBC count WBC Exposed vs. Unexposed: 0.58 (–0.19, 1.35)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Comparison of means analysis N = 68, Exposed vs. Unexposed analysis N = 141, Continuous regression analysis N = 56			Regression coefficient (exposed workers vs. all workers) Regression coefficient per unit increase in PFOA	Continuous: 0.029 (–0.011, 0.071)
	Confounding: Age, job seniority, body mass index, smoking and alcohol consumption. Additional confounding for continuous regression analyses: year of observation.						

Notes: BMI = body mass index; CI = confidence interval; DNBC = Danish National Birth Cohort; IQR = interquartile range; IRR = incidence rate ratio; LLOQ = lower limit of quantitation; LWBC = Laizhou Wan Birth Cohort; SE = standard error; BMI = body mass index; LRTI = lower respiratory tract infection; OCC = Odense Child Cohort; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; RSV = respiratory syncytial virus; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; T2 = tertile 2; T3 = tertile 3; WBC = white blood cell; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-9. Associations Between PFOA Exposure and Asthma in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Children			
Dong et al. (2013) *Medium*	Taiwan, 2009–2010	Case-control and cross-sectional	Children from GBCA with (cases) or without (controls)	Serum Cases: 1.2 (0.50–2.2) Controls: 0.5 (0.4–1.3)	Asthma, Asthma Control Test score, asthma severity score, IgE in serum (IU/mL),	Asthma: OR by quartiles of PFOA Asthma Control Test score,	Asthma Q2: 1.58 (0.89, 2.8) Q3: 2.67 (1.49, 4.79) Q4: 4.05 (2.21, 7.42) p-trend < 0.001

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			asthma, ages 10–15 yr, N = 231 (cases), N = 225 (controls)		AEC (10^6/L), ECP in serum (µg/L)	asthma severity score, IgE, AEC, ECP: mean values by quartiles	IgE Q1:512.1 (329.4, 694.8) Q2: 604.6 (422.1, 787.1) Q3: 788.2 (607.1, 969.2) Q4: 836.4 (652, 1,020.8) p-trend = 0.05 AEC Q1: 325.9 (253.7, 398.1) Q2: 339.7 (266.8, 412.6) Q3: 422.1 (349.9, 494.2) Q4: 498 (423.7, 572.3) p-trend < 0.001 ECP Q1: 30.3 (14.3, 46.3) Q2: 34.8 (18.9, 50.7) Q3: 44.3 (28.4, 60.2) Q4: 57.8 (42.2, 73.4) p-trend = 0.010 Asthma Control Test score, asthma severity score: trends across quartiles not statistically significant
	Results: Lowest quartile used as reference group. **Confounding:** age, sex, BMI, parental education, ETS exposure, and month of survey.						
Humblet et al. (2014) *Medium*	United States, 1999–2008	Cross-sectional	Adolescents, ages 12–19 yr old from NHANES N = 1,877	Serum Never asthma 4.0 (2.8–5.4) Ever asthma 4.3 (3.1–5.7) No current asthma 4.0 (2.8–5.4)	Asthma, wheeze	OR per doubling in PFOA or per unit increase in PFOA	Ever asthma Per doubling: 1.18 (1.01, 1.39), p-value = 0.04 Per unit increase: 1.06 (1.00, 1.11), p-value = 1.11 Current asthma Per doubling: 1.12 (0.92, 1.36), p-value = 0.26

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
				Current asthma 4.2 (2.9–5.6) No wheezing 4.0 (2.9–5.5) Wheezing 4.4 (2.9–5.6)			Per unit increase: 1.03 (0.97, 1.10), p-value = 0.30 Wheeze Per doubling: 1.0 (0.80, 1.23), p-value = 0.98 Per unit increase: 1.01 (0.94, 1.07), p-value = 0.87
	Exposure: No wheezing defined as no wheezing in the past 12 mo. Wheezing defined as history of wheezing in the past 12 mo. **Confounding:** Sex, smoking, age, race/ethnicity, survey cycle, poverty-income ratio, health insurance.						
Smit et al. (2015) *Medium*	Ukraine and Greenland, Exposure: 2002–2004, Outcome: 2010–2012	Cohort	Mother-child pairs with follow-up when the children were 5–9 yr of age, N = 1,024	Maternal blood Ukraine: GM = 0.97 (P5–P95: 0.45–2.34) Greenland: GM = 1.79 (P5–P95: 0.80–3.66)	Asthma	OR per SD increase in PFOA	Asthma ever (combined): 0.8 (0.62, 1.04) Ukraine: 0.93 (0.47, 1.84) Greenland: 0.79 (0.60, 1.03)
	Confounding: Maternal allergy, smoking during pregnancy, education level, maternal age, child sex, child age at follow-up, gestational age at blood sample, parity, breastfeeding, and birthweight.[c]						
Impinen et al. (2018) *Medium*	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 and 10 yr of age, N = 641	Cord blood 1.6 (1.2–2.1)	Asthma	OR per log2-unit increase PFOA	Current asthma (10 yr): 1.06 (0.82, 1.37); p-value = 0.649 Asthma ever (10 yr): 1.1 (0.78, 1.54); p-value = 0.589
	Confounding: Sex.						
Beck et al. (2019) *Medium*	Denmark, Enrollment: 2010–2012	Cohort	Children, early pregnancy to 5 yr N = 970 (507 boys, 363 girls)	Maternal blood 1.68 (1.13–2.35)	Wheeze, self-reported asthma, doctor-diagnosed asthma	OR per doubling in maternal serum PFOA	Wheeze All: 0.98 (0.78, 1.23) Boys 0.94 (0.71, 1.23) Girls: 1.08 (0.75, 1.55) Self-reported asthma All: 1.57 (0.93, 2.68) Boys: 2.17 (1.07, 4.42) Girls: 1.06 (0.49, 2.30)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Doctor-diagnosed asthma All: 0.81 (0.53, 1.22) Boys: 0.72 (0.46, 1.12) Girls: 1.70 (0.63, 4.56)
	Confounding: Parity, maternal education level, maternal pre-pregnancy BMI, asthma predisposition, child sex.						
Gaylord et al. (2019) *Medium*	New York City, NY 2014–2016	Case-control	Children with (cases) or without (controls) asthma aged 13–22, N = 118 (cases), N = 169 (controls)	Serum Cases: 1.80 (Range: 0.56–5.03) Controls: 1.38 (Range: 0.36–4.28)	Asthma	OR per log-unit increase in PFOA	1.34 (0.55, 3.29)
	Comparison: Logarithm base not specified. **Confounding:** Sex, race/ethnicity, age, BMI, tobacco smoke exposure.						
Impinen et al. (2019) *Medium*	Oslo, Norway, Enrollment: 1999–2008	Cohort	Pregnant women and their infants (followed to age 7), N = 921	Maternal blood 2.54 (1.86–3.30)	Asthma	OR per IQR increase in PFOA	Current asthma: Total: 1.11 (0.69, 1.79); p-value = 0.657 Boys: 1.34 (0.70, 2.60); p-value = 0.38 Girls: 0.91 (0.46, 1.82); p-value = 0.799 Ever asthma: Total: 0.99 (0.70, 1.39); p-value = 0.933 Boys: 0.98 (0.63, 1.54); p-value = 0.945 Girls: 0.99 (0.58, 1.70); p-value = 0.982
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Manzano-Salgado et al. (2019) *Medium*	Spain, 2003–2008	Cohort	Children, 4 yr, N = 1,184 7 yr, N = 1,068	Maternal blood 2.35 (1.63–3.30)	Asthma	OR or RR per log2-unit increase in maternal PFOA	4-yr follow-up: OR = 0.77 (0.50, 1.17) 7-yr follow-up: OR = 0.77 (0.54, 1.10) 4 and 7 yr Girls: RR = 1.01 (0.61, 1.68) Boys: RR = 0.74 (0.49, 1.13)
	Confounding: OR assessment: Age at follow-up of the child; RR assessment: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth.						
Zeng et al. (2019a) *Medium*	Shanghai, China, 2012–2015	Cohort	Enrolled in pregnancy, follow-up at 5 yr N = 358 (187 boys, 171 girls)	Cord blood Boys: 7.13 (5.15–9.97) Girls: 6.51 (4.57–8.73)	Asthma	OR per log10-unit increase in PFOA	All: 0.98 (0.22, 4.49), p-value = 0.98 Boys: 0.32 (0.04, 2.36), p-value = 0.26 Girls: 5.6 (0.22, 145.87), p-value = 0.30
	Confounding: Child weight at age 5, gestational age, breastfeeding during the first 6 mo, maternal education, maternal pre-pregnancy BMI, and annual household income.						
Huang et al. (2020) *Medium*	China Recruitment: 2011–2013, Follow-up at 5 yr	Cohort	Children ages 1–5 yr N = 344 (182 boys, 162 girls)	Cord blood 6.68 (4.82–9.13)	IgG, IgE levels	Regression coefficient per log10-unit increase in PFOA	IgG 0.01 (–0.05, 0.06), p-value = 0.856 IgE –0.30 (–0.64, 0.04), p-value = 0.084 Results stratified by age and sex not statistically significant
	Confounding: Infant sex, maternal age, maternal education level, birth weight.						
Jackson-Browne et al. (2020) *Medium*	NHANES, United States, 2013–2014	Cross-sectional	Children, ages 3–11 yr, N = 607	Serum GM = 1.9 (1.4–2.7)	Asthma	OR per ln-SD increase in PFOA	p.1 (0.9, 1.4) By age: 3–5 yr: 1.6 (1.0, 2.7) 6–11 yr: 1.0 (0.7, 1.3)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							p-value for interaction by age = 0.47 By sex: Females: 1.1 (0.6, 1.7) Males: 1.1 (0.9, 1.4) p-value for interaction by sex = 0.65 By race/ethnicity: White, non-Hispanic: 1.3 (0.9,2.0) Black, non-Hispanic: 0.9 (0.7, 1.3) Hispanic: 1.3 (0.9, 1.9) Other: 1.1 (0.6, 1.7) p-value for interaction by race = 0.41
	Confounding: Sex, age, race/ethnicity, serum cotinine, poverty to income ratio.						
Kvalem et al. (2020) *Medium*	Norway Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, 10 yr N = 378 (193 boys, 185 girls) Children, 10–16 yr N = 375 (191 boys, 184 girls) Children, 16 yr N = 375 (191 boys, 184 girls)	Serum All: 4.36 (IQR: 1.77) Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)	Asthma	RR per IQR increase in PFOA	10 yr All: 1.06 (0.93, 1.21) Boys: 0.99 (0.84, 1.16) 10–16 yr All: 1.04 (0.88, 1.23) Boys: 0.95 (0.72, 1.26) Girls: 1.36 (0.98, 1.89) 16 yr All: 1.04 (0.87, 1.24) Boys: 0.99 (0.76, 1.27) Girls: 1.21 (0.81, 1.82)
	Confounding: 10 yr: Age at follow-up, physical activity, mothers' education; 16 yr: BMI at 16 yr, puberty status at 16 yr, mothers' education, physical activity level at 16 yr.						
Okada et al. (2012)	Japan 2002–2005	Cohort	Pregnant women and	Maternal serum 1.3 (0.8–1.7)	IgE levels (log10-IU/mL)	Regression coefficients per	Linear regression 0.766 (0.104, 1.428)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Medium			children from the Hokkaido Study on Environment and Children's Health; follow-up at 18 mo N = 128			log10-unit increase in PFOA	Quadratic regression −1.429 (−2.416, −0.422) Cubic regression −3.078 (−5.431, −0.726) Results stratified by gender not statistically significant for boys and combined
	Confounding: Maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period.						
Stein et al. (2016) *Medium*	United States, 1999–2000, 2003–2004, 2005–2006	Cross-sectional	Children aged 12-19 years, NHANES N = 638	Serum GM = 3.59 (95% CI: 3.26, 3.96)	Asthma and wheeze	OR [per IQR(lnPFOA) increase (0.78 ln-ng/mL)]	Asthma 1.28 (0.81, 2.04) Wheeze 0.94 (0.51, 1.73)
	Confounding: Age, sex, race/ethnicity, survey year.						
Xu et al. (2020a) *Medium*	United States 2007–2012	Cross-sectional	Adults from NHANES, ages 20–79 yr N = 3,630	Serum Mean (SD) = 3.87 (3.13) µg/L	Fractional exhaled nitric oxide (ppb)	Percent change per doubling of PFOA, or by tertile	Fractional exhaled nitric oxide 2.64 (0.38, 4.96), p-value < 0.05 T2: 5.29 (1.88, 8.81), p-value < 0.01 T3: 6.34 (2.81, 10.01), p-value < 0.001 p-trend < 0.001
	Results: Lowest tertile used as reference group. **Confounding:** Age, sex, race/ethnicity, BMI, annual family income, education level, serum cotinine, recent respiratory symptom, and smoking status.						
Zhou et al. (2017b) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without	Serum Case boys: 1.3 (0.5–2.3)	Asthma	Asthma: Comparison of PFOA	Asthma: Increased PFOA among asthmatics, p-value < 0.001

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			(controls) asthma ages 10−15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123	Case girls: 0.8 (0.5–1.8) Control boys: 0.5 (0.4–1.4) Control girls: 0.5 (0.4–1.2)		distributions (Wilcoxon rank-sum test)	
	Confounding: Cases and controls were matched on age and sex.						
Zhu et al. (2016) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10−15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123	Serum Case boys: 1.26 Case girls: 0.81 Control boys: 0.52 Control girls: 0.54	Asthma	OR for highest vs. lowest quartiles of PFOA exposure	Boys: 4.24 (1.81, 9.42); p-value for trend = 0.001 Girls: 3.68 (1.43, 9.48); p-value for trend = 0.005
	Confounding: Age, BMI, parental education, ETS, parental asthma, month of survey.						
Zhou et al. (2017c) *Low*	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10−15 from the GBCA	Serum Cases: 1.16 (0.48–2.16) Controls: 0.52 (0.44–1.27)	Asthma	OR per unit increase in PFOA	Females with high testosterone: 3.16 (1.47, 6.78) Females with low testosterone: 2.88 (1.39, 5.97) Males with high testosterone: 2.42 (1.47, 3.99)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123 Sexes evenly divided into high/low hormone classifications				Males with low testosterone: 2.82 (1.60, 4.97) Females with high estradiol: 2.56 (1.27, 5.12) Females with low estradiol: 3.54 (1.61, 7.79) Males with high estradiol: 2.93 (1.64, 5.24) Males with low estradiol: 1.85 (1.12, 3.06) No statistically significant interactions for low/high hormone levels in either sex
	Confounding: Age, sex, BMI, parental education, environmental tobacco smoke exposure, physical activity, month of survey.						
Timmermann et al. (2017a) *Low*	Faroe Islands, recruitment: 1997–2000	Cohort	Pregnant women and infants, follow-up at ages 5, 7, and 13 yr, N = 559	Maternal serum 3.3 (2.5–4.0)	Asthma	OR per doubling of maternal PFOA	Asthma (age 5): Total: 1.37 (0.81, 2.32) No MMR vaccine before age 5: 10.37 (1.06, 101.93) Yes MMR vaccine before age 5: 0.76 (0.41, 1.39) Asthma (age 13): Total: 1.12 (0.67, 1.88) No MMR vaccine before age 5: 9.92 (1.06, 93.22) Yes MMR vaccine before age 5: 0.65 (0.35, 1.20)
	Confounding: Family history of eczema in children, allergic eczema, and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, daycare attendance at age 5, birth weight, and family history of chronic bronchitis/asthma.						
Averina et al. (2019)	Norway 2010–2011	Cohort	Adolescents in their first year	Serum	Asthma, self-reported,	OR by quartiles of PFOA	TFF1 Q4 vs. Q1: 2.07 (1.01, 4.23); p-value = 0.046

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low			of high school from TFF1 and TFF2 N = 675	Girls: GM = 2.1 (IQR = 1.2) Boys: GM = 1.9 (IQR = 0.7)	doctor-diagnosed		No other statistically significant associations
	Confounding: Sex, age, BMI, physical activity, unemployment/disability of parents, living with adoptive parents, fish intake.						
Workman et al. (2019) *Low*	Canada 2010–2012	Cohort	Mothers and their infants N = 85	Maternal plasma 0.89 (Range: 0.16–7.1)	Recurrent wheezing episodes	Difference in prenatal PFOA levels for wheezing vs. no wheezing (Mann-Whitney test)	No significant differences
	Confounding: None reported.						

Notes: AEC = absolute eosinophil counts; BMI = body mass index; CI = confidence interval; ECP = eosinophilic cationic protein; GBCA = Genetics and Biomarkers Study for Childhood Autism; ETS = environmental tobacco smoke; GM = geometric mean; IgE = immunoglobulin E; IQR = interquartile range; MMR = measles, mumps, rubella; mo = months; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; TFF1 = Tromsø Fit Futures; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-10. Associations Between PFOA Exposure and Allergies in Recent Epidemiologic Studies

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Wang et al. (2011) *Medium*	Taiwan 2004	Cohort and cross-sectional	Pregnant women and their children at age 2 hr N = 244 (133 boys, 111 girls)	Cord blood 1.71 (0.75–17.40)	Atopic dermatitis, IgE levels (log-KU/L)	Atopic dermatitis: OR by quartiles of PFOA exposure IgE:	Atopic dermatitis Q2: 0.84 (0.28, 2.48) Q3: 1.03 (0.42, 2.56) Q4: 0.58 (0.22, 1.58) IgE in cord blood at birth

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						Regression coefficient per ln-unit change in PFOA	All: 0.134 (SE = 0.115), p-value = 0.047 Boys: 0.206 (SE = 0.164), p-value = 0.025 Girls: 0.067 (SE = 0.231), p-value = 0.823 IgE in serum at age 2 All: 0.027 (SE = 0.244), p-value = 0.870 Boys: 0.097 (SE = 0.345), p-value = 0.710 Girls: 0.001 (SE = 0.452), p-value = 0.998
	Results: Lowest quartile used as reference group. **Confounding:** Gender, gestational age, maternal age. Additional confounding for atopic dermatitis: maternal history of atopy, duration of breast feeding, pre-natal ETS exposure. Additional confounding for IgE: parity.						
Okada et al. (2012) *Medium*	Japan 2002–2005	Cohort	Pregnant women and children from the Hokkaido Study on Environment and Children's Health; follow-up at 18 mo N = 343	Maternal serum 1.3 (0.8–1.7)	Food allergy, eczema, otitis media, and wheezing	OR per log10-unit increase in PFOA	Food allergy 1.67 (0.52, 5.37) Eczema 0.96 (0.23, 4.02) Otitis media 1.51 (0.45, 5.12) Wheezing 1.27 (0.27, 6.05)
	Confounding: maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant gender, breastfeeding period, environmental tobacco exposure, daycare attendance and blood sampling period.						
Okada et al. (2014) *Medium*	Japan 2003–2009	Cohort	Japanese women who had	Maternal blood 2.01 (1.31–3.26)	Total allergic diseases (eczema,	OR by quartiles of PFOA exposure	Total allergic diseases Q2: 1.05 (0.81, 1.37) Q3: 0.80 (0.61, 1.06)

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			singleton births and their infants N = 2,062		wheezing, and allergic rhinoconjunctivitis symptoms)		Q4: 0.79 (0.59, 1.04) p-value for trend = 0.030
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, number of siblings, day care attendance, and ETS exposure in infancy at 24 months.						
Buser et al. (2016) *Medium*	United States 2005–2016	Cross-sectional	Adolescents aged 12–19 yr from NHANES N by cycle: 2005–2006: 637 2007–2010: 701	Serum 2005–2006: GM = 3.59 (2.46–5.36) 2007–2010: GM = 3.27 (2.43–4.47)	Food allergy or sensitization	OR by quartiles of PFOA exposure	Food allergy, 2007–2010 cycle Q2: 2.84 (0.83, 9.73) Q3: 1.70 (0.51, 5.65) Q4: 9.09 (3.32, 24.9) p-value for trend < 0.001 Food sensitization, 2005–2006 cycle Q2: 0.91 (0.47, 1.76) Q3: 1.28 (0.59, 2.76) Q4: 1.23 (0.57, 2.65) p-value for trend = 0.74
	Outcome: Food sensitization defined as at least 1 food specific IgE level ≥0.35 kU/L. **Results:** Lowest quartile used as reference. **Confounding:** Age, sex, race/ethnicity, BMI, serum cotinine.[c]						
Goudarzi et al. (2016a) *Medium*	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study N = 1,558 (765 girls, 793 boys)	Maternal blood 2.01 (1.31–3.35)	Allergic diseases, total	OR by quartiles of PFOA exposure	Q2: 1.07 (0.79, 1.47) Q3: 0.95 (0.70, 1.31) Q4: 0.83 (0.59, 1.16) p-value for trend = 0.208 No statistically significant associations, trends, or interactions by sex
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, maternal educational level, sex, parental allergic history, number of older siblings, breastfeeding, daycare attendance, ETS exposure.						

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Stein et al. (2016) *Medium*	United States, 1999–2000, 2003–2004, 2005–2006	Cross-sectional	Children aged 12-19 years, NHANES N = 638	Serum GM = 3.59 (95% CI: 3.26, 3.96)	Allergy and rhinitis	OR [per IQR(lnPFOA) increase (0.78 ln-ng/mL)]	Allergy 1.12 (0.85, 1.47) Rhinitis 1.35 (1.10, 1.66)
	Confounding: Age, sex, race/ethnicity, survey year.						
Timmermann et al. (2017a) *Medium*	Faroe Islands, Recruitment: 1997–2000	Cohort	Pregnant women and infants, follow-up at ages 5, 7, and 13 yr, N = 559	Maternal serum 3.3 (2.5–4.0)	Allergy, allergic rhino-conjunctivitis in past 12 mo, positive skin prick test, IgE	OR per doubling of PFOA IgE: Percent change per doubling of PFOA	Allergy at age 5 0.92 (0.53, 1.57) Allergic rhino-conjunctivitis in past 12 mo, at age 13 1.18 (0.65, 2.15) Positive skin prick test, age 13 1.16 (0.76, 1.77) IgE, age 7: −5.15 (−31.92, 32.14)
	Confounding: Maternal parity, family history of eczema in children, allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal fish intake during pregnancy, and duration of breastfeeding; for IgE: family history of eczema in children, allergic eczema, and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, fish intake at age 5, number of siblings, and daycare attendance at age 5.						
Impinen et al. (2018) *Medium*	Oslo, Norway, 1992–2002	Cohort, Nested case-control	Infants followed up at 2 yr and 10 yr of age, N = 641	Cord blood 1.6 (1.2–2.1)	Rhinitis, rhino-conjunctivitis, SPT	OR per log2-unit increase in PFOA	Rhinitis, current, 10 yr 1.30 (0.97, 1.74); p-value = 0.083 Rhinitis, ever, 10 yr 1.29 (0.95, 1.74); p-value = 0.098 Rhino-conjunctivitis, ever, 10 yr 1.32 (0.97, 1.79); p-value = 0.079 Rhinitis, ever, spes IgE > 0.35, 10 yr 1.24 (0.90, 1.71); p-value = 0.185

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							SPT, any pos, 10 yr 0.97 (0.75, 1.24); p-value = 0.788 SPT + and/or sIgE > 0.35, 10 yr 1.03 (0.81, 1.30); p-value = 0.815
	Confounding: Sex.						
Impinen et al. (2019) *Medium*	Oslo, Norway, Enrollment: 1999–2008	Cohort	Pregnant women and their infants (followed to age 7), N = 921	Maternal blood 2.54 (1.86–3.30)	Allergy, food or inhaled	OR per IQR-unit increase in PFOA	Allergy, food, current All: 1.32 (0.92, 1.90); p-value = 0.136 Boys: 1.49 (0.89, 2.50); p-value = 0.131 Girls: 1.15 (0.68, 1.94); p-value = 0.602 Allergy, food, ever All: 1.10 (0.77, 1.57); p-value = 0.613 Boys: 1.04 (0.63, 1.73); p-value = 0.867 Girls: 1.14 (0.68, 1.91); p-value = 0.626 Allergy, inhaled, current All: 0.96 (0.55, 1.67); p-value = 0.887 Boys: 1.0 (0.46, 2.15); p-value = 0.994 Girls: 0.88 (0.39, 2.01); p-value = 0.765 Allergy, inhaled, ever All: 1.25 (0.88, 1.78); p-value = 0.213

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Boys: 1.13 (0.71, 1.80); p-value = 0.597 Girls: 1.44 (0.84, 2.47); p-value = 0.189
	Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy, nursey attendance.						
Ait Bamai et al. (2020) *Medium*	Hokkaido, Japan, 2003–2012	Cohort	Early pregnancy to 7 yr, N = 2,689	Maternal blood 1.94 (1.30–2.95)	Rhino-conjunctivitis	RR per ln-unit increase in PFOA, from birth to 7 yr old	0.95 (0.83, 1.09); p-value = 0.487
	Confounding: Sex, parity, maternal age at delivery, maternal smoking during pregnancy, pre-pregnancy BMI, and annual household income during pregnancy.						
Kvalem et al. (2020) *Medium*	Norway, Enrollment: 1992–1993; Follow-up: 2002–2009	Cohort and cross-sectional	Children, age 10 yr: N = 377 Age 16 yr: N = 375	Serum All: 4.36 (IQR: 1.77) Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)	Rhinitis, skin prick test (SPT)	Change in RR per IQR increase in PFOA	Rhinitis 10 yr All: 0.84 (0.61, 1.15); p-value = 0.28 Boys: 0.77 (0.53, 1.11); p-value = 0.16 Girls: 0.84 (0.48, 1.49); p-value = 0.56 16 yr All: 1.08 (1.01, 1.14); p-value = 0.02 Boys: 1.06 (0.84, 1.32); p-value = 0.63 Girls: 1.16 (0.90, 1.50); p-value = 0.25 SPT 10 yr All: 1.11 (1.07, 1.15); p-value < 0.0001 Boys: 1.02 (0.82, 1.27); p-value = 0.84

Reference, Confidence	Location, years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Girls: 1.19 (0.79, 1.80); p-value = 0.39 16 yr All: 1.07 (1.05, 1.08); p-value < 0.0001 Boys: 1.05 (1.03, 1.06); p-value < 0.0001 Girls: 1.13 (0.86, 1.47); p-value = 0.38
	Confounding: 10 yr: Physical activity at 10 yr, mothers' education, BMI at 10 yr; 16 yr: BMI at 16 yr, puberty status at 16 yr, mothers' education, physical activity level at 16 yr.						

Notes: BMI = body mass index; CI = confidence interval; ETS = environmental tobacco smoke; GM = geometric mean; IgE = immunoglobulin E; IQR = interquartile range; MMR = measles, mumps, rubella; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; SE = standard error; SPT = skin prick test; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-11. Associations Between PFOA Exposure and Eczema in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
General Population							
Goudarzi et al. (2016a) *Medium*	Japan 2003–2013	Cohort	Children at age 4 from the Hokkaido Study N = 1,558 (765 girls, 793 boys)	Maternal blood 2.01 (1.31–3.35)	Eczema	OR by quartiles of PFOA	Q2: 1.10 (0.76, 1.59) Q3: 0.92 (0.623, 1.34) Q4: 0.84 (0.56, 1.27) p-value for trend = 0.287 Girls Q2: 0.88 (0.50, 1.55) Q3: 1.16 (0.67, 2.03)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: 1.21 (0.68, 2.17) p-value for trend = 0.356 Boys Q2: 1.31 (0.80, 2.18) Q3: 0.74 (0.43, 1.27) Q4: 0.59 (0.32, 1.08) p-value for trend = 0.022 p-value for interaction by sex = 0.039
	Results: Lowest quartile used as reference. **Confounding:** Maternal age, maternal educational level, sex, parental allergic history, number of older siblings, breastfeeding, daycare attendance, ETS exposure.[c]						
Okada et al. (2014) *Medium*	Japan 2003–2009	Cohort	Japanese women who had singleton births and their infants N = 2,062	Maternal blood 2.01 (1.31–3.26)	Eczema	OR by quartiles of PFOA exposure	Eczema Q2: 1.03 (0.75, 1.41) Q3: 0.86 (0.62, 1.19) Q4: 0.72 (0.51, 1.00) p-value for trend = 0.032
	Results: Lowest quartile used as reference group. **Confounding:** Maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, and ETS exposure in infancy at 24 months.						
Timmermann et al. (2017a) *Medium*	Denmark 1997–2000	Cohort	Pregnant women and infants from the CHEF study at ages 5, 7, and 13 yr N = 559	Serum Prenatal at birth: 3.3 (2.5–4.0) Age 5/7: 4.0 (3.3–5.0)	Atopic eczema at age 13	OR per doubling of PFOA at age 13	Age 5: 0.72 (0.42, 1.25) Age 13: 1.36 (0.85, 2.19) MMR vaccination before age 5 Yes: 4.48 (0.42, 47.69) No: 0.82 (0.49, 1.36)
	Confounding: Family history of eczema in children., allergic eczema and hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breastfeeding, and fish intake at age 13, birth weight, and family history of chronic bronchitis/asthma, maternal parity.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Chen et al. (2018) *Medium*	China 2012–2015	Cohort	Infants followed up at 6, 12, and 24 mo N = 687 children (328 female and 359 male)	Cord blood All: 6.98 (Range = < 0.09 –29.97) Female: 7 (Range = 0.70–29.97) Male: 6.89 (Range = < 0.09 –25.99)	Atopic dermatitis	OR per log-unit increase in PFOA, or by quartiles	All: 1.35 (0.93, 1.97) Q2: 1.48 (0.87, 2.52) Q3:1.16 (0.67, 2) Q4:1.74 (1.02, 2.95) Female: 2.07 (1.13, 3.8) Q2: 1.23 (0.52, 2.93) Q3:1.81 (0.79, 4.14) Q4: 2.52 (1.12, 5.68) Male: 0.98 (0.58, 1.64) Q2: 1.57 (0.76, 3.23) Q3: 0.81 (0.37, 1.78) Q4: 1.34 (0.64, 2.82)
	Comparison: Logarithm base not specified. **Results:** Lowest quartile used as reference group. **Confounding:** Maternal age, maternal pre-pregnancy BMI, gestational week at delivery, birth weight, maternal education, paternal education, parity, mode of delivery, family history of allergic disorders, infant sex, family income, maternal ethnicity, paternal smoking, breastfeeding.						
Impinen et al. (2018) *Medium*	Norway 1992–2002	Cohort, Nested case-control	Children from the ECA study at 0, 2, and 10 yr N = 641	Cord blood 1.6 (Q1–Q3 = 1.2−2.1)	Atopic dermatitis diagnosed anytime between 0–2 yr old, or between 0–10 yr old	OR per log2-unit increase PFOA	Ages 0–2: 1.18 (0.94, 1.5) Ages 0–10: 0.99 (0.59, 1.67)
	Confounding: Sex.						
Manzano-Salgado et al. (2019) *Medium*	Spain 2003–2015	Cohort	Pregnant women and children followed up at ages 1.5, 4, and 7 from the INMA study N = 1,188 at 1.5 and 4 yr,	Maternal plasma 2.35 (1.63–3.30)	Eczema	OR or RR per log2-unit increase in PFOA	Age 1.5: 1.1 (0.91, 1.31) Age 7: 0.96 (0.81, 1.14) Follow-up at age 4: 0.97 (0.81, 1.17) Boys at ages 1.5, 4, and 7: 0.98 (0.81, 1.18) Girls at ages 1.5, 4, and 7: 0.9 (0.75, 1.07)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 1,071 at 7 yr				From ages 1.5 to 7 yr: 0.96 (0.85, 1.08) No statistically significant associations
	Confounding: Age at follow-up of the child, maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth.						
Wen et al. (2019a) *Medium*	Taiwan 2001–2005	Cohort	Children at age 2 yr N = 839	Cord blood 0.65 (0.23–1.96)	Atopic dermatitis	OR by tertiles of PFOA	T2: 0.75 (0.26, 1.89) T3: 2.58 (1.27, 5.32); p-value < 0.01
	Results: Lowest tertile used as reference. **Confounding:** Sex, family income, maternal atopy, breast feeding, and maternal age at childbirth.						
Wen et al. (2019b) *Medium*	Taiwan 2001–2005	Cohort	Infants followed from birth up to 5 yr of age N = 863	Cord blood 0.65 (0.23–1.96)	Atopic dermatitis	Hazard ratio for PFOA ≥1.96 ng/mL *vs*. < 1.96 ng/mL	1.89 (1.1, 3.16); p-value < 0.05
	Confounding: Sex, parental education, parental atopy, breast feeding, and maternal age at childbirth.						

Notes: BMI = body mass index; CHEF = Children's Health and Environment in the Faroe Islands; ECA = Environment and Childhood Asthma; ETS = environmental tobacco smoke; INMA = Spanish Environment and Childhood (Infancia y Medio Ambiente); MMR = measles, mumps, rubella; OR = odds ratio; Q2 = Quartile 2, Q3 = Quartile 3, Q4 = Quartile 4; T2 = tertile 2; T3 = tertile 3; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Table D-12. Associations Between PFOA Exposure and Autoimmune Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Steenland et al. (2013) *Medium*	West Virginia 1952–2011	Cohort	Males and females from C8 Health Project,	Serum 26 (13–68)	Occurrence of conditions with and without a 10-yr lag:	RR by quartiles of PFOA	RA, no lag Q2: 1.24 (0.85, 1.79) Q3: 1.40 (0.96, 2.03) Q4: 0.99 (0.68, 1.43)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Ages ≥20, N = 32,254		rheumatoid arthritis (RA), lupus, multiple sclerosis (MS), ulcerative colitis (UC), Crohn's disease (CD)		p-trend = 0.84 RA, with lag Q2: 1.53 (0.61, 2.58) Q3: 1.73 (1.10, 2.71) Q4: 1.35 (0.87, 2.11) p-trend = 0.73 Lupus, no lag Q2: 1.49 90.68, 3.34) Q3: 1.01 (0.44, 2.30) Q4: 0.71 (0.31, 1.65) p-trend = 0.94 Lupus, with lag Q2: 0.79 (0.27, 2.34) Q3: 1.26 (0.40, 4.03) Q4: 0.61 (0.19, 1.91) p-trend = 0.93 MS, no lag Q2: 0.85 (0.44, 1.63) Q3: 1.56 (0.81, 3.00) Q4: 1.26 (0.65, 2.42) p-trend = 0.22 MS, with lag Q2: 1.16 (0.54, 2.47) Q3: 1.62 (0.74, 3.52) Q4: 1.32 (0.61, 2.84) p-trend = 0.59 UC, no lag Q2: 1.76 (1.04, 2.00) Q3 2.63 (1.56, 4.43)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: 2.86 (1.65, 4.96) p-trend < 0.0001 UC, with lag Q2: 1.71 (0.89, 3.27) Q3: 2.05 (1.07, 3.91) Q4: 3.05 (1.56, 5.96) p-trend < 0.0001 CD, no lag Q2: 1.25 (0.61, 2.58) Q3: 1.15 (0.55, 2.41) Q4: 1.00 (0.48, 2.09) p-trend = 0.73 CD, with lag Q2: 0.80 (0.32, 1.99) Q3: 0.97 (0.36, 2.60) Q4: 0.69 (0.26, 1.82) p-trend = 0.79
	Results: Lowest quartile used as reference. **Confounding:** Sex, race/ethnicity, smoking, BMI, alcohol consumption.[c]						
Gaylord et al. (2020) *Medium*	United States	Case-control	Children and adolescents younger than 21 yr with (cases) and without (controls) celiac disease N = 88 (42 girls, 46 boys)	Serum Cases: 1.26 (IQR = 0.76) Controls: 0.99 (IQR = 0.51)	Celiac disease	OR per ln-unit change in PFOA	3.85 (0.71, 21.1) Girls: 20.6 (1.13, 375); p-value < 0.05 Boys: 1.05 (0.11, 9.59)
	Confounding: Genetic susceptibility score, albumin, BMI, age, race (non-Hispanic white vs. other race/ethnicity) and sex.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Steenland et al. (2018b) *Low*	United States 1999–2012	Case-control	Patients with UC, CD, or healthy controls N = 114 UC, 60 CD, 75 neither	Serum UC: 2.93 CD: 1.78 Controls: 1.33	UC	OR of UC vs. CD and/or neither per ln-unit increase in PFOA, or by quintiles	UC vs. CD: 1.68 (1.07, 2.23) UC vs. neither: 2.00 (1.08, 3.67) UC vs. CD and neither: 1.60 (1.14, 2.24) Q2: 0.81 (0.22, 2.93) Q3: 40.98 (11.67, 150.34) Q4: 33.36 (11.32, 119.36) Q5: 2.86 (0.94, 8.75)
	Results: Lowest quintile used as reference. **Confounding:** Age, sex, ethnic group (white or non-white).						
Sinisalu et al. (2020) *Low*	Finland 1999–2005	Cohort	Pregnant women and infants at birth and 3 mo from the Type 1 Diabetes Prediction and Prevention Study in Finland (DIPP) N = 33 (17 celiac disease, 16 controls)	Cord blood Case: 2.32 (min–max: 1.31–4.80) Control: 2.43 (min–max: 1.23–4.46) 3-mo serum Case: 4.34 (min–max: 1.23–9.17) Control: 4.05 (min–max: 0.98–6.25)	Celiac disease	Comparison of mean PFOA exposure levels	No significant differences in exposure between cases and control at birth or 3 mo
Xu et al. (2020d) *Low*	Sweden 2014–2016	Cohort	Residents of Ronneby municipality Ronneby panel study: N = 57	Serum Ronneby panel study: 20 (11–29) Ronneby resampling: 16 (9–23)	CD, UC	HR for exposure period vs. not exposed (1980–1984)	CD: 1.58 (1.00−2.49) for early (1985−94) exposure period No associations for the later years UC: No associations any exposure periods

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Ronneby resampling: N = 113 Karlshamn: N = 19	Karlshamn: 2 (1–2)			
	Confounding: Age, gender, calendar year.						
Ammitzbøll et al. (2019) *Low*	Denmark 2019	Case-control	Adults with (cases) or without (controls) RRMS or CIS N = 162 (92 women, 70 men)	Serum Cases: 1.88 (1.34–2.32) Controls: 1.94 (1.38–3.01)	Relapsing remitting multiple sclerosis (RRMS)	Percent change in PFOA comparing MS cases vs. healthy controls	−12 (−24, 2); p-value = 0.099 Females: 7 (−13, 32); p-value = 0.526 Males: −28 (−42, −9); p-value = 0.006
	Confounding: Age, sex, breastfeeding.						

Notes: BMI = body mass index; CD = Crohn's disease; CIS = clinically isolated serum syndrome; DIPP = Diabetes Prediction and Prevention Study in Finland; HR = hazard ratio; IQR = interquartile range; MS = multiple sclerosis; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RA = rheumatoid arthritis; RR = risk ratio; RRMS = relapsing remitting multiple sclerosis; UC = ulcerative colitis.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted

[c] Confounding indicates factors the models presented adjusted for.

D.5 Cardiovascular

D.5.1 Cardiovascular Endpoints

Table D-13. Associations Between PFOA Exposure and Cardiovascular Effects in Recent Epidemiological Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Children and Adolescents							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Li et al. (2021) *High* for gestation, birth, and childhood exposures (3-yr and 8-yr) *Medium* for exposure at 12-yr follow-up	United States 2003–2006	Cohort	Pregnant women and their children followed up at birth and ages 3, 8, and 12 from HOME Study Gestation: N = 203 At birth: N = 124 Age 3: N = 137 Age 8: N = 165 Age 12: N = 190	Maternal serum Gestation: 5.3 (3.7–7.2) Cord serum At birth: 3.2 (2.4–4.7) Serum At age 3: 5.4 (3.7–7.4) At age 8: 2.4 (1.8–3.2) At age 12: 1.3 (1.0–1.6)	SBP (z-score), mean of SBP and DBP (z-score)	Regression coefficient per log2-unit IQR increase in PFOA	SBP (z-score) Gestation: 0.1 (−0.1, 0.2) At birth: 0.1 (−0.1, 0.3) Age 3: 0 (−0.2, 0.3) Age 8: 0 (−0.4, 0.5) Age 12: 0.2 (−0.1, 0.6) Mean of SBP and DBP (z-score) Gestation: 0 (−0.1, 0.2) At birth: 0.1 (−0.1, 0.2) Age 3: 0.1 (−0.1, 0.3) Age 8: 0.1 (−0.2, 0.4) Age 12: 0.2 (0.0, 0.5)
	Confounding[c]: visit, visit*PFAS, maternal age, maternal education, maternal pre-pregnancy BMI, gestational serum cotinine concentrations, and parity; and child age, sex, race, and pubertal stage. Additional confounding for analyses at age 3, age 8, and age 12: Breastfeeding duration.						
Ma et al. (2019) *Medium*	United States 2003–2012	Cross-sectional	Adolescents aged 12–20 from NHANES N = 2,251 (1,048 female, 1,203 male)	Serum Levels not provided	DBP, SBP	Regression coefficient per log10-unit increase in PFOA	DBP Total cohort: 0.008 (−0.009, 0.026) Females: −0.005 (−0.027, 0.016) Males: 0.018 (−0.01, 0.046) SBP Total cohort: −0.003 (−0.01, 0.004) Females: −0.005 (−0.015, 0.004) Males: −0.004 (−0.014, 0.007)
	Confounding: Age, gender, race, BMI, cotinine, dietary calcium, caloric intake, sodium consumption, potassium consumption, sampling year.						
Warembourg et al. (2019) *Medium*	France, Spain, Lithuania, Norway, Greece,	Cohort	Pregnant women and their children at ages 6 and 11 from	Maternal blood 2.3 (1.4–3.3) Plasma 1.5 (1.2–2.0)	DBP, SBP	Regression coefficient per log2-unit IQR increase PFOA	DBP Maternal PFOA: 0.29 (−0.55, 1.13) Childhood PFOA: 0.23 (−0.45, 0.91) SBP

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	United Kingdom 1999–2015		the HELIX Project N = 1,277 Prenatal exposure Postnatal exposure				Maternal PFOA: −0.1 (−1, 0.8) Childhood PFOA: 0.39 (−0.34, 1.12)
	Confounding: Cohort of inclusion, maternal age, maternal education level, maternal pre-pregnancy BMI, parity, parental country of birth, child age, child sex, child height.						
Canova et al. (2021) *Medium*	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 yr and children aged 8 to 11 yr from health surveillance program in Veneto Region Adolescents: N = 6,669 Children: N = 2,693	Serum Adolescents: 38.9 (20.1–68.8) Children: 20.9 (12.9–33.5)	DBP, SBP	Regression coefficient per ln-unit increase in PFOA, or by quartiles	DBP Adolescents Per ln-unit increase: −0.11 (−0.37, 0.15) Q2: −0.23 (−0.84, 0.39) Q3: −0.28 (−0.93, 0.36) Q4: −0.08 (−0.77, 0.61) Children Per ln-unit increase: 0.16 (−0.23, 0.54) Q2: 0.58 (−0.28, 1.44) Q3: 0.37 (−0.50, 1.24) Q4: 0.68 (−0.21, 1.57) SBP Adolescents Per ln-unit increase: −0.16 (−0.53, 0.20) Q2: −0.44 (−1.31, 0.43) Q3: −1.01 (−1.92, −0.10) Q4: −0.44 (−1.42, 0.54) Children Per ln-unit increase: −0.51 (−1.02, −0.01) Q2: −0.08 (−1.20, 1.05) Q3: −0.22 (−1.35, 0.91) Q4: −0.98 (−2.14, 0.18)
	Results: Lowest quartile used as the reference group.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Age, gender, country of birth, data on food consumption, degree of physical activity, salt intake, smoking status (for adolescents only), time lag between the beginning of the study and the date of enrollment.						
Papadopoulou et al. (2021) *Medium*	United Kingdom, France, Spain, Lithuania, Norway, Greece Recruitment 1999–2010, Follow-up: 2013–2015	Cohort	Mother-child pairs from the HELIX Project, children followed up around age 8 (range 6–12) N = 1,101	Maternal plasma (prenatal) 2.22 (1.34–3.29) Plasma (childhood) 1.53 (1.17–1.96)	DBP (z-score), SBP (z-score)	Regression coefficient per doubling in PFOA, or by quartiles	DBP Maternal PFOA: −0.01 (0.10, 0.09) Q2: 0.04 (−0.14, 0.21) Q3: 0.00 (−0.22, 0.21) Q4: 0.08 (−0.17, 0.33) p-trend = 0.614 Childhood PFOA: 0.01 (−0.11, 0.13) Q2: −0.01 (−0.16, 0.14) Q3: 0.00 (−0.16, 0.16) Q4: 0.09 (−0.09, 0.27) p-trend = 0.390 SBP Maternal PFOA: 0.03 (−0.08, 0.14) Q2: 0.08 (−0.11, 0.28) Q3: 0.04 (−0.19, 0.28) Q4: 0.06 (−0.22, 0.33) p-trend = 0.910 Childhood PFOA: 0.03 (−0.11, 0.16) Q2: −0.05 (−0.22, 0.11) Q3: −0.05 (−0.23, 0.13) Q4: 0.10 (−0.10, 0.30) p-trend = 0.388
	Comparison: Maternal quartiles are defined as follows (in µg/L PFOA): Q1: 0.02–1.33; Q2: 1.34–2.22; Q3: 2.22–3.29; Q4: 3.29–31.64; childhood quartiles are defined as follows (in µg/L PFOA): Q1: 0.21–1.17; Q2: 1.17–1.53; Q3: 1.53–1.96; Q4: 1.96–6.66. **Results:** Lowest quartile used as the reference group. **Confounding:** Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, child gender, PFHxS, PFNA, PFOS.						
Manzano-Salgado et al. (2017b) *Medium*	Spain 2003–2008	Cohort	Pregnant women and their children at ages 4 and 7 from INMA study	Maternal blood GM = 2.32 (1.63–3.31)	Blood Pressure (BP) (z-score) Cardiometabolic Risk Score (CMR)	Regression coefficient per log2-unit increase in PFOA	BP All age 4: −0.06 (−0.16, 0.04) Girls: −0.04 (−0.18, 0.1) Boys: −0.08 (−0.23, 0.07) All age 7: −0.02 (−0.11, 0.07) Girls: −0.08 (−0.21, 0.04)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Age 4 N = 839 (412 girls, 427 boys) Age 4 N = 386 (197 girls, 189 boys) for CMR score measurements Age 7 N = 1,086 (535 girls, 551 boys)				Boys: 0.04 (−0.08, 0.16) CMR All age 4: 0.27 (−0.35, 0.89) Girls: −0.22 (−1.1, 0.66) Boys: 0.72 (−0.17, 1.62)
	Confounding: Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child.						
Lin et al. (2013a) *Medium* for CIMT *Low* for Systolic BP	Taiwan 2006–2008	Cross-sectional	Adolescents and young adults ages 12–30 N = 637	Serum 3.49 (75th percentile = 6.54)	SBP, CIMT	Mean by PFOA exposure group	SBP: No associations CIMT: No associations
	Comparison: Groups were defined as follows: (1) up to 50th percentile; (2) 50th–75th percentile; (3) 75th–90th percentile; (4) above 90th percentile. **Confounding:** Age, gender, smoking status, alcohol drinking, body mass index; for CIMT, also includes SBP, LDL, triglyceride, high-sensitivity CRP, homeostasis model assessment of insulin resistance.						
Geiger et al. (2014b) *Medium*	United States 1999–2000, 2003–2008	Cross-sectional	Children ages ≤18 yr from NHANES N = 1,655	Serum Mean (SE) = 4.4 (0.1)	Hypertension	OR per ln-unit increase in PFOA, or by quartile	Hypertension Per ln-unit increase: 0.76 (0.53, 1.10) Q2: 0.89 (0.53, 1.49) Q3: 0.96 (0.53,1.73) Q4: 0.69 (0.41, 1.17) p-trend = 0.2477
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, race-ethnicity, BMI categories, annual household income categories, moderate activity, TC, and serum cotinine.						
Averina et al. (2021) *Medium*	Norway 2010–2011	Cross-sectional	First level high school students ages 15–19 yr from TFF1	Serum Girls: GM (IQR) = 2.14 (1.26)	Hypertension	OR by quartiles	Hypertension Q2: 1.28 (0.74, 2.22), p-value = 0.37 Q3: 1.45 (0.85, 2.49), p-value = 0.175 Q4: 2.08 (1.17, 3.69), p-value = 0.013

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 940	Boys: GM (IQR) = 1.86 (0.67)			
	Outcome: Hypertension defined as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg. **Comparison:** Quartiles are defined as follows (in ng/mL PFOA): Q1: 0.28–1.56; Q2: 1.57–1.92; Q3: 1.93–2.44; Q4: 2.45–13.97. **Results:** Lowest quartile used as the reference group. **Confounding:** Sex, age, BMI and physical activity outside school.						
Lin et al. (2016) *Medium*	Taiwan 1992–2000	Cross-sectional	Adolescents and young adults ages 12–30 N = 848	Serum GM = 3.21 (95% CI: 3.00–3.46)	8-OHDG (log-µg/g creatinine) CIMT CD31+ / CD42a− (log count/µL) CD31+ / CD42a+ (log count/µL) CD62E (log count/µL) CD62P (log count/µL)	Mean by PFOA exposure level group	8-OHDG: Borderline statistically significant increase across exposure groups, 7.55–7.68 (Group 3); p-trend = 0.059 CIMT: No associations across exposure groups; p-trend = 0.2868 CD31+ / CD42a-: Statistically significant decrease across exposure groups, 5.14–4.77; p-trend = 0.036 CD31+ / CD42a+, CD62E, CD62P: No statistically significant associations across exposure groups
	Comparison: Groups were defined as follows: (1) up to 50th percentile; (2) 50th–75th percentile; (3) 75th–90th percentile; (4) above 90th percentile. **Confounding:** Age, gender, smoking status, BMI, systolic blood pressure, low-density lipoprotein, triglyceride, homeostasis model assessment of insulin resistance, and high-sensitivity CRP.						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children ages 8–12 N = 48	Serum 0.99 (IQR = 0.45)	DBP, SBP	Regression coefficient per unit increase in PFOA	DBP: 7.75 (−0.25, 15.7) SBP: 7.99 (−2.29, 18.3)
	Confounding: Age, race, sex.						
Koshy et al. (2017) *Low*	United States 2011–2012	Cross-sectional	Children and adolescents from the World	Serum 1.81 (IQR = 0.90)	Augmentation Index (AI)	Regression coefficient per	AI: –1.41 (−4.59, 1.78) BAD: 0.45 (0.04, 0.87) PWV: 0.05 (−0.17, 0.28)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			Trade Center Health Registry (WTCHR) N = 308	Comparison: 1.39 (IQR = 0.75)	Brachial Artery Distensibility (BAD) Pulse Wave Velocity (PWV)	ln-unit increase in PFOA	
	Confounding: BMI category, caloric intake, cotinine concentration, physical activity, race, sex.						
				Pregnant Women			
Matilla-Santander et al. (2017) *Medium*	Spain 2003–2008	Cohort	Pregnant women from INMA study N = 1,240	Plasma 2.35 (1.63–3.30)	CRP (log10 mg/dL)	Percent median change by quartiles and per log10-unit increase in PFOA	CRP 2.86 (−8.12, 14.3) By quartile: Q2: −12.19 (−27.3, 6.18) Q3: −3.92 (−22.1, 17.3) Q4: 3.05 (−17.3, 28.4)
	Results: Lowest quartile as the reference group. **Confounding:** Sub-cohort, country of birth, pre-pregnancy body mass index, previous breastfeeding, parity, gestational week at blood extraction, physical activity, relative Mediterranean Diet Score.						
				General Population			
Liao et al. (2020) *High*	United States 2003–2012	Cross-sectional	Adults ages 20+ from NHANES N = 6,967 (3,439 females, 3,528 males)	Serum 3.33 (2.13–5.10)	DBP, SBP, hypertension	DBP and SBP: Regression coefficient per log10-unit increase in PFOA Hypertension: OR by tertiles or regression coefficient around inflection point (1.80 ng/mL)	DBP: −0.34 (−1.43, 7.55) SBP: 1.83 (0.40, 3.25) Hypertension T2: 1.03 (0.89, 1.2) T3: 1.32 (1.13, 1.54), p-value < 0.01, p-trend < 0.001 No significant interactions by age Females T2: 0.96 (0.77, 1.19) T3: 1.42 (1.12, 1.79), p-value < 0.001, p-trend = 0.003 Males: No statistically significant associations, or trends Ages > 60 yr T2: 0.84 (0.66, 1.06)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							T3: 1.32 (1.03, 1.68) p-trend = 0.003 Ages ≤ 60 yr: No statistically significant associations or trends Levels ≤ 1.80 ng/mL: 0.56 (0.32, 0.99) Levels > 1.80 ng/mL: 1.32 (1.03, 1.68)
	Outcome: Hypertension defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed antihypertensive medication. **Comparison:** Tertiles are defined as follows (in ng/mL PFOA): T1 ≤ 2.5; 2.5< T2 ≤ 4.4; 4.4< T3. **Results:** Lowest tertile used as the reference group. **Confounding:** Age, sex, education level, race, diabetes mellitus, consumption of at least 12 alcohol drinks/year, current smoking status, body mass index, waist circumference, hemoglobin, TC, estimated glomerular filtration rate (eGFR), dietary intake of sodium, dietary intake of potassium, and dietary intake of calcium.						
Mattsson et al. (2015) *High*	Sweden 1990–1991, 2002–2003	Case-control	Rural men N = 462	Serum Cases: 4.2 (IQR = 1.8) Controls: 4.0 (IQR = 2.0)	CHD	OR by quartiles	CHD Q2: 0.79 (0.44, 1.43) Q3: 1.18 (0.67, 2.06) Q4: 0.88 (0.5, 1.55)
	Results: Lowest quartile used as reference group. **Confounding:** BMI, systolic blood pressure, TC, HDL, tobacco use.						
Mobacke et al. (2018) *High*	Sweden Years not reported	Cross-sectional	Adults aged 70 from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study N = 801	Serum Mean (SD) = 3.59 (1.69)	Left Ventricular End-Diastolic Diameter (LVEDD) (mm) Left Ventricular Mass Index (LVMI) ($g/m^{2.7}$) Relative Wall Thickness (RWT)	Regression coefficient per ln-unit increase in PFOA	LVEDD: 0.58 (−0.03, 1.18) LVMI: −0.65 (−1.94, 0.65) RWT: −0.12 (−0.22, −0.001)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Sex, SBP, antihypertensive medication, HDL and LDL, blood glucose, waist circumference, triglycerides, BMI, education levels, exercise habits, smoking, energy, alcohol intake.						
Bao et al. (2017) *Medium*	China 2015–2016	Cross-sectional	Adults aged 22–96 N = 1,612 (408 females, 1,204 males)	Serum 6.19 (4.08–9.31)	DBP, SBP, hypertension	Regression coefficient per ln-unit increase in PFOA Hypertension: OR per ln-unit increase PFOA	DBP Total: 2.18 (1.35, 2.98) SBP Total: 1.69 (0.25, 3.13) Females: 2.91 (0.1, 5.72) Males: No association Hypertension: No statistically significant associations
	Outcome: Hypertension defined as mean SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, and/or use of antihypertensive medications. **Confounding:** Age, sex, BMI, education, income, exercise, smoking, drinking, family history of hypertension.						
Liu et al. (2018a) *Medium*	United States 2004–2007	Controlled trial	Overweight and obese adults ages 30−70 in the POUNDS Lost Study N = 621 (384 females, 237 males)	Plasma Females: 4.1 (2.8–5.6) Males: 5.2 (3.9–6.8)	DBP, SBP	Partial Spearman correlation coefficient	DBP: 0.1; p-value < 0.05 SBP: 0.04
	Confounding: Age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), dietary intervention groups.						
Lin et al. (2020b) *Medium*	United States 1996–2014	Cohort	Adults from the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) N = 957 at baseline, 956 at year 2, and 346 at year 14	Serum Baseline: 4.9 (3.5–6.7) Year 2: 5.7 (4.0–8.0) Year 14: 2.8 (2.0–3.8)	DBP, SBP, pulse pressure (mmHg), and hypertension	Regression coefficient per log2-unit increase in PFOA or by quartiles Hypertension: HR or RR per log2-unit	DBP: No statistically significant associations by timepoint, by quartiles, or by sex (p-value for interaction by sex = 0.81) SBP: Baseline: 1.49 (0.29, 2.70) Baseline males: 2.36 (0.13, 4.60); p-value for interaction by sex = 0.28

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
						increase PFOA or by quartiles	No statistically significant associations by follow-up timepoint or by quartiles Pulse Pressure: No statistically significant associations by timepoint, by quartiles, or by sex (p-value for interaction by sex = 0.24) Hypertension Baseline males: 1.27 (1.06, 1.53); p-value for interaction by sex = 0.07165 No statistically significant associations by timepoint or by quartiles
	Outcome: Hypertension defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in those without diabetes, SBP ≥ 30 mmHg, and DBP ≥ 80 mmHg in those with diabetes, self-reported hypertension diagnosis, or use of antihypertensive medication. **Confounding:** Sex, age, race/ethnicity, treatment assignment, education, income, marital status, alcohol intake, smoking, and DASH diet score.						
Mi et al. (2020) *Medium*	China 2015–2016	Cross-sectional	Shenyang residents ages 23–94 N = 1238 (559 women, 679 men)	Serum 4.8 (3.6–7.4)	DBP, SBP, hypertension	DBP, SBP: egression coefficient per ln-unit increases in PFOA Hypertension: OR per ln-unit increase in PFOA	DBP 1.49 (0.34, 2.64) Females: 0.38 (−0.75, 1.51) Males: 1.82 (−0.04, 3.67) p-interaction = 0.05 Ages > 60: 1.96 (0.62, 3.31) Ages 23–60: No associations No statistically significant sex interactions within age groups SBP: No statistically significant associations or interactions by sex or age Hypertension 1.72 (1.27, 2.31) Females: 2.32 (1.38, 3.91) p-interaction = 0.22 Ages > 60: 3.58 (2.14, 5.98)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Ages 23–60: No associations No statistically significant sex interactions within age groups
	Outcome: Hypertension defined as mean SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or use of antihypertensive medicines for previous two weeks. **Confounding:** Age, sex, ethnicity, career, education, smoking, alcohol drinking, physical activity, annual household income, and seafood consumption.						
Mitro et al. (2020) *Medium*	United States 1999–2005	Cohort	Pregnant women and their children at age 3 from Project Viva N = 761 mothers (496 ages <35, 265 ages ≥35)	Plasma 5.6 (4.0–7.6)	DBP, SBP, CRP (mg/L)	Percent difference per log2-unit increase in PFOA Regression coefficient per log2-unit increase in PFOA	DBP, SBP, CRP: No statistically significant associations
	Population: For measurements of CRP, N = 454 mothers (247 ages <35, 207 ages ≥35). **Confounding:** age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity; breastfeeding in a prior pregnancy for BP measurements only.						
Pitter et al. (2020) *Medium*	Italy 2017–2019	Cross-sectional	Adults aged 20–39 yr from Veneto Region with PFAS-contaminated drinking water DBP and SBP: N = 15,380 (7,428 males, 7,952 females) Hypertension risk: N = 15,786 (7,667 males, 8,119 females)	Serum 35.8 (13.7–78.9) Male: 58.3 (25.1–114.7) Female: 22.6 (8.8–49.4)	DBP, SBP, hypertension risk	DBP, SBP: Regression coefficient per ln-unit increase in PFOA, or by quartiles Hypertension risk: OR per ln-unit increase in PFOA, or by quartiles	DBP 0.34 (0.21, 0.47) Q2: 0.24 (−0.16, 0.64) Q3: 0.78 (0.36, 1.20) Q4: 0.97 (0.53, 1.42) Males: 0.23 (0.04, 0.42) Females: 0.39 (0.21, 0.57) SBP 0.37 (0.19, 0.54) Q2: 0.26 (−0.29, 0.81) Q3: 0.74 (0.16, 1.31) Q4: 1.07 (0.46, 1.68) Males: 0.46 (0.19, 0.73)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Females: 0.31 (0.08, 0.55) Hypertension risk 1.06 (1.01, 1.12) Q2: 1.00 (0.85, 1.16) Q3: 1.02 (0.87, 1.20) Q4: 1.16 (0.99, 1.37) Males: 1.08 (1.02, 1.15) Females: 1.06 (0.97, 1.15)
	Outcome: Hypertension defined as any self-reported diagnosis, use of antihypertensive drugs, or elevated systolic blood pressure (SBP ≥ 140 mmHg)/diastolic blood pressure (DBP ≥ 90 mmHg). **Results:** Lowest quartile used as the reference group. **Confounding:** Age, BMI, time lag between the enrollment and the beginning of the study, gender, physical activity, smoking habits, food consumption, salt habit, country of birth, alcohol consumption, education level and center in charge of the BP measurement.						
Min et al. (2012) *Medium*	United States 2003–2006	Cross-sectional	Adults ages 20+ from NHANES N = 1,415	Serum GM = 4.0 (3.86–4.13)	Hypertension	OR by quartile	Hypertension Q4: 1.84 (1.07, 3.18)
	Outcome: Hypertension defined as SBP > 140 mmHg or DBP > 90 mmHg or as a self-reported medical diagnosis of hypertension. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, sex, race/ethnicity, education, income, smoking habits, alcohol use, obesity status, total saturated fatty acid intake, physical activity, serum folate, TC, and poor kidney function.						
Winquist and Steenland (2014a) *Medium*	United States 2008−2011	Cohort	Workers at a Mid-Ohio Valley chemical plant and residents of the surrounding community from C8 Health Project N = 32,254	Serum 26.1 (12.8–68.1)	Hypertension	HR by quintiles	Hypertension Q2: 1.10 (1.02, 1.19) Q3: 1.10 (1.02, 1.18) Q4: 1.05 (0.97, 1.12) Q5: 0.98 (0.91, 1.06)
	Outcome: Hypertension cases were identified based on self-reported diagnosis. **Results:** Lowest quintile used as the reference group.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Age, sex, years of schooling, race, smoking, smoking duration, smoking pack-years, regular alcohol consumption, BMI, self-reported type-2 diabetes.						
Liu et al. (2018b) *Medium*	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1,871	Serum GM (SE) = 1.86 (1.02)	Hypertension	OR per ln-unit increase in PFOA	Hypertension: 1.13 (0.81, 1.58)
	Outcome: Hypertension defined as average SBP ≥ 130 mmHg and average DBP ≥ 85 mmHg, or self-reported use of prescribed antihypertensive medication. **Confounding:** Age, gender, ethnicity, lifestyle variables (smoking status, alcohol intake and household income), medications (antihypertensive, antihyperglycemic, and antihyperlipidemic agents), other components of the metabolic syndrome.						
Christensen et al. (2019) *Medium*	United States 2007–2014	Cross-sectional	Adults ages 20+ from NHANES N = 2,975	Serum 2.8 (1.8–4.3)	Hypertension	OR by quartiles	Hypertension No statistically significant associations
	Outcome: Hypertension defined as SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, or use of antihypertensive drug in a patient with a history of hypertension. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, alcohol intake, family income, MPAH, PFDE, PFHxS, PFOS, PFUnDA, race/ethnicity, smoking status, survey cycle.						
Donat-Vargas et al. (2019b) *Medium*	Sweden 1990–2013	Cohort	Adults aged 30–60 at baseline N = 187	Plasma Baseline: 2.9 (2.2–4.2) Follow-up: 2.7 (1.9–3.6)	Hypertension	OR by tertiles or per SD-unit increase in PFOA	Hypertension Baseline: OR per increase: 1.12 (0.78, 1.59) Follow-up: OR for T3: 1.14 (0.51, 2.58) Prospective: No statistically significant associations
	Outcome: Hypertension defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, self-reported diagnosis, or use of antihypertensive drugs **Results:** Results by tertile use lowest tertile as the reference group. **Confounding:** Gender, age, education, sample year, body mass index, smoking habit, alcohol consumption, physical activity, healthy diet score.						
Jeddi et al. (2021a) *Medium*	Italy 2017–2019	Cross-sectional	Residents aged 20–39 from the PFAS-contaminated Veneto region N = 15,876	Serum GM (range): 67.66 (0.70–1400.0)	Elevated blood pressure	OR per ln-unit increase in PFOA	Elevated blood pressure: 1.05 (1.01, 1.08), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Outcome: Elevated blood pressure defined as SBP ≥ 130 mmHg or DBP ≥ 85 mmHg. **Confounding:** Age, gender, time lag between the beginning of the study and blood sampling center where BP has been measured, education, number of deliveries, physical activity, country of birth, diet, alcohol intake, and smoking status, and other components of metabolic syndrome.						
Shankar et al. (2012) *Medium*	United States 1990–2000, 2003–2004	Cross-sectional	Adults ages 40+ from NHANES N = 1,216 (623 females, 593 males)	Serum Female: 3.9 (2.9, 5.6) Male: 4.3 (3.0, 6.1)	CVD, cardiovascular heart disease (CVHD), peripheral arterial disease (PAD), stroke, CVD or PAD Cardiovascular Disease (CVD)	OR by quartiles	CVD Q3: 1.77 (1.04, 3.02) Q4: 2.01 (1.12, 3.60) Increasing trend by quartiles; p-trend = 0.01 CVHD Q4: 2.24 (1.02, 4.94) Increasing trend by quartiles; p-trend = 0.007 PAD Q4: 1.78 (1.03, 3.08) Increasing trend by quartiles; p-trend = 0.04 Stroke Q2: 4.39 (1.44, 13.37) Q3: 3.94 (1.48, 10.05) Q4: 4.26 (1.84, 9.89) p-trend = 0.02 CVD or PAD: Q3: 1.72 (1.13, 2.64) Q4: 2.28 (1.40, 3.71) Increasing trend by quartiles; p trend < 0.001 Females: Q4: 2.99 (1.53, 5.81) Increasing trend by quartiles; p-trend = 0.004

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Males: Q3: 1.75 (1.04, 2.96) Q4: 1.83 (1.02, 3.28) Increasing trend by quartiles; p-trend = 0.04
	Results: Lowest quartile used as reference. **Confounding:** Age, sex, race/ethnicity, educational level, smoking status, alcohol intake, body mass index, hypertension, diabetes mellitus, serum TC level; serum high-sensitivity CRP level and serum uric acid level for CVD and PAD outcomes only.						
Fry and Power (2017) *Medium*	United States 2003–2006	Cohort	Adults ages 60+ from NHANES N = 1,023	Serum 23.7 ng/g (SE = 0.7 ng/g)	Mortality by cerebrovascular or heart diseases	HR per SD-unit increase in PFOA	Mortality 0.98 (0.81, 1.17)
	Confounding: Age, education, gender, race/ethnicity, smoking status.						
Lind et al. (2017b) *Medium*	Sweden 2001–2004	Cross-sectional	Adults ages 70+ in Uppsala, Sweden N = 1,016 (509 females and 507 males)	Plasma 3.3 (2.52–4.39)	CIMT, carotid artery intima-media complex gray scale median (CIM-GSM), carotid artery atherosclerotic plaque	CIMT, CIM-GSM: Regression coefficient per ln-unit increase in PFOA Plaque: OR per ln-unit increase in PFOA	CIMT, CIM-GSM, atherosclerotic plaque: no statistically significant associations; all p-values > 0.25
	Confounding: Sex, HDL, LDL and serum triglycerides, BMI, blood pressure, smoking exercise habits, energy and alcohol intake, diabetes, educational level.						
Huang et al. (2018) *Medium*	United States 1999–2014	Cross-sectional	Adults from NHANES ages 18+ N = 10,859	Serum 3.17 (1.97–4.90)	CVD, angina pectoris, congestive heart disease, CHD, heart attack, stroke, CRP (mg/L)	OR by quartiles CRP: Spearman correlation coefficient	CVD: No association by quartiles, no significant trend; p-trend = 0.703 No associations, trend, or interaction by age groups Females Q2: 0.76 (0.49, 1.18) Q3: 1.04 (0.66, 1.66) Q4: 1.14 (0.75, 1.75)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Males Q2: 1.49 (0.98, 2.26) Q3: 1.56 (1.02, 2.40) Q4: 1.45 (0.92, 2.28) No trend or interaction by sex Angina pectoris: No association by quartiles, no significant trend; p-trend = 0.391 Congestive heart disease: No association by quartiles, no significant trend; p-trend = 0.670 CHD: No association by quartiles, no significant trend; p-trend = 0.097 Heart attack Q2: 1.57 (1.06, 2.34) Q3: 1.62 (1.04, 2.53) Q4: 1.47 (0.91, 2.37) p-trend = 0.231 Stroke Q2: 1.01 (0.70, 1.44) Q3: 1.42 (0.94, 2.13) Q4: 1.37 (0.92, 2.05) p-trend = 0.045 CRP: −0.068; p-value < 0.001
	Comparison: Age groups were defined as < 50 yr and ≥50 yr. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, sex, race/ethnicity, family poverty-income ratio, education levels, physical activity levels, BMI, alcohol drinking status, smoking status, diabetes, hypertension, family history of CVD, total energy intake, log-transformed levels of serum cotinine, log-transformed levels of serum TC.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Cardenas et al. (2019) *Medium*	United States 1996–2014	Controlled trial	Prediabetic adults ages 25+ from DPP and DPPOS N = 877	Plasma GM (IQR) = 4.82 (3.20)	MVD, nephropathy, neuropathy, retinopathy	OR per log2-unit increase in baseline PFOA	MVD, nephropathy, neuropathy, retinopathy: No statistically significant associations
	Confounding: Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment; baseline fasting glucose and HbA1c levels for microvascular disease only.						
Hutcheson et al. (2020) *Medium*	United States 2005–2006	Cross-sectional	Adults from C8 Health Project N = 48,206	Serum With diabetes: 28.7 (12.9–73.6) Without diabetes: 27.6 (13.4–70.4)	Stroke	OR per ln-unit increase in PFOA	0.96 (0.91, 1.01)
	Confounding: Age, BMI, CRP, diabetes duration, eGFR, HDL, LDL, history of smoking, race, sex.						
Osorio-Yanez et al. (2021) *Medium*	United States 1999	Cohort	Prediabetic adults ages 25+ enrolled in the DPP trial N = 666	Plasma 5.35 (IQR = 3.60)	CAC (Agastston score)	OR per doubling in PFOA	CAC (11–400): 1.17 (0.91, 1.50) CAC (> 400): 1.05 (0.71, 1.57)
	Results: CAC < 11 used as reference group. **Confounding:** Sex, age, body mass index, race/ethnicity, cigarette smoking, education, treatment assignment, statin use.						
He et al. (2018) *Low*	United States 2003–2012	Cross-sectional	Adults ages 20+ from NHANES N = 3,948 (females) and 3,956 (males)	Serum Female Mean (SE) = 3.46 (0.04) Male Mean (SE) = 4.50 (0.06)	DBP, SBP	Percent difference per interquartile ratio increase in PFOA by quartiles	DBP: No associations in men or women. No significant trend (p-trend = 0.390 and 0.167 among females and males, respectively) SBP: No associations in men or women. No significant trend (p-trend = 0.096 and 0.642 among females and males, respectively)
	Results: Lowest quartile used as the reference group. Interquartile ratio = 75th/25th percentiles of serum PFOA: 2.43 ng/mL. **Confounding:** None listed.						
Yang et al. (2018)	China	Cross-sectional	Adult men N = 148	Serum	DBP, SBP, hypertension	Regression coefficient per	DBP: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Low	Years not reported			1.90 (Range: 0.6–5.0)		log-unit increase in n-PFOA Hypertension: OR for elevated pressure (DBP ≥ 90 or SBP ≥ 140 mm Hg) comparing above or below median	SBP: 12.94 (−1.46, 27.35) OR: 10.8 (1.31, 90)
	Outcome: Hypertension evaluated by individual BP components. **Comparison:** Logarithm base not specified. **Confounding:** Age.						
Chen et al. (2019a) *Low*	Croatia 2007–2008	Cross-sectional	Adults aged 44–56 N = 122	Plasma GM (range) = 2.87 (1.03–8.02)	DBP, SBP	Regression coefficient per ln-unit increase in PFOA	DBP: −1.00 (−4.11, 2.11) SBP: −2.15 (−8.49, 4.18)
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, physical activity.						
Graber et al. (2019) *Low*	United States 2016–2017	Cross-sectional	Members of community with exposed water supply (Paulsboro, NJ) ages 12+ N = 105	Serum 2.98 (1.94–4.69)	Cardiovascular conditions, self-reported	OR per unit increase in PFOA	Any condition 0.97 (0.9, 1.05)
	Confounding: Age, BMI.						
Honda-Kohmo et al. (2019) *Low*	United States 2005–2006	Cross-sectional	Adults ages 20+ from C8 Health Project N = 5,270 with diabetes and	Serum 28.4 (12.6–74.9)	CHD	OR per ln-unit increase in PFOA or by quintiles	CHD Diabetic adults: 0.9 (0.85, 0.96) Q2: 0.92 (0.71, 1.18) Q3: 0.86 (0.67, 1.11) Q4: 0.74 (0.58, 0.96)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			49,191 without diabetes				Q5: 0.73 (0.57, 0.94) Diabetic females: 0.88 (0.80, 0.96) Diabetic males: 0.93 (0.85, 1.00) Non-diabetic adults: 0.95 (0.92, 0.98)
	Results: Results by quintile use lowest quintile as the reference group. **Confounding:** Age, BMI, CRP, diabetes duration, eGFR, HDL, LDL, hemoglobin, iron, sex, smoking history, uric acid, white blood cell count.						
Occupational Populations							
Simpson et al. (2013) *Medium*	United States 2008–2011	Cohort	Adults 20 years and older who lived near a PFOA contamination event. Worker cohort worked at DuPont plant between January 1948 and December 2002. N = 32,254	Modeled 26.1 (SD: 278.9)	Stroke	Hazard ratio by PFOA quintiles, per 1 unit increase in baseline PFOA, and per ln-unit increase in baseline PFOA	Stroke Retrospective, no lag Q2 (>178-319 ng/mL): 1.39 (1.11, 1.76); p = 0.005 Q3 (>319-912 ng/mL): 1.36 (1.08, 1.71); p = 0.010 Q4 (>912-4490 ng/mL): 1.45 (1.15, 1.82); p = 0.002 Q5 (>4490 ng/mL): 1.13 (0.90, 1.44); p = 0.30 Per 1 ng/mL increase: 1.00 (0.99, 1.01); p = 0.52 Per 1-ln ng/mL increase: 1.01 (0.97, 1.06); p = 0.59 Retrospective, 5-yr lag Q2 (>130-217 ng/mL): 1.39 (1.09, 1.77); p = 0.009 Q3 (>217-560 ng/mL): 1.42 (1.12, 1.81); p = 0.004 Q4 (>560-3420 ng/mL): 1.41 (1.11, 1.80); p = 0.005 Q5 (>3420 ng/mL): 1.17 (0.91, 1.49); p = 0.22

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Per 1 ng/mL increase: 1.00 (0.99, 1.01); p = 0.44 Per 1-ln ng/mL increase: 1.01 (0.96, 1.05); p = 0.79 Prospective, no lag Q2 (>244-460 ng/mL): 1.07 (0.73, 1.59); p = 0.72 Q3 (>460-1240 ng/mL): 1.07 (0.72, 1.58); p = 0.74 Q4 (>1240-5500 ng/mL): 1.18 (0.79, 1.75); p = 0.42 Q5 (>5500 ng/mL): 0.87 (0.58, 1.30); p = 0.50 Per 1 ng/mL increase: 0.99 (0.97, 1.01); p = 0.28 Per 1-ln ng/mL increase: 0.97 (0.89, 1.05); p = 0.41
	Results: Lowest quintile used as reference group. **Confounding:** Hypertension, self-reported diabetes, race (white/non-white), smoking status, and alcohol consumption.						
Steenland et al. (2015) *Low*	United States 2008–2011	Cohort	Current and former workers at a chemical plant N = 3,713	Serum Cumulative exposure IQR with or without 10-yr lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	CHD, hypertension, stroke	Incidence rate ratio (RR) by quartiles	CHD: No associations with or without lag; RRs ranging from 0.93 to 1.23. No significant trend. Hypertension: No association with or without lag; RRs ranging from 0.91 to 1.04 No significant trend. Stroke No lag Q2: 2.63 (1.06, 6.56)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							No associations with lag; RRs ranging from 2.63 to 2.07. No significant trend.
	Outcome: Hypertension was self-reported and only analyzed if participants reported taking medication for it. **Results:** Lowest quartiles used as the reference group. **Confounding:** Gender, race, education, BMI, smoking, alcohol consumption.						
Christensen et al. (2016a) *Low*	United States 2012–2013	Cross-sectional	Male anglers ages 50+ N = 154	Serum 2.50 (1.80–3.30)	Cardiovascular condition (any), CHD, hypertension	OR per unit increase of PFOA	Any condition: 0.96 (0.72, 1.29) CHD: 0.97 (0.61, 1.45) Hypertension: 0.74 (0.52, 1.01)
	Outcome: Hypertension was self-reported. **Confounding:** Age, BMI, work status, and alcohol consumption.						
Girardi and Merler (2019) *Low*	Italy 1960–2018	Cohort	Male workers N = 154	Serum GM by tertiles = 1,700; 13,051; and 81,934 ng/mL-years	Mortality by circulatory disease, ischemic heart disease, or stroke (ictus)	Standardized Mortality Ratio by tertiles Mortality Risk Ratio (for PFAS plant workers vs. nearby metal factory workers)	Mortality: No statistically significant associations
	Exposure: Tertiles of cumulative serum PFOA were defined as follows (in ng/mL-years): T1 ≤ 4,034; 4,034< T2 ≤ 16,956; 16,956< T3. **Confounding:** Age at risk, calendar period.						

Notes: AI = augmentation index; BAD = brachial artery distensibility; BMI = body mass index; BP = blood pressure; CAC = coronary artery calcium; CHD = coronary heart disease; CI = confidence interval; CIM-GSM = carotid artery intima-media complex gray scale median; CIMT = carotid artery intima-media thickness (mm); CMR = cardiometabolic risk score; CRP = C-reactive protein; CVD = cardiovascular disease; CVHD = cardiovascular heart disease; DBP = diastolic blood pressure (mmHg); DPPOS = Diabetes Prevention Program Outcomes Study; DPP = Diabetes Prevention Program; eGFR = estimated glomerular filtration rate; GM = geometric mean; HDL = high density lipoprotein cholesterol; HELIX = Human Early-Life Exposome; HOME = Health Outcomes and Measures of the Environment; HR = hazard ratio; INMA = Infancia y Medio Ambiente (Environment and Childhood) Project; IQR = Interquartile Range; LDL = low-density lipoprotein cholesterol; LVEDD = left ventricular end-diastolic diameter (mm); LVMI = left ventricular mass index (g/m^2); MPAH = 2-(N-methyl-PFOSA) acetate; MVD = microvascular disease; NHANES = National Health and Nutrition Examination Survey; NJ = New Jersey; OR = odds ratio; PAD = peripheral arterial disease; PFDE = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnDA = perfluoroundecanoic acid; PIVUS = Prospective Investigation of the Vasculature in Uppsala Seniors; POUNDS = Preventing Overweight Using Novel Dietary Strategies; PWV = pulse wave velocity; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; RWT = relative wall

thickness; SBP = systolic blood pressure (mmHg); SD = standard deviation; SE = standard error; T2 = tertile 2; T3 = tertile 3; TC = total cholesterol; TFF1 = Tromsø Fit Futures 1; WTCHR = World Trade Center Health Registry; yr = years.
[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.
[c] Confounding indicates factors the models presented adjusted for.

D.5.2 Serum Lipids

Table D-14. Associations Between PFOA Exposure and Serum Lipid Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Children							
Li et al. (2021) *High* for gestation, birth, and childhood exposures (3-yr and 8-yr) *Medium* for exposure at 12-yr follow-up	United States 2003–2006	Cohort	Pregnant women and their children followed up at birth and ages 3, 8, and 12 yr from HOME Study Gestation: N = 203 At birth: N = 124 Age 3: N = 137 Age 8: N = 165 Age 12: N = 190	Maternal serum Gestation: 5.3 (3.7–7.2) Cord serum At birth: 3.2 (2.4–4.7) Serum At age 3: 5.4 (3.7–7.4) At age 8: 2.4 (1.8–3.2) At age 12: 1.3 (1.0–1.6)	Levels (mg/dL) of triglycerides and HDL; triglycerides to HDL ratio	Regression coefficient per log2-unit IQR increase in PFOA	Triglycerides Gestation: 0.0 (−0.2, 0.2) At birth: 0.1 (−0.1, 0.3) Age 3: −0.2 (−0.4, 0.1) Age 8: 0 (−0.3, 0.2) Age 12: 0.1 (−0.2, 0.3) HDL Gestation: −1.5 (−4.7, 1.7) At birth: −2.1 (−5.6, 1.4) Age 3: 0.4 (−3.5, 4.4) Age 8: 2.1 (−3.0, 7.3) Age 12: 3.1 (−1.6, 7.8) Triglycerides to HDL ratio Gestation: 0.0 (−0.2, 0.3) At birth: 0.2 (−0.1, 0.4) Age 3: −0.2 (−0.5, 0.0) Age 8: −0.1 (−0.4, 0.2) Age 12: 0.0 (−0.3, 0.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: visit, visit*PFAS, maternal age, maternal education, maternal pre-pregnancy BMI, gestational serum cotinine concentrations, and parity; and child age, sex, race, and pubertal stage. Additional confounding for analyses at age 3, age 8, and age 12: Breastfeeding duration.						
Lin et al. (2009) *Medium*	United States 1999–2000 and 2003–2004	Cross-sectional	Adolescents ages 12–20 yr from NHANES N = 474	Serum Mean (SEM) = 1.51 (0.05) log10-ng/mL	Metabolic syndrome HDL cholesterol and metabolic syndrome triglycerides	OR per log10-unit increase in PFOA	Metabolic syndrome HDL cholesterol Model 4: 1.20 (0.60, 2.39) Model 5: 1.50 (0.67, 3.36) Metabolic syndrome triglycerides Model 4: 1.64 (0.72, 3.73) Model 5: 1.15 (0.54, 2.47)
	Outcome: Metabolic syndrome HDL cholesterol defined as HDL ≤1.04 mmol/L; metabolic syndrome triglycerides defined as triglycerides ≥ 1.24 mmol/L. **Confounding:** Model 4: Age, sex, race, health behaviors (smoking status, alcohol intake, and household income), measurement data (CRP and HOMA/insulin) and medications; additional confounding for model 5: Other components of the metabolic syndrome.						
Nelson et al. (2010) *Medium*	United States 2003–2004	Cross-sectional	Adolescent girls ages 12–19 yr from NHANES N not reported	Serum Level not reported	Level (mg/dL) of HDL	Regression coefficient by quartiles	HDL Q4: 4.3 (0.1, 8.5)
	Results: Results by quartiles use lowest quartile as the reference group. Quartile analyses discussed in-text only and values provided for Q4 only. **Confounding:** Not reported.						
Geiger et al. (2014a) *Medium*	United States 1999–2008	Cross-sectional	Adolescents ages 12–18 yr from NHANES N = 815	Plasma Mean (SE) = 4.2 (0.2)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides; elevated TC; elevated LDL; depressed HDL; elevated triglycerides	Lipid levels: Regression coefficient per ln-unit increase in PFOA, Mean change by tertiles Elevated or depressed: OR per	TC: 4.55 (0.90, 8.20) T2: 4.72 (−1.23, 10.67) T3: 7.0 (1.40, 12.60) p-trend = 0.170 HDL: −1.52 (−3.02, −0.03) T2: 0.53 (−1.23, 2.30) T3: −1.19 (−2.94, 0.56) p-trend = 0.177

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
						ln-unit increase in PFOA, or by tertiles	LDL: 5.75 (2.16, 9.33) T2: 3.61 (−1.13, 8.36) T3: 8.18 (3.04, 13.32) p-trend = 0.0027 TG: 1.74 (−2.88, 6.36) T2: 3.0 (−5.68, 11.68) T3: 0.09 (−6.11, 6.30) p-trend = 0.994 Elevated TC: 1.44 (1.11, 1.88) T2: 1.49 (0.97, 2.29) T3: 1.49 (1.05, 2.12) p-trend = 0.025 Depressed HDL: 1.32 (0.82, 2.13) T2: 1.06 (0.65, 1.73) T3: 1.45 (0.87, 2.41) p-trend = 0.149 Elevated LDL: 1.61 (1.14, 2.27) T2: 1.26 (0.74, 2.15) T3: 1.56 (0.98, 2.48) p-trend = 0.054 Elevated TG: 1.10 (0.64, 1.89) T2: 1.35 (0.60, 3.01) T3: 0.86 (0.46, 1.64) p-trend = 0.598

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Outcome: Elevated TC defined as TC > 170 mg/dL; elevated LDL defined as LDL > 110 mg/dL; depressed HDL defined as HDL < 40 mg/dL; elevated triglycerides defined as triglycerides > 150 mg/dL. **Results:** Lowest tertile used as the reference group. **Confounding:** Age, sex, race-ethnicity, BMI categories, annual household income categories, activity level, and serum cotinine.						
Frisbee et al. (2010) *Medium* for TC, GDL-C, fasting TG; *low* for LDL	United States 2005–2006	Cross-sectional	Children and adolescents ages 1.0 to 17.9 yr in the C8 Health Project N = 12,470	Serum Mean (SD) = 69.2 (111.9)	Abnormal TC, abnormal HDL, abnormal LDL, and abnormal fasting triglycerides	OR by quintiles	Abnormal TC Q2: 1.1 (1.0, 1.3) Q3: 1.2 (1.0, 1.4) Q4: 1.2 (1.1, 1.4) Q5: 1.2 (1.1, 1.4) Abnormal HDL Q2: 1.0 (0.8, 1.2) Q3: 1.0 (0.8, 1.2) Q4: 1.0 (0.9, 1.2) Q5: 0.9 (0.8, 1.1) Abnormal LDL Q2: 1.2 (1.0, 1.5) Q3: 1.2 (1.0, 1.4) Q4: 1.2 (1.0, 1.4) Q5: 1.4 (1.2, 1.7) Abnormal fasting triglycerides Q2: 1.0 (0.7, 1.5) Q3: 1.3 (0.9, 1.9) Q4: 1.6 (1.1, 2.3) Q5: 1.0 (0.7, 1.6)
	Outcomes: Abnormal TC defined as TC ≥ 170 mg/dL; abnormal HDL defined as HDL < 40 mg/dL; abnormal LDL calculated for participants with a triglyceride level < 400 mg/dL regardless of fasting status and defined as LDL ≥ 110 mg/dL; fasting triglycerides defined as self-reported fasting > 6 hr before phlebotomy, and abnormal fasting triglycerides defined as fasting triglycerides ≥ 150 mg/dL. **Results:** Lowest quintile used as the reference group. **Confounding:** Age, estimated time of fasting, BMI z-score, sex, regular exercise.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Timmermann et al. (2014) *Medium*	Denmark 1997	Cross-sectional	Children ages 8–10 from Danish component of EYHS N = 400 normal weight, N = 59 overweight	Plasma 9.3 (Range = 0.8–35.2)	Triglycerides (mmol/L)	Percent change per 10-unit increase PFOA	Normal weight: 1.4 (−9.0, 13.0), p-value = 0.79 Overweight: 76.2 (22.8, 153), p-value = 0.002 p-value for PFOA-BMI interaction = 0.004
	Confounding: Sex, age, ethnicity, paternal income, fast-food consumption, and fitness.						
Maisonet et al. (2015b) *Medium* for TC and HDL at age 7 and all lipids at age 15 *Low* for Triglycerides and LDL at age 7	United Kingdom 1991–1992	Case-control	Pregnant women and their daughters followed up at ages 7 and 15 from ALSPAC Age 7: N = 111 Age 15: N = 88	Serum 3.6 (Range = 1.2–16.4)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides (ln-mg/dL)	Regression coefficient per unit increase in PFOA by tertiles	TC Age 7 T1: 13.75 (0.05, 27.45) T2: −0.53 (−15.39, 14.33) T3: −1.53 (−4.61, 1.54) Age 15 T1: 17.19 (0.45, 33.93) T2: −1.22 (−16.45, 14.01) T3: −2.09 (−5.59, 1.40) LDL Age 7 T1: 14.01 (3.26, 24.76) T2: −5.56 (−17.22, 6.10) T3: 0.03 (−2.38, 2.45) Age 15 T1: 14.26 (0.25, 28.26) T2: −1.29 (−14.03, 11.45) T3: −1.41 (−4.33, 1.51) HDL Age 7 T1: 0.50 (−5.78, 6.79) T2: 4.49 (−2.33, 11.30)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							T3: −0.40 (−1.82, 1.01) Age 15 T1: 0.56 (−7.02, 8.15) T2: 1.04 (−5.87, 7.94) T3: −0.52 (−2.10, 1.06) Triglycerides Age 7 T1: −0.063 (−0.278, 0.153) T2: −0.150 (−0.384, 0.084) T3: −0.020 (−0.068, 0.029) Age 15 T1: 0.135 (−0.049, 0.319) T2: −0.047 (−0.215, 0.120) T3: −0.013 (−0.051, 0.025)
	Confounding: Previous live births, maternal education, and maternal age at delivery.						
Zeng et al. (2015) *Medium*	Taiwan 2009–2010	Cross-sectional	Children ages 12–15 N = 225	Serum Median = 0.5	Levels (ng/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per ln-unit increase PFOA	TC: 6.57 (2.72, 10.42) p-value = 0.001 LDL: 4.66 (1.67, 7.65) p-value = 0.002 HDL: −1.56 (−3.20, 0.08) p-value = 0.06 Triglycerides: 19.63 (14.82, 24.34) p-value < 0.001
	Confidence: Results for triglycerides and LDL considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Age, gender, BMI, parental education level, exercise, ETS exposure.[c]						
Domazet et al. (2016)	Denmark 1997–2009	Cohort	Members of the European Youth	Plasma	Levels (mmol/L) of triglycerides	Percent change in triglycerides at ages	Age 9 and 15: −1.46 (−17.84, 18.22)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Medium			Study (EYHS) evaluated at ages 9 and 15 (N = 260), 9 and 21 (N = 175), or 15 and 21 (N = 171)	Median at age 9 = 9.7 (male) or 9.0 (female) Median at age 15 = 3.7 (male) or 3.4 (female) Median at age 21 = 3.1 (male) or 2.7 (female)		9 and 15, or age 9 and 21, or age 15 and 21 per 10 ng/mL increase in PFOA at age 9 or 15	Age 9 and 21: −8.07 (−30.3, 20.9) Age 15 and 21: 2.54 (−31.18, 84.56)
	Confounding: Sex, age, and triglycerides levels at baseline age; ethnicity, maternal parity, and maternal income in 1997 (9 yr of age). Waist circumference was adjusted for height in order to account for body size.						
Manzano-Salgado et al. (2017b) *Medium*	Spain 2003–2008	Cohort	Pregnant women and their children (age 4) from INMA study N = 627	Maternal plasma during first trimester GM = 2.32	Levels (z-score) of TC, LDL, HDL, and triglycerides	Regression coefficient per log2-unit increase PFOA	TC: 0.02 (−0.10, 0.15) LDL: 0.03 (−0.08, 0.15) HDL: −0.04 (−0.15, 0.08) Boys: −0.20 (−0.37, −0.03) Girls: No association Triglycerides: 0.04 (−0.07, 0.15)
	Confidence: Results for triglycerides and LDL considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Maternal region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI; age/sex of child.						
Jain et al. (2018) *Medium*	United States 2013–2014	Cross-sectional	Children ages 6–11 N = 458	Serum GM = 1.78	Levels (log10-mg/dL) of TC, HDL, and non-HDL	Regression coefficient per log10-unit increase linear PFOA	TC: −0.0085 p-value = 0.46 Non-HDL: −0.0016 p-value = 0.61 HDL: 0.0223 p-value = 0.45
	Confounding: Gender, race/ethnicity, age, poverty-income ratio, body mass index percentiles, fasting time, and exposure to secondhand smoke.						
Kang et al. (2018) *Medium*	Korea 2012–2014	Cross-sectional	Children ages 3–18 from KorEHS-C	Serum Median = 1.88	Levels of TC (mg/dL), LDL (mg/dL), and triglycerides (ln-mg/dL)	Regression coefficient per ln-unit increase PFOA	TC: −2.26 (−11.49, 6.98) LDL: 3.90 (−4.81, 12.61)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 147				Triglycerides: 0.02 (−0.13, 0.18)
	Results: LDL and triglycerides evaluated at ages 7–18 only (N = 117). **Confounding:** Age, sex, BMI z-score, household income, secondhand smoking.						
Mora et al. (2018) *Medium*	United States 1999–2010	Cohort and cross-sectional	Pregnant women and their children from Project Viva N = 512 prenatal, 596 mid-childhood	Prenatal maternal plasma Median = 5.4 Mid-childhood plasma Median = 4.3	Levels (mg/dL) of TC, HDL, LDL, and triglycerides	Regression coefficient per IQR increase in PFOA	No statistically significant prenatal exposure associations Mid-childhood: TC: 2.6 (−0.5, 5.7) Boys: 1.2 (−3.0, 5.4) Girls: 5.2 (0.4, 9.9) HDL: 1.5 (0.1, 2.9)
	Confounding: maternal education, prenatal smoking, gestational age at blood draw (for prenatal data), and child's sex, race/ethnicity, and age at lipids/ALT measurements.						
Jensen et al. (2020a) *Medium*	Denmark 2010–2012	Cohort	Pregnant women and their children assessed at 3 mo and 18 mo N = 260 at 3 mo, 83 at 18 mo	Maternal serum Median = 1.62	Levels (standard deviation score) of TC, LDL, HDL, and triglycerides	Regression coefficient per unit increase in PFOA	Regression coefficients for all children were between −0.07 and 0.1, all with p-values > 0.05 LDL at 18 mo Boys: −0.29 (−0.58, −0.003) p-value for interaction with sex = 0.01 Triglycerides at 18 mo Boys: 0.43 (0.16, 0.70) p-value for interaction with sex < 0.01
	Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI2, education, smoking, sex, and lipid outcome at 3 mo.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Spratlen et al. (2020b) *Medium*	United States 2001–2002	Cross-sectional	Pregnant women and their children from the Columbia University World Trade Center birth cohort N = 222	Cord blood Median = 2.46	Levels (mg/dL) of TC, total lipids, and triglycerides in cord blood	Percent change per 1% increase in PFOA Geometric mean ratios (GMRs) by quartiles	TC: 0.038 (−0.032, 0.109) GMR p-trend = 0.39 Total lipids: 0.087 (0.021, 0.153) GMR p-trend = 0.04 Triglycerides: 0.256 (0.129, 0.383) GMR p-trend = 0.001
	Confounding: Maternal age, child sex, maternal education, maternal race, parity, pre-pregnancy BMI, marital status, family smoking, and gestational age.						
Blomberg et al. (2021) *Medium* for HDL and TC *Low* for LDL and TG	Faroe Islands Recruitment: 2007–2009	Cohort and cross-sectional	Children from the Faroese Birth Cohort 5 at birth, 18 mo, and 9 yr Birth: N = 459 (219 female, 240 male) 18 mo: N = 334 9 yr: N = 366	Serum PFAS at birth: 0.9 (0.63–1.34) Female: 0.93 (0.65–1.42) Male: 0.87 (0.61–1.22) PFAS at 18 mo: 2.74 (1.19–1.74) PFAS at 9 yr: 1.43 (1.19–1.74) Levels at 5 yr and by sex at 18 mo and 9 yr not reported	Levels (mmol/L) of TC, HDL	Regression coefficient per log2-unit increase in PFOA	TC: Regression coefficients were between −0.14 and 0.18, all with p-values > 0.05 HDL: Regression coefficients were between −0.031 and 0.041, all with p-values > 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Child sex and maternal education; analyses except PFAS at 9 yr additionally adjusted for maternal smoking during pregnancy, maternal pre-pregnancy BMI, and parity.						
Canova et al. (2021) *Medium* for TC, HDL, BP; *Low* for LDL, TG	Italy 2017–2019	Cross-sectional	Adolescents aged 14 to 19 yr and children aged 8 to 11 yr from health surveillance program in Veneto Region Adolescents: N = 6,669 Children: N = 2,693	Serum Adolescents: 38.9 (20.1–68.8) Children: 20.9 (12.9–33.5)	Levels (ng/mL) of TC, HDL, LDL, triglycerides	Regression coefficient per ln-unit increase in PFOA	TC Adolescents: 1.05 (0.31, 1.80) Children: 0.85 (−0.44, 2.14) HDL Adolescents: −0.17 (−0.47, 0.14) Children: 0.64 (0.09, 1.19) LDL Adolescents: 1.03 (0.39, 1.66) Children: 0.17 (−0.98, 1.32) Triglycerides Adolescents: 0.01 (0.00, 0.03) Children: 0.00 (−0.02, 0.02)
	Confounding: Age, gender, country of birth, data on food consumption, degree of physical activity, salt intake, smoking status (for adolescents only), time lag between the beginning of the study and the date of enrollment.						
Papadopoulou et al. (2021) *Medium*	United Kingdom, France, Spain, Lithuania, Norway, Greece	Cohort	Mother-child pairs from the HELIX Project, children followed up around age 8 (range 6–12) N = 1,101	Maternal plasma (prenatal) 2.22 (1.34–3.29)	Levels (z-scores) of HDL, LDL, and triglycerides	Regression coefficient per doubling in PFOA, or by quartiles	HDL Maternal PFOA: −0.01 (−0.13, 0.10) Q2: −0.11 (−0.32, 0.10) Q3: −0.06 (−0.31, 0.19) Q4: −0.05 (−0.35, 0.24) p-trend = 0.821

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Recruitment 1999–2010, Follow-up: 2013–2015			Plasma (childhood) 1.53 (1.17–1.96)			Childhood PFOA: 0.17 (0.03, 0.32) Q2: 0.04 (−0.14, 0.22) Q3: 0.11 (−0.08, 0.31) Q4: 0.18 (−0.03, 0.40) p-trend = 0.160 LDL Maternal PFOA: −0.04 (−0.08, 0.15) Q2: −0.07 (−0.28, 0.14) Q3: −0.14 (−0.40, 0.11) Q4: −0.14 (−0.44, 0.16) p-trend = 0.394 Childhood PFOA: −0.17 (−0.32, −0.03) Q2: 0.03 (−0.15, 0.21) Q3: −0.03 (−0.23, 0.16) Q4: −0.10 (−0.32, 0.12) p-trend = 0.195 Triglycerides Maternal PFOA: 0.09 (−0.03, 0.21) Q2: 0.20 (−0.01, 0.41) Q3: 0.17 (−0.08, 0.43) Q4: 0.28 (−0.02, 0.58) p-trend = 0.244 Childhood PFOA: −0.06 (−0.21, 0.08) Q2: −0.15 (−0.33, 0.03) Q3: −0.21 (−0.41, −0.01) Q4: −0.13 (−0.35, 0.09) p-trend = 0.345
	Results: Lowest quartile used as the reference group.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, child gender, PFHxS, PFNA, PFOS.						
Tian et al. (2020) *Medium*	China 2012	Cohort	Pregnant women and their newborn children from the S-MBCS N = 306	Maternal plasma 19.6 (14.6–27.2)	Levels (ln-mg/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per ln-unit increase in PFOA, or by tertile	TC: Per ln-unit: −0.06 (−0.17, 0.05), p-value = 0.259 T2: −0.10 (−0.22, 0.02) T3: −0.07 (−0.18, 0.05) LDL: Per ln-unit: 0.0 (−0.14, 0.14), p-value = 0.982 T2: −0.06 (−0.22, 0.09) T3: −0.02 (−0.17, 0.13) HDL: Per ln-unit: −0.09 (−0.22, 0.03), p-value = 0.153 T2: −0.14 (−0.28, 0.01) T3: −0.11 (−0.25, 0.03) Triglycerides: Per ln-unit: 0.03 (−0.09, 0.16), p-value = 0.586 T2: −0.03 (−0.17, 0.11) T3: 0.04 (−0.09, 0.18)
	Results: Lowest tertile used as reference group. **Confounding:** Maternal age, pre-pregnancy BMI, household income, infant sex, gestational age.						
				Pregnant Women			
Starling et al. (2014b) *Medium* for TC, HDL, and LDL *Low* for Triglycerides	Norway 2003–2004	Cross-sectional	Women in mid pregnancy (median = 18 wk of gestation) from MoBa N = 891	Plasma 2.25 (1.66–3.03)	Levels (mg/dL) of TC, HDL, LDL, and triglycerides (ln-mg/dL)	Regression coefficient per ln-unit or IQR increase in PFOA, or by quartiles	TC Per ln-unit: 2.58 (−4.32, 9.47) Per IQR: 1.55 (−2.60, 5.69) Q2: 1.49 (−6.49, 9.48) Q3: 3.54 (−4.51, 11.59)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Q4: 3.90 (−5.00, 12.80) HDL Per ln-unit: 2.13 (−0.26, 4.51) Per IQR: 1.28 (−0.15, 2.71) Q2: 0.22 (−2.38, 2.83) Q3: 2.31 (−0.59, 5.20) Q4: 3.42 (0.56, 6.28) LDL Per ln-unit: 2.25 (−3.97, 8.48) Per IQR: 1.36 (−2.38, 5.10) Q2: 0.94 (−6.08, 7.96) Q3: 4.16 (−3.19, 11.50) Q4: 3.35 (−4.35, 11.06) Triglycerides Per ln-unit: 0 (−0.07, 0.06) Per IQR: 0 (−0.04, 0.04) Q2: 0.03 (−0.04, 0.11) Q3: 0.01 (−0.08, 0.09) Q4: −0.04 (−0.12, 0.04)
	Results: Lowest quartile used as reference group. **Confounding:** Age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, and oily fish consumed daily.						
Skuladottir et al. (2015) *Medium*	Denmark 1988–1989	Cross-sectional	Pregnant women N = 854	Serum Mean = 4.1	Levels (mmol/L) of TC	Regression coefficient by quintile	TC: Q2: 0.10 (−0.19, 0.39) Q3: 0.39 (0.10, 0.67) Q4: 0.24 (−0.05, 0.54) Q5: 0.45 (0.15, 0.75)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							p-trend = 0.003
	Results: Lowest quintile used as the reference group. **Confounding:** Age, parity, education, smoking and pre-pregnancy BMI, total caloric intake, and intake of vegetables, meat, and meat products.						
Matilla-Santander et al. (2017) *Medium*	Spain 2003–2008	Cohort	Pregnant women from the Spanish INMA birth cohort N = 1240	Plasma Median = 2.35	Levels of TC (mg/dL), triglycerides (log10-mg/dL), and C-reactive protein (log10-mg/dL)	Percent change in median lipid level per log10-unit increase in PFOA	TC: 1.26 (0.01, 2.54) Triglycerides: −2.78 (−6.15, 1.42) with inverted U-shaped dose response
	Confidence: Triglycerides results considered *low* confidence because of a lack of fasting prior to blood sample collection. **Confounding:** Sub-cohort, country of birth, pre-pregnancy body mass index, previous breastfeeding, parity, gestational week at blood extraction, physical activity, and relative Mediterranean Diet Score.						
Starling et al. (2017) *Medium*	United States 2009–2014	Cohort	Pregnant women ages 16−45 from the Healthy Start study N = 598	Serum Median = 1.1	Levels of HDL (mg/dL) and triglycerides (ln-mg/dL)	Regression coefficient per ln-unit increase PFOA	HDL: 1.90 (0.22, 3.59) Triglycerides: −0.006 (−0.049, 0.036)
	Confounding: Maternal age, race/ethnicity, pre-pregnancy body mass index, education, gravidity, smoking, and gestational age at blood draw.						
Yang et al. (2020b) *Medium*	China 2013–2014	Cohort	Pregnant women ages 20–40 yr in early pregnancy N = 436	Serum 5.41 (3.40–9.08)	Levels (ln-mmol/L) of TC, triglycerides, HDL, and LDL; LDL/HDL ratio	Regression coefficient per ln-unit increase in PFOA, or by quartiles	TC Per ln-unit: −0.013 (−0.156, 0.131) Q2: 0.41 (0.11, 0.71) Q3: 0.26 (−0.12, 0.64) Q4: −0.20 (−0.59, 0.19) p-trend = 0.523 Triglycerides Per ln-unit: 0.044 (−0.131, 0.217) Q2: 0.33 (−0.10, 0.76) Q3: 0.23 (−0.22, 0.68) Q4: 0.07 (−0.40, 0.54)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							p-trend = 0.484 HDL Per ln-unit: 0.018 (−0.025, 0.062) Q2: 0.06 (−0.02, 0.14) Q3: 0.05 (−0.01, 0.11) Q4: 0.01 (−0.10, 0.12) p-trend = 0.837 LDL Per ln-unit: −0.046 (−0.143, 0.051) Q2: 0.23 (−0.01, 0.47) Q3: 0.07 (−0.18, 0.32) Q4: −0.24 (−0.50, 0.02) p-trend = 0.090 LDL/HDL ratio Per ln-unit: −0.042 (−0.075, −0.009) p-value < 0.05 Q2: 0.01 (−0.06, 0.07) Q3: −0.02 (−0.10, 0.06) Q4: −0.11 (−0.21, −0.01) p-trend = 0.019
	Results: Results by quartiles use lowest quartile as reference group. **Confounding:** Age, body mass index (BMI) at baseline, husband smoking, GDM, parity (nulliparous, multiparous), education, career, income, energy intake and physical activity in the late term of pregnancy, gestational weeks, carbohydrate, protein, SFA, MUFA, and PUFA intake in the late term of pregnancy.						
Dalla Zuanna et al. (2021) *Medium* for TC HDL; *low* for LDL	Italy 2017–2020	Cross-sectional	Pregnant women ages 18–44 from an area exposed to PFAS through drinking water	Serum 16.0 (6.7–35.5)	Levels (mg/dL) of TC, HDL, and LDL	Regression coefficient per ln-unit increase in PFOA, or by quartiles	TC Per ln-unit: −4.25 (−8.26, −0.23), p-value< 0.05 Q2: −1.12 (−13.24, −11.00)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 319 I Trimester: N = 101 II Trimester: N = 88 III Trimester: N = 130	I Trimester: 17.7 (8.9–35.9) II Trimester: 15.4 (4.7–35.5) III Trimester: 14.5 (6.5–34.4)			Q3: −12.65 (−25.25, −0.06), p-value< 0.05 Q4: −13.76 (−26.68, −0.83), p-value< 0.05 HDL Per ln-unit: 2.01 (0.53, 3.48), p-value< 0.05 Q2: 4.56 (0.13, 9.00), p-value< 0.05 Q3: 3.74 (−0.88, 8.37) Q4: 6.88 (2.14, 11.62), p-value< 0.05 LDL Per ln-unit: −6.74 (−10.15, −3.34), p-value< 0.05 Q2: −4.70 (−15.02, 5.62) Q3: −15.81 (−26.55, −5.07), p-value< 0.05 Q4: −21.17 (−32.22, −10.12), p-value< 0.05 First Trimester TC: 7.62 (−1.33, 16.57) HDL: 2.88 (−1.03, 6.80) LDL: 3.45 (−3.30, 10.22) Second Trimester TC: −0.55 (−7.20, 6.08) HDL: 1.34 (−1.85, 4.54) LDL: −1.80 (−6.93, 3.31) Third Trimester TC: −11.02 (−18.07, −3.96), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							HDL: 1.98 (−0.15, 4.13) LDL: −13.92 (−20.31, −7.52), p-value < 0.05
	Results: Results by quartile use lowest quartile as the reference group. **Confounding:** Age, number of previous deliveries, BMI, physical activity, smoking habits, country of birth, education level, laboratory in charge of the analyses of serum lipids, gestation weeks and reported fish consumption (in tertiles).						
			General Population				
Lin et al. (2009) *Medium*	United States 1999–2000 and 2003–2004	Cross-sectional	Adults ages 20+ years from NHANES N = 969	Serum Mean (SEM) = 1.48 (0.04) log10-ng/mL	Metabolic syndrome HDL cholesterol and metabolic syndrome triglycerides	OR per log10-unit increase in PFOA	Metabolic syndrome HDL cholesterol Model 4: 1.14 (0.84, 1.55) Model 5: 1.22 (0.86, 1.71) Metabolic syndrome triglycerides Model 4: 0.91 (0.69, 1.20) Model 5: 0.86 (0.65, 1.13)
	Outcome: Metabolic syndrome HDL cholesterol defined as HDL < 1.03 mmol/L in men and HDL < 1.29 mmol/L in women; metabolic syndrome triglycerides defined as triglycerides ≥ 1.69 mmol/L. **Confounding:** Model 4: Age, sex, race, health behaviors (smoking status, alcohol intake, and household income), measurement data (CRP and HOMA/insulin) and medications; additional confounding for model 5: Other components of the metabolic syndrome.						
Nelson et al. (2010) *Medium*	United States 2003–2004	Cross-sectional	Adults ages 20–80 yr from NHANES N = 860	Serum 3.9 (Range = 0.1–37.3)	Levels (mg/dL) of TC, HDL, non-HDL, LDL	Regression coefficient per unit increase in PFOA, or by quartiles	TC Per unit increase: 1.22 (0.04, 2.40) Q4: 9.8 (−0.2, 19.7) p-trend by quartiles = 0.07 HDL 20–80 yr Per unit increase: −0.12 (−0.41, 0.16) 60–80 yr Q4: −8.7 (−16.3, −1.1)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Non-HDL Per unit increase: 1.38 (0.12, 2.65) LDL Per unit increase: −0.21 (−1.91, 1.49)
	Results: Results by quartile use lowest quartile as the reference group. **Confounding:** Age, sex, race/ethnicity, SES, saturated fat intake, exercise, time in front of a TV or computer, BMI, alcohol consumption, and smoking.						
Liu et al. (2018b) *Medium*	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1871	Serum GM = 1.86	Levels of TC (mg/dL), LDL (mg/dL), HDL (mg/dL), triglycerides (ln-mg/dL)	Regression coefficient (SE) per ln-unit increase in PFOA	TC: 5.58 (2.03) p-value < 0.05 LDL: 4.47 (2.47) HDL: 1.93 (0.64) p-value < 0.01 Triglycerides: −0.08 (0.04)
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, antihyperglycemic, and antihyperlipidemic agents).						
Dong et al. (2019) *Medium*	United States 2003–2014	Cross-sectional	Adults aged 20–80 from NHANES N = 8,849	Serum Mean = 3.7	Levels (mg/dL) of TC, LDL, HDL	Regression coefficient per unit increase PFOA	TC all cycles: 1.48 (0.2, 2.8) Inconsistent associations with LDL or HDL across NHANES cycles.
	Confounding: Age, gender, race, family income index, BMI, waist circumference, physical activities, diabetes status, smoking status, number of alcoholic drinks per day.						
Jain et al. (2019d) *Medium*	United States 2004–2015	Cross-sectional	Members of NHANES Non-obese N = 1,053 females (NF) and 1,237 males (NM)	Serum GMs: Female = 2.5 Male = 3.4	Levels (mg/dL) of TC, LDL, HDL, triglycerides	Regression coefficient per log10-unit increase PFOA	TC OM: 0.0519 (0.0128, 0.0911) p-value = 0.01 No clear associations in NF, NM, or OF LDL

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			Obese N = 699 females (OF) and 640 males (OM)				OM: 0.0822 (0.0098, 0.1546) p-value = 0.03 No clear associations in NF, NM, or OF HDL: No clear associations Triglycerides: No clear associations
	Confounding: race/ethnicity, smoking status, age, PIR, fasting time, use of lipid-lowering medicine, physical exercise, survey year, daily dietary intake of TC, daily intake of total saturated fat, calories, caffeine, alcohol, protein intake.						
Fan et al. (2020) *Medium*	United States 2011–2014	Cross-sectional	Adults age 20+ from NHANES N = 1,067	Serum Median = 2.05 ng/mL	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per log10-unit increase in PFOA	TC: 6.74 (3.23, 10.2) p-value < 0.001 LDL: 4.67 (1.57, 7.77) p-value = 0.003 HDL: 2.23 (0.97, 3.49) p-value = 0.001 Triglycerides: 0 (−0.05, 0.04) p-value = 0.891
	Confounding: Age, gender, race, education level, PIR, BMI, smoking status, alcohol use, energy intake levels, screen time.						
Jain and Ducatman (2020) *Medium*	United States 2007–2014	Cross-sectional	Adults age 20+ from NHANES Non-diabetic non-LLM users: N = 2,872 Diabetic non-LLM users: N = 316 Non-diabetic LLM users: N = 519 Diabetic LLM users: N = 293	Serum Levels not reported	Apolipoprotein B (log10-mg/dL)	Regression coefficient per log10-unit increase in PFOA	Apolipoprotein B Non-diabetic non-LLM users: 0.03878, p-value < 0.01 Diabetic non-LLM users: −0.02055, p-value = 0.52 Non-diabetic LLM users: −0.01042, p-value = 0.59 Diabetic LLM users: −0.00058, p-value = 0.98

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Population: LLM = Lipid-lowering medication. **Confounding:** Gender, age, age squared, race/ethnicity, poverty-income ratio, fasting time in hours, log10-transformed BMI, smoking status, survey year, daily intake of cholesterol, caffeine, alcohol, total calories, total protein, and total fat.						
Steenland et al. (2009) *Medium* for TC, HDL *Low* for TG, LDL	United States 2005–2006	Cross-sectional	Adults ages 18+ from the C8 Health Project, current or former residents from areas supplied with contaminated water N = 46,494	Serum 26.6 (range: 0.25–17,556.6)	Levels (ln-mg/dL) of TC, LDL, HDL, non-HDL cholesterol, and triglycerides; TC/HDL ratio; high TC	Lipid levels, ratios: Regression coefficient per ln-unit increase in PFOA, or by deciles High TC: OR by PFOA quartiles	TC Per ln-unit: 0.01112 (SD = 0.00076) Decile 2: 0.01 (SE = 0.004), p-value = 0.0026 Decile 3: 0.02 (SE = 0.004), p-value < 0.0001 Decile 4: 0.03 (SE = 0.004), p-value < 0.0001 Decile 5: 0.04 (SE = 0.004), p-value < 0.0001 Decile 6: 0.03 (SE = 0.004), p-value < 0.0001 Decile7: 0.04 (SE = 0.004), p-value < 0.0001 Decile 8: 0.04 (SE = 0.004), p-value < 0.0001 Decile 9: 0.04 (SE = 0.004), p-value < 0.0001 Decile 10: 0.05 (SE = 0.004), p-value < 0.0001 HDL

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							0.00276 (SD = 0.00094) LDL 0.01499 (SD = 0.00121) Triglycerides 0.00169 (SD = 0.00219) TC/HDL ratio 0.00831 (SD = 0.0011) Non-HDL 0.01406 (SD = 0.03476) High TC Q2: 1.21 (1.12, 1.31) Q3: 1.33 (1.23, 1.43) Q4: 1.38 (1.28, 1.50) p-trend < 0.0001
	Outcome: High TC defined as ≥240 mg/dL. **Results:** Results by quartile use lowest quartile as the reference group; results by decile use lowest decile as the reference group. **Confounding:** Age, male gender, smoking status, education level, drinks alcohol, currently exercises, and BMI.						
Eriksen et al. (2013) *Medium*	Denmark 1993–1997	Cross-sectional	Adults ages 50–65 from DCH N = 753	Plasma Mean = 7.1	Levels of TC (mg/dL)	Regression coefficient per IQR increase in PFOA	4.4 (1.1, 7.8) p-value = 0.01
	Confounding: Sex, education, age, BMI, smoking status, intake of alcohol, egg, and animal fat and physical activity.						
Fisher et al. (2013) *Medium*	Canada 2007–2009	Cross-sectional	Adults ages 18–74 yr from CHMS, cycle 1 N = 2,700 TC, HDL, Non-HDL, TC/HDL ratio: N = 2,345	Plasma GM (SD) = 2.46 (1.83)	Levels (ln-mmol/L) of TC, HDL, LDL, non-HDL, triglycerides; TC/HDL ratio (ln-transformed); high cholesterol	Lipid levels, TC/HDL ratio: Regression coefficient per ln-unit increase in PFOA	TC 0.03 (−0.017, 0.07), p-value = 0.22 HDL 0.0009 (−0.04, 0.04), p-value = 0.96

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			LDL, triglycerides: N = 1,168 High cholesterol: N = 1,042			High cholesterol: OR per ln-unit increase in PFOA, or by quartiles	LDL 0.02 (−0.06, 0.091), p-value = 0.63 Non-HDL 0.036 (−0.01, 0.08), p-value = 0.13 Triglycerides −0.003 (−0.13, 0.12), p-value = 0.94 TC/HDL ratio 0.02 (−0.016, 0.0), p-value = 0.22 High cholesterol per ln-unit increase: 1.22 (0.89, 1.67) Q2: 1.61 (1.02, 2.53) Q3: 1.26 (0.76, 2.07) Q4: 1.50 (0.86, 2.62) p-trend = 0.10
	Outcome: High cholesterol defined as TC > 5.2 mmol/L. **Results:** Lowest quartile used as the reference group. **Confounding:** Lipid levels, TC/HDL ratio : Age, sex, marital status, BMI alcohol, smoking status and physical activity index; High cholesterol: Age, gender and alcohol consumption.						
Fitz-Simon et al. (2013) *Medium* for TC, HDL *Low* for TG, LDL	United States Baseline: 2005–2006; Follow-up: 2010	Cohort	Adults ages 20–60 from C8 Short-Term Follow-up Study living in West Virginia and Ohio with PFOA-contaminated drinking water	Serum Baseline GM (SD) = 74.8 (208.7) Follow-up GM (SD) = 30.8 (143.9)	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Percentage decrease (log10 of final and initial ratio change per log10 of ratio change in PFOA)	TC: 1.65 (0.32, 2.97) R^2 = 0.03 LDL: 3.58 (1.47, 5.66) R^2 = 0.06 HDL: 1.33 (−0.21, 2.85) R^2 = 0.04 Triglycerides: −0.78 (−5.34, 3.58)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 560 (N = 521 for LDL analysis)				R^2 = 0.08
	Confounding: Age, sex, interval between measurements, and fasting status.						
Winquist and Steenland (2014a) *Medium*	United States 2008−2011	Cohort	Workers at a Mid-Ohio Valley chemical plant and residents of the surrounding community from C8 Health Project N = 32,254	Serum 26.1 (12.8–68.1)	Hypercholesterolemia	HR by quintiles	Hypercholesterolemia Whole cohort Q2: 1.24 (1.15, 1.33) Q3: 1.17 (1.09, 1.26) Q4: 1.19 (1.11, 1.27) Q5: 1.19 (1.11, 1.28) p-trend = 0.005 Men 40–59 yr of age Q2: 1.38 (1.21, 1.56) Q3: 1.32 (1.17, 1.50) Q4: 1.31 (1.16, 1.48) Q5: 1.44 (1.28, 1.62) p-trend < 0.001
	Outcome: Hypercholesterolemia cases were identified based on self-reported diagnosis. **Results:** Lowest quintile used as the reference group. **Confounding:** Age, sex, years of schooling, race, smoking, smoking duration, smoking pack-years, regular alcohol consumption, BMI, self-reported type-2 diabetes.						
Donat-Vargas et al. (2019b) *Medium*	Sweden 1990–2013	Cohort	Non-diabetic adults ages 30–60 at baseline in Västerbotten Intervention Programme (VIP) N = 187	Plasma Baseline median = 2.9 Median at 10-yr follow-up = 2.7	Levels (mmol/L) of TC and triglycerides	Regression coefficient per 1-SD increase in PFOA, or by tertiles	Per change in PFOA TC Baseline: −0.19 (−0.36, −0.02) Follow-up: −0.03 (−0.21, 0.15) Prospective: −0.12 (−0.23, 0) Triglycerides Baseline: −0.03 (−0.14, 0.07) Follow-up: −0.08 (−0.20, 0.04)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Prospective: −0.07 (−0.13, −0.01) Overall non-significant inverse association using tertiles
	Confounding: Gender, age, education, sample year, body mass index, smoking habit, alcohol consumption, physical activity and healthy diet score.						
Lin et al. (2019) *Medium*	United States 1996–2014	Cohort and cross-sectional	Prediabetic adults age 25+ from the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) N = 940 (888 not on metformin)	Plasma Median = 4.9	Levels (mg/dL) of TC, LDL, HDL, triglycerides, non-HDL, and very low-density lipids (VLDL); hypercholesterolemia, hypertriglyceridemia	Regression coefficient per doubling PFOA HR or OR for hypercholesterolemia or hypertriglyceridemia per doubling of PFOA	<u>Cross-sectional</u> TC: 6.09 (3.14, 9.04); p < 0.01 LDL: 2.93 (0.22, 5.63); p-value < 0.05 HDL: −0.49 (−1.38, 0.40) Triglycerides: 17.75 (9.77, 25.74); p-value < 0.01 VLDL: 3.66 (2.18, 5.15); p-value < 0.01 Hypercholesterolemia at baseline OR: 1.29 (1.05, 1.57) Hypertriglyceridemia at baseline OR: 1.48 (1.21, 1.81) <u>Prospective</u> Hypercholesterolemia HR: 1.06 (0.94, 1.19) Greater effect in the placebo group Hypertriglyeridemia HR: 1.23 (1.04, 1.45) Greater effect in the placebo group

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Confounding: Age, sex, race and ethnicity, marital status, educational attainment, drinking, smoking, percent of daily calorie from fat intake, daily fiber intake, physical activity level, and waist circumference at baseline.						
Canova et al. (2020) *Medium*	Italy 2017–2019	Cross-sectional	Residents of PFAS "Red Area" with contaminated public water supply ages 20–39 N = 15,720 (7,620 female, 8,100 male)	Serum Median = 35.8 Female = 22.65 Male = 58.3	Levels (mg/dL) of TC, LDL, HDL, non-HDL, and triglycerides	Regression coefficient per ln-unit increase PFOA, or by decile	TC 1.94 (1.48, 2.41) p-value for interaction with sex = 0.15 Associations for deciles 2–10 consistently increase from 2.83 to 9.10 LDL 7.79 1.12 (0.71, 1.52) p-value for interaction with sex = 0.577 Associations for deciles 2–10 moderately increase from 1.4 to 5.3 HDL 0.49 (0.32, 0.67) Male: 0.13 (−0.11, 0.37) Female: 0.83 (0.57, 1.1) p-value for interaction with sex < 0.001 Associations for deciles 2–10 moderately increase from 0.45 to 2.07 Triglycerides 0.02 (0.01, 0.03) p-value for interaction with sex = 0.815 Associations for deciles 2–10 increase from 0.04 to 0.09

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Results: Lowest decile used as the reference group. **Confounding:** Age, BMI, time lag between enrollment and beginning of study, physical activity, smoking habits, country of birth, alcohol consumption, education level, laboratory in charge of analyses, reported food consumption.						
Lin et al. (2020c) *Medium*	Taiwan 2016–2017	Cross-sectional	Adults aged 55 to 75 that resided in the study area for more than 10 yr and not taking lipid-lowering medication N = 352	Serum 8.6 (6.2–11.6)	Levels (mg/dL) of TC, HDL, LDL, and triglycerides	Regression coefficient by quartiles	TC Q2: 2.48 (−8.00, 12.96) Q3: 2.88 (−7.64, 13.40) Q4: 4.04 (−6.65, 14.73) p-trend = 0.47 HDL Q2: 0.45 (−3.57, 4.48) Q3: −3.36 (−7.40, 0.68) Q4: −1.72 (−5.82, 2.38) p-trend = 0.18 LDL Q2: 4.79 (−4.65, 14.23) Q3: 8.72 (−0.76, 18.20) Q4: 8.06 (−1.57, 17.69) p-trend = 0.07 Triglycerides Q2: 0.55 (−17.93, 19.03) Q3: 14.43 (−4.13, 32.98) Q4: 15.45 (−3.40, 34.30) p-trend = 0.05
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, smoking status, and drinking status.						
Liu et al. (2020a) *Medium*	United States 2004–2007	Randomized clinical trial	Adults from POUNDS Lost study ages 20+ N = 326	Plasma Median = 4.6	Levels (mg/dL) of TC, triglycerides, and apolipoproteins log10-ApoB, ApoE, and ApoC-III	Least-squared means (LSM) by tertile PFOA	TC T1: 189.1 (7.9) T2: 189.3 (7.6) T3: 188.4 (7.7) p-trend = 0.67 Triglycerides T1: 111.1 (11.2) T2: 137.3 (10.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							T3: 131.8 (10.9) p-trend = 0.06
	Results: LSM are presented with standard error in parentheses. **Confounding:** Age, sex, race, educational attainment, smoking status, alcohol consumption, physical activity, BMI, regular lipid-lowering medication use, dietary intervention groups.						
Han et al. (2021) *Medium*	China 2016–2017	Case-control	Adults ages 25 to 74 including type 2 diabetes cases and healthy controls N = 304	Serum Cases: 10.05 (6.75–17.05) Controls: 11.40 (9.20–17.40)	Levels (log10-mmol/L) of TC, HDL, LDL, and triglycerides	Regression coefficient per log10-unit increase in PFOA	TC: 0.01 (−0.05, 0.07) HDL: −0.03 (−0.09, 0.04) LDL: 0.02 (−0.07, 0.10) Triglycerides: 0.09 (−0.06, 0.23)
	Confounding: Age, sex, BMI.						
Jeddi et al. (2021a) *Medium*	Italy 2017–2019	Cross-sectional	Residents aged 20–39 from the PFAS-contaminated Veneto region N = 15,876	Serum GM (range): 67.66 (0.70–1400.0)	Reduced HDL, elevated triglycerides	OR per ln-unit increase in PFOA	Reduced HDL: 0.93 (0.89, 0.97), p-value < 0.05 Elevated triglycerides: 1.10 (1.05, 1.16), p-value < 0.05
	Outcome: Reduced HDL defined as HDL < 40 mg/L for male or HDL < 50 mg/L for female; elevated triglycerides defined as triglycerides ≥ 175 mg/dL. **Confounding:** Age, gender, time lag between the beginning of the study and blood sampling center where BP has been measured, education, number of deliveries, physical activity, country of birth, diet, alcohol intake, and smoking status, and other components of metabolic syndrome.						
			Occupational Populations				
Olsen et al. (2003) *Medium*	United States, Belgium 1994–2000	Cross-sectional	Current and former workers at two fluorochemical production plants Male N = 421, Female	Serum Antwerp Mean (SD) = 1.03 ppm (1.09); Decatur = 1.90 ppm (1.59)	Levels of cholesterol (ln-mg/dL)	Regression coefficient per unit increase in PFOA	Cholesterol 0.032 (0.013, 0.051)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 97, Regression analysis N = 174				
	Confounding: Age, BMI, drinks/day, cigarettes/day, location, entry period, baseline years worked.						
Costa et al. (2009) *Medium*	Italy 2007	Cross-sectional	Current and former male employees of an Italian chemical production plant, Comparison of means analysis N = 68, Exposed vs. Unexposed analysis N = 141, Continuous regression analysis N = 56	Serum Production workers (2007): 3.89 µg/mL (2.18–18.66 µg/mL)	Levels of TC and HDL (mg/dL)	Comparison of mean outcome (Exposed vs. unexposed workers) Regression coefficient (exposed workers vs. all workers) Regression coefficient per unit increase in PFOA	No significant differences in comparison of mean HDL Comparison of mean TC p-value = 0.003 TC Exposed vs. Unexposed: 21.7 (6.83, 36.6), p-value = 0.005 Continuous: 0.028 (0.002, 0.055), p-value < 0.05 HDL Exposed vs. Unexposed: 2.42 (−2.30, 7.13) Continuous: −0.018 (−0.047, 0.012)
	Confounding: Age, job seniority, body mass index, smoking and alcohol consumption. Additional confounding for continuous regression analyses: year of observation.						
Sakr et al. (2007a) *Medium*	United States 2004	Cross-sectional	Active employees at a Washington Works site where APFO is used N = 1,019 Workers not on lipid-lowering medications	Serum Mean (SD) = 0.428 (0.860) ppm, Range = 0.005 –9.550 ppm	Levels (mg/dL) of TC, LDL, HDL and levels (ln-mg/dL) of VLDL and triglycerides	Regression coefficient per unit increase PFOA	TC 4.036 (1.284) p-value = 0.002 Workers not on lipid-lowering medications: 5.519 (1.467) p-value < 0.001

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 840				LDL 2.834 (1.062) p-value = 0.008 Workers not on lipid-lowering medications: 3.561 (1.213) p-value = 0.003 HDL −0.178 (0.432) p-value = 0.680 Workers not on lipid-lowering medications: 0.023 (0.508) p-value = 0.964 VLDL 0.045 (0.021) p-value = 0.031 Workers not on lipid-lowering medications: 0.055 (0.025) p-value = 0.026 Triglycerides 0.018 (0.021) p-value = 0.384 Workers not on lipid-lowering medications: 0.030 (0.024) p-value = 0.207
	Results: Reported as effect estimate (standard error). **Confounding:** Age, gender, BMI.						
Olsen et al. (2000) *Low*	United States	Cross-sectional	Male workers involved in	Serum	Level (mg/dL) of HDL	Regression coefficient per	HDL

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	1993–1997		ammonium perfluorooctanoate production N = 265	1993: 1.1 (Range = 0.0–80.0) ppm 1995: 1.2 (Range = 0.0–114.1) ppm 1997: 1.3 (Range = 0.1–81.3) ppm		1 ppm increase in PFOA	1993: −0.14 (SD = 0.33), p-value = 0.67 1995: −0.10 (SD = 0.08), p-value = 0.18 1997: −0.19 (SD = 0.13), p-value = 0.16
	Confounding: Age, alcohol and cigarette use, BMI, testosterone.						

Notes: APFO = ammonium perfluorooctanoate; ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; BP = blood pressure; CHMS = Canadian Health Measures Survey; CRP = C-reactive protein; DCH = Diet, Cancer and Health; DPP = Diabetes Prevention Program; DPPOS = Diabetes Prevention Program Outcomes Study; ETS = environmental tobacco smoke; EYHS = European Youth Study; GM = geometric mean; GMR = geometric mean ratio; HDL = high-density lipids; HELIX = Human Early-Life Exposome; HR = hazard ratio; IQR = interquartile range; LDL = low-density lipids; HOME = Health Outcomes and Measures of the Environment; INMA = Infancia y Medio Ambiente (Environment and Childhood) Project; KorEHS-C = Korea Environmental Health Survey in Children and Adolescents; LLM = Lipid-lowering medication; mo = months; MoBa = Norwegian Mother and Child Cohort Study; MUFA = monounsaturated fatty acid; NF = non-obese female; NHANES = National Health and Nutrition Examination Survey; NM = non-obese male; OF = obese female; OM = obese male; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PIR = poverty-income ratio; POUNDS = Preventing Overweight Using Novel Dietary Strategies; PUFA = polyunsaturated fatty acid; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; S-MBCS = Shanghai-Minhang Birth Cohort Study; SD = standard deviation; SE = standard error; SEM = standard error of the mean; SFA = saturated fatty acid; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; TC = total cholesterol; TG = triglycerides; VIP = Västerbotten Intervention Programme; VLDL = very low-density lipoprotein; yr = years.

[a] Exposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

D.6 Endocrine

Table D-15. Associations Between PFOA Exposure and Endocrine Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
General Population							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Lebeaux et al. (2020) *High* for Cord serum thyroid hormones; *Medium* for maternal serum thyroid hormones	United States 2003–2007	Cohort	Mother-infant pairs from Health Outcome Measures of the Environment (HOME) Study N = 256 for cord serum N = 185 for maternal serum	Cord serum 5.6 Maternal serum 5.5	Levels of TSH (µIU/L), TT4 (µg/dL), TT3 (ng/dL), FT4 (ng/dL), and FT3 (pg/mL)	Regression coefficient per log2-unit increase in PFOA	Cord serum TSH: 0.06 (–0.08, 0.19) TT4: 0.03 (–0.02, 0.08) TT3: –0.01 (–0.09, 0.06) FT4: –0.01 (–0.04, 0.03) FT3: –0.01 (–0.06, 0.03) Maternal serum TSH: 0.09 (–0.14, 0.33) TT4: –0.03 (–0.10, 0.04) TT3: –0.01 (–0.05, 0.04) FT4: –0.01 (–0.06, 0.03) FT3: –0.01 (–0.04, 0.01)
	Confounding: Individual PFAS, maternal age at delivery, race/ethnicity, marital status at baseline, maternal education level, household income, mean log10 cotinine, maternal alcohol usage during pregnancy, nulliparity, maternal BMI based on pre-pregnancy weight in pounds, child's sex, gestational week at blood draw for PFAS measurement, and (for cord serum only) delivery mode.						
Blake et al. (2018) *Medium*	Fernand, Ohio, USA 1991–2008	Cohort	Fernald Community Cohort, Median age 38 yr at enrollment, N = 122 for TSH measurements; 47 male and 75 female N = 144 for TT4 measurements; 63 males and 81 females	Drinking water Serum 12.7	Levels of TSH (ln-µIU/mL) and TT4 (ln-µg/dL)	Percent change per IQR increase in PFOA	TSH −0.48 (−9.68, 9.65) p-value = 0.92 Males: 9.38 (−7.47, 29.3) p-value = 0.47 Females:−6.64 (−17.8, 5.97); p-value = 0.31 TT4 −1.18 (−5.12, 2.92); p-value = 0.57 Males: −2.71 (−9.05, 4.08); p-value = 0.43 Females: −1.62 (−6.88, 3.94); p-value = 0.56
	Confounding: Age, year of measurement, sex, education, income, marital status, BMI.[c]						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Jain and Ducatman (2019b) *Medium*	United States 2007–2012	Cross-sectional	Adults from NHANES aged 20+ Glomerular filtration (GF) status: GF-1 = 1,653 GF-2 = 720 GF-3A = 114 GF-3B/4 = 62	Serum Levels not reported	Levels of TSH (log-µIU/mL), TGN (log-ng/mL), TT4 (log-µg/dL), FT4 (log-ng/dL), TT3 (log-ng/dL), FT3 (log-pg/mL)	Regression coefficient per log10-unit increase in PFOA	TSH GF-1: −0.004, p-value = 0.89 GF-2: 0.085, p-value < 0.01 GF-3A: −0.229, p-value = 0.04 GF-3B/4: 0.012, p-value = 0.88 FT4 GF-1: −0.010, p-value = 0.17 GF-2: −0.020, p-value = 0.08 GF-3A: 0.038, p-value = 0.07 GF-3B/4: −0.040, p-value = 0.15
	GF Stages: GF-1: GFR ≥ 90 mL/min/1.73m^2; GF-2: GFR between 60 and 90 mL/min/1.73m^2; GF-3A: GFR between 45 and 60 mL/min/1.73m^2; GF-3B/4: GFR between 15 and 45 mL/min/1.73m^2. **Confounding:** Gender, race/ethnicity, iodine deficiency status, age, BMI, fasting time, poverty-income ratio, total calories consumed during the last 24 hr, smoking status, use of drugs.						
Jain (2013) *Low*	United States 2007–2008	Cohort	Adults and children from NHANES aged 12+ N = 1,540 including children	Serum Total cohort Lowest tertile Tertile 1 ≤ 3.3 Highest tertile Tertile3 ≥ 5.1	Levels of TSH (µIU/L), FT3 (pg/L), TT3 (fg/dL), FT4 (pg/L), TT4 (pg/L), TGN	Regression coefficient per log10-unit increase in PFOA, or by tertiles	TSH: Significantly increased levels (Tertile 3 vs. Tertile 1), p-value < 0.01 TT3: 0.032, p-value = 0.01 FT3, FT4, TT4, TGN: No statistically significant associations
	Results: Lowest tertile used as the reference group. **Confounding:** Gender, race, age, iodine deficiency, iodine replete.						
Lewis et al. (2015) *Low*	United States 2011–2012	Cross-sectional	Children and adults from NHANES, aged 12–80 145 females 12 to <20 680 females 20–80	Serum Females 12–20: 1.53 Females 20–40: 1.49 Females 40–60: 1.62 Females 60–80: 2.55	Levels of TSH (µIU/mL), TT3 (ng/dL), FT3 (pg/mL), TT4 (µg/mL), FT4 (ng/dL)	Percent change per doubling of PFOA	TSH Females 12–20: 16.6 (2.6, 28.6) 20–80: No associations Males, all age groups: No associations TT3 Females

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			158 males 12 to < 20 699 men	Males 12–20: 1.85 Males 20–40: 2.35 Males 40–60: 2.31 Males 60–80: 2.48			60–80: 3.3 (0.6, 6) Younger than 60: No associations Males, all age groups: No associations FT3 Females 60–80: 1.8 (0.2, 3.4) Younger than 60: No associations Males, all age groups: No associations TT4 Females 12–20: 4.1 (0.6, 8.9), p-value < 0.1 20–80: No associations Males 40–60: −3.1 (−6.2, 0.1), p-value < 0.10 12–40 or 60–80: No associations FT4 Females 20–40: 2.0 (0, 4.1) 12–20 or 40–80: no associations Males, all age groups: No associations
	Confounding: Age, BMI, poverty-income ratio, serum cotinine, and race/ethnicity.						
Byrne et al. (2018) *Low*	St. Lawrence Island, Alaska, USA 2013–2014	Cross-sectional	Alaska Natives, aged 18–45 N = 85 38 men 47 women	Serum 1.01 Male: 1.47 Female: 0.772	Levels of TSH (ln-µIU/mL), TT3 (pg/mL), FT3 (ng/dL), TT4 (µg/dL),	Regression coefficient per ln-unit increase in PFOA	TSH Total cohort: 0.63 (0.22, 1.03), p-value < 0.005 TT3

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
					FT4 (ng/dL)		Total cohort: −7.67 (−18.61, 3.27), p-value = 0.17 Males: −14.24 (−26.24, −2.24), p-value = 0.02 Females: 11.29 (−5.25, 27.83) p-value for sex interaction = 0.18 FT3, TT4, FT4: No statistically significant associations
	Confounding: Age, sex, smoking status.						
Convertino et al. (2018) *Low*	Scotland 2008–2011	Controlled trial	Adults, Solid-tumor cancer patients 49	Serum Median PFOA ranging from 9 to 1,530 nmol/mL	Levels of FT4 (mmol/L)	Regression coefficient per unit increase in PFOA Median and mean FT4 levels by exposure categories	0.003, p-value = 0.21 Increasing trend in FT4 by exposure categories
	Confounding: None given.						
Heffernan et al. (2018) *Low*	United Kingdom 2015	Cross-sectional	Women aged 20–45 yr, with (cases) or without (controls) polycystic ovarian syndrome (PCOS) N = 59	Serum Geometric mean = 2.49 for both cases and controls	Levels of TSH (mU/L), FT3 (ln-pmol/L), FT4 (ln-pmol/L)	Regression coefficient per ln-unit increase in PFOA	TSH PCOS cases: 0.86, p-value < 0.01 PCOS controls: −0.13, p-value = 0.75 FT3, FT4: No statistically significant associations
	Confounding: Serum albumin.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Zhang et al. (2018b) *Low*	China 2013–2016	Cross-sectional	Women aged 20–40 yr, with (cases) or without (controls) POI N = 120	Plasma Cases:11.10 Controls: 8.35	Levels (ng/mL) of TSH, FT3, FT4	Regression coefficient per log-unit increase in PFOA	TSH POI cases: 1.39 (0.18, 2.59) POI controls: 1.65 (0.86, 2.44) FT4: POI cases: −3.42 (−5.39, −1.46) POI controls: No association FT3 No statistically significant associations
	Comparison: Logarithm base not specified. **Confounding:** Age, BMI, education, income, sleep, and parity.						
				Children			
Xiao et al. (2019) *High*	Faroe Islands, Denmark 1994–1995	Cohort	Pregnant women and their infant children N = 172 and 153 for measurements in maternal and cord serum, respectively	Maternal blood Geometric mean = 2.37 µg /g	Cord serum levels of TSH (log-IU/L), T4 (log-pmol/L), FT3 (log-pmol/L), FT4, (log-pmol/L) FT3 resin uptake, FT4 index (FTI) (log-IU/L)	Regression coefficient per log2-unit increase in PFOA	TSH :23.1 (1.9, 48.6) T4: 1.9 (−4.1, 8.3) FT3: 0.5 (−5.6, 6.9) FT4: 1.9 (−11.5, 17.2)
	Confounding: Child sex (in detailed results), parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury.						
Kim et al. (2020a) *High*	South Korea 2012–2017	Cohort	Children, aged 2, 4, 6 yr N = 181–660	Serum Age 2: 4.39 Age 4: 3.65 Age 6: 3.83	Levels of TSH, FT4 (ng/dL), and T3 (ng/dL) at age 6	Regression coefficient per ln-unit increase in PFOA	FT4 at age 6 All: 0.07, p-value < 0.05 Boys: 0.04, p-value < 0.05 No interaction with sex

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
					Subclinical hypothyroidism	Subclinical hypothyroidism: OR per increase in PFOA	TSH, T3: No statistically significant associations between or within age groups
	Confounding: Age, sex, dietary iodine intake.						
Kang et al. (2018) *Medium*	Korea 2012–2014	Cross-sectional	Children from Seoul and Gyeonggi aged 3–18 N = 147	Serum 1.88	Levels (ng/dL) of TSH, FT4	Regression coefficient per ln-unit increase in PFOA	TSH: −0.14 (−0.62, 0.34), p-value = 0.341 FT4: 0.04 (−0.01, 0.09), p-value = 0.075
	Confounding: Age, sex, BMI z-score, household income, secondhand smoking.						
Aimuzi et al. (2019) *Medium*	China 2012–2013	Cross-sectional	Pregnant women and their children N = 567 Male children = 305 Female children = 262	Cord blood 7.57	Levels of TSH (ln-mIU/L), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per ln-unit increase in PFOA	FT4 All children: 0.14 (0.02, 0.26) Boys: 0.25 (0.08, 0.42) Girls: 0.01 (−0.16, 0.18)
	Confounding: Maternal age, fish intake, parity infant sex, gestational age at delivery, and maternal pre-pregnancy BMI.						
Itoh et al. (2019) *Medium*	Japan 2003–2005	Cohort	Pregnant women and their children 259 male children 240 female children	Plasma 2.00	Levels of TSH (ln-µU/mL), FT3 (ln-pg/mL), FT4 (ln-pg/mL), TPOAb (ln-IU/mL), TgAb (ln-IU/mL)	Regression coefficient per ln-unit increase in PFOA	TgAb Boys, maternal TA-negative: −0.13 (−0.27, −0.002), p-value = 0.047 All boys or maternal TA-positive: no association Girls, maternal TA-positive: 0.27 (0.95, 0.44), p-value = 0.007 All girls or maternal TA-negative: no association TSH, FT3, FT4, TPOAb: No statistically significant associations
	Confounding: Age at delivery, parity, educational level, alcohol consumption, smoking during pregnancy, pre-pregnancy BMI.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			Pregnant Women				
Dreyer et al. (2020) *High*	Denmark 2010–2012	Cohort	Pregnant women from Odense Child Cohort (OCC) N = 1,048	Serum 1.64	Levels of diurnal urinary (dU) cortisol (nmol/24-hr), dU-cortisone (nmol/24-hr), dU-cortisol/cortisone, serum cortisol (nmol/L)	Percent change per 2-fold increase in PFOA	Serum cortisol: –15.8 (–33.1, 1.5) Tertile 2: –19.6 (–51.0, 11.8) Tertile 3: –35.1 (–69.4, –0.7), p-value < 0.05 p-trend = 0.05 dU-cortisol, dU-cortisone, dU-cortisol/cortisone: No statistically significant associations
	Confounding: Age, parity, and offspring sex.						
Xiao et al. (2019) *High*	Faroe Islands, Denmark 1994–1995	Cross-sectional	Pregnant women and their children, Maternal age 28 (SD = 5.6) N = 172 and 153 for measurements in maternal and cord serum, respectively	Maternal blood Geometric mean = 2.37 µg/g	Maternal serum levels of TSH (log-IU/L), T4 (log-pmol/L), FT3 (log-pmol/L), FT4 (log-pmol/L) FT3 resin uptake FT4 index	Regression coefficient per log2-unit increase in PFOA	TSH: 12.6 (−4.5, 32.8) T4: 0.7 (−5.5, 7.3) FT3: 3.1 (−1.2, 7.6) FT4: −0.4 (−5.4, 4.8)
	Confounding: Child sex (in detailed results), parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury.						
Preston et al. (2018) *Medium*	United States 1999–2002	Cross-sectional	Pregnant women and their children N = 726 and 718 for free T4 and TSH	Maternal plasma 5.6	Levels of TSH (mIU/mL), FT4 µg/dL), TT4 (µg/dL)	Percent difference in hormone level per IQR increase in PFOA	FT4: −1.87 (3.4, −0.31) TSH: 0.28 (−9.26, 10.8) TSH TPOAb-negative: 0.88 (−9.22, 12.1) TSH TPOAb-positive: −19 (−35.1, 1.15)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			measures, respectively				p-value for effect modification by TPOAb status = 0.08
	Confounding: Maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw.						
Itoh et al. (2019) *Medium*	Japan 2003–2005	Cross-sectional	Pregnant women and their children N = 499	Plasma 2.00	Levels of TSH (ln-μU/mL), FT3 (ln-pg/mL), FT4 (ln-pg/mL), TPOAb (ln-IU/mL), TgAb (ln-IU/mL)	Regression coefficient per ln-unit increase in PFOA	TPOAb: −0.23 (−0.44, −0.02), p-value = 0.033 TgAb: −0.01 (−0.21, 0.19), p-value = 0.929
	Confounding: Age at delivery, parity, pre-pregnancy BMI, educational level, alcohol consumption, and smoking habits.						
Aimuzi et al. (2020) *Medium*	Shanghai, China 2013–2016	Cross-sectional	Pregnant women prior to 16 wk of gestation N = 1877 1,615 TPOAb-negative 222 TPOAb-positive	Serum Total cohort: 12.32 TPOAb-negative: 12.32 TPOAb-positive: 12.3	Levels of TSH (ln-mIU/L), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per ln-unit increase in PFOA	FT4 Total cohort: 0.12 (0.02, 0.23) TPOAb-negative: 0.11 (−0.01, 0.22) TPOAb-positive: 0.14 (−0.20, 0.48) TSH, FT3: All associations not statistically significant
	Confounding: Pre-pregnancy BMI, gestational age at thyroid hormone (TH) measurement, fish intake, maternal age, hospital indicators, maternal education, difference between PFAS and THs measured gestational weeks.						

Notes: BMI = body mass index; FT3 = free triiodothyronine; FT4 = free thyroxine; GF = glomerular filtration; GFR = glomerular filtration rate; HOME = Health Outcomes and Measures of the Environment; NHANES = National Health and Nutrition Examination Survey; POI = premature ovarian insufficiency; T3 = triiodothyronine; T4 = thyroxine; TA = thyroid antibodies; TgAb = thyroglobulin antibody; TGN = thyroglobulin; TPOAb = thyroid peroxidase antibody; TSH = thyroid stimulating hormone; TT3 = total triiodothyronine; TT4 = total thyroxine; USA = United States of America; yr = years.

[a] Exposure levels are reported as median unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.7 Metabolic/Systemic

Table D-16. Associations Between PFOA Exposure and Metabolic Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Ashley-Martin et al. (2017) *High*	Canada, Recruitment 2008–2011	Cohort	Pregnant women and their children, from the MIREC Study N = 1,176	Maternal blood 1.7	Adiponectin, leptin	Regression coefficient per log10-unit increase in PFOA	Adiponectin, leptin: No statistically significant associations
	Confounding: Maternal age, pre-pregnancy body mass index, sex, and parity.[c]						
Buck et al. (2018) *High*	United States, 2003–2006	Cohort	Pregnant women and their children in the HOME study N = 230	Maternal serum 5.6	Adiponectin, leptin	Percent change per doubling of PFOA	Adiponectin, leptin: No statistically significant associations
	Confounding: Maternal age, race, education, income, parity, maternal body mass index, serum cotinine, delivery mode, and infant sex.						
Chen et al. (2019b) *High*	China, 2012–2017	Cohort	Infants followed up at age 5, N = 404	Cord blood 6.74	BMI, WC, body fat, waist-to-height ratio	Regression coefficient per ln-unit increase in PFOA, or by tertile	BMI, WC, body fat, waist-to-height ratio: No statistically significant association
	Confounding: Maternal age, maternal pre-pregnancy BMI, gestational week at delivery, maternal education, paternal smoking during pregnancy, and parity.						
Jensen et al. (2020a) *High*	Denmark, 2010–2012	Cohort	Pregnant women and their infants assessed at birth, 3 mo, and 18 mo, Odense Child Cohort N = 593	Maternal serum 1.62	BMI z-score, WC	Regression coefficient per unit increase in PFOA	BMI z-score, WC: No statistically significant associations
	Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI^2, education, smoking, sex, visit, adiposity marker at birth.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Minatoya et al. (2017) *High*	Japan, 2002–2005	Cohort	Pregnant women and their children N = 168	Serum 1.4	Adiponectin, leptin	Regression coefficient per log10-unit increase in maternal serum PFOA	Adiponectin, leptin: No statistically significant associations
	Confounding: Maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age, infant sex.						
Alderete et al. (2019) *Medium*	United States, 2001–2012	Cohort	Obese Hispanic children, 8–14 yr N = 39	Plasma GM = 2.78	Glucose (fasting, 2 hr, AUC), Insulin (fasting, 2 hr, AUC), HOMA-IR	Regression coefficient per ln-unit increase in PFOA	Glucose (2 hr): 30.6 (8.8, 52.4), p-value < 0.05 Glucose (fasting, AUC), insulin, HOMA-IR: No statistically significant association
	Confounding: sex, baseline social position (categorical), baseline outcome, baseline and change in age at follow-up, pubertal status (categorical), baseline and change in body fat percent at follow-up.						
Braun et al. (2016) *Medium*	United States, recruitment 2003–2006	Cohort	Pregnant women and their children N = 285	Serum 5.3	Overweight/obese, BMI z-score, WC, body fat percentage, weight-for-age	BMI z-score: Regression coefficient by Terciles Other outcomes: Mean change between 2 and 8 yr by tercile	BMI z-score: 0.44 (0.13, 0.74) T2: 0.44 (0.23, 0.64) T3: 0.37 (0.14, 0.6) WC: 4.3 (1.7, 6.9) Body fat percent: 3.6 (1.8, 5.5) Weight-for-age T2: 0.49 (0.31, 0.67) T3: 0.43 (0.23, 0.64) Overweight/obese: No statistically significant association
	Results: Lowest tercile used as the reference group. Tercile 1 (0.5–4.3 ng/mL), tercile 2 (4.4–6.7 ng/mL), tercile 3 (6.8–26 ng/mL) maternal PFOA. **Confounding:** Maternal age, race, education, income, parity, employment, marital status, depressive symptoms, BMI at 16 wk gestation, fruit/vegetable consumption, fish consumption, prenatal vitamin use, maternal serum cotinine concentrations, child age in months.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Conway et al. (2016) *Medium*	United States, 2005–2006	Cross-Sectional	Children living in six PFOA-contaminated water districts with type 1 diabetes N = 39	Serum Mean = 68.4 ng/L	T1D, T2D, and uncategorized diabetes	OR per ln-unit increase in PFOA	T1D: 0.52 (0.54, 0.97) T2D and uncategorized diabetes: No statistically significant association
	Confounding: Age, sex, race, BMI, eGFR, hemoglobin, iron.						
Domazet et al. (2016) *Medium*	Denmark, 1997–2009	Cohort	Children followed through ages 9, 15, and 21, N = 176	Blood, plasma, glucose Age 15 Males: 9.7 Females: 9.0 Age 21 Males: 3.1 Females: 2.7	WC (cm), HOMA-B, HOMA-IR, insulin, glucose, skinfold thickness, BMI	Percent change in WC at 21 yr old in higher levels of PFOA at age 21 Percent change in HOMA-B at age 15 per 10-unit increase in PFOA exposure at age 9	WC: –11.11 (−19.90, 1.36), p-value = 0.03 HOMA-B: −10.93 (−19.67, −1.11) HOMA-IR, insulin, glucose, skinfold thickness, BMI: No statistically significant association
	Confounding: sex, age, and outcome levels at baseline (9 yr of age), and ethnicity, maternal parity, and maternal income in 1997 (9 yr of age). Waist circumference was adjusted for height in order to account for body size.						
Domazet et al. (2020) *Medium*	Denmark, 1997	Cross-sectional	Children from the European Youth Heart Study aged 9 yr N = 242	Plasma Boys: 9.5 Girls: 9.5	Leptin, fat mass, adiponectin	Percent change per 10% increase in PFOA	Body fat: −1.22 (−2.91, 0.5), p-value = 0.161 Adiponectin: 1.7 (−0.15, 3.59), p-value = 0.071 Leptin: −4.44 (−8.74, 0.06), p-value = 0.053
	Confounding: age, sex, parity, maternal income level.						
Gyllenhammar et al. (2018b) *Medium*	Sweden, 1996–2011, children followed up at age 5	Cohort	Mothers and their children from the POPUP Study N = 193	Maternal serum 2.3	BMI z-score	Regression coefficient per IQR increase in maternal PFOA	BMI z-score: Ages 36 and 48 mo: Positive statistically significant associations.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
							Age 60 mo: Non-significant positive association (numeric results not provided)
	Confounding: Sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, and total time of breastfeeding.						
Hartman, (2017) *Medium*	United Kingdom, recruitment 1991–1992	Cohort	Pregnant women and their daughters N = 319	Maternal serum 3.7	WC (cm), Trunk fat (%), BMI (kg/m^2)	Regression coefficient per unit increase in PFOA	WC: −0.54 (−0.9, 0.11), p-value = 0.01 Trunk fat: −0.27 (−0.55, 0.0), p-value = 0.05 BMI: −0.16 (−0.32, 0.0), p-value = 0.05 Body fat percentage: No statistically significant associations
	Confounding: sampling design, pre-pregnancy BMI (kg/m^2) and maternal educational status.						
Kang et al. (2018) *Medium*	Korea, 2012–2014	Cross-sectional	Children from KorEHS-C Seoul and Gyeonggi, 3–18 yr of age, N = 147	Plasma 5.68	Fasting blood glucose (mg/dL)	Regression coefficient per ln-unit increase in PFOA	Blood glucose: 1.262 (−1.108, 3.633), p-value = 0.294
	Confounding: Age, sex, BMI z-score, household income, secondhand smoking.						
Kobayashi et al. (2017) *Medium*	Japan, 2002–2005	Cross-sectional	Infants from Hokkaido Study on Environment and Children's Health N = 177	Maternal serum 1.4	Ponderal index at birth	Regression coefficient per ln-unit increase in PFOA	Ponderal index: −0.44 (−0.99, 0.12), p-value = 0.123
	Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, gestational age, infant sex, and maternal blood sampling period.						
Karlsen et al. (2017) *Medium*	Faroe Islands, recruited 2007–2009 (at birth)	Cohort	Mother-child pairs N = 444	Serum	BMI z-score, Overweight	Regression coefficient or RR per log10–	BMI z-score at age 5: −0.27 (−0.52, −0.02), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			follow-up at child ages 18 mo and 5 yr	Maternal 2-week serum: 1.40 Child 5-yr serum: 2.20		unit increase in child or maternal PFOA, or by tertiles	Overweight at age 5: RR: 1.5 (1.01, 2.24), p-value < 0.05 T3: 1.88 (1.05, 3.35), p-value < 0.05
	Results: Lowest tertile used as reference. **Confounding:** Maternal nationality, age at delivery, pre-pregnancy BMI, smoking during pregnancy, child sex, exclusive breastfeeding duration, child's fish intake at age 5 yr.						
Lauritzen et al. (2018) *Medium*	Norway and Sweden, Recruitment 1986–1988	Cohort	Pregnant women and their children at 5-yr follow-up N = 412	Serum Norway: 1.64 Sweden: 2.33	BMI, triceps skin fold, subscapular skinfold, overweight	Regression coefficient or OR per ln-unit increase in maternal PFOA	BMI, triceps skin fold, subscapular skinfold, overweight: No statistically significant associations
	Confounding: Age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 wk, interpregnancy interval, previous breastfeeding duration and country of residence.						
Lopez-Espinosa et al. (2016) *Medium*	United States, 2005–2006	Cohort	Children ages 6–9 yr N = 1123 (girls) N = 1169 (boys)	Serum Girls: 30.1 Boys: 34.8	Insulin-like growth factor–1 (IGF-1) (ln-ng/mL)	Percent difference by quartiles.	IGF-1 Girls: Q3: −3.6 (−6.6, −0.5) Boys Q3: −7.4 (−12.8, −1.6) No other statistically significant associations
	Results: Lowest quartile used as the reference group. **Confounding:** age and month of sampling.						
Manzano-Salgado et al. (2017b) *Medium*	Spain, Recruitment 2003−2008	Cohort	Mother-child pairs, followed for 8 yr, INMA Study N = 1,230	Maternal blood GM = 2.32	BMI, WC, overweight, waist-to-hip ratio	Regression coefficient per-log2-unit increase in PFOA	BMI, waist circumference, overweight, waist-to-hip ratio: No statistically significant associations
	Confounding: Maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI), age of child.						
Martinsson et al. (2020) *Medium*	Sweden, 2003–2008	Case-control	Pregnant women and their children at	Serum 3.1	Overweight	OR by quartiles	OW: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			age 4, Southern Sweden Maternity Cohort N = 1,048				
	Results: Lowest quartile used as reference. **Confounding:** Risk strata, difference from strata-specific mean, sex.						
Mora et al. (2017) *Medium*	United States, 1999–2002	Cohort	Early childhood N = 992	Maternal Plasma 5.6	WC (cm), Skinfold thickness, BMI, waist-to-hip ratio, obesity, overweight, total fat mass index, total fat-free mass index	Regression coefficient per IQR-unit increase in PFOA	WC All: 0.31 (0.04, 0.57) Boys: 0.5 (0.06, 0.93) Skinfold thickness, BMI, waist-to-hip ratio, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association
	Confounding: maternal age, race/ethnicity, education, parity, pre-pregnancy BMI, timing of blood draw, household income, child sex, age at outcome assessment.						
Pinney et al. (2019) *Medium*	Greater Cincinnati and the San Francisco Bay Area, Recruitment 2004–2007, followed annually or semi–annually until 2014	Cohort	Girls, age 6–8 N = 667	Serum 6.4	BMI, waist-height ratio, waist-hip ratio	Regression coefficient by quintiles or per ln-unit increase in PFOA	BMI: Quintile 4 vs. Quintile 1: −0.248 (−0.489, 0.007), p-value = 0.044 Quintile 5 vs. Quintile 1: −0.436 (−0.685, 0.187), p-value = 0.001 Per ln-unit increase −0.264 (−0.416, 0.112), p-value = 0.001 Waist-height Per ln-unit increase: −0.009 (−0.017, 0.002), p-value = 0.013 Waist-hip ratio: No statistically significant association
	Results: Lowest quintile used as the reference group.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
	Confounding (BMI): Race, parental education, average kcal, physical activity. **Confounding (Waist-height ratio):** Age at exam, race, parental education, average kcal, physical activity.						
Scinicariello et al. (2020a) *Medium*	United States, 2013–2014	Cross-sectional	Children aged 3–11 yr from NHANES N = 600	Serum GM = 1.95 (SE = 0.08)	BMI z-score (BMIZ), height-for-age z-score (HAZ), weight-for-age z-score (WAZ)	Regression coefficient per ln-unit increase in PFOA and by tertiles	BMIZ: −0.19 (−0.5, 0.12) T2: −0.3 (−0.6, 0.01) T3: −0.15 (−0.49, 0.2) Females: –0.45 (–1, 0.1) T2: –0.2 (–0.68, 0.29) T3: –0.31 (–0.9, 0.28) Males: –0.02 (–0.35, 0.3) T2: –0.38 (–0.7, –0.05) T3: –0.07 (–0.5, 0.37) HAZ: −0.31 (−0.67, 0.04) T2: −0.17 (−0.38, 0.03) T3: −0.28 (−0.65, 0.08) Females: –0.36 (–0.87, 0.14) T2: –0.25 (–0.45, –0.05) T3: –0.35 (–0.88, 0.17) Males: –0.28 (–0.7, 0.14) T2: –0.2 (–0.53, 0.13) T3: –0.23 (–0.64, 0.19) WAZ: −0.34 (−0.68, −0.01) T2: −0.33 (−0.63, −0.04) T3: −0.28 (−0.65, 0.08) Females: –0.53 (–1.18, 0.12) T2: –0.28 (–0.73, 0.16) T3: –0.43 (–1.08, 0.23) Males: –0.22 (–0.51, 0.08) T2: –0.42 (–0.77, –0.07) T3: –0.21 (–0.56, 0.15) No statistically significant association trends by sex

NHANES = National Health and Nutrition Examination Survey.
Results: Lowest tertile used as reference.

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
	Confounding: Age, quadratic age, race/ethnicity, poverty-income ratio, serum cotinine, birthweight, maternal smoking during pregnancy, hematocrit, sex.						
Fleisch et al. (2017) *Medium* for metabolic function *Low* for HOMA-IR	United States, Pregnant women recruited 1999–2002, outcome assessed at mid-childhood follow-up	Cohort	Mid-childhood, 7.7 yr N = 584	Plasma GM = 4.2	Leptin, Adiponectin, HOMA-IR	Percent change by quartiles	Leptin Q3: −23.3 (−37, −6.5) Q4: −20.1 (−35.1, −1.6) Adiponectin Q2: 16.3 (1.8, 32.9) Q3: 22.7 (6.9, 40.8) HOMA-IR: No statistically significant association
	Results: Lowest quartile used as reference. **Confounding:** Characteristics of child (age, sex, race/ethnicity), mother (age, education), and neighborhood census tract at mid-childhood (median household income, percent below poverty).						
				Pregnant Women			
Mitro et al. (2020) *High*	United States, Recruitment 1999–2002	Cohort	Pregnant women N = 786	Plasma 5.6	WC(cm), BMI, Adiponectin, skinfold thickness, arm circumference, leptin	Percent change (%) or Regression coefficient per log2–unit increase in PFOA	WC: 1.1% (0.1, 2.2), p-value < 0.05 BMI: 0.3 (0.0, 0.6), p-value < 0.05 Adiponectin, skinfold thickness, arm circumference, hemoglobin, leptin: No statistically significant associations
	Confounding: age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, breastfeeding in a prior pregnancy.						
Preston et al. (2020) *High*	United States, 1999–2002	Cohort	Pregnant women from the Project Viva cohort N = 1,533	Plasma 5.9	GDM, impaired glucose tolerance, isolated hyperglycemia, blood glucose levels	Regression coefficient by quartiles OR by quartiles	Gestational diabetes, impaired glucose tolerance, isolated hyperglycemia, blood glucose levels: No statistically significant association
	Confounding: Maternal age, pre-pregnancy BMI, prior history of gestational diabetes/parity, race/ethnicity, smoking, and education.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Starling et al. (2017) *High*	United States, 2009–2014	Cohort	Pregnant women and their children N = 628	Maternal serum 1.1	Maternal glucose (ln(mg/dl))	Regression coefficient by tertiles	Maternal glucose: T3: −0.025 (−0.046, 0.004) Maternal glucose (continuous) and T2: No statistically significant association
	Confounding: Maternal age, pre-pregnancy body mass index (BMI), race/ethnicity, education, smoking during pregnancy, gravidity, and gestational age at blood draw.						
Ashley-Martin et al. (2016) *Medium*	Canada, Pregnant women recruited 2008–2011, outcome assessed at birth	Cohort	Pregnant women from MIREC N = 1,609	Serum 15.2	GWG (kg)	Regression coefficient per log2-unit increase in PFOA	No statistically significant associations
	Confounding: Age, income, parity.						
Jaacks et al. (2016) *Medium*	United States, 2005–2007	Cohort	Pregnant women N = 218	Serum Mean = 3.66	GWG (kg)	Regression coefficient and OR per SD-unit increase in PFOA	GWG 0.09 (−0.84 1.02) OR for excessive GWG: 1.06 (0.76, 1.47)
	Confounding: Pre-pregnancy non-fasting serum lipids, BMI.						
Jensen et al. (2018) *Medium*	Denmark, recruitment 2010–2012, outcome assessed 12–20 wk later	Cohort	Pregnant women, Odense Child Cohort N = 158	Serum 1.67	Blood glucose, insulin, c-peptide, 2-hr glucose, insulin resistance, beta-cell function, insulin sensitivity	Percent change per log2-unit increase in PFOA	No statistically significant associations
	Confounding: Age, parity, education level, pre-pregnancy BMI.						
Liu et al. (2019) *Medium*	China, 2013–2015	Case-control	Pregnant women without history or family history of diabetes	Serum 2.25	GDM, glucose homeostasis	Regression coefficient per ln-unit increase, or by tertiles of	GDM: Per ln-unit increase sum m-PFOA: 1.23 (0.92, 1.64) T2: 0.91 (0.4, 2.07) T3: 2.01 (0.92, 4.37)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			N = 189			sum m-PFOA or L-PFOA	Per ln-unit increase sum m-PFOA: 2.04 (0.99, 4.21) T2: 1.04 (0.47, 2.34) T3: 2.04 (0.94, 4.46) Per ln-unit increase sum L-PFOA: Glucose homeostasis (1 hr): 0.55 (0.01, 1.1), p-value = 0.049 Glucose homeostasis (2 hr): 0.73 (0.27, 1.18), p-value = 0.002 Glucose homeostasis (fasting, 1 hr, 2 hr) for m-PFOA and glucose homeostasis (fasting) for L-PFOA: No statistically significant association
	Confounding: Maternal age, BMI in early pregnancy, fetal sex, serum triglyceride, TC.						
Marks et al. (2019) *Medium*	United Kingdom 1991–1992	Cohort	Mothers from ALSPAC N = 905	Serum Mothers of sons: 3.0 Mothers of daughters: 3.7	GWG (absolute)	Regression coefficient per 10% increase in log-unit PFOA	GWG: No statistically significant associations
	Comparison: Logarithm base not specified. **Confounding:** Maternal education, prenatal smoking, maternal age at delivery, parity, pre-pregnancy BMI, gestational age at delivery, gestational age at sample.						
Rahman et al. (2019) *Medium*	United States, 2009–2013	Cohort	Pregnant women with singleton pregnancies N = 2,292	Plasma GM = 1.99	GDM	Risk Ratio per SD-unit increase in PFOA	GDM (family history of T2D): 1.27 (1.11, 1.45) Overall cohort, no family history of T2D, normal pre-pregnancy BMI, overweight pre-pregnancy BMI: No statistically significant association
	Confounding: Maternal age, enrollment BMI, education, parity, race/ethnicity, serum cotinine.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Ren et al. (2020) *Medium*	China, 2012	Cross-sectional	Pregnant women enrolled in the Shanghai-Minhang Birth Cohort Study N = 705	Plasma 20.2	Glucose (1 hr, fasting)	Regression coefficient per ln-unit increase in PFOA	Glucose (1 hr tolerance test): 0.31 (0.03, 0.52), p-value = 0.031 Glucose after fasting, glucose after 1 hr tolerance test by gestational weeks: No statistically significant association
	Confounding: maternal age at enrollment, pre-pregnancy BMI, per capita household income, education level, passive smoking, pregnancy complication, history of abortion and stillbirth, parity.						
Shapiro et al. (2016) *Medium*	Canada, 2008–2011	Cohort	Pregnant women N = 1,195	Urine Normal glucose GM = 1.68 Gestational impaired glucose tolerance GM = 1.70 Women with GDM GM = 1.64	GDM, gestational impaired glucose tolerance	OR per quartile PFOA	Gestational diabetes, gestational impaired glucose tolerance: No statistically significant association
	Confounding: Maternal age, race, pre-pregnancy BMI, and education.						
Valvi et al. (2017) *Medium*	Faroe Islands, 1997–2000	Cohort	Pregnant women and their children N = 604	Maternal serum 3.31	Gestational diabetes	OR per doubling of PFOA, or by tertiles	Gestational diabetes: Per doubling: 0.79 (0.44, 1.41) T2: 1.01 (0.5, 2.06) T3: 0.66 (0.3, 1.48)
	Results: Lowest tertile used as the reference group. **Confounding:** Maternal age at delivery, education, parity, pre-pregnancy BMI, smoking during pregnancy.						
Wang et al. (2018c) *Medium*	China 2013	Case-control	Pregnant women with (cases) and without (controls) GDM	Serum Cases: 1.38 Controls: 1.30	Fasting blood glucose, GDM	Fasting blood glucose: OR by tertiles of n-PFOA	Fasting blood glucose, GDM: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			N = 242			GDM: OR per unit increase in n-PFOA	
	Confounding: Fasting blood glucose: BMI, age, GDM status; GDM: BMI, GWG, ethnic groups, maternal education, parity, maternal drinking during pregnancy, household income.						
Wang et al. (2018a) *Medium*	China, 2013–2014	Cohort	Pregnant women aged 20–40 N = 385	Serum 7.3	Fasting blood glucose, fasting insulin, HOMA-IR, gestational diabetes, oral glucose tolerance	LSM by tertiles	No statistically significant associations
	Results: Lowest tertile used as reference. **Confounding:** Pregnant age, diabetes mellitus history of relatives, husband smoking status, family per capita income, baby sex, averaged intake of meat, vegetable, and aquatic products, averaged physical activity, and averaged energy intake, pre-pregnant maternal BMI.						
Xu et al. (2020b) *Medium*	China, 2017–2019	Nested case-control	Pregnant women N = 165 cases, 330 controls	Serum Cases: 8.19 Controls: 7.91	Gestational diabetes mellitus	OR per unit increase in PFOA; OR per log10-unit increase in PFOA	Gestational diabetes mellitus Q2: 1.05 (0.45, 2.04) Q3: 1.12 (0.46, 2.20) Q4: 1.20 (0.28, 2.21) p-trend = 0.60 log-PFOA: 1.51 (0.63, 3.84), p-value = 0.33
	Confounding: Maternal age, sampling time, parity, BMI, educational level, and serum lipids.						
				General Population			
Cardenas et al. (2017) *High*	United States, Recruitment July 1996–May 1999, outcome assessed annually until May 2001	Cohort	Adults at high risk of Type-2 diabetes N = 956	Plasma GM = 4.82	Adiponectin (µg/mL), HbA1c (%), Insulin (fasting) (µU/mL), Glucose (fasting) (µU/mL), HOMA-IR, Insulin (30 min, µU/mL),	Regression coefficient per doubling of PFOA	Adiponectin: –0.29 (–0.54, –0.04), p-value = 0.02 HbA1c: 0.04 (0.001, 0.07), p-value = 0.05 Insulin (fasting): 2.26 (1.16, 3.35), p-value = 0.000056 Glucose (fasting): 0.66 (0.07, 1.24), p-value = 0.03

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					Proinsulin (fasting, pM), HOMA-B (beta), Insulin (corrected response), Insulinogenic index, Diabetes, HOMA-IR, glucose (30 min), glucose (2 hr), BMI		HOMA-IR: 0.64 (0.34, 0.94), p-value = 0.000031 Insulin (30 min): 7.85 (3.63, 12.07), p-value = 0.00028 Proinsulin (fasting): 1.17 (0.72, 2.71), p-value = 0.00070 HOMA-B: 15.93 (6.78, 25.08), p-value = 0.00066 Insulin (corrected): 0.04 (0.01, 0.07), p-value = 0.01 Insulinogenic index: 0.08 (0.01, 0.15), p-value = 0.02 Diabetes, HOMA-IR, glucose (30 min), glucose (2 hr), BMI: No statistically significant association
	Confounding: Sex, race/ethnicity, BMI, age, marital status, education, smoking history.						
Blake et al. (2018) *Medium*	United States, 1991–2008	Cohort	Adults living in a community with water supply from a PFAS-contaminated aquifer N = 192	Serum 12.7	BMI	Percent change per IQR increase in PFOA	BMI: No statistically significant associations
	Confounding: Age, year of measurement, sex, education, income, marital status, and BMI.						
Cardenas et al. (2019) *Medium*	United States, 1996–2014	Controlled trial	Adults older than 25 without diabetes and	Plasma GM = 4.82	T2D	Hazard ratio per log2-unit increase in	Diabetes: HR: 1.05 (0.94, 1.18)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
			with elevated fasting and postload glucose, Diabetes Prevention Program N = 956			baseline PFOA and by PFOA tertiles	T2: 0.94 (0.75, 1.17) T3: 0.94 (0.75, 1.18)
	Results: Lowest tertiles used as the reference group. **Confounding:** Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment.						
Christensen et al. (2019) *Medium*	United States, 2007–2014	Cross-sectional	Adults from NHANES age 20+ N = 2,975	Serum 2.8	Elevated waist circumference (Males: ≥102 cm. Females: ≥88 cm), metabolic syndrome, glucose	OR by quartiles	WC Q2: 0.66 (0.46, 0.92), p-value < 0.05 Q3: 0.62 (0.39, 0.98), p-value < 0.05 Metabolic syndrome, glucose level: No statistically significant association
	Confounding: PFDE, PFOS, PFHxS, MPAH, PFNA, PFUnDA, survey cycle, age, sex, race/ethnicity, family income, alcohol intake, and smoking status.						
Conway et al. (2016) *Medium*	United States, 2005–2006	Cross-sectional	Adults working or living in six PFOA-contaminated water districts with diabetes N = 6,460	Serum All participants mean = 68.4	T1D, T2D, Uncategorized Diabetes	OR per ln-unit increase in PFOA	All T1D: 0.76 (0.71, 0.8) T2D: 0.94 (0.92, 0.97) Uncategorized DM: 0.94 (0.9, 0.99) Adults Type 1 DM: 0.74 (0.7, 0.79) Type 2 DM: 0.91 (0.89, 0.94) Uncategorized DM: 0.92 (0.88, 0.96)
	Confounding: Age, sex, race, BMI, eGFR, hemoglobin, iron.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Donat-Vargas et al. (2019a) *Medium*	Sweden, 1990–2003, 2001–2012	Case-control	Adults with (cases) and without (controls) type-2 diabetes living in Sweden N = 248	Plasma Cases: 2.8 Controls: 3.0	Type 2 Diabetes, HOMA-IR, HOMA-B	OR per 1-log10 SD increase in baseline PFOA	T2D: 0.65 (0.43, 0.97) HOMA-IR, HOMA-B: No statistically significant association
	Confounding: gender, age, sample year, red and processed meat intake, fish intake, BMI.						
Duan et al. (2020) *Medium*	China, 2017	Cross-sectional	Adults, 19 to 87 yr old N = 252	Serum 14.83	Fasting glucose (nmol/L), HbA1c	Regression coefficient per 1% increase in PFOA	Glucose (fasting): 0.018 (0.004, 0.033), p-value = 0.014 HbA1c: No statistically significant association
	Confounding: sex, age, body mass index, smoking and alcohol-drinking status, exercising status, education level, and family history of diabetes.						
Jain et al. (2019e) *Medium*	United States, 2011–2014	Cross-sectional	Adults from NHANES, age 20+ N = 2,883	Serum GM = 2.2 (non-obese); 2.0 (obese)	Obesity	Comparison of geometric mean PFOA levels non-obese vs. obese	Obesity: p-value = 0.02
	Confounding: Sex, race, age, poverty-income ratio, physical activity, BMI, and serum cotinine.						
Jeddy et al. (2018) *Medium*	England, mothers recruited 1991–1002, outcome assessed at age 17	Nested case-control studies	Pregnant mothers and their 17-yr-old daughters, ALSPAC N = 221	Maternal serum 3.8	Fat mass	Regression coefficient per unit increase in PFOA	105.88 (−621.59, 833.34)
	Confounding: Maternal pre-pregnancy BMI, maternal education, maternal age at delivery, gestational age at sample collection, and ever breastfed status at 15 mo.						
Liu et al. (2018a) *Medium* for adiposity/weight change	Boston, Massachusetts and Baton Rouge, Louisiana, 2004–2007	Controlled Trial	Overweight and obese patients from the POUNDS Lost Trial, ages 30–70 yr	Plasma, glucose Males: 27.2 Females: 22.3	Leptin, HOMA-IR, insulin, resting metabolic rate, body weight, HbA1c, glucose,	Partial Spearman correlation coefficient with baseline PFOA	Spearman correlations Leptin: 0.09, p-value < 0.05 HOMA-IR: 0.1, p-value < 0.05 Resting metabolic rate, body weight, HbA1c, glucose, VAT fat

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Uninformative for insulin resistance			N = 621		VAT fat mass, whole body fat, BMI, waist circumference	Regression coefficient log10-unit increase in PFOA, or by tertile	mass, whole body fat, BMI, waist circumference: No statistically significant association
	Confounding: age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups.						
Liu et al. (2018b) *Medium*	United States, 2013–2014	Cross-sectional	Adults from NHANES N = 1,871	Serum GM = 1.86	Fasting blood glucose, 2-hr glucose, HbA1c, insulin levels, HOMA-IR, beta-cell function, metabolic syndrome, WC	Regression coefficient per ln-unit increase in PFOA	HbA1c: −0.12 (0.05), p-value < 0.05 Beta-cell function: 0.12 (0.05); p-value < 0.05 Fasting blood glucose, 2-hr glucose, insulin levels, HOMA-IR, metabolic syndrome, WC: No statistically significant associations
	Results: Effect estimates are reported with SE in parentheses. **Confounding:** Age, gender, ethnicity, smoking status, alcohol intake, household income, WC, and medications (antihypertensive, antihyperglycemic, and antihyperlipidemic agents).						
Mancini et al. (2018) *Medium*	France, 1990–2012	Cohort	Women, 40–60 N = 71,294	Dietary Mean = 0.86 ng/kg body weight/day	T2D	Hazard ratio by deciles	T2D Decile 4: 1.21 (1.06, 1.46), p-value < 0.05 Decile 5: 1.35 (1.15, 1.59), p-value < 0.05 Decile 6: 1.19 (1.05, 1.41), p-value < 0.05
	Results: Lowest decile used as the reference group. **Confounding:** smoking status, physical activity, education level, hypertension, hypercholesterolemia, family history of diabetes, energy intake, alcohol intake, adherence to the Western diet and adherence to the Mediterranean diet, water consumption, dairy product consumption.						
Su et al. (2016) *Medium*	Taiwan, 2009–2011	Cross-sectional	Adults aged 20–60 living in Taiwan N = 571	Plasma 8.0	Diabetes, Fasting blood glucose (ng/mL),	OR by quartiles, per doubling of PFOA	Diabetes: Q2: 0.39 (0.16, 0.96) Q3: 0.2 (0.07, 0.58) Q4: 0.16 (0.05, 0.5) Total: 0.56 (0.43, 0.75)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
					blood glucose (120 min) (ln) (ng/mL), glucose AUC (ng/mL), HbA1c (ln) (%)	Geometric mean ratio (GMR) by quartiles, or per doubling of PFOA	Glucose (Fasting): Q2: 0.96 (0.93, 0.99) Q3: 0.95 (0.92, 0.97) Q4: 0.95 (0.92, 0.98) Per doubling PFOA: 0.98 (0.97, 0.99) Glucose (120 min) Q2: 0.87 (0.82, 0.94) Q3: 0.9 (0.94, 0.95) Q4: 0.85 (0.79, 0.91) Per doubling PFOA: 0.96 (0.94, 0.98) Glucose AUC: Q2: 0.9 (0.85, 0.95) Q3: 0.9 (0.86, 0.95) Q4: 0.88 (0.84, 0.93) Per doubling PFOA: 0.97 (0.95, 0.99) HbA1c: Q2: 0.98 (0.96, 1.0) Q4: 0.97 (0.95, 1.0) Per doubling PFOA: 0.99 (0.98, 1.0)
	Results: Lowest quartile used as reference group. **Confounding (Diabetes):** age, sex, education, smoking (ever vs. never), alcohol (ever vs. never), BMI, hypertension, TC, regular exercise **Confounding (Other):** age, sex, education, smoking, alcohol, BMI, hypertension, TC, regular exercise.						
Sun et al. (2018a) *Medium*	United States, 1989–2011[d]	Case-control	Female nurses drawn from the Nurses' Health Study II cohort study N = 1,586	Plasma Cases: 4.96 Controls: 4.57	Type 2 Diabetes, HbA1c, fasting insulin, adiponectin	Regression coefficient per SD log10-unit increase in PFOA	T2D Per increase: 1.24 (1.06, 1.45), p-value = 0.009 OR for T3: 1.54 (1.04, 2.28)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
						OR by tertiles	HbA1c, fasting insulin, adiponectin: No statistically significant association
	Confounding: Age, month of sample collection, fasting status, menopausal status, postmenopausal hormone use, family history of diabetes, oral contraceptive use, breastfeeding duration at blood draw, number of children delivered after 1993, states of residence, smoking status, alcohol intake, physical activity, baseline BMI, and Alternative Healthy Eating Index (AHEI) score.						
Chen et al. (2019a) *Medium* for metabolic syndrome *Low* for all other outcomes	Croatia 2007–2008	Cross-sectional	Residents of Hvar ages 44–56 yr N = 122	Plasma GM = 2.87 (Range: 1.03–8.02)	BMI, fasting insulin (µIU/mL), fasting plasma glucose (mmol/L), glycated HbA1c (%), hip circumference (cm), homeostatic model assessment of beta-cell function (HOMA-B, homeostatic model assessment of insulin resistance (HOMA-IR), metabolic syndrome defined by the ATP III criteria, waist circumference (cm)	Metabolic syndrome: OR per ln-unit increase in PFOA All other outcomes: regression coefficient per ln-unit increase in PFOA	Metabolic syndrome: 2.19 (0.88, 4.42); p-value = 0.09 All other outcomes: No statistically significant associations
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels[a]	Outcome	Comparison	Results[b]
Occupational Populations							
Steenland et al. (2013) *Medium*	United States 2005–2006	Retrospective Occupational Cohort	Adult residents and workers from the C8 Health Project N = 32,254	Serum 26	Type 1 diabetes, with and without a 10-yr lag	RR by quartiles	T1D, validated and self-reported No lag: No statistically significant associations or trends by quartiles With lag Q2: 0.42 (0.09, 2.00) Q3: 0.70 (0.14, 0.35) Q4: 0.38 (0.08, 1.93) p-trend = 0.84 T1D, validated cases only: No statistically significant associations or trends by quartiles
	Confounding: Sex, race/ethnicity, smoking, BMI, alcohol consumption.						

Notes:AHEI = Alternative Healthy Eating Index; ATP III = Adult Treatment Panel III; AUC = area under the curve; BMI = body mass index; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; EYHS = European Youth Heart Study; ; GDM = gestational diabetes mellitus; GM = geometric mean; GMR = geometric mean ratio; GWG = gestational weight gain; HAZ = height-for-age z-score; HbA1c = hemoglobin A1c; HOMA-B = homeostatic model assessment of β-cell function; HOMA-IR = homeostatic model assessment for insulin resistance; HOME = Health Outcomes and Measures of the Environment; hr = hour/s; HR = hazard ratio; IGF = insulin-like growth factor; INMA = Infancia y Medio Ambiente (Environment and Childhood) Project; IR = insulin resistance; IQR = interquartile range; KorEHS-C: Korea Environmental Health Survey in Children and Adolescents; LSM = least square mean; MIREC = Maternal-Infant Research on Environmental Chemicals; MPAH = 2-(N-methyl-PFOSA) acetate; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; OW = overweight; PFDE = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnDA = perfluoroundecanoic acid; POUNDS = Preventing Overweight Using Novel Dietary Strategies; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; SOLAR = Study of Latino Adolescents at Risk of Type 2 Diabetes; T1D = type 1 diabetes; T2D = type 2 diabetes; T2 = tertile 2; T3 = tertile 3; WAZ = weight-for-age z-score; WC = waist circumference; wk = week/s; yr = year/s.

[a] Exposure levels are reported as median in ng/mL unless otherwise noted.

[b] Results are reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

[d] Recruitment 1989, blood sample collection 1995–2000, outcome assessed during biennial follow-up through June 2011.

D.8 Nervous

Table D-17. Associations Between PFOA Exposure and Neurological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Children and Adolescents							
Harris et al. (2018) *High*	United States, Recruitment: 1999–2002; Follow-up at early- and mid-childhood	Cohort	Pregnant women and their children from Project Viva N = 853	Plasma Maternal: 5.6 (4.1–7.7) Child: 4.4 (3.1–6.0)	Both age groups: Wide Range Assessment of Visual Motor Abilities (WRAVMA) score Early childhood only: Peabody Picture Vocabulary Test (PPVT-III) score Mid-childhood only: Kaufman Brief Intelligence Test Second Edition (KBIT-2) nonverbal and verbal IQ, (WRAML2) design memory and picture memory	Mean differences by quartiles of PFOA exposure	Visual-motor Early childhood Q2: 1.0 (−1.0, 2.9) Q3: 0.5 (−1.6, 2.6) Q4: 2.3 (0.1, 4.5) Mid-childhood (maternal plasma) Mid-childhood (child plasma) Q2: −4.1 (−8.0, −0.2) Q3: −0.4 (−4.5, 3.7) Q4: −6.1 (−10.5, −1.6) Nonverbal IQ Mid-childhood (maternal plasma) Q2: −0.7 (−3.8, 2.3) Q3: −1.8 (−5.0, 1.4) Q4: 1.6 (−1.8, 4.9) Mid-childhood (child plasma) Q2: 0.4 (−3.3, 4.1) Q3: −1.5 (−5.4, 2.3) Q4: −3.2 (−7.4, 1.0) Verbal IQ Mid-childhood (maternal plasma) Q2: −3.3 (−5.7, −1.0) Q3: −2.7 (−5.2, −0.2) Q4: −2.4 (−5.1, 0.2) Mid-childhood (child plasma) Q2: −1.0 (−3.9, 2.0) Q3: −2.0 (−5.11, 1.1)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Q4: −2.8 (−6.2, −0.6) Design memory Mid-childhood (maternal plasma) Q2: 0.2 (−0.3, 0.8) Q3: 0.3 (−0.3, 0.8) Q4: 0.7 (0.1, 1.3) Mid-childhood (child plasma) Q2: 0 (−0.6, 0.6) Q3: −0.4 (−1.1, 0.2) Q4: −0.4 (−1.1, 0.3) Picture memory Mid-childhood (maternal plasma) Q2: −0.6 (−1.2, 0) Q3: 0.1 (−0.5, 0.7) Q4: −0.1 (−0.7, 0.5) Mid-childhood (child plasma) Q2: −0.3 (−1.0, 0.4) Q3: 0.2 (−0.5, 1.0) Q4: 0 (−0.8, 0.7) PPVT-III: No statistically significant associations
	Results: Lowest quartile used as reference. **Confounding:** Year of pregnancy blood collection gestational age at time of pregnancy blood collection, estimated glomerular filtration rate at blood draw, maternal race/ethnicity, age, education, KBIT-2 score, pre-pregnancy BMI, smoking status, paternal education, annual household income in mid-childhood, HOME-SF score, child's sex and age at mid-childhood cognitive testing, proxy for breastfeeding of a prior child.[c]						
Niu et al. (2019) *High*	China, Recruitment: 2012; Follow-up at age 4 yr	Cohort	Pregnant women and their children from the Shanghai-Minhang Birth Cohort	Maternal plasma 19.9 (15.3–27.4)	ASQ-3 skill scales: communication, gross motor, fine motor, problem solving, personal-social	RR per ln-unit increase in PFOA and by tertiles	Communication 0.84 (0.59, 1.19) Females: 0.64 (0.34, 1.19) T2: 0.86 (0.49, 1.50) T3: 0.55 (0.28, 1.10) p-trend < 0.10 Males: 1.07 (0.70, 1.62)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			N = 533 (236 Females; 297 Males)				T2: 1.02 (0.65, 1.6) T3: 0.96 (0.61, 1.52) p-value for interaction by sex = 0.255 Gross Motor 0.86 (0.47, 1.58) Females: 2.31 (0.75, 7.10) T2: 1.08 (0.33, 3.57) T3: 1.90 (0.66, 5.44) Males: 0.47 (0.25, 0.89); p-value < 0.05 T2: 0.51 (0.23, 1.11) T3: 0.45 (0.19, 1.04) p-trend < 0.10 p-value for interaction by sex = 0.002 Fine Motor 0.99 (0.53, 1.84) No statistically significant associations, trends, or interactions by sex Problem Solving 1.26 (0.73, 2.15) No statistically significant associations, trends, or interactions by sex Personal-Social Skills 1.67 (0.89, 3.14) Females: 9.00 (3.82, 21.21); p-value < 0.05 Males: 1.03 (0.53, 2.01)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							T2: 1.60 (0.80, 3.19) T3: 1.50 (0.77, 2.93) p-value for interaction by sex = 0.002
	Outcome: Neuropsychological problems defined as scores ≤10th percentile. **Results:** Lowest tertile used as reference. For personal-social skills, no cases of neuropsychological problems were present among the lowest tertile of PFOA exposure among girls; as a result, the Poisson regression model did not converge. **Confounding:** Maternal age at enrollment, pre-pregnancy BMI, maternal education, paternal education parity, per capita household income, maternal passive smoking, maternal prenatal depressive symptoms, gestational age, child sex.						
Oulhote et al. (2016) *High*	Faroe Islands, Recruitment: 1997–2000, Follow-up at ages 5 and 7 yr	Cohort	Children at 5 yr (n = 508) and 7 yr (n = 491)	Serum Maternal: 3.34 (2.56–4.01) 5 yr: 4.06 (3.33–4.98) 7 yr: 4.37 (3.53–5.66)	Strengths and Difficulties Questionnaire (SDQ) scores: Total score (hyperactivity/inattention, conduct problems, peer relationship problems, emotional symptoms), prosocial behavior, internalizing problem, externalizing problems, autism screening (peer-problems minus pro-social)	Mean difference (autism, internalizing, externalizing, total) or mean ratio (hyperactivity/inattention, conduct, peer relationship, emotional, prosocial) per doubling of PFOA	SDQ total score Prenatal exposure: −0.37 (−1.34, 0.61), p-value = 0.46 5-yr serum: 1.03 (0.11, 1.95), p-value = 0.03 7-yr serum: 0.1 (−0.83, 1.03), p-value = 0.84 Hyperactivity/Inattention Prenatal exposure: 0.93 (0.76, 1.13), p-value = 0.43 5-yr serum: 1.1 (0.91, 1.32), p-value = 0.33 7-yr serum: 0.97 (0.8, 1.16), p-value = 0.71 Conduct Prenatal exposure: 0.86 (0.71, 1.04), p-value = 0.12 5-yr serum: 1.19 (0.99, 1.44), p-value = 0.06 7-yr serum: 1.01 (0.84, 1.22), p-value = 0.92 Peer Relationship

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Prenatal exposure: 0.99 (0.71, 1.38), p-value = 0.96 5-yr serum: 1.54 (1.16, 2.06), p-value < 0.01 7-yr serum: 1.23 (0.92, 1.65), p-value = 0.17 Emotional Prenatal exposure: 1.04 (0.84, 1.3), p-value = 0.7 5-yr serum: 1.09 (0.88, 1.34), p-value = 0.45 7-yr serum: 0.98 (0.8, 1.21), p-value = 0.85 Prosocial Prenatal exposure: 1.02 (0.95, 1.1), p-value = 0.58 5-yr serum: 0.97 (0.9, 1.04), p-value = 0.4 7-yr serum: 1 (0.93, 1.07), p-value = 0.95 Internalizing Prenatal exposure: 0 (−0.55, 0.55), p-value = 0.99 5-yr serum: 0.59 (0.06, 1.13), p-value = 0.03 7-yr serum: 0.19 (−0.34, 0.72), p-value = 0.49 Externalizing Prenatal exposure: −0.37 (−0.99, 0.24), p-value = 0.24

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							5-yr serum: −0.09 (−0.69, 0.5), p-value = 0.15 7-yr serum: −0.09 (−0.69, 0.5), p-value = 0.76 Autism screening Prenatal exposure: −0.22 (−0.67, 0.23), p-value = 0.35 5-yr serum: 0.68 (0.25, 1.11) 7-yr serum: 0.18 (−0.25, 0.6), p-value = 0.42
	Confounding: Age, sex, maternal age, pre-pregnancy BMI, parity, socioeconomic status, alcohol, and smoking during pregnancy.						
Braun et al. (2014) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 4–5 yr	Cohort	Pregnant women and their children from the HOME study N = 175 (80 Females; 95 Males)	Maternal Serum 5.5 (3.8–7.6)	Social Responsiveness Scale (SRS) total score	Regression coefficient per log10-unit/2SD increase in PFOA	SRS −0.9 (−3.1, 1.4) Females: −1.8 (−4.6, 1.0) Males: 0.7 (−2.5, 3.8) p-value for interaction by sex = 0.22
	Confounding: Maternal race, maternal age, maternal education, marital status, annual household income, maternal depressive symptoms, maternal IQ, child sex, caregiving environment score, maternal serum.						
Chen et al. (2013) *Medium*	Taiwan, Recruitment: 2004–2005; Follow-up at age 2 yr	Cohort	Pregnant women and their children from the Taiwan Birth Panel Study N = 239	Cord blood Mean = 2.6 (SD = 2.5)	Comprehensive Developmental Inventory (CDI) skill quotients: cognitive, fine motor, gross motor, language, self-help, social, whole test	Regression coefficient per IQR increase in ln-unit PFOA	Cognitive: −0.3 (−3.3, 2.7) Fine Motor: −0.1 (−3.1, 2.9) Gross Motor: −1.1 (−4.7, 2.3) Language: 0.8 (−2.4, 3.9) Self Help: −1.7 (−5.6, 2.2) Social: 0.8 (−3.2, 4.9) Whole Test: −0.6 (−3.7, 2.4)
	Confounding: Maternal education, family income, infant sex and gestational age, breastfeeding, HOME score at 24 mo of age, cord blood cotinine levels, postnatal environmental tobacco smoke exposure.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Ghassabian et al. (2018) *Medium*	United States, 2008–2010	Cohort	Children aged 7 yr from Upstate KIDS Study N = 788	Blood 1.12 (IQR = 0.96)	SDQ scores: total behavioral difficulties – total score, borderline problems; hyperactivity, conduct, peer, or emotional problems; difficulties in prosocial behavior	Regression coefficient (total behavioral difficulties, problem scores) and OR (borderline behavioral difficulties, problem scores, difficulties in prosocial behavior) per log-SD increase in PFOA and by quartiles	Total Behavioral Difficulties (β) −0.01 (−0.06, 0.05) Q2: −0.05 (−0.19, 0.10) Q3: 0.03 (−0.12, 0.17) Q4: −0.05 (−0.21, 0.12) Difficulties in Prosocial Behavior (OR) 1.35 (1.03, 1.75) Q2: 2.63 (0.97, 7.14) Q3: 2.93 (1.03, 8.28) Q4: 3.23 (1.04, 10.07) All other outcomes: No statistically significant associations
	Outcome: Borderline behavioral difficulties were defined as having SDQ Total Difficulties Score within the borderline/abnormal range. **Comparison:** Logarithm base not specified. **Results:** Lowest quartile used as reference. **Confounding:** Child's age and sex, maternal age, pre-pregnancy BMI, race/ethnicity, education, marital status, history of smoking in pregnancy, having private insurance, parity, and infertility treatment.						
Goudarzi et al. (2016b) *Medium*	Japan, 2002–2005	Cohort	Pregnant women and their infants at 6 and 18 mo from the Hokkaido Study on Environment and Children's Health N = 90 Females; 83 Males	Maternal serum 1.2 (0.8–1.7)	Bayley Scales of Infant Development, Second Edition (BSID-II) mental development index (MDI), psychomotor development index (PDI)	Regression coefficient log10-unit increase in PFOA and by quartiles, least square means by quartiles	MDI Females (6 mo) −0.296 (−11.96, −0.682) Q1: 89.25 (82.03, 96.47) Q2: 89.68 (82.14, 97.23) Q3: 89.03 (81.35, 96.72) Q4: 84.19 (76.11, 92.28), p-trend = 0.045 β Q2: 0.43 (−4.39, 5.25) β Q3: −0.21 (−5.29, 4.86) β Q4: −5.05 (−10.66, 0.55) Males (6 mo) 0.110 (−3.31, 7.14)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							No statistically significant trend by quartiles, p-trend = 0.615 β Q2: 0.23 (−5.29, 5.77) β Q3: 2.44 (−2.39, 7.29) β Q4: 0.44 (−4.91, 5.81) PDI 6 mo: −0.006 (−5.93, 5.50) Females: 0.055 (−8.37, 12.93) Males: 0.068 (−5.56, 9.26) 18 mo: 0.002 (−7.66, 7.85)
	Confounding: Gestational age, parity, maternal age, smoking during pregnancy, alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education level, blood sampling period, breast feeding, total dioxin levels.						
Jeddy et al. (2017) *Medium*	Great Britain. Recruitment: 1991–1992; Follow-up at age 15 and 18 mo	Cohort	Mothers and daughters aged 15 and 38 mo from ALSPAC N = 353	Maternal serum 3.7 (2.8–4.8)	MacArthur Communicative Development Inventories (MCDI): communicative, intelligibility, language, nonverbal communication, social development, verbal comprehension, and vocabulary comprehension scores	Regression coefficient per unit change in PFOA	Nonverbal, 15 mo.: 0.10 (−0.07, 0.27) Social, 15 mo.: −0.06 (−0.36, 0.23) Verbal, 15 mo.: 0.24 (0.12, 0.36) Maternal age ≤30: No statistically significant associations Maternal age > 30: 0.35 (0.15, 0.55) Vocabulary, 15 mo.: 0.29 (−2.07, 2.64) Maternal age < 25: −11.39 (−22.76, −0.02) Maternal age ≥25: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Communicative, 38 mo.: −0.02 (−0.08, 0.04) Maternal age < 25: 0.29 (0.03, 0.54) Maternal age ≥25: No statistically significant associations Intelligibility, 38 mo.: −0.04 (−0.08, −0.01) Maternal age ≤ 30: No statistically significant associations Maternal age >30: −0.06 (−0.11, −0.01) Language, 38 mo.: −0.83 (−2.21, 0.54) Nonverbal, social, language: No statistically significant associations stratified by maternal age at delivery
	Confounding: Parity, maternal age, maternal education, maternal smoking status, gestational age at sample collection, total maternal Crown-Crisp Experiential Factor.						
Liew et al. (2015) *Medium*	Denmark, Recruitment: 1996–2002; Follow-up at average age 10.7 yr	Case-Control	Mother-child pairs from Danish National Birth Cohort 215 Cases (39 Females; 176 Males)	Maternal plasma Cases: 4.06 (3.08–5.50) Controls: 4.00 (3.01–5.42)	ADHD, ASD	RR and OR (stratified by quartile or by sex) per ln-unit increase in PFOA or by quartiles	ADHD: 0.98 (0.82, 1.16) ASD: 0.98 (0.73, 1.31) No statistically significant associations by quartiles or by sex

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
			545 Controls (33 Females; 180 Males)				
	Results: Lowest quartile used as reference. **Confounding:** Maternal age at delivery, SES, parity, smoking and drinking during pregnancy, psychiatric illnesses, gestational week of blood drawn, child's sex, birth year.						
Liew et al. (2018) *Medium*	Denmark, Recruitment: 1996–2002; Follow-up at age 5 yr	Cohort	Pregnant women and their children from the Danish National Birth Cohort N = 1,592	Maternal plasma 4.28 (3.15–5.49)	Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) full-scale IQ, performance IQ, verbal IQ	Regression coefficient for mean difference per ln-unit increase in PFOA and by quartiles	Full-Scale IQ: −0.1 (−2.7, 2.4) Performance IQ: 0.5 (–2.1, 3.0) Verbal IQ: −1.1 (−3.7, 1.6) No statistically significant associations or trends by quartiles
	Results: Lowest quartile used as reference. **Confounding:** Maternal age at childbirth, parity, maternal socioeconomic status, maternal IQ, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal pre-pregnancy BMI, gestational week of blood draw.						
Long et al. (2019) *Medium*	Denmark, Recruitment: 1982–1999; Follow-Up: 1993–2009	Case-Control	Pregnant women and their children from the Historic Birth Cohort at Statens Serum Institut 37 Cases (7 Females; 29 Males) 50 Controls (15 Females; 35 Males)	Amniotic fluid Cases: 0.29 (Range: 0.10–0.78) Controls: 0.32 (Range: 0.10–1.86)	ASD	OR per unit increase in PFOA	0.164 (0.013, 2.216), p-value = 0.167 Females: 0.001 (0, 192.7), p-value = 0.275 Males: 0.270 (0.020, 3.634), p-value = 0.536
	Confounding: Child's birth year, child sex, mother's age at delivery, father's age at childbirth, birth weight, gestational week at sampling, gestational age at birth, Apgar score, parity, congenital malformation.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Lyall et al. (2018) *Medium*	United States, 2007–2009	Case-Control	Children and adolescents aged 4.5–9 yr from EMA study N = 985 (553 Cases; 432 Controls)	Maternal serum Cases: GM = 3.58 (95% CI: 3.41–3.76) Controls: GM = 3.67 (95% CI: 3.49–3.86)	ASD measured by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR), intellectual disability	OR per ln-unit increase in PFOA and by quartiles	ASD: 0.78 (0.60, 1.01) Q2: 0.56 (0.39, 0.81) Q3: 0.58 (0.40, 0.86) Q4: 0.62 (0.41, 0.93), p-trend = 0.05 Intellectual Disability: 0.63 (0.43, 0.92) Q2: 0.44 (0.26, 0.76) Q3: 0.67 (0.39, 1.14) Q4: 0.48 (0.26, 0.88), p-trend = 0.06
	Results: Lowest quartile used as reference. **Confounding:** Matching factors, parity, maternal age, race/ethnicity, weight at sample collection, and maternal birthplace.						
Oulhote et al. (2019) *Medium*	Faroe Islands, Recruitment: 1997–2000; Follow-up at age 7 yr	Cohort	Children N = 419	Maternal blood 3.25 (2.54–3.99)	Boston Naming Test with and without cues, SDQ total score	Regression coefficient per IQR increase in PFOA	Boston Naming Test With Cues Prenatal: −0.14 (−0.26, 0.05) 5-yr serum: −0.01 (−0.07, 0.05) Without Cues Prenatal: −0.07 (−0.16, 0.00) 5-yr serum: −0.01 (−0.07, 0.05) SDQ Prenatal: 0.11 (0.02, 0.26) 5-yr serum: 0 (−0.06, 0.06)
	Confounding: None reported.						
Quaak et al. (2016) *Medium*	Netherlands, Recruitment: 2011–2013; Follow-up through age 18 mo	Cohort	Pregnant women and their children from LINC 54 (20 Females; 34 Males)	Cord blood 870.0 ng/L (Range: 200–2,300 ng/L)	Child Behavior Checklist 1.5–5 (CBCL 1.5–5) measures of ADHD, externalizing behavior	Regression coefficient by tertiles	ADHD Slightly negative, not statistically significant associations for overall population and males. Slightly positive for females. No interactions reported by sex. Externalizing Behavior

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							T2: −3.33 (−7.65, 0.29), p-value = 0.12 T3: −2.30 (−6.88, 1.55), p-value = 0.31 Females T2: −5.24 (−12.82, 0.00), p-value = 0.10 T3: 0.71 (−3.83, 5.21), p-value = 0.74 Males T2: −5.87 (−10.76, −0.43), p-value = 0.05 T3: −5.54 (−11.57, −0.29), p-value = 0.09
	Results: Lowest tertile used as reference. **Confounding:** Alcohol use, smoking, family history of ADHD, education.						
Shin et al. (2020) *Medium*	United States, Recruitment: 2002–2009; Follow-up: 2009–2017	Case-Control	Mother-child pairs from the CHARGE study, with children aged 2–5 yr 453 (239 Cases; 214 Controls; 88 Females; 365 Males)	Maternal serum 2.33 (1.59–3.32)	ASD measured by Autism Diagnostic Interview-Revised (ADI-R)	OR per increase (ln-unit or linear scale) in modeled, maternal, prenatal PFOA or measured, maternal, postnatal PFOA and by quartiles	By modeled prenatal exposure Ln-unit: 0.94 (0.59, 1.49) Linear: 1.01 (0.89, 1.14) By measured postnatal levels Ln-unit: 1.09 (0.71, 1.67) Linear: 1.06 (0.84, 1.33) No statistically significant associations, trends, or interactions by quartiles or by sex
	Results: Lowest quartile used as reference. **Confounding:** Child's age, child's sex, regional center, child's birth year, parity, gestational age at delivery, maternal race/ethnicity, maternal birthplace, mother's age at delivery, maternal pre-pregnancy BMI, periconceptional maternal vitamin intake, homeownership, breastfeeding duration.						
Skogheim et al. (2019) *Medium*	Norway, Recruitment: 1999–2008;	Cohort	Mother-child pairs from MoBa	Maternal plasma 2.50 (1.77–3.21)	Nonverbal and Verbal Working Memory measured	Regression coefficient per unit increase in	Nonverbal Working Memory Q2: −0.12 (−0.32, 0.09) Q3: −0.19 (−0.41, 0.03)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up: 2007–2011		N = 943		by Stanford Binet Intelligence Scales	PFOA and by quintiles	Q4: −0.18 (−0.41, 0.05) Q5: −0.38 (−0.61, −0.15), p-value < 0.01 Verbal Working Memory Q2: 0.17 (−0.05, 0.40) Q3: 0.32 (0.07, 0.56) Q4: 0.24 (−0.01, 0.49) Q5: 0.24 (−0.01, 0.50)
	Results: Lowest quintile used as reference. **Confounding:** Maternal education, age, parity, fish intake, child sex, child age at testing, maternal ADHD symptoms.						
Spratlen et al. (2020a) *Medium*	United States, Recruitment: 2001–2001; Follow-up at age 1, 2, and 3 yr	Cohort	Pregnant women and their children from the Columbia University Birth Cohort N = 302 (150 Females; 152 Males)	Cord blood GM = 2.31 (Range: 0.18–8.14)	BSID-II scores: Mental and Psychomotor Development Index (MDI and PDI), Full IQ, Performance IQ, Verbal IQ	Regression coefficient of mean difference per log-unit increase in maternal PFOA	MDI Year 1: −1.10 (−3.83, 1.63) Year 2: 1.26 (−2.64, 5.16) Year 3: 3.93 (0.08, 7.77) PDI Year 1: −1.05 (−6.02, 3.92) Year 2: 0.23 (−3.27, 3.74) Year 3: 2.35 (−2.84, 7.54) Full IQ Year 4: 2.50 (−1.15, 6.15) Year 6: 0.87 (−3.89, 5.63) Performance IQ Year 4: 0.64 (−4.12, 5.4) Year 6: −1.37 (−6.25, 3.51) Verbal IQ Year 4: 3.99 (−0.34, 8.32) Females: 5.97 (0.34, 11.6) Males: 1.92 (−4.76, 8.60) Interaction p-value = 0.29 Year 6: 3.02 (−2.49, 8.53)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							No other statistically significant associations or interactions by sex
	Comparison: Logarithm base not specified. **Confounding:** Maternal age, material hardship, parity, pre-pregnancy BMI, maternal IQ, maternal race, maternal education, family smoking status, child age at testing, child's gestational age at birth, maternal demoralization, trimester on 9/11, child's sex, child's breastfeeding history.						
Stein et al. (2013) *Medium*	United States, Recruitment: 2005–2006, Follow-Up: 2009–2010	Cohort	Pregnant mothers and their children aged 6–12 yr from the C8 Health Project Modeled = 284 Measured = 319	Modeled in utero exposure 43.7 (11.7–110.8) Serum 35.0 (15.3–93.2)	NEPSY-II scores: comprehension of instructions, design copying, narrative memory free and cued recall, word generation semantic/initial letter Wechsler Abbreviated Scale of Intelligence: Full-scale IQ, performance IQ, verbal IQ Conners' Continuous Performance test scores: clinical confidence index, commissions T-score, hit reaction time T-score, omissions T-score	Regression coefficient per ln-unit increase in PFOA and by quartiles	Comprehension of instructions Prenatal: 0.14 (−0.08, 0.36) By serum: 0.03 (−0.22, 0.28) Design copying Prenatal: 0.21 (−0.06, 0.48) Q4: 1.02 (0, 2.04) By serum: 0.26 (−0.04, 0.55) Narrative memory free and cued Recall Prenatal: −0.14 (−0.36, 0.08) By serum: −0.07 (−0.31, 0.17) Word generation semantic/initial letter Prenatal: 0.10 (−0.09, 0.30) By serum: 0.03 (−0.19, 0.25) Full-scale IQ Prenatal: 0.83 (−0.13, 1.79) Q4: 4.61 (0.68, 8.54) By serum: 0.99 (−0.06, 2.04) Performance IQ Prenatal: 0.58 (−0.39, 1.55) By serum: 0.94 (−0.14, 2.01)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Wechsler Individual Assessment Test-II (WIAT-II) scores: word reading/pseudoword decoding, numeral operations		Verbal IQ Prenatal: 0.41 (−0.60, 1.42) By serum: 0.29 (−0.83, 1.40) Clinical confidence index Prenatal: −2.37 (−4.24, −0.50) Q2: −2.14 (−9.86, 5.57) Q3: −7.68 (−15.32, −0.04) Q4: −8.49 (−16.14, −0.84) By serum: −2.15 (−4.19, −0.10) Q2: −5.62 (−12.52, 1.27) Q3: −3.23 (−10.37, 3.91) Q4: −6.90 (−14.04, 0.25) Commissions t-score Prenatal: −0.17 (−0.89, 0.55) Q2: 1.52 (−1.46, 4.51) Q3: 0.16 (−2.79, 3.12) Q4: 0.03 (−2.93, 2.99) By serum: 0.12 (−0.66, 0.91) Q2: 0.95 (−1.71, 3.61) Q3: −0.32 (−3.08, 2.44) Q4: 0.60 (−2.16, 3.36) Hit reaction time t-score Prenatal: −0.37 (−1.22, 0.49) Q2: −1.69 (−5.24, 1.86) Q3: −1.88 (−5.40, 1.63) Q4: −1.38 (−4.90, 2.14) By serum: −0.70 (−1.63, 0.24) Q2: −1.67 (−4.84, 1.49) Q3: −1.76 (−5.04, 1.52) Q4: −1.73 (−5.01, 1.55)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Omissions t-score Prenatal: −0.02 (−1.06, 1.03) Q2: 0.10 (−4.21, 4.42) Q3: −0.40 (−4.68, 3.88) Q4: 0.10 (−4.19, 4.38) By serum: 0.12 (−0.66, 0.91) Q2: −2.20 (−5.95, 1.55) Q3: 0.07 (−3.82, 3.95) Q4: −0.57 (−4.46, 3.31) Word reading Prenatal: 0.50 (−0.40, 1.41) Q2: 1.72 (−2.05, 5.48) Q3: 0.61 (−3.07, 4.30) Q4: 2.27 (−1.43, 5.96) By serum: −0.02 (−1.01, 0.98) Q2: −1.32 (−4.70, 2.06) Q3: −1.91 (−5.34, 1.52) Q4: −1.09 (−4.54, 2.36) Numerical operations Prenatal: 0.65 (−0.48, 1.78) Q2: 4.45 (−0.25, 9.14) Q3: 4.75 (0.13, 9.36) Q4: 3.12 (−1.51, 7.76) Females: −0.6 (−5.0, 3.9) Males: 4.4 (0.4, 9.2) p-value for interaction by sex = 0.14 By serum: 0.15 (−1.17, 1.46) Q2: 0.36 (−4.17, 4.88) Q3: 1.11 (−3.51, 5.73) Q4: −0.41 (−5.06, 4.25) Females: −4.1 (−8.6, 0.3) Males: 3.9 (0.2, 9.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							p-value for interaction by sex = 0.01 No other statistically significant interactions by sex
	Results: Lowest quartile used as reference. For brevity, only statistically significant associations by quartiles are included for NEPSY-II and Wechsler Abbr. **Confounding:** Child age at neuropsychological assessment, child sex, test examiner, HOME score, maternal Full-Scale IQ, child BMI at neuropsychological assessment.						
Strøm et al. (2014) *Medium*	Denmark Recruitment: 1988–1999 Follow-up: 2010	Cohort	Pregnant women and their children, from the DaFO88 cohort N = 876	Maternal serum 3.7 (IQR = 2.0)	Depression, ADHD, scholastic achievement	Depression, ADHD: Hazard ratio (depression and ADHD) by tertile Scholastic achievement: Regression coefficient per unit increase in PFOA and by tertiles	Depression T2: 1.37 (0.85, 2.21) T3: 1.03 (0.61, 1.73) p-value for trend = 0.28 ADHD T2: 0.48 (0.18, 1.28) T3: 0.74 (0.29, 1.87) p-value for trend = 0.45 Scholastic Achievement: −0.07 (−0.15, 0.001), p-value = 0.18 T3: −0.25 (−0.64, 0.14), p-value = 0.21
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, pre-pregnancy BMI, parity, maternal smoking during pregnancy, maternal education, maternal cholesterol, maternal triglycerides, offspring sex.						
Vuong et al. (2016) *Medium*	United States, Recruitment: 2003–2006; Follow-up at ages 5 and 8 yr	Cohort	Children ages 5 and 8 yr from the HOME study N = 218	Maternal serum 5.4 (3.6–7.5)	Behavior Rating Inventory of Executive Function (BRIEF) scores for behavioral regulation index,	All outcomes: OR for score ≥60 per unit increase in PFOA	Behavioral Regulation: 1.11 (−1.22, 3.44) Metacognition: 0.58 (−1.77, 2.93) Global Executive Function: 1.06 (−1.33, 3.45)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					metacognition index, global executive composite, inhibit, shift, emotional control, working memory, plan/organize, initiate, organization of materials, monitor	Index and compositive scores only: Regression coefficient per ln-unit increase in PFOA and by quartiles	No statistically significant associations or interactions by age; no statistically significant associations or trends by quartiles Inhibit: 1.45 (0.76, 2.77) Shift: 1.01 (0.51, 1.98) Emotional control: 1.33 (0.62, 2.84) Working memory: 0.84 (0.47, 1.47) Plan/organize: 1.43 (0.74, 2.76) Initiate: 2.13 (0.89, 5.10) Organization: 1.83 (0.81, 4.16) Monitor: 1.80 (0.86, 3.78)
	Confounding: Maternal age, race, education, income, maternal serum cotinine, maternal depression, HOME score, maternal IQ, marital status, child sex.						
Vuong et al. (2018b) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 3 and 8 yr	Cohort	Children from the HOME study N = 204	Serum 3 yr: 5.4 (3.7–7.4) 8 yr: 2.4 (1.8–3.2)	BRIEF measures of behavioral regulation, metacognition, global executive composite indices	OR per ln-unit increase in PFOA	Behavioral Regulation 3 yr: 1.01 (0.29, 3.53) 8 yr: 1.56 (0.49, 4.92) Metacognition 3 yr: 1.30 (0.47, 3.57) 8 yr: 3.18 (1.17, 8.60) Global Executive Function 3 yr: 1.39 (0.45, 4.24) 8 yr: 2.69 (0.92, 7.90)
	Confounding: Maternal age, race/ethnicity, household income, maternal smoking status, maternal alcohol consumption, maternal depression, HOME score, marital status, maternal marijuana use, maternal IQ, maternal serum PCBs, maternal blood lead levels, child sex.						
Vuong et al. (2018a) *Medium*	United States, Recruitment: 2003–2006;	Cohort	Mother-child dyads from the HOME study	Serum Prenatal: 5.2 (3.6–7.6)	Conners' Continuous Performance Test-	Regression coefficient per	Conners' Commissions Prenatal: −2.0 (−3.8, −0.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Follow-up at age 3 and 8 yr		N = 204	3 yr: 5.4 (3.7–7.4) 8 yr: 2.5 (1.7–13.2)	II commissions t-score, omissions t-score, hit reaction time, tau (ms) Virtual Morris Water Maze (VMWM) scores for visual-spatial learning distance (pool units), learning time (s), memory retention distance (%), and memory retention time (s)	ln-unit increase in PFOA	3 yr: −0.1 (−2.3, 2.1) 8 yr: −0.01 (−2.4, 2.4) Omissions Prenatal: −2.3 (−7.1, 2.6) 3 yr: −1.9 (−7.8, 3.9) 8 yr: 1.0 (−5.8, 7.8) Hit reaction time Prenatal: −0.7 (−3.5, 2.2) 3 yr: 0.2 (−3.5, 4.0) 8 yr: −2.3 (−6.8, 2.3 Tau Prenatal: −10.6 (−43.6, 22.3) 3 yr: 22 (−16.5, 60.6) 8 yr:14.6 (−21.9, 51.1) Visual-spatial scores (VMWM) Learning distance Prenatal: −0.1 (−1.7, 1.5) 3 yr: 0.5 (−1.2, 2.2) 8 yr: 0.1 (−1.8, 2.0) Learning time Prenatal: 0.5 (−2.0, 3.0) 3 yr: 1.4 (−1.4, 4.2) 8 yr: −0.1 (−3.5, 3.3) Memory retention distance Prenatal: 2.8 (−1.7, 7.4) 3 yr: −0.9 (−7.1, 5.4) 8 yr: 1.1 (−5.8, 8.0) Memory retention time Prenatal: −0.3 (−2.0, 1.3) 3 yr: −1.5 (−3.3, 0.2) 8 yr: −0.1 (−2.4, 2.1)
	Confounding: Maternal age, race/ethnicity, household income, maternal smoking status, maternal alcohol consumption, maternal depression, HOME score, marital status, maternal marijuana use, maternal IQ, maternal serum ΣPCBs, maternal blood lead levels, child sex.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Vuong et al. (2019) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 3 and 8 yr	Cohort	Pregnant women and their children from the HOME study N = 221	Serum Maternal: GM = 5.2 8 yr: GM = 2.4	Wechsler Intelligence Scale for Children–Fourth Edition (WISC-IV): full-scale IQ, perceptual reasoning, processing speed, verbal comprehension, working memory	Regression coefficient per ln-unit increase in PFOA	Full-Scale IQ Prenatal: 3.3 (−0.4, 6.9) 3 yr: 2.4 (−1.5, 6.4) 8 yr: 2.3 (−3.3, 7.9) Perceptual Reasoning Prenatal: 0.7 (−3.2, 4.6) 3 yr:1.2 (−3.0, 5.4) 8 yr: 2.3 (−3.7, 8.2) Processing Speed Prenatal: 3.3 (−0.8, 7.5) 3 yr: 1.7 (−2.6, 6) 8 yr: 2.8 (−3.0, 8.5) Verbal Comprehension Prenatal: 2.3 (−1.1, 5.6) 3 yr: 1.0 (−2.9, 4.8) 8 yr: −1.8 (−6.9, 3.2) Working Memory Prenatal: 4.1 (0.3, 8.0) 3 yr: 2.9 (−1.0, 6.7) 8 yr: 4.3 (−0.7, 9.3)
	Confounding: Maternal age, race/ethnicity, household income, maternal marijuana use, maternal blood lead, maternal serum ΣPCBs and cotinine, maternal depression, vitamin use, maternal IQ, marital status, HOME score, child sex, breastfed.						
Vuong et al. (2020a) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 8 yr	Cohort	Mother-child pairs with children aged 8 yr from the HOME study N = 161	Maternal serum Mean = 6.1 (SD = 3.8)	Wide Range Achievement Test 4 (WRAT-4) reading composite score	Regression coefficient per log10-unit increase in PFOA	12.6 (3.0, 22.2)
	Confounding: Maternal age, race/ethnicity, education, household income, marital status, maternal depression, maternal serum cotinine, maternal blood lead levels, maternal fish consumption, maternal IQ, child sex, HOME score.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Wang et al. (2015) *Medium*	Taiwan, Recruitment: 2000–2001; Follow-up at age 5 yr	Cohort	Pregnant women and their children aged 5 and 8 yr from TMICS N = 120	Serum 5 yr: 2.50 (1.54–3.35) 8 yr: 2.50 (1.54–3.33)	Full-Scale IQ, Performance IQ, Verbal IQ	Regression coefficient per log2-unit increase in PFOA	Full-Scale IQ 5 yr: 1.2 (−1.0, 3.5) 8 yr: −0.4 (−2.5, 1.7) Performance IQ 5 yr: 1.0 (−1.4, 3.4) 8 yr: −1.1 (−3.2, 1.0) Verbal IQ 5 yr: 0.9 (−1.4, 3.3) 8 yr: 0.5 (−1.5, 2.5)
	Confounding: Maternal education, family annual income, children's age, sex, HOME score at IQ assessment.						
Zhang et al. (2018a) *Medium*	United States, Recruitment: 2003–2006; Follow-up at age 3, 5, and 7 yr	Cohort	Pregnant women and their children aged 3, 5, and 7 yr from the HOME study N = 167	Serum Maternal: 5.4 (3.6–7.3) 3 yr: 5.5 (3.9–7.7) 8 yr: 2.4 (1.8–3.2)	Basic reading, brief reading, letter word identification, passage comprehension measured by Woodcock Johnson Test of Achievement-III (WJ-III) Reading composite, word reading, sentence comprehension measured by Wide Range Achievement Test 4 (WRAT-4)		Basic Reading Maternal Serum: 0.7 (−4.9, 6.2) Year 3 Serum: 6.4 (−1.6, 14.1) Brief Reading Maternal Serum: 3.7 (−1.8, 9.3) Year 3 Serum: 10.4 (2.8, 18.1) Letter Word Identification Maternal Serum: 2.0 (−3.1, 7.1) Year 3 Serum: 9.2 (2.1, 16.3) Passage Comprehension Maternal Serum: 3.8 (0.1, 7.7) Year 3 Serum: 8.5 (3.3, 13.7) Word Attack Maternal Serum: 0.5 (−5.1, 6.1) Year 3 Serum: 4.9 (−2.0, 11.8) Reading Composite Maternal Serum: 3.5 (−1.1, 8.2) Year 3 Serum: 2.8 (−3.1, 8.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							Year 8 Serum: 2.6 (−3.1, 8.2) Word Reading Maternal Serum: 2.3 (−2.1, 6.7) Year 3 Serum: 1.0 (−4.7, 6.7) Year 8 Serum: 6.1 (0.9, 11.3) Sentence Comprehension Maternal Serum: 3.7 (−1.6, 9.0) Year 3 Serum: 3.1 (−4.1, 10.1) Year 8 Serum: −0.1 (−6.6, 6.4)
	Confounding: Maternal age, race, education, household income, parity, smoking (serum cotinine concentration, ng/mL), maternal IQ, breastfeeding duration (weeks), HOME score.						
			General Population				
Ding and Park (2020) *Medium*	United States, 2003–2016	Cross-sectional	Adults aged 20–69 yr from NHANES N = 2,731	Serum 2.0 (1.3–2.9)	High and low frequency hearing impairment (HFHI and LFHI)	OR per log2-unit increase in PFOA and ≥90th percentile vs. < 90th percentile	HFHI OR (per doubling): 0.97 (0.82, 1.14) OR (90th percentiles): 1.05 (0.61, 1.81) LFHI OR (per doubling): 0.98 (0.73, 1.32) OR (90th percentiles): 1.40 (0.48, 4.07)
	Confounding: Age, age square, sex, race/ethnicity, education level, poverty-income ratio, smoking status, BMI, noise exposures (occupational, recreational, firearm noise), NHANES cycles.						
Gallo et al. (2013) *Medium*	United States, 2005–2006	Cross-sectional	Adults aged 50+ years from the C8 Health Project N = 21,024	Serum Range: 0.25–22,412	Memory impairment (self-reported)	OR per doubling of PFOA and by quartiles	OR: 0.96 (0.94, 0.98) Q2: 0.88 (0.79, 0.97) Q3: 0.83 (0.75, 0.92) Q4: 0.79 (0.71, 0.88) Q5: 0.79 (0.71, 0.88) p-trend < 0.001
	Comparison: Logarithm base not specified.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Results: Lowest quartile used as reference. **Confounding:** Age, ethnicity, gender and school level, household income, physical activity, alcohol consumption, cigarette smoking.						
Lenters et al. (2019) *Medium*	Norway, Recruitment: 2003–2009; Follow-up: 2008–2016	Cohort	Children and adults from HUMIS N = 1,199	Breast milk 40.000 ng/L (26.809–61.256 ng/L)	ADHD	OR per IQR increase in ln-unit PFOA	1.35 (0.87, 2.11), p-value = 0.183
	Confounding: Maternal age, childbirth year, maternal education, parity, smoking during pregnancy, small-for-gestational age, preterm birth, maternal pre-pregnancy BMI, single mother around perinatal period, maternal fish intake.						
Li (2020) *Medium*	United States, 1999–2006	Cross-sectional	Adults aged 20+ years from NHANES N = 2,525	Serum 2.25 (Range: 0.07–51.1)	Hearing threshold > 25 dB by frequency	OR by quartiles	2,000 Hz Q2: 1.41 (0.95, 2.10) Q3: 1.26 (0.85, 1.87) Q4: 1.76 (1.20, 2.60), p-trend < 0.01 3,000 Hz Q2: 1.39 (0.98, 1.98) Q3: 1.38 (0.98, 1.96) Q4: 1.64 (1.16, 2.34), p-trend = 0.02 4,000 Hz Q2: 1.31 (0.95, 1.83) Q3: 1.12 (0.81, 1.56) Q4: 1.41 (1.01, 1.98), p-trend = 0.11
	Results: Lowest quartile used as reference. **Confounding:** Age, sex, BMI, education, ethnicity group, family income, sample weights.						
Shrestha et al. (2017) *Medium*	United States, 2000–2002	Cross-sectional	Residents aged 55–74 yr who lived adjacent to Hudson River N = 126	Serum 8.1 (5.9–11.9)	Affective state: Beck Depression Inventory (BDI) total score, State-Trait Anxiety	Regression coefficient per IQR increase in ln-unit PFOA	Depression: 0.08 (−0.85, 1.02), p-value = 0.86 CVLT Total Score: 2.63 (0.20, 5.06), p-value = 0.03

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Inventory state and trait t-scores Attention: Trail-making test Part A (ln-transformed time to complete) Executive function: Stroop color word test t-score, Trail-making test part B (ln-transformed time to complete), Wisconsin Card Sorting Test preservative ln-transformed error and response Memory and learning: California Verbal Learning Test total and subscores, Wechsler Memory Scale logical memory and visual reproduction immediate and delayed recall scores		Wisconsin Card Sorting Test Perseverative Error: −0.18 (−0.34, −0.01), p-value = 0.04 Perseverative Response: −0.20 (−0.38, −0.02), p-value = 0.03 Wechsler Memory Scale Logical Memory Immediate Recall: 0.28 (−0.85, 1.42), p-value = 0.62 Delayed Recall: 0.09 (−0.98, 1.15), p-value = 0.87 Visual Reproduction Immediate Recall: −0.11 (−0.79, 0.56), p-value = 0.74 Delayed Recall: −0.12 (−0.83, 0.59), p-value = 0.74 No statistically significant associations: State-Trait Anxiety Inventory, Stroop color word test, trail-making tests, motor function outcomes, visuospatial outcomes

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Motor function (dominant and non-dominant hands): finger tapping test average scores, grooved pegboard test ln-transformed times to completion, static motor steadiness test ln-transformed total numbers of contacts and times touching, dominant hand reaction time Visuospatial function: Wechsler Adult Intelligence Scale-Revised total scores for block design and digit symbol coding		
	Confounding: Age, sex, education, serum total PCB.						
			Pregnant Women				
Vuong et al. (2020b) *Medium*	United States Recruitment: 2003–2006 Follow-up: ~20 wk gestation and postpartum	Cohort	Pregnant women from the HOME study N = 300	Maternal serum 5.4 (3.6–7.6)	Beck Depression Inventory-II (BDI-II)	Relative risk per ln-unit increase in PFOA	Medium Score Trajectory: 1.3 (0.8, 2.0) High Score Trajectory: 0.9 (0.5, 1.9) OR for score > 13 from pregnancy to 8 yr postpartum: 1.1 (0.7, 1.6)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	(4 wk, 1, 2, 3, 4, 5, and 8 yr)						

Notes: ADHD = attention deficit hyperactivity disorder; ALSPAC = Avon Longitudinal Study of Parents and Children; ASD = autism spectrum disorder; ASQ = Ages and Stages Questionnaire; BDI = Beck Depression Inventory; BMI = body mass index; BRIEF = Behavior Rating Inventory of Executive Function; BSID-II = Bayley Scales of Infant Development second edition; CDI = Comprehensive Developmental Inventory; CHARGE = Childhood Autism Risk from Genetics and Environment; CVLT = California Verbal Learning Test; DaFO88 = Danish Fetal Origins 1988; EMA = Early Markers for Autism; GM = geometric mean; HFHI = high-frequency hearing impairment; HOME = Health Outcomes and Measures of the Environment; HUMIS = Human Milk Study; ID = intellectual disability; IQ = intelligence quotient; IQR = interquartile range; KBIT-2 = Kaufman Brief Intelligence Test Second Edition; LFHI = low-frequency hearing impairment; LINC = Linking Maternal Nutrition to Child Health; MCDI = MacArthur-Bates Communicative Development Inventories; MDI = Mental Development Index; mo = month/s; MoBa = Mother, Father, and Child Cohort Study; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; PDI = Psychomotor Development Index; PPVT-III = Peabody Picture Vocabulary Test – Third Edition; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire; SES = socioeconomic status; SRS = Social Responsiveness Scale; T2 = tertile 2; T3 = tertile 3; TMICS = Taiwan Maternal and Infant Cohort Study; VMWM = Virtual Morris Water Maze; WIAT-II = Wechsler Individual Achievement Test-II; WISC-IV = Wechsler Intelligence Scale for Children–Fourth Edition; WJ-III = Woodcock Johnson Test of Achievement-III; WPPSI-R = Wechsler Primary and Preschool Scales of Intelligence-Revised; WRAML = Wide Range Assessment of Memory & Learning 2; WRAT-4 = Wide Range Achievement Test 4; WRAVMA = Wide Range Assessment of Visual Motor Abilities; 2yr = year/s.

[a] Exposure levels are reported as median unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.9 Renal

Table D-18. Associations Between PFOA Exposure and Renal Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
General Population							
Dhingra et al. (2016b) High	United States, 1951–2011	Cohort	Adults from C8 Health Project/C8 Science Panel, >20 yr,	Serum Cumulative PFOA exposure at failure or end of follow-up: Mean = 3.32 ng/	CKD	HR by PFOA quintiles, at 0-, 5-, 10-, and 20-yr lags	Main cohort 0-yr lag: Quintile 2: 1.26 (0.9, 1.75), p-value = 0.18 Quintile 3: 1.12 (0.8, 1.55), p-value = 0.52

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			Main cohort = 28,240, prospective cohort = 27,952	mL-yr (SD = 7.26)			Quintile 4: 1.12 (0.81, 1.56), p-value = 0.49 Quintile 5: 1.24 (0.88, 1.75), p-value = 0.21 p-value for trend = 0.80 5-, 10-, and 20-yr lag: No statistically significant associations or trends Prospective cohort Quintile 2: 1.36 (0.89, 2.09), p-value = 0.16 Quintile 3: 0.94 (0.62, 1.45), p-value = 0.79 Quintile 4: 1.12 (0.72, 1.75), p-value = 0.6 Quintile 5: 1.08 (0.7, 1.66), p-value = 0.74 p-value for trend = 0.77
	Outcome: CKD was self-reported then confirmed by medical records or presence in United States Renal Data System renal failure registry (nonneoplastic, non-genetic, and diagnosed after age 20). **Results:** Lowest quintile used as reference group. **Confounding:** Gender, time-varying self-reported hypertension, time-varying self-reported diabetes diagnosis, time-varying self-reported high cholesterol diagnosis, time-varying smoking, category of BMI, and education category.[c]						
Dhingra et al. (2017) Medium	United States, 2005–2006	Cross-sectional	Women from C8 Science Panel, 30–65 yr, N = 29,641	Serum Measured: 60th percentile = 36.3 µg/mL (20th–80th percentile = 11.1–88.0 µg/mL)	eGFR	Regression coefficient per ln-unit increase in PFOA, or by quintiles, or by deciles	Modeled serum PFOA Per ln increase: 0.05 (0.01), p-value = 0.43 Quintile 2: −0.08 (0.27), p-value = 0.77 Quintile 3: 0.37 (0.27), p-value = 0.17 Quintile 4: 0.21 (0.27), p-value = 0.44

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Modeled: 60th percentile = 26.8 µg/mL (20th–80th percentile = 5.8–82.4 µg/mL)			Quintile 5: 0.23 (0.27), p-value = 0.41 Dose response by deciles: decreased until the 4th decile and remained approximately flat thereafter Measured serum PFOA Per ln increase: −0.14 (0.07), p-value = 0.03 Quintile 2: −0.64 (0.27), p-value = 0.018 Quintile 3: −1.03 (0.27), p-value = 0.0001 Quintile 4: −0.84 (0.27), p-value = 0.0019 Quintile 5: −0.98 (0.27), p-value = 0.0003
	Results: Lowest quintile used as reference group. Effect estimates are provided with standard deviation in parentheses. **Confounding:** Smoking status, BMI, education level, race, sex, and birth year.						
Lin et al. (2013a) *Low*	Taiwan, 2006–2008	Cross-sectional	Adolescents and young adults from YOTA study, 12–30 yr, N = 644	Serum 3.49 (75th percentile = 6.54)	Uric acid (mg/dL)	Mean concentration by PFOA percentiles	≤50th percentile: 6.08 (0.1) 50th–75th: 6.08 (0.11) 75th–90th: 6.11 (0.14) >90th: 6.13 (0.17) p-value for trend = 0.983
	Results: Effect estimates are provided with standard error in parentheses. **Confounding:** Age, gender, smoking status, alcohol drinking, BMI.						
Blake et al. (2018) Medium	United States, 1991–2008	Cohort	Adults and children, Fernald Community Cohort (FCC)	Serum 12.7 (7.83–19.5)	eGFR	Percent change per IQR increase in PFOA	All: Repeated-measures model: −0.83 (−2.44, 0.77); p-value = 0.31 Latent model: −0.74 (−2.45, 0.96); p-value = 0.39

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
			N = 192 (115 females, 77 males)				Female: −1.38 (−3.41, 0.65), p-value = 0.18 Male: 0.95 (−3.08, 4.98), p-value = 0.21 p-value for interaction by sex = 0.38
	Confounding: Age, year of measurement, sex, education, income, marital status, and BMI.						
Conway et al. (2018) *Low*	United States, 2005–2006	Cohort	Adults, C8 Health Project, Diabetic = 5,210, non-diabetic = 48,440	Serum Diabetic: 28.6 (12.6–72.7) Non-diabetic: 28.0 (13.6–71.4)	CDK (eGFR of < 60 mL/min/1.73 m^2)	OR per ln-unit increase in PFOA	Diabetics: 0.92 (0.86, 0.98) Non-diabetic: 0.99 (0.96, 1.03)
	Confounding: Age, sex, BMI, HDL, LDL, white blood cell count, CRP, hemoglobin, and iron.						
Covertino et al. (2018) *Low*	United Kingdom, 2008–2011	Controlled trial	Adults, solid-tumor cancer patients N = 49	Plasma Exposure levels non reported	Creatinine (µmol/L), urea (µmol/L)	Regression coefficient per 1-µM increase in PFOA	No observable differences with measured plasma PFOA concentrations
	Confounding: None reported.						
Arrebola et al. (2019) *Low*	Spain, 2009–2010	Cross-sectional	Adults, BIOAMBIENT.ES study N = 342	Serum 1.83 (1.34–2.53)	Uric acid (mg/dL), hyperuricemia	OR(hyperuricemia) or regression coefficient per log-unit increase in PFOA	Uric acid Wet-basis and lipid-basis models: 0.04 (−0.06, 0.14); p-value = 0.425 Wet-basis model with adjustment for serum lipids: 0.04 (−0.06, 0.14); p-value = 0.459 Hyperuricemia (OR) Wet-basis and lipid-basis models: 1.83 (0.93, 3.68); p-value = 0.083 Wet-basis model with adjustment for serum lipids: 1.78 (0.90, 3.45); p-value = 0.095

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Outcome: Hyperuricemia defined as at least one of a) serum uric acid levels ≥7.0 mg/dL in males or ≥6.0 mg/dL in females, at recruitment or in previous screenings, b) had been prescribed any pharmacological treatment for lowering uric acid levels, and/or c) had been diagnosed with gout by a clinician. **Comparison:** Logarithm base not specified. **Confounding:** Sex, age, body mass index, weight loss during the last 6 mo, region of recruitment, smoking habit, alcohol consumption, education, place of residence.						
Liu et al. (2018b) *Low*	United States, 2013–2014	Cross-sectional	Adults from NHANES, 18+ years, N = 1,871	Serum GM = 1.86 (SE = 1.02)	Total protein (g/dL)	Regression coefficient per ln-unit increase in PFOA	0.05 (SE = 0.03)
	Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (antihypertensive, antihyperglycemic, and antihyperlipidemic agents).						
Chen et al. (2019a) *Low*	Croatia, 2007–2008	Cross-sectional	Adults, 44–56 yr N = 122	Plasma GM = 2.87 (range = 1.03–8.02)	Uric acid (µmol/L), creatinine (µmol/L)	Regression coefficient per ln-unit increase in PFOA	Uric acid: 5.02 (−22.09, 32.09) Creatinine: 0.46 (−5.60, 6.52)
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity.						
Jain and Ducatman (2019c) *Low*	United States, 2005–2014	Cross-sectional	Adults from NHANES, ≥20 yr, N = 8,220	Serum Levels not reported	Levels of albumin in urine (log10-µg/mL), creatinine in urine (log10-mg/dL), albumin-to-creatinine ratio in urine (log10-mg/g), albumin in serum (log10-mg/dL), creatinine in serum (log10-mg/dL)	Regression coefficient per log10-unit increase in PFOA, or percent change per 10% increase in PFOA	Albumin in urine Per log10-unit increase: −0.17 p-value < 0.01 Negative associations across GF stages Percent change per 10% increase: −1.59 p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.15 Creatinine in urine Per log10-unit increase: 0.02 p-value = 0.2 No significant associations across eGFR stages

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Percent change per 10% increase: 0.22 p-value for gender and race/ethnicity interaction = 0.02 Albumin- to-creatinine ratio in urine Per log10-unit increase: −0.19 p-value < 0.01 Negative associations across GF stages Percent change per 10% increase: −1.82 p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.88 Albumin in serum Per log10-unit increase: 0.02 p-value < 0.01 Positive associations across eGFR stages Percent change per 10% increase: 0.17 p-value < 0.05 p-value for gender and race/ethnicity interaction = 0.74 Creatinine in serum Per log10-unit increase: 0.01 p-value = 0.19 Positive associations in GF-1 Negative associations in GF-3B/4 Percent change per 10% increase: 0.07

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							p-value for gender and race/ethnicity interaction < 0.01
	GF Stages: GF-1: GFR ≥ 90 mL/min/1.73m^2; GF-2: GFR between 60 and 90 mL/min/1.73m^2; GF-3A: GFR between 45 and 60 mL/min/1.73m^2; GF-3B/4: GFR between 15 and 45 mL/min/1.73m^2 **Confounding:** Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), poverty-income ration, NHANES survey period.						
Jain and Ducatman (2019a) Low	United States, 2007–2014	Cross-sectional	Adults from NHANES, ≥20 yr, Males = 3,330, females = 3,506	Serum Males: GM = 2.36 (2.24–2.48) Females: GM = 3.19 (3.06–3.32)	Uric acid (mg/dL) by glomerular filtration (GF) stage	Regression coefficient per log10-unit increase in PFOA	Males GF-1: 0.04, p-value < 0.01 GF-2: 0.05, p-value < 0.01 GF-3A: 0.03, p-value = 0.27 GF-3B: −0.07, p-value < 0.01 Females GF-1: 0.03, p-value = 0.01 GF-2: 0.02, p-value = 0.11 GF-3A: 0.09, p-value < 0.01 GF-3B: 0.004, p-value = 0.91
	GF Stages: GF-1: eGFR > 90 mL/min per 1.73 m^2, GF-2: 60 < eGFR ≤ 90 mL/min per 1.73 m^2, GF-3A: 45< eGFR ≤ 60 mL/min per 1.73 m^2; GF-3B/4: 15< eGFR ≤ 45 mL/min per 1.73 m^2 **Confounding:** Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), poverty-income ration, NHANES survey period.						
Wang et al. (2019b) *Low*	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project N = 1,612 (males = 1,204, females = 408)	Serum 6.19 (4.08–9.31)	CKD, eGFR	OR(CKD), or regression coefficient per ln-unit increase in PFOA, or by quartiles	CKD (OR) Per ln-unit increase: 0.73 (0.57, 0.95), p-value = 0.019 Q2: 0.72 (0.45, 1.13) Q3: 0.83 (0.52, 1.31) Q4: 0.60 (0.36, 1.01) p-value for trend = 0.234 eGFR Per ln-unit increase: All: 1.23 (0.30, 2.17), p-value = 0.008 Males: 1.29 (0.21, 2.36), p-value = 0.019

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Females: 1.54 (−0.36, 3.44), p-value = 0.111 p-value for interaction by sex = 0.999 Q2: 1.00 (−0.8, 2.81) Q3: 0.63 (−1.2, 2.46) Q4: 2.07 (0.22, 3.91) p-value for trend = 0.050
	Outcome: CKD defined as eGFR < 60 mL/min per 1.73 m^2. **Results:** Lowest quartile used as reference group. **Confounding:** Age, sex, BMI, education, annual income, regular exercise, cigarette smoking, drinking alcohol, family history of CKD, TC.						
Zeng et al. (2019c) *Low*	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project N = 1,612 (males = 1,204, females = 408)	Serum 6.19 (4.08–9.31)	Uric acid (mg/dL), hyperuricemia	OR (hyperuricemia) or regression coefficient (uric acid) per log10-unit increase in PFOA	Hyperuricemia (OR) All: 1.29 (1.08, 1.54) Males: 1.21 (1, 1.46) Females: 1.76 (1.06, 2.94) p-value for interaction by sex = 0.183 Uric acid All: 0.18 (0.09, 0.26), p-value < 0.001 Males: 0.17 (0.06, 0.27) Females: 0.14 (0.01, 0.27) p-value for interaction by sex = 0.988
	Outcome: Hyperuricemia defined as serum uric acid levels > 7.0 mg/dL in males or > 6.0 mg/dL in females. **Confounding:** Age, sex, BMI, income, drinking, smoking, career, exercise, offal consumption, fish and seafood consumption, serum creatinine.						
Lee et al. (2020) *Low*	United States, 1999–2016	Cross-sectional	Adults from NHANES, 18+ years, N = 46,748	Serum Exposure levels not reported	Albuminuria	OR per SD-unit increase in log10-PFOA	Discovery data set: 0.69 (0.57, 0.83). FDR = 0.006 Validation data set: 0.68 (0.58, 0.80), p-value = 0.029
	Outcome: Albuminuria defined as urine albumin-to-creatinine ratio ≥30 mg/g. **Confounding:** Age, age squared, sex, diabetes mellitus, hypertension, BMI, race/ethnicity, smoking, and socioeconomic status.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Scinicariello et al. (2020b) *Low*	United States, 2009–2014	Cross-sectional	Adults from NHANES N = 4,915 (no CKD = 4,103; CKD = 874)	Serum GM = 2.37 (SE = 0.06)	Uric acid (mg/dL), hyperuricemia, gout	OR (hyperuricemia, gout), or regression coefficient (uric acid) by quartiles	Uric acid Overall population Q2: 0.17 (0.06, 0.29) Q3: 0.24 (0.11, 0.37) Q4: 0.42 (0.26, 0.57) p-value for trend = 0.0001 Participants with CKD Q2: 0.14 (−0.38, 0.65) Q3: −0.05 (−0.63, 0.53) Q4: 0.6 (−0.04, 1.24) p-value for trend = 0.02 Participants without CKD Q2: 0.08 (−0.03, 0.2) Q3: 0.31 (0.17, 0.46) Q4: 0.16 (0.01, 0.31) p-value for trend = 0.001 Hyperuricemia (OR) Overall population Q2: 1.05 (0.77, 1.44) Q3: 1.21 (0.87, 1.69) Q4: 1.81 (1.29, 2.55) p-value for trend = 0.004 Participants with CKD Q2: 1.15 (0.69, 1.92) Q3: 0.95 (0.53, 1.69) Q4: 1.82 (0.96, 3.47) p-value for trend = 0.21 Participants without CKD Q2: 0.96 (0.64, 1.44) Q3: 1.19 (0.75, 1.88) Q4: 1.65 (1.1, 2.46) p-value for trend = 0.02 Gout (OR)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Overall population Q2: 1.75 (0.9, 3.31) Q3: 2.34 (1.32, 4.15) Q4: 3.17 (1.68, 5.98) p-value for trend = 0.01 Participants with CKD Q2: 1.83 (0.79, 4.19) Q3: 3.02 (1.28, 7.15) Q4: 2.73 (1.28, 5.84) p-value for trend = 0.04 Participants without CKD Q2: 2.11 (0.72, 6.23) Q3: 2.57 (1, 6.59) Q4: 3.88 (1.46, 10.33) p-value for trend = 0.05
	Outcomes: CKD defined as eGFR < 60 mL/min per 1.73 m^2 and/or albuminuria. Hyperuricemia defined as serum uric acid levels ≥7.0 mg/dL in males or ≥6.0 mg/dL in females. Gout was self-reported diagnosis from a health professional. **Results:** Lowest quartile used as reference group. **Confounding:** Race/ethnicity, age, sex, education, alcohol, smoking, serum cotinine, BMI, diabetes, hypertension, CKD.						
	Children and Adolescents						
Geiger et al. (2013) *Low*	United States, 1999–2000; 2003–2008	Cross-sectional	Children and adolescents from NHANES, 12–18 yr, N = 1,772	Serum Mean = 4.3 (SE = 0.1)	Uric acid (mg/dL), hyperuricemia	OR (hyperuricemia) or regression coefficient (uric acid) per ln-unit increase in PFOA or by quartiles	Hyperuricemia (OR) Per ln increase: 1.59 (1.19, 2.13) Q2: 0.94 (0.58, 1.53) Q3: 1.01 (0.62, 1.63) Q4: 1.62 (1.1, 2.37) p-value for trend = 0.007 Uric acid Per ln increase: 0.2 (0.11, 0.29) Q2: 0.02 (−0.10, 0.14) Q3: 0.03 (−0.11, 0.17) Q4: 0.3 (0.17, 0.43) p-value for trend = 0.0001
	Outcome: Hyperuricemia defined as serum uric acid levels ≥6 mg/dL.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	Results: Lowest quartile as reference group. **Confounding:** Age, sex, race/ethnicity, BMI, annual household income, moderate activity, TC, serum cotinine.						
Kataria et al. (2015) *Low*	United States, 2003–2010	Cross-sectional	Children and adolescents from NHANES, 12–19 yr, N = 1,962	Serum 3.5 (2.5–4.7)	eGFR (min/mL/1.73 m^2), uric acid (mg/dL), creatinine (mg/dL)	Regression coefficient by quartiles	eGFR Q2: −2.63 (−7.07, 1.81) Q3: −5.42 (−11.46, 0.61) Q4: −6.61 (−11.39, −1.83), p-value < 0.01 Uric acid Q2: 0.17 (−0.033, 0.37) Q3: 0.13 (−0.03, 0.28) Q4: 0.21 (0.056, 0.37), p-value < 0.01 Creatinine Q2: 0.007 (−0.012, 0.027) Q3: 0.021 (−0.008, 0.05) Q4: 0.029 (0.004, 0.054), p-value < 0.05
	Results: Lowest quartile used as reference group. **Confounding:** Sex, poverty-income ratio, caregiver education, serum cotinine, prehypertension, insulin resistance, BMI, hypercholesterolemia, race/ethnicity categories.						
Qin et al. (2016) *Low*	Taiwan 2009–2010	Cross-sectional	Children from GBCA Study, 12–15 yr, N = 225 (123 girls, 102 boys)	Serum All: 0.5 (0.4–1.3) Boys: 0.5 (0.4–1.4) Girls: 0.5 (0.4–1.2)	Uric acid (mg/dL), hyperuricemia	Regression coefficient per ln-unit increase in PFOA (uric acid), and by quartiles; OR scaled with increasing quartiles (hyperuricemia)	Uric acid All: 0.15 (0.01, 0.28), p-value = 0.032 Boys: 0.24 (0.06, 0.42), p-value = 0.011 Increasing trend in mean uric acid levels by quartiles; Q1 = 4.85 (4.53, 5.17) vs. Q4 = 5.65 (5.33, 5.96); p-value for trend = 0.033 Girls: 0.01 (−0.19, 0.22), p-value = 0.892

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							No trend in mean uric acid levels by quartiles; Q1 = 4.64 (4.43, 4.94) vs. Q4 = 4.73 (4.41, 5.06); p-value for trend = 0.756 Hyperuricemia (OR) All: 2.16 (1.29, 3.61), p-value < 0.05 Boys: 2.76 (1.37, 5.56), p-value < 0.05 Girls: 1.64 (0.69, 3.85)
	Outcome: Hyperuricemia defined as uric acid level ≥6 mg/dL. **Results:** Lowest quartile used as the reference group. **Confounding:** Age, gender, BMI, parental education level, exercise, environmental tobacco smoke exposure, and serum creatinine.						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children, 8–12 yr N = 40	Serum 0.99 (IQR = 0.45)	Creatinine (mg/dL)	Regression coefficient per unit increase in PFOA	−0.02 (−0.15, 0.11)
	Confounding: Age, sex, race.						
			Pregnant Women				
Gyllenhammar et al. (2018b) Medium	Sweden; 1996–2011	Cohort	Mothers and infants follow-up to 5 yr of age, POPUP study N = 381	Maternal serum 2.3 (1.6–3.0)	Cystatin C (GFRcc) (mL/min/1.73 m^2)	Regression coefficient per IQR increase in maternal PFOA	0.004 (SD = 0.002), p-value = 0.022
	Confounding: Sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, and total time of breastfeeding.						
Nielsen et al. (2020) *Low*	Sweden, 2009–2014	Cohort	Pregnant women, PONCH study N = 73	Serum Early pregnancy: 1.8 (5th–95th percentile = 0.8–4.4)	eGFR: LMrev, CKD-EPI(creatinine), CAPA, CKD-EPI(cystatin C), mean of LMrev	Spearman's correlation coefficient	Cross-sectional correlations consistently weak and non-significant Early to late pregnancy changes: No significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				Late pregnancy: 1.5 (5th–95th percentile = 0.7 –3.1)	and CAPA, mean of CKD-EPI$_{creatinine}$ and CKD-EPI$_{cystatin\ C}$ Glomerular pore size		eGFR: LMrev: 0.002, p-value = 0.99 CKD-EPI(creatinine): 0.03, p-value = 0.83 CAPA: 0.06, p-value = 0.64 CKD-EPI(cystatin C): 0.03, p-value = 0.83 mean of LMrev and CAPA: 0.04, p-value = 0.76 mean of CKD-EPI(creatinine) and CKD-EPI(cystatin C): 0.002, p-value = 0.98 Glomerular pore size: CAPA/LMrev: 0.09, p-value = 0.47 CKD-EPI(cystatin C)/CKD-EPI(creatinine): −0.003, p-value = 0.98
	Outcome: Glomerular pore size is estimated as the ratio between eGFR(cystatin C) and eGFR(creatinine) and was calculated by the two ratios provided. **Confounding:** Number of days between sampling, pregnancy-induced change in BMI.						
			Occupational Populations				
Rotander et al. (2015) *Low*	Australia, 2013	Cross-sectional	Firefighters with past exposure to AFFF, 17–66 yr old N = 137 (97% male)	Serum 4.2 (range = 0.3–18)	Uric acid (µmol/L)	Regression coefficient per log10-unit increase in PFOA	0.021 (SE = 0.032), p-value = 0.508
	Confounding: Age, sex, BMI, smoking status, total serum protein, PFOS, PFHxS.						

Notes: FCC = Fernald Community Cohort; YOTA = Young Taiwanese Cohort Study; GBCA = Genetic Biomarkers Study for Childhood Asthma; ; eGFR = estimated glomerular filtration rate (mL/min per 1.73 m^2); FDR = false discovery rate; GF = glomerular filtration; CKD = chronic kidney disease; BMI = body mass index; GM = geometric mean; HR = hazard ratio; IQR = interquartile range; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; OR = odds ratio; SD = standard deviation; SE = standard error; NHANES = National Health and Nutrition Examination Survey; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; PONCH = Pregnancy Obesity Nutrition and Child Health study; LMrev = Lund Malmö Revised; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration study; CAPA = Caucasian Asian Pediatric Adult; AFFF = aqueous film-forming foam; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.
[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.
[c] Confounding indicates factors the models presented adjusted for.

D.10 Hematological

Table D-19. Associations Between PFOA Exposure and Hematological Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
General Population							
Etzel et al. (2019) *Medium*	United States, 2003–2010	Cross-sectional	Children and adults from NHANES, ≥12 yr of age, N = 7,040	Serum Median = 3.9 (2.6–5.5)	Vitamin D deficiency (< 50 ng/mL), 25-hydroxy Vitamin D ([25(OH)D], nmol/L)	Regression coefficient or prevalence OR (POR) per doubling of PFOA, or by quintiles	Per doubling of PFOA: Vitamin D deficiency POR: 1.02 (0.93, 1.11) 25-hydroxy Vitamin D −0.3 (−1.0, 0.4) No significant associations or trends
	Results: Lowest quintile used as reference group. **Confounding:** Gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty to income ratio, smoking status, 6-mo examination period.[c]						
Chen et al. (2019a) *Medium*	Croatia 2007–2008	Cross-sectional	Adults, 44–56 yr of age, N = 122	Plasma GM = 2.87 (min = 1.03, max = 8.02)	Calcium in serum (mmol/L)	Regression coefficient per ln-unit increase PFOA	–0.02 (–0.07, 0.03)
	Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity.						
Jain (2020a) *Medium*	United States 2003–2016	Cross-sectional	Adults from NHANES, ≥20 yr of age, N = 11,251	Adult serum non-anemic males: GM = 3.3 (95% CI: 3.2, 3.4) non-anemic females: GM = 2.5 (95% CI: 2.4, 2.6)	Whole blood hemoglobin (WBHGB) (log10-g/dL)	Regression coefficient per log10-unit increase in PFOA	Non-anemic males: 0.009, p-value < 0.01 Non-anemic females: 0.006, p-value < 0.01 Anemic males: 0.026, p-value < 0.01 Anemic females: 0.034, p-value < 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				anemic males: GM = 2.4 (95 % CI: 2.1, 2.7) anemic females: GM = 1.6 (95% CI: 1.4, 1.7)			
	Outcome: Anemia defines as whole blood hemoglobin concentrations < 12 g/dL (females) and < 13 g/dL (males). **Confounding:** Age, BMI, poverty-income ratio, serum cotinine, survey year, daily alcohol intake.						
Convertino et al. (2018) *Low*	United Kingdom, 2008–2011	Controlled trial	Solid-tumor cancer patients ≥18 yr of age, N = 49	Plasma Range = 0–~633,527 µM	aPTT (s) Fibrinogen (g/L) PPT (s)	Regression coefficient per unit increase in PFOA	"Almost no observable differences" (statistical results not provided)
	Confounding: By design, randomly assigned exposure levels and excluded patients with life expectancy < 3 mo, anticancer therapy within the last 4 wk, HIV infection, hepatitis B or hepatitis C, inadequate hematologic function, inadequate renal function, abnormal liver function tests, lack of physical integrity of the gastrointestinal tract, uncontrolled cardiac disease, or use of warfarin, phenytoin, or tolbutamide.						
Khalil et al. (2018) *Low*	United States, 2016	Cross-sectional	Children with obesity, 8–12 yr of age, N = 47	Serum, median = 0.99 (IQR = 0.45)	25-hydroxy Vitamin D (ng/mL)	Regression coefficient (per unit increase in PFOA)	1.90 (–5.49, 9.30)
	Confounding: Age, sex, race.						

Notes: aPTT = activated partial thromboplastin time. HIV = human immunodeficiency virus. PPT = prothrombin time; GM = geometric mean; BMI = body mass index; IQR = interquartile range; mo = months; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; WBHGB = whole blood hemoglobin; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.11 Respiratory

Table D-20. Associations Between PFOA Exposure and Respiratory Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Agier et al. (2019) *Medium*	France, Greece, Lithuania, Norway, Spain, United Kingdom 2019	Cohort	Pregnant women and their children, ages 6–12 yr, N = 1,033	Maternal and child's serum, plasma, or whole blood Prenatal (maternal) Median = 2.4 (IQR = 2) Postnatal (child) Median = 1.5 (IQR = 0.8)	FEV1	Regression coefficient per log2-unit increase in PFOA	Prenatal: –1.4 (–2.7, –0.1), p-value = 0.03 Postnatal: 0.5 (–0.6, 1.5), p-value = 0.33
	Confounding: Centre of recruitment, child's sex, child's age, child's height, parental country of birth, breastfeeding duration, season of conception, presence of older siblings, parental education level, maternal age, maternal pre-pregnancy body mass index, postnatal passive smoking status, prenatal maternal active, and passive smoking status.[c]						
Gaylord et al. (2019) *Medium*	New York, US 2014–2016	Cross-sectional	Adolescents and young adults ages 13–22 yr, N = 287	Adolescents and young adults' serum Comparison group: median = 1.38 (min = 0.36, max = 4.28) WTCHR group: median = 1.80 (min = 0.56, max = 5.03)	FEV1 FVC FEV1/FVC TLC RV FRC Resistance at an oscillation frequency of 5 Hz, 5–20 Hz, 20 Hz	Regression coefficient per log-unit increase in PFOA	No statistically significant differences observed between exposure groups for the measured outcomes, p-value > 0.05
	Comparison: Logarithm base not specified. **Confounding**: Sex, race/ethnicity, age, BMI, tobacco smoke exposure.						
Impinen et al. (2018) *Medium*	Norway 1992–2002	Cohort	Infants followed up at 2 yr and 10 yr, N = 641	Cord blood, Median = 1.6 (1.2, 2.1)	Oslo Severity Score (1–5 vs. 0)	OR per log2-unit increase in PFOA	1.43 (1.03, 1.98), p-value = 0.033

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					Oslo Severity Score (6–12 vs. 0) Reduced lung function at birth		1.25 (0.83, 1.89), (p-value = 0.276 1.08 (0.56, 2.07), p-value = 0.819
	Outcome: Reduced lung function at birth: Lung function (tPTEF/tE) with standardized z-score, and binary variable of decreased lung function (cutoff < 0.20). **Confounding**: Sex.						
Manzano-Salgado et al. (2019) *Medium*	Spain 2003–2015	Cohort	Pregnant women and children followed up at ages 1.5, 4, and 7 yr, N = 503 (4 yr) N = 992 (7 yr)	Maternal blood, Median = 2.35 (1.63, 3.30)	FEV1, FVC FEV1/FVC, FEF25%–75%	Regression coefficient per log2-unit increase PFOA	FVC (4 yr): –0.17 (–0.34, –0.01) p-value not reported FEV1, FEV1/FVC, FEF25%–75%: No statistically significant associations
	Confounding: Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth.						
Qin et al. (2017) *Medium*	Taiwan, 2009–2010	Case-control	Children with asthma and without asthma, aged 10–15, N = 132 (with asthma) N = 168 (without asthma)	Serum, Children with asthma: Median = 1.02 (0.48, 2.13) Children without asthma: Median = 0.50 (0.43, 0.69)	FEV1 FVC FEF25%–75% PEF	Regression coefficient per ln-unit increase PFOA, or by quartiles	Children with asthma: FEV1: –0.10 (–0.19, –0.02), p-value < 0.05 Quartile analysis: p-value for trend = 0.002 FEF25%–75%: –0.22 (–0.40, –0.05), p-value < 0.05 Quartile analysis p-value for trend = 0.014 FVC, PEF: No statistically significant associations Children without asthma:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
							No statistically significant associations for any outcomes
	Confounding: age, sex, BMI, parental education level, exercise, environmental tobacco smoke exposure, and month of survey.						
Steenland et al. (2015) *Low*	United States, 2008–2011	Cohort	Adult workers and former workers at a chemical plant, N = 146	No lag cumulative exposure, 3.03–11.42 µg/mL-year 10-yr lag cumulative exposure, 0.8–7.04 µg/mL-year	COPD no lag and 10-yr lag	Rate ratio (RR) by quartiles	No lag: Q2: 1.2 (0.64, 2.27) Q3: 1.25 (0.65, 2.37) Q4: 1.13 (0.59, 2.17) 10-yr lag: Q2: 0.75 (0.38, 1.48) Q3: 1.16 (0.6, 2.26) Q4: 0.77 (0.38, 1.57)
	Results: Lowest quartile used as reference group. **Confounding:** Gender, race, education, BMI, smoking, alcohol consumption.						

Notes: FEF25%–75% = Forced Expiratory Flow at 25%−75%; FEV1 = Forced Expiratory Volume in 1 s; FRC = Functional Residual Capacity; FVC = Forced Vital Capacity; PEF = Peak Expiratory Flow rate; RV = Residual Volume; TLC = Total Lung Capacity; WTCHR = World Trade Center Health Registry; BMI = body mass index; OR = odds ratio; RR = rate ratio; IQR = interquartile range; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

D.12 Musculoskeletal

Table D-21. Associations Between PFOA Exposure and Musculoskeletal Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
Children and Adolescents							
Jeddy et al. (2018) *Medium*	England, 1991–2009	Cohort	Females from the ALSPAC Study, Age 17, N = 221	Maternal serum 3.8 (2.9–4.9)	Area adjusted BMC (g), bone area (cm^2), BMC (g), BMD, cortical bone	Regression coefficient per unit increase in PFOA	Height: −0.6 (−1.06, −0.14) Bone area: −15.48 (−29.40, −1.55)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
					area (cm^2), cortical BMC (mg), cortical BMD (mg/cm^2), cortical thickness (mm), endosteal circumference (mm), height (cm), periosteal circumference (mm), total femoral neck BMD (g/cm^2), total hip BMD (g/cm^2), total lean mass (g)		No other statistically significant associations
	Confounding: Maternal pre-pregnancy BMI, maternal education, maternal age at delivery, gestational age at sample collection, ever breastfed status at 15 mo.[c]						
Cluett et al. (2019) *Medium*	United States, 1999–2010	Cross-sectional	Children from Project Viva, Ages 6–10, Overall N = 531 Male N = 296 Female N = 280	Plasma Overall: 4.4 (IQR = 3.2)	Areal bone mineral density (aBMD) z-score, bone mineral content (BMC) z-score	Regression coefficient per log2-unit increase in PFOA	aBMD z-score −0.16 (−0.25, −0.06) Males: −0.11 (−0.23, 0.00) Females: −0.24 (−0.4, −0.07) p-value for interaction by sex = 0.27 BMC z-score: No statistically significant associations
	Confounding: Maternal age, education, census tract median household income, individual household income, and child age, sex, race/ethnicity, year of blood draw, dairy intake, physical activity.						
Khalil et al. (2018) *Low*	United States 2016	Cross-sectional	Obese children, ages 8–12 N = 23	Serum 0.99 (IQR = 0.45)	BMD measured as broadband ultrasound attenuation (dB/MHz) and speed of sound	Regression coefficient per unit increase in PFOA	BMD (broadband ultrasound attenuation) −0.08 (−24.2, 24) BMD (speed of sound) −31.2 (−64, 1.54)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
					(m/s), stiffness index (%)		Stiffness index −8.79 (−28.1, 10.5)
	Confounding: Age, sex, race.						
Di Nisio et al. (2019) *Low*	Italy 2017–2018	Cross-sectional	Male high school students N = 100 (50 controls, 50 exposed)	Serum Controls: 4.70 (3.5–6.6) Exposed: 7.35 (4.7–14.9) Semen Controls: 0.1 (0.08–0.11) Exposed: 0.24 (0.11–0.99)	Arm span (cm)	Mann-Whitney test (Exposed vs. Controls)	Arm span Controls: 182.75 (178.0, 185.8) Exposed: 179.00 (174.2, 187.0) Adjusted p-value for comparison of medians = 0.738
	Results: Values for each outcome are reported as median (25th–75th percentile). **Confounding:** None reported.						
				General Population			
Uhl et al. (2013) *Medium*	United States, 2003–2008	Cross-sectional	Females from NHANES, Ages 20–84, N = 1,921 Ages 20–49 N = 1,104 (All adults N = 3,809)	Serum Females 20–84: Weighted mean = 4.22 Females 20–49: Weighted mean = 4.83	Osteoarthritis	OR per ln-unit increase in PFOA and by quartiles	Females ages 20–84 1.35 (1.02, 1.79), p-value < 0.05 Q2: 1.44 (0.80, 2.62) Q3: 1.18 (0.67, 2.08) Q4: 1.98 (1.24, 3.19), p-value < 0.01 Females ages 20–49 2.23 (0.81, 6.12) Q2: 2.71 (0.93, 7.91) Q3: 1.52 (0.36, 6.39) Q4: 4.95 (1.27, 19.4), p-value < 0.05 All adults ages 20–49 Q4: 3.76 (1.25, 11.4) No other statistically significant associations
	Results: Lowest quartiles used as the reference group.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	Confounding: Age, race/ethnicity, SES, smoking, BMI, vigorous reactional activity, prior wrist, hip, or spine fracture.						
Lin et al. (2014) *Medium*	United States, 2005–2006, 2007–2008	Cross-sectional	Adults from NHANES Ages ≥20, Males N = 1,192, Females N = 842, Females in menopause N = 305	Serum GM = 3.96 (SD = 3.86)	Total BMD (g/cm^2) in hip or lumbar spine; fractures in hip, wrist, spine, or all types	OR per ln-unit increase in PFOA	All fracture types Males: 0.84 (0.67, 1.07) Females: 0.98 (0.75, 1.28) Females in menopause: 1.53 (0.63, 3.74) Other outcomes: no statistically significant associations
	Confounding: Age, race/ethnicity, BMI, smoking, drinking, treatment for osteoporosis, use of prednisone or cortisone daily.						
Khalil et al. (2016) *Medium*	United States, 2009–2010	Cross-sectional	Adolescents and adults from NHANES, Ages 12–80, N = 958 females, 956 males	Serum Mean = 3.7 (SE = 0.18)	BMD (g/cm^2) of total femur, femoral neck, lumbar spine; Osteoporosis among females	BMD: Regression coefficient per ln-unit increase in PFOA and by quartiles Osteoporosis: OR per ln-unit increase in PFOA and by quartiles	Total femur Females: −0.017 (−0.038, 0.003) Q2: −0.02 (−0.04, −0.001), p-value < 0.05 Q3: −0.002 (−0.038, 0.034) Q4: −0.03 (−0.063, 0.003) Males: Not statistically significant Femoral neck Females: −0.017 (−0.033, −0.001) No statistically significant associations by quartiles Males: Not statistically significant Osteoporosis: 1.84 (1.17, 2.90), p-value = 0.008 Q2: 1.25 (0.38, 4.06) Q3: 1.23 (0.37, 4.05) Q4: 2.59 (1.01, 6.67), p-value = 0.049 Lumbar spine: No statistically significant associations
	Results: Lowest quartile used as the reference group.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	Confounding: Age, ethnicity, BMI, serum cotinine, physical activity, milk consumption, blood lead concentration.						
Hu et al. (2019) *Medium*	United States, 2004–2007	Cohort and cross-sectional	Adults from the POUNDS Lost study, Ages 30–70, N = 294	Plasma Cross-sectional: Mean = 5.2 (3.5–6.5) Cohort: Mean = 5.4 (3.7–6.6)	BMD and 2-yr ΔBMD (g/cm^2) of spine, total hip, femoral neck, hip trochanter, hip intertrochanteric area, and Ward's triangle area	Regression coefficient per SD increase in PFOA	Spine BMD analyses Cross-sectional: −0.021 (−0.038, −0.004) 2-yr ΔBMD: −0.002 (−0.007, 0.004) Total hip BMD analyses Cross-sectional: −0.015 (−0.029, −0.001) 2-yr ΔBMD: −0.004 (−0.008, 0.000) Femoral neck BMD analyses Cross-sectional: −0.016 (−0.03, −0.002) 2-yr ΔBMD: −0.001 (−0.007, 0.004) Hip trochanter BMD analyses Cross-sectional: −0.015 (−0.029, −0.002) 2-yr ΔBMD: −0.003 (−0.007, 0.001) Hip intertrochanteric area BMD analyses Cross-sectional: −0.016 (−0.032, 0.000) 2-yr ΔBMD: −0.006 (−0.011, −0.001), p-value < 0.05 Ward's triangle area BMD analyses Cross-sectional: −0.015 (−0.033, 0.003) 2-yr ΔBMD: −0.004 (−0.012, 0.005) No statistically significant associations or interactions by sex

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels[a] (ng/mL)	Outcome	Comparison	Results[b]
	Confounding: For cross-sectional, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group; For cohort, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group, baseline BMD, 2-yr weight change.						
	Occupational Populations						
Steenland et al. (2015) *Low*	United States 2008–2011	Retrospective occupational cohort	DuPont plant workers from the C8 Health Project N = 3,713	Drinking water/ occupational, serum Median = 113; Cumulative exposure, 25th–75th percentiles with or without 10-yr lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	Osteoarthritis	Incidence rate ratio by quartiles	Osteoarthritis no lag Q2: 0.88 (0.58, 1.34) Q3: 0.97 (0.71, 1.54) Q4: 0.97 (0.59, 1.59) p-trend log-PFOA cumulative exposure = 0.92 p-trend via quartiles = 0.48 Osteoarthritis with lag Q2: 0.74 (0.49, 1.10) Q3: 0.56 (0.34, 0.93) Q4: 0.67 (0.39, 1.14) p-trend log-PFOA cumulative exposure = 0.13 p-trend via quartiles = 0.15
	Results: Lowest quartile used as the reference group. **Confounding:** Gender, race, education, BMI, smoking, alcohol consumption.						

Notes: aBMD = areal bone mineral density; ALSPAC = Avon Longitudinal Study of Parents and Children; BMC = bone mineral content; BMD = bone mineral density; BMI = body mass index; GM = geometric mean; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POUNDS Lost = Prevention of Obesity Using Novel Dietary Strategies Lost clinical trial; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; SD = standard deviation; SE = standard error; SES = socioeconomic status.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

D.13 Gastrointestinal

Table D-22. Associations Between PFOA Exposure and Gastrointestinal Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Timmerman et al. (2020) *Medium*	Guinea-Bissau 2012–2015	Cohort	Children aged < 2 yr previously enrolled in a RCT for measles vaccination N = 236 (113 girls, 123 boys)	Serum 0.68 (0.53–0.92)	Diarrhea	OR per doubling of PFOA at inclusion or 9-mo visit	At inclusion: 1.09 (0.56, 2.09) At 9 mo: 1.54 (0.72, 3.29) No statistically significant associations or interactions by sex
	Confounding: Weight and age at inclusion, sex, maternal education, breastfeeding without solids.[c]						
Dalsager et al. (2016) *Low*	Denmark 2010–2015	Cohort	Pregnant women and their children from the Odense Child Cohort, Ages 1–4 yr N = 346	Serum 1.68 (Range: 0.32–10.12)	Diarrhea, vomiting (number of days with symptom or proportion of days under/above median)	Incidence rate ratio (number of days) or OR (proportion of days) by tertiles of PFOA exposure	Diarrhea Number of days with symptom T2: 1.07 (0.61, 1.89) T3: 1.08 (0.55, 2.13) Proportion of days under/above median T2: 1.10 (0.64, 1.89) T3: 0.94 (0.51, 1.74) Vomiting Number of days with symptom T2: 0.89 (0.61, 1.32) T3: 0.95 (0.62, 1.47) Proportion of days under/above median T2: 1.05 (0.62, 1.78) T3: 0.95 (0.52, 1.72)
	Results: Lowest tertile used as reference. **Confounding:** Maternal age, maternal educational level, parity, and child age.						
Hammer et al. (2019) *Low*	Faroe Islands Enrollment: 1986–2009;	Cohort	Children and adults from CHEF	Blood	Inflammatory bowel disease	Incidence rate ratio for highest vs. lowest tertile	0.60 (0.23, 1.56)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	follow-up until 2017		N = 2,843	Low exposure: GM = 0.95 (0.76–1.34) High exposure: GM = 4.42 (3.55–4.98)		of PFOA exposure	
	Confounding: Age, calendar period.						
Xu et al. (2020d) *Low*	Sweden 2014–2016	Cohort	Residents of Ronneby municipality Ronneby panel study: N = 57 Ronneby resampling: N = 113 Karlshamn: N = 19	Serum Ronneby panel study: 20 (11–29) Ronneby resampling: 16 (9–23) Karlshamn: 2 (1–2)	Inflammatory bowel disease (ln-ng/mL levels of calprotectin or zonulin)	Regression coefficient per unit increase in PFOA	Calprotectin Panel study: −0.006 (−0.03, 0.02) Resampling: −0.01 (−0.03, 0.005) Karlshamn: −0.15 (−0.84, 0.55) Zonulin Panel study: −0.002 (−0.02, 0.02) Resampling: −0.01 (−0.02, 0.01) Karlshamn: −0.29 (−0.85, 0.27)
	Confounding: Age, BMI, gender.						

Notes: RR = risk ratio; BMI = body mass index; RCT = randomized controlled trial; CHEF = Children's Health and the Environment in the Faroes; OR = odds ratio; GM = geometric mean; T2 = tertile 2; T3 = tertile 3; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.

[b] Results reported as effect estimate (95% confidence interval) unless otherwise specified.

[c] Confounding indicates factors the models presented adjusted for.

Table D-23. Associations Between PFOA Exposure and Dental Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Ramesh et al. (2019) *Medium*	United States 1999–2002	Cross-sectional	Adolescents from NHANES aged 12–19 yr N = 2,869	Serum Median = 3.5 (2.3–4.9)	Dental caries	OR per log2-unit increase in PFOA and by quartiles	1.00 (0.91, 1.12) Q2: 0.95 (0.74, 1.20) Q3: 1.04 (0.82, 1.32) Q4: 0.95 (0.74, 1.21)
	Results: Lowest quartile used as reference.						

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
	Confounding: Gender, race, education level of parent/guardian, family-poverty-to-income ratio, blood lead level, serum cotinine level.[c]						
Wiener & Waters (2019) *Medium*	United States 2013–2014	Cross-sectional	Children from NHANES aged 3–11 yr N = 629	Serum GM = 1.92 (95% CI: 1.74, 2.11)	Dental caries experience	OR per IQR increase in PFOA	1.33 (0.70, 2.53); p-value = 0.352
	Confounding: Age, sex, race/ethnicity, ratio of family-income-to-poverty guidelines, tooth brushing frequency, dental visit, percentages of sugar in the diet, fluoride in the water.						

Notes: PFOA = perfluorooctanoic acid; GM = geometric mean; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; CI = confidence interval; IQR = interquartile range; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4.
[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results are reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.14 Ocular

Table D-24. Associations Between PFOA Exposure and Ocular Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Zeeshan et al. (2020) *Medium*	China, 2016	Cross-sectional	Adults, from the Isomers of C8 Health Project, ages 22–96 yr, N = 1,202	Serum Median = 6.06 (3.97–9.12)	Visual impairment, synechia, macula disorder, corneal pannus, shallow anterior chamber, vitreous disorder, retinal disorder, lens opacity, conjunctival disorder,	OR per ln-unit increase in PFOA	Visual impairment 1.8 (1.37, 2.37); p-value < 0.05 Eye disease, combined ≤65 yr: 1.25 (1.01, 1.56); p-value < 0.05 >65 yr: 1.19 (0.71, 1.98) All other outcomes: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
					combined eye disease		
	Confounding: Age, sex, BMI, education, income, career, exercise time, drinking, smoking.[c]						

Notes: BMI = body mass index; OR = odds ratio.
[a] Exposure levels reported as median (25th–75th percentile) unless otherwise specified.
[b] Results are reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.15 Dermal

Table D-25. Associations Between PFOA Exposure and Dermal Health Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Results[b]
Ernst et al. (2019) *Medium*	Denmark 1999–2017	Cohort	Pregnant women and their children from the Puberty Cohort within the DNBC N = 555 girls, 565 boys	Maternal blood (first trimester) Girls Sample 1: 4.8 (2.7–8.2) Girls Sample 2: 4.1 (2.3–6.4) Boys Sample 1: 5.1 (2.8–8.3) Boys Sample 2: 4.3 (2.2–6.7)	Acne, age at occurrence (months)	Regression coefficient per log2-unit increase in PFOA, or by tertiles	Girls: −5.16 (−8.50, −1.82) T3: −6.09 (−12.10, −1.70) Boys: −1.06 (−3.62, 1.49); p-value = 0.58
	Results: Lowest tertile used as a reference group. **Confounding:** Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy body mass index, daily number of cigarettes smoked in first trimester.[c]						

Notes: DNBC = Danish National Birth Cohort.
[a] Exposure levels reported as median (10th–90th percentile).
[b] Results reported as effect estimate (95% confidence interval).
[c] Confounding indicates factors the models presented adjusted for.

D.16 Cancer

Table D-26. Associations Between PFOA Exposure and Cancer in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Eriksen et al. (2009) *Medium*	Denmark 1993–2006	Cohort	Adults with no previous cancer diagnosis, Ages 50–65 at enrollment, Prostate cancer, 1,393; Bladder cancer, 1,104; Pancreatic cancer, 900; Liver cancer, 839	Serum Mean (5th–95th percentile): Cases, men: 6.8 (3.1–14.0); Controls, men: 6.9 (3.2–13.3); Cases, women: 6.0 (2.6–11.0); Controls, women: 5.4 (2.2–11.6)	Cancers: prostate, bladder, pancreatic, liver	IRR per unit increase in PFOA, or by quartiles	Prostate cancer: Q2: 1.09 (0.78, 1.53) Q3: 0.94 (0.67, 1.32) Q4: 1.18 (0.84, 1.65) Per unit increase: 1.03 (0.99, 1.07) Bladder cancer: Q2: 0.71 (0.46, 1.07) Q3: 0.92 (0.61, 1.39) Q4: 0.81 (0.53, 1.24) Per unit increase: 1.00 (0.95, 1.05) Pancreatic cancer: Q2: 0.88 (0.49, 1.57) Q3: 1.33 (0.74, 2.38) Q4: 1.55 (0.85, 2.80) Per unit increase: 1.03 (0.98, 1.1) Liver cancer: Q2: 1.0 (0.44, 2.23) Q3: 0.49 (0.22, 1.09) Q4: 0.60 (0.26, 1.37) Per unit increase: 0.95 (0.86, 1.06)
	Results: Lowest quartile used as the reference group. **Confounding:** Prostate cancer: years of school attendance, BMI, dietary fat intake, and vegetable intake; Bladder cancer: smoking status, smoking intensity, smoking duration, years of school attendance, occupation associated with risk for bladder cancer; Pancreatic cancer: smoking status, smoking intensity, smoking duration, dietary fat intake, and fruit and vegetable intake; Liver cancer: smoking status, years of school attendance, alcohol intake, and occupation associated with risk for liver cancer.[c]						
	Greenland 2000–2003	Case-control	Greenlandic Inuit women with and	Plasma	Breast cancer	OR per ln-unit increase in PFOA	1.2 (0.77, 1.88), p-value = 0.43

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Bonefeld-Jorgensen et al. (2011) *Medium*			without breast cancer, 76	Cases: 2.5 (Range = 0.2–7.2) Controls: 1.6 (Range = 0.2–7.6)			
	Confounding: Age, BMI, pregnancy, cotinine, breastfeeding, and menopausal status.						
Barry et al. (2013) *Medium*	United States 2005–2006	Cohort	Adults from C8 Health Project, Ages ≥20 yr, 32,254	Modeled Community: 19.4 (Range = 2.8–9,217) Worker: 174.4 (Range = 5.2–3,683)	Cancers (no-lag and 10-yr lag): kidney, testicular, thyroid, breast, lung	HR per unit increase in PFOA, or by quartiles	Kidney cancer (no lag): Q2: 1.23 (0.70, 2.17) Q3: 1.48 (0.84, 2.60) Q4: 1.58 (0.88, 2.84) p-trend = 0.18 Per unit increase: 1.1 (0.98, 1.24), p-value = 0.1 Kidney cancer (10-yr lag): Q2: 0.99 (0.53, 1.85) Q3: 1.69 (0.93, 3.07) Q4: 1.43 (0.76, 2.69) p-trend = 0.34 Per unit increase: 1.09 (0.97, 1.21), p-value = 0.15 Testicular cancer (no lag): Q2: 1.04 (0.26, 4.22) Q3: 1.91 (0.47, 7.75) Q4: 3.17 (0.75, 13.45) p-trend = 0.04 Per unit increase: 1.34 (1.00, 1.79), p-value = 0.05 Testicular cancer (10-yr lag): Q2: 0.87 (0.15, 4.88) Q3: 1.08 (0.20, 5.90)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Q4: 2.36 (0.41, 13.65) p-trend = 0.02 Per unit increase: 1.28 (0.95, 1.73), p-value = 0.10 Thyroid cancer (no lag): Q2: 1.54 (0.77, 3.12) Q3: 1.48 (0.74, 2.93) Q4: 1.73 (0.85, 3.54) p-trend = 0.25 Thyroid cancer (10-yr lag): Q2: 2.06 (0.93, 4.56) Q3: 2.02 (0.90, 4.52) Q4: 1.51 (0.67, 3.39) p-trend = 0.65 Breast cancer (no lag): Per unit increase: 0.94 (0.89, 1.00), p-value = 0.05 Breast cancer (10-yr lag): Per unit increase: 0.93 (0.88, 0.99), p-value = 0.03 Lung cancer (no lag): Per unit increase: 0.88 (0.78, 1.00), p-value = 0.05 Lung cancer (10-yr lag): Per unit increase: 0.92 (0.81, 1.04), p-value = 0.17
	Results: Lowest quartile used as the reference group. **Confounding:** Time-varying smoking, time-varying alcohol consumption, sex, education, and stratified by 5-yr period of birth year.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Steenland and Woskie (2012) *Medium*	United States 1948–2009	Cohort	Exposed DuPont chemical plant workers in West Virginia, 5,791	Serum 4.3 ng/mL-years	Mortality: bladder cancer, kidney cancer, mesothelioma	SMR by quartiles, or for all quartiles	Bladder cancer mortality (no lag): Q1: 1.24 (0.15, 4.47) Q2: 2.49 (0.97, 5.78) Q3: 0.39 (0.01, 2.17) Q4: 0.36 (0.10, 2.01) All quartiles: 1.08 (0.52, 1.99) Kidney cancer mortality (no lag): Q1: 1.07 (0.02, 3.62), p-value < 0.05 Q2: 1.37 (0.28, 3.99), p-value < 0.05 Q3: 0.00 (0.00, 1.42), p-value < 0.05 Q4: 2.66 (1.15, 5.24), p-value < 0.05 All quartiles: 1.28 (0.66, 2.24) Kidney cancer mortality (10-yr lag): Q1: 1.05 (0.13, 3.79), p-value < 0.05 Q2: 0.87 (0.11, 3.15), p-value < 0.05 Q3: 0.44 (0.01, 2.44), p-value < 0.05 Q4: 2.82 (1.13, 5.81), p-value < 0.05 Kidney cancer mortality (20-yr lag) Q1: 1.34 (0.28, 3.91), p-value < 0.05 Q2: 0.46 (0.01, 2.55), p-value < 0.05 Q3: 0.00 (0.00, 2.03), p-value < 0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							Q4: 3.67 (1.48, 7.57), p-value < 0.05 Mesothelioma mortality (no lag): Q1: 0.00 (0.00, 15.4), p-value < 0.05 Q2: 0.00 (0.00, 7.51), p-value < 0.05 Q3: 1.73 (0.04, 9.65), p-value < 0.05 Q4: 6.27 (2.04, 14.63), p-value < 0.05 All quartiles: 2.85 (1.05, 6.20), p-value < 0.05 Mesothelioma mortality (10-yr lag): Q1: 0.00 (0.00, 17.80) Q2: 0.00 (0.00, 9.55) Q3: 3.08 (0.37, 11.12) Q4: 4.66 (1.27, 11.93) Mesothelioma mortality (20-yr lag): Q1: 9.09 (0.23, 50.60) Q2: 0.00 (0.00, 15.24) Q3: 2.60 (0.31, 9.39) Q4: 3.44 (0.71, 10.05)
	Results: Other DuPont workers from the region were used as the reference group. **Confounding:** Not reported.						
Vieira et al. (2013) *Medium*	United States 1996–2005	Case-control	Adults living near the Dupont Teflon-manufacturing plant, 7,869	Modeled Low: Range = 3.7–12.8 Medium: Range = 12.9–30.7	Cancers: kidney, prostate	OR by exposure category	Kidney cancer: Low: 0.8 (0.4, 1.5) Medium: 1.2 (0.7, 2.0) High: 2.0 (1.3, 3.2) Very high: 2.0 (1.0, 3.9) Prostate cancer:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
				High: Range = 30.8–109 Very high: Range = 110–655			Low: 1.1 (0.8, 1.5) Medium: 0.8 (0.6, 1.0) High: 0.8 (0.5, 1.1) Very high: 1.5 (0.9, 2.5)
	Results: Unexposed population used as the reference group. **Confounding:** Age, race, sex, diagnosis year, insurance provider, and smoking status.						
Ghisari et al. (2014) *Medium*	Greenland 2000–2003	Case-control	Women of Greenland Inuit descent aged 18–80 years. Cases were diagnosed with breast cancer N = 89	Serum	Breast cancer	OR (for high serum PFOA vs low)	CYP17; A1/A2 + A2/A2: 8.79 (1.22, 63.5), p = 0.031
	Confounding: Age and cotinine.						
Ducatman et al. (2015) *Medium*	United States 2005–2006	Cross-sectional	Men from C8 Health Study, Ages 20–49, 9,169; Ages 50–69, 3,819	Serum Mean (SD): 40.22 (3.50)	Prostate-specific antigen (PSA) level	Regression coefficient (β) per ln-unit increase in PFOA GM ratio (GMR) (PSA < 4.0 ng/mL vs. PSA ≥ 4.0 ng/mL)	Age 20–49 β = 1, p-value = 0.9 GMR = 1.15 (0.67, 1.98) Age 50–69 β = 1, p-value = 0.72 GMR = 0.96 (0.77, 1.2)
	Confounding: Age, smoking status, average alcohol intake, and body mass index.						
Ghisari et al. (2017) *Medium*	Denmark 1996–2002	Nested case-control	Adult women, 283	Serum Cases: 4.87 Controls: 4.90	Breast cancer	Relative risk ratio (RR) per ln-unit increase in PFOA, compared across genotypes: CYP1A1 (Ile462Val), CYP1B1 (Leu432Val), COMT (Val158Met),	Cohort RR = 1.17 (0.63, 2.17) CYP19 CC RR = 7.24 (1.00, 52), p-value < 0.05 No significant associations observed for remaining genotypes

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
						CYP17 (−34T > C), CYP19 (C > T)	
	Confounding: Age at blood draw, BMI before pregnancy, total number of gravidities, oral contraceptives use, age of menarche, smoking status and alcohol intake during pregnancy, physical activity, maternal education.						
	Results: Lowest tertile used as the reference group. **Confounding:** Age, BMI, cotinine levels, parity, and breastfeeding.						
Hurley et al. (2018) *Medium*	California, US 2011–2015	Nested case-control	Adult women, 1,760	Serum Median (min–max): Cases: 2.350 (0.042–39.100) Controls: 2.475 (0.096–20.200)	Breast cancer (invasive)	OR per log10-unit increase in PFOA, or by tertiles	T2: 0.901 (0.705, 1.152) T3: 0.925 (0.715, 1.197) Per unit increase: 0.733 (0.496, 1.081), p-value = 0.11
	Results: Lowest tertile used as the reference group. **Confounding:** Age at baseline enrollment, race/ethnicity, region of residence, date of blood draw, season of blood draw, total smoking pack-years, BMI, family history of breast cancer, age at first full-term pregnancy, menopausal status at blood draw, and pork consumption.						
Cohn et al. (2020) *Medium*	United States 1959–1967	Nested case-control	Adult daughters of women in CHDS cohort, 310 controls, 102 cases	Perinatal serum Cases: 30.5 (14.1–55.8) Controls: 0.4 (0.2–0.6)	Breast cancer	OR per log2-unit increase in PFOA	"Found no associations;" No results reported
	Confounding: Maternal: cholesterol, age at pregnancy, history of breast cancer, primiparity, overweight at first prenatal visit, serum levels of DDTs and metabolite DDE, African American status, whether daughter was breastfed.						
Mancini et al. (2020) *Medium*	France 1990–2013	Nested case-control	Postmenopausal women, Ages 40–65 in 1990, 194 cases, 194 controls	Serum 6.64 (1.29–21.39)	Breast cancer	ORs by quartiles, and by estrogen (ER) or progesterone receptor (PR) status	Overall: Q2: 1.69 (0.89, 3.21) Q3: 0.88 (0.43, 1.8) Q4: 0.92 (0.43, 1.98) p-trend = 0.43 ER negative:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							ORs of 3–7 p-trend = 0.59 PR negative: ORs of 1–4 p-trend = 0.90
	Results: Lowest quartile used as the reference group. **Confounding:** Total serum lipids, BMI, smoking status, physical activity, education level, personal history of benign breast disease, family history of breast cancer, parity/age at first full-term pregnancy, total breastfeeding duration, age at menarche, age at menopause, use of oral contraceptives, current use of menopausal hormone therapy.						
Shearer et al. (2021) *Medium*	United States 1993–2014	Nested case-control	Adults, 55–74, 648 Ages 55–59, 190 Ages 60–65, 224 Ages 65+, 234 Males 432 Females 216	Serum 5.5 (4.0–7.3)	Renal cell carcinoma	ORs per log2-unit increase in PFOA or by quartiles (total cohort only)	Q2: 1.47 (0.77, 2.8) Q3: 1.24 (0.64, 2.41) Q4: 2.63 (1.33, 5.2) p-trend = 0.007 Per unit increase: 1.71 (1.23, 2.37) 55–59: 2.1 (1.21, 3.34) 60–65: 1.6 (1, 2.45) 65+: 1.79 (1.21, 2.77) p-heterogeneity = 0.66 Males: 1.7 (1.31, 2.35) Females: 1.79 (1.1, 2.95) p-heterogeneity = 0.87
	Results: Lowest quartile used as the reference group. **Confounding:** BMI, smoking, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, calendar year of blood draw; sex, race and ethnicity, study year of blood draw, study center.						
Fry and Power (2017) *Medium*	US NHANES 2003–2006	Cohort	Adults, Ages 60+, 1,032	Serum Median (SE): 23.7 (0.7) ng/g lipid	Cancer mortality	Hazard ratio per SD-unit increase in PFOA	0.94 (0.8, 1.11), p-value = 0.45
	Confounding: Age, gender, race/ethnicity, and smoking status.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Goodrich et al. (2022) *Medium*	United States MEC Study Recruitment: 1993–1996	Nested case-control	Adults, 100 (50 cases, 50 controls)	Plasma GSM (GSD): Cases: 4.21 (2.13) Controls: 4.78 (1.89)	Hepatocellular carcinoma	OR for >8.6 µg/L vs. ≤8.6 µg/L PFOA, or per SD increase in PFOA	1.20 (0.52, 2.80), p-value = 0.67 Per SD increase: 0.86 (0.64, 1.20), p-value = 0.36
	Results: PFOA cutoff of 8.6 µg/L is the 85th percentile of PFOA in the study. **Confounding:** Conditional logistic regression matched for age, sex, race, and study site.						
Steenland et al. (2015) *Low*	United States 2008–2011	Retrospective occupational cohort	Adult workers, 3,713	Drinking water/occupational, serum Median = 113; Cumulative exposure, 25th–75th percentiles with or without 10-yr lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	Cancers with and without a 10-yr lag: bladder, colorectal, melanoma, prostate	IRR by quartiles	Bladder cancer no lag: Q2: 0.32 (0.08, 1.33) Q3: 0.95 (0.28, 3.14) Q4: 0.23 (0.05, 0.93) p-trend log-PFOA cumulative exposure = 0.04 p-trend via quartiles = 0.19 Bladder cancer with lag: Q2: 0.55 (0.12, 2.61) Q3: 0.47 (0.1, 2.21) Q4: 0.31 (0.06, 1.54) p-trend log-PFOA cumulative exposure = 0.06 p-trend via quartiles = 0.03 Colorectal, melanoma and prostate cancers report p-trends of 0.10 or greater
	Results: Lowest quartile used as the reference group. **Confounding:** Gender, race, education, BMI, smoking, alcohol consumption.						
Christensen et al. (2016a) *Low*	Wisconsin, US, 2012–2013	Cross-sectional	Male anglers, Ages 50+, 154	Serum 2.50 (1.80–3.30)	Cancer (any)	OR per unit increase in PFOA	1.5 (1.08, 2.17)
	Confounding: Age, BMI, work status, alcohol consumption.						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
Girardi and Merler (2019) *Low*	Italy 1960–2018	Occupational Retrospective Cohort	Male workers, 154	Occupational, serum GM by tertiles = 1,700 ; 13,051; and 81,934 ng/mL-years	Mortality: Liver cancer, liver cancer or cirrhosis, lung cancer, malignant neoplasm, malignant neoplasms of lymphatic and hematopoietic tissues	Mortality risk ratio (RR) by tertiles for PFAS plant workers vs. nearby metal factory workers Standardized mortality ratio in each cumulative PFOA tertile	Malignant neoplasms of lymphatic and hematopoietic tissues RR T1: 1.44 (0.18, 11.8) RR T2: 1.8 (0.22, 14.6) RR T3: 5.06 (1.61, 16) p-trend < 0.001 Any malignant neoplasm p-trend = 0.008 All other mortalities not significant
	Confounding: Age at risk, calendar period.						
Lin et al. (2020a) *Low*	China 2014–2017	Case-control	Children, Ages < 16, 84	Serum 13.89 (8.05–21.37)	Germ cell tumors	OR per unit increase in PFOA	1.03 (0.99, 1.08)
	Confounding: Infectious disease, cosmetics usage, barbecued food consumption, filtered water use, indoor decorating, living near farmland (maternal behaviors/factors during pregnancy).						
Tsai et al. (2020) *Low*	Taiwan 2014–2016	Case-control	Adult women, 239 Age 50 or younger, 120 Age over 50, 119	Plasma Mean (GM): 2.15 (1.77)	Breast cancer	OR per ln-unit increase in PFOA	Total cohort: 1.14 (0.66, 1.96) Age 50 or younger: 0.78 (0.4, 1.51) Age over 50: 0.89 (0.59, 1.34)
	Confounding: Pregnancy history, oral contraception use, abortion, BMI, menopause, and education level.						
Itoh et al. (2021) *Low*	Japan 2001–2005	Case-control	Adult women, Ages 20–74, 802 (401 breast cancer cases, 401 controls)	Serum 5.57 (3.98–7.62)	Breast cancer	OR by quartiles	Q2: 0.37 (0.19, 0.73), p-value < 0.05 Q3: 0.39 (0.18, 0.84), p-value < 0.05 Q4: 0.20 (0.08. 0.51), p-value < 0.05 p-trend = 0.001
	Results: Lowest quartile used as the reference group. **Confounding:** Age, residential area, BMI, height, menopausal status, age at menopause, age at first childbirth, family history of breast cancer, smoking status, strenuous physical activity in the past 5 year, moderate physical activity in the past 5 year, age at menarche, number of births,						

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
	breastfeeding duration, alcohol intake, isoflavone intake, education level, serum total concentrations of PCBs, fish and shellfish intake, vegetable intake, and calendar year of blood sampling.						
Liu et al. (2021) *Low*	China 2016–2017	Case-control	Adult men, 96 Adult women, 223	Serum Case: 7.7 (4.4–12.8); Control: 10.9 (7.9–16.1)	Thyroid cancer	OR by quartiles	Total Q2: 0.24 (0.12, 0.50) Q3: 0.24 (0.11, 0.49) Q4: 0.20 (0.09, 0.44) p-trend < 0.001 Male: Q2: 0.15 (0.03, 0.76) Q3: 0.18 (0.04, 0.85) Q4: 0.32 (0.08, 1.34) P-trend = 0.313 Female: Q2: 0.31 (0.14, 0.71) Q3: 0.28 (0.12, 0.63) Q4: 0.25 (0.10, 0.59) p-trend = 0.006
	Results: Lowest quartile used as the reference group. **Confounding:** Age, sex, and diabetes status.						
Omoike et al. (2021) *Low*	United States 2005–2012	Cross-sectional	Adults from NHANES, Ages ≥20 yr, 6,652	Serum 3.20 (2.00–4.90)	Cancers: ovarian, breast, uterine, and prostate	OR per unit increase in PFOA, or by quartiles	Ovarian cancer: Q2: 0.07 (0.07, 0.072) Q3: 0.69 (0.68, 0.70) Q4: 1.77 (1.75, 1.79) p-trend < 0.001 Per unit increase: 1.015 (1.013, 1.017) Breast cancer: Q2: 2.40 (2.38, 2.42) Q3: 1.39 (1.38, 1.40) Q4: 2.30 (2.28, 2.31) p-trend < 0.001 Per unit increase:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL)[a]	Outcome	Comparison	Select Results[b]
							1.089 (1.089, 1.090) Uterine cancer: Per unit increase: 0.912 (0.910, 0.914) Prostate cancer: Per unit increase: 0.944 (0.943, 0.944)
	Results: Lowest quartile used as the reference group **Confounding:** Age, sex, education, race/ethnicity, PIR, BMI, and serum cotinine.						
Cao et al. (2022) *Low*	China 2019–2021	Case-control	Adults and children, Ages 12–84 yr, 406 (203 cases, 203 controls)	Serum Cases: 6.6 (4.5–11) Controls: 5.4 (3.75–8.53)	Liver cancer	OR per log-ng/mL increase in PFOA	1.036 (1.002, 1.070) p-trend = 0.07
	Results: Logarithm base not specified. **Confounding:** Age, education level, BMI, annual household income, sex, smoking habit, and medical history.						

Notes: BMI = body mass index; CHDS = The Child Health and Development Studies; ER = estrogen receptor; GM = geometric mean; GMR = geometric mean ratio; GSD = geometric standard deviation; HR = hazard ratio; IRR = incidence rate ratio; MEC = Multiethnic Cohort study; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; RR = risk ratio; SD = standard deviation; SE = standard error; SMR = standardized mortality ratio; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; yr = years.

[a] Exposure levels reported as median (25th–75th percentile) in ng/mL unless otherwise noted.

[b] Results reported as effect estimate (95% confidence interval), unless otherwise noted.

[c] Confounding indicates factors the models presented adjusted for.

Appendix E. Benchmark Dose Modeling

E.1 Epidemiology Studies

For the epidemiological studies considered for dose-response assessment, the U.S. Environmental Protection Agency (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 prevalence of the outcome 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, 2012) because EPA was able to define a level considered clinically adverse for these outcomes. Details are provided in the following sections. In addition, EPA re-expressed the reported β coefficients when modeling results for decreased birthweight when regression coefficients were reported per log-transformed units of exposure (see details in Section E.1.2). Sensitivity analyses to evaluate the potential impact of re-expression in a hybrid approach when modeling hepatic and serum lipid studies for perfluorooctane sulfonic acid (PFOS) showed little impact on benchmark doses (lower confidence limit) (BMDLs) (see details in Sections E.1.3 and E.1.4).

EPA also performed benchmark dose (BMD) modeling and provided study lowest-observed-adverse-effect levels/no-observed-adverse-effect levels (LOAELs/NOAELs) for the hepatic and serum lipid dose-response studies as sensitivity analyses of the hybrid approach. For the immune studies, where a clinically defined adverse level is not well defined, EPA used the results from the multivariate models provided in the studies and determined a benchmark response (BMR) according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012) (see details in Section E.1.1). For specific approaches used to determine PODs please see Table E-1.

Table E-1. Summary of Modeling Approaches for POD Derivation From Epidemiological Studies

Endpoint	Studies[a]	Reported Result or Beta (Units)	LogPFOA	Re-Expression (Yes/No)	Approach	BMR (SD or Cutoff)
Anti-tetanus and anti-diphtheria antibody response	**Budtz-Jørgensen and Grandjean (2018a)**	BMD = $\log_2(1-BMR)/\beta$ $\log_2$(tetanus or diphtheria) per ng/mL PFOA	Yes	No	BMD modeling BMD = $\log_2(1-BMR)/\beta$	0.5 SD and 1 SD
Anti-tetanus and anti-diphtheria antibody response	Timmerman et al. (2021)	Percent difference $=(10^{\beta}-1)*100$ log10(tetanus or diphtheria) per ng/mL PFOA	No	No	BMD modeling BMD = log10(1−BMR)/β	0.5 SD and 1 SD
Anti-rubella antibody response	Granum et al. (2013)	Rubella(IU/mL) per ng/mL PFOA	No	No	BMD modeling	0.5 SD and 1 SD

Endpoint	Studies[a]	Reported Result or Beta (Units)	LogPFOA	Re-Expression (Yes/No)	Approach	BMR (SD or Cutoff)
Decreased birth weight	**Wikström et al. (2020)** Chu et al. (2020) Sagiv et al. (2018) Starling et al. (2017) Darrow et al. (2013)	BW per ln(ng/mL) PFOA or per IQR PFOA	Yes	Yes	Hybrid	5% and 10%
Elevated ALT	**Nian et al. (2019)**	Percent difference $=(e^{\beta}-1)*100$ ln(ALT) per ln(ng/mL) PFOA	Yes	No	Hybrid	5% and 10%
Elevated ALT	Gallo et al. (2012)	ln(ALT) per ln(ng/mL) PFOA	Yes	No	Hybrid	5% and 10%
Elevated ALT	Darrow et al. (2016)	ln(ALT) per ln(ng/mL) PFOA	Yes	No	Hybrid	5% and 10%
Increased total cholesterol	**Dong et al. (2019)**	TC per ng/mL PFOA	No	No	Hybrid	5% and 10%
Increased total cholesterol	Steenland et al. (2009)	ln(TC) per ln(ng/mL) PFOA	Yes	No	Hybrid	5% and 10%
Increased total cholesterol	Lin et al. (2019)	mean difference in TC (mg/dL) per quartile of PFOA (ng/mL)	No	No	BMDS	0.5 SD and 1 SD

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDS = Benchmark Dose Software; BMR = benchmark response; BW = birth weight; IQR = interquartile range; POD = point of departure; SD =standard deviation; TC = total cholesterol.

[a] Bolded study name identifies study result that advanced as the POD.

For the non-cancer 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, 2012). However, as there is currently no prescriptive hierarchy, selection of model types was often based on the goodness of fit.

For cancer endpoints, multistage models are generally preferred (U.S. EPA, 2012).

E.1.1 Modeling Results for Immunotoxicity

E.1.1.1 Modeling Results for Decreased Tetanus Antibody Concentrations

E.1.1.1.1 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Tetanus Antibody Concentrations at 7 Years of Age and PFOA Exposure Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of perfluorooctanoic acid (PFOA) measured at age 5 years, against $\log_2$-transformed anti-tetanus antibody concentrations measured at the 7-year-old examination controlling for sex, exact age at the 7-year-old examination, and booster type at age 5 years. Models were evaluated with additional control for PFOS (as $\log_2$(PFOS)) (also called multi-PFAS models), and without PFOS (also called single-PFAS models). Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median PFOA concentration, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). The piecewise-linear model did not fit better than the linear model for the PFOA exposure without adjustment for PFOS using a likelihood ratio test ($p = 0.76$; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS ($\log_2$(PFOS)) ($p = 0.69$).

Table E-2 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for PFOA at age 5 years and tetanus antibodies at age 7 years. These regression coefficients (β) and their standard errors (SE) were obtained by EPA from the authors Budtz-Jørgensen and Grandjean (2022, 2018a). Since Budtz-Jørgensen and Grandjean (2018a) $\log_2$-transformed the outcome variable, the BMR measured in unit of $\log_2$(tetanus antibody concentration) was $\log_2(1-0.05) = 0.074$ $\log_2$(IU/mL)).

Table E-2. Results Specific to the Slope From the Linear Analyses of PFOA Measured at Age 5 Years and Log_2(Tetanus Antibody Concentrations) Measured at Age 7 Years From Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOS Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope (β_{LB}) ng/mL
PFOA at Age 5	Linear	No[a]	−0.197	0.0630	$p = 0.002$	−0.301
PFOA at Age 5	Linear	Yes[b]	−0.185	0.0697	$p = 0.008$	−0.299

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOA), and sex, exact age at the 7-year-old examination, and booster type at age 5 years.
[b] Multi-PFAS model: adjusted for PFOA and PFOS, and sex, exact age at the 7-year-old examination, and booster type at age 5 years.

Interpretation of results in Table E-2:

- PFOA is a significant predictor in the single-PFAS model ($\beta = -0.197$; $p = 0.002$).

- Effects of PFOA in the single-PFAS model are attenuated when $\log_2$(PFOS) is included in the model ($\beta = -0.185$; $p = 0.008$).
- The point estimate results for PFOA (β) in the single-PFAS model are potentially confounded by PFOS since there was a 5% reduction in the effect size for PFOA from −0.197 to −0.185 when controlling for PFOS.
- One explanation is that PFOS was a confounder of the PFOA effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.
- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which point estimate is the best representation of any effect of PFOA.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. This is because there is only 5% difference in the BMD and a negligible difference in the BMDL when PFOS is included in the model.

E.1.1.1.1.1 Selection of the Benchmark Response

The BMD approach involves dose-response modeling to obtain BMDs, i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data and the BMDLs to serve as potential PODs for deriving quantitative estimates below the range of observation (U.S. EPA, 2012). Selecting a BMR to estimate the BMDs and BMDLs involves making judgments about the statistical and biological characteristics of the dataset and about the applications for which the resulting BMDs and BMDLs will be used. An extra risk of 10% is recommended as a standard reporting level for quantal data for toxicological data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects as the basis of the POD for a reference value. However, a BMR of 1% has typically been used for quantal human data from epidemiology studies (U.S. EPA, 2012), although this is more typically used for epidemiologic studies of cancer mortality within large cohorts of workers, which can support the statistical estimation of small BMRs.

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), which used a 5% fixed change in the distribution of antibody concentrations as the BMR to derive BMDs and BMDLs. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome.

A blood concentration for tetanus antibodies of 0.1 IU/mL is sometimes cited in the tetanus literature as a 'protective level' and (Grandjean et al., 2017b) noted that the Danish vaccine

producer Statens Serum Institut recommended the 0.1 IU/mL "cutoff" level "to determine whether antibody concentrations could be considered protective," and Galazka and Kardymowicz (1989) mention the same concentration. However, the 2018 WHO update (WHO, 2018) argues that:

> *"…the minimum amount of circulating antitoxin that in most cases ensures immunity to tetanus is assay specific. Within in vivo neutralization tests, modified ELISAs or bead-based immunofluorescence assays, concentrations at or exceeding 0.01 IU/mL are usually considered protective against disease, whereas antitoxin concentrations of at least 0.1–0.2 IU/mL are defined as positive when ELISA techniques are used for the assessment. Cases of tetanus have been documented, however, in persons with antitoxin concentrations above these thresholds. Hence, a "protective antibody concentration" may not be considered a guarantee of immunity under all circumstances."*

In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 or ½ SD change from the control mean may be selected (U.S. EPA, 2012). As noted above, a lower BMR can also be used if it can be justified on a biological and/or statistical basis. Figure E-1 replicates a figure in the Technical Guidance (page 23) (U.S. EPA, 2012) to show 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.

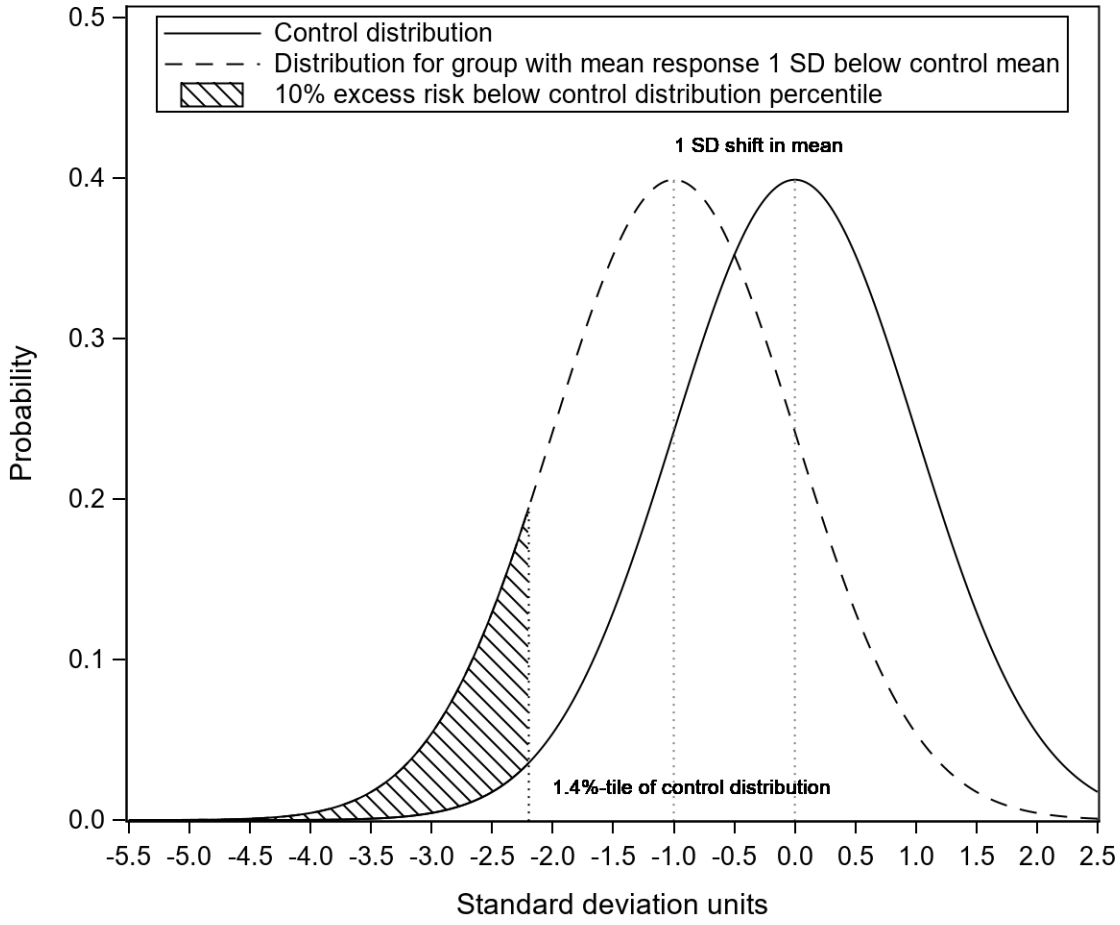


Figure E-1. Difference in population tail probabilities resulting from a one standard deviation shift in the mean from a standard normal distribution, illustrating the theoretical basis for a baseline BMR of 1 SD

BMR = benchmark response; SD =standard deviation.

Statistically, the Technical Guidance additionally suggests that studies of developmental effects can support lower BMRs. Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Biologically, a BMR of ½ SD is a reasonable choice as anti-tetanus antibody concentrations prevent against tetanus, which is a rare, but severe and sometimes fatal infection, with a case-fatality rate in the United States of 13% during 2001–2008 (CDC, 2011). The case-fatality rate can be more than 80% for early lifestage cases (Patel and Mehta, 1999). Selgrade (2007)

suggests 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 tetanus antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFOA. By contrast, a BMR of 1 SD may be more appropriate for an effect that would be considered 'minimally adverse.' A BMR smaller than ½ SD is generally selected for severe effects (e.g., 1% extra risk of cancer mortality); decreased antibody concentrations offer diminished protection from severe effects but are not themselves severe effects.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with both a 1 SD change in the distribution of $\log_2$(tetanus antibody concentrations) and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(tetanus antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the 25th and 75th percentiles of the tetanus antibody concentrations at age 7 years in IU/mL was (0.65, 4.6). Log_2-tranforming these values provides the 25th and 75th percentiles in $\log_2$(IU/mL) as (−0.62, 2.20). Assuming that these $\log_2$-transformed values are reasonably represented by a normal distribution, the width of the IQR is approximately 1.35 SDs (Rosner, 2015). Thus, SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (2.20−(−0.62))/1.35 = 2.09 $\log_2$(IU/mL).

While there was not a clear definition of the size of an adverse effect for a continuous endpoint like antibody concentrations, the value of 0.1 IU/mL is sometimes cited. As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies at age 7 years in $\log_2$(IU/mL), EPA calculated that 2.8% of those values would be below the cutoff value of 0.1 IU/mL (i.e., −3.32 $\log_2$(IU/mL)). A BMR of ½ SD resulted in 7.9% of the values being below that cutoff, which is 5.1% extra risk. This demonstrates the generic guidance that a BMR of ½ SD can provide a reasonably good estimate of 5% extra risk. Figure E-2 shows an example of this.

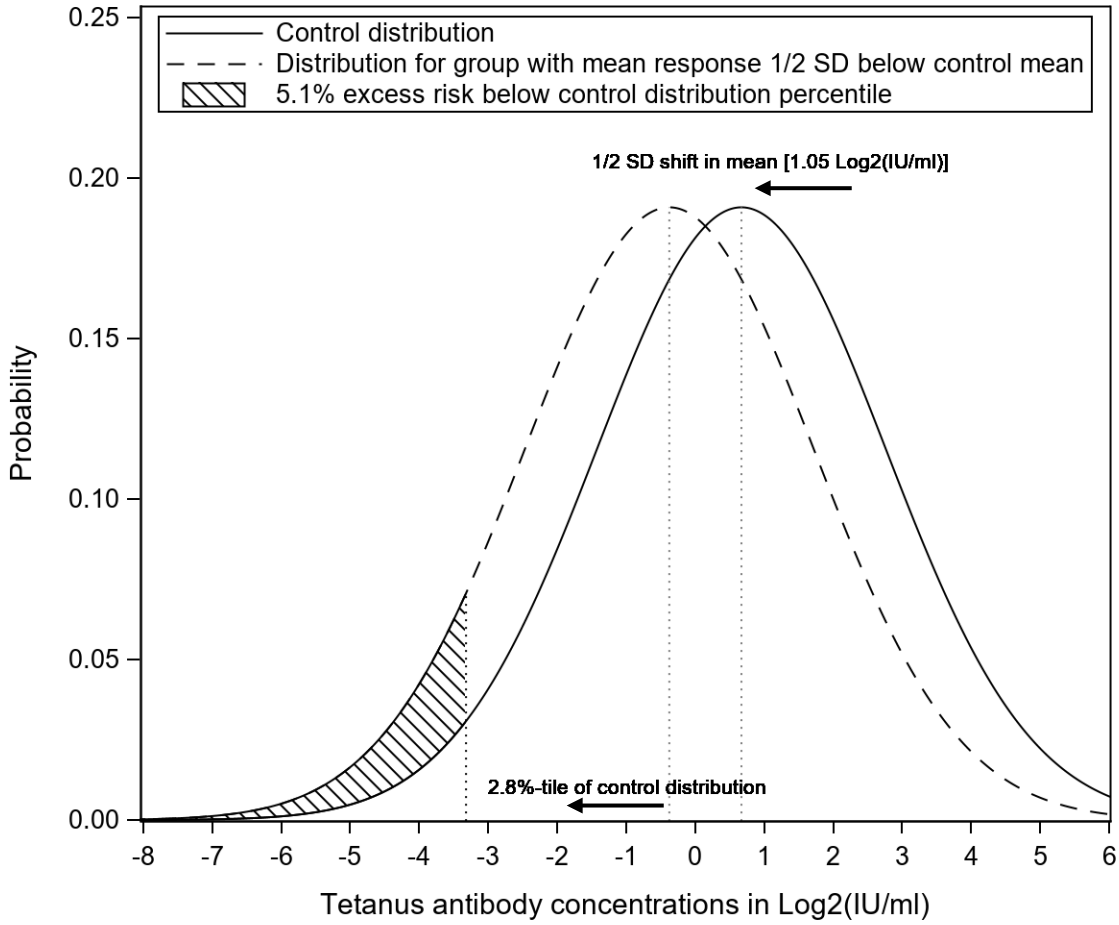


Figure E-2. Difference in Population Tail Probabilities Resulting From a ½ Standard Deviation Shift in the Mean From an Estimation of the Distribution of Log_2(Tetanus Antibody Concentrations at Age 7 Years)

IU = international units; SD =standard deviation

Table E-3. BMDs and BMDLs for Effect of PFOA at Age 5 Years on Anti-Tetanus Antibody Concentrations at Age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a BMR of ½ SD Change in Log_2(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_2(Tetanus Antibodies Concentration)

BMR	Estimated Without Control of PFOS		Estimated With Control of PFOS	
	BMD (ng/mL) $\beta = -0.197$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.301$ per ng/mL	BMD (ng/mL) $\beta = -0.185$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.299$ per ng/mL
½ SD	5.30	3.47[a]	5.66	3.49
1 SD	10.6	6.94	11.3	6.98

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOA concentration measured at age 5 years was 0.8 ng/mL, the 5th percentile was 2.4 ng/mL, and the 10th percentile was 2.8 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDL for a BMR of ½ SD ($BMDL_{½\ SD}$ = 3.47 ng/mL) in the single-PFAS model is well within the observed range (Table E-3). No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFOA well (p = 0.002).

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 5% higher than the $BMD_{½\ SD}$ estimate from the models with just PFOA, and the $BMDL_{½\ SD}$ estimates are the same. The change in BMD estimates may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which PFAS model provided the 'better' estimate of the point estimate of the effect of PFOA in light of potential confounding, the two $BMDL_{½\ SD}$ estimates are the same (3.53 ng/mL). EPA advanced the derivation based on results that did not control for PFOS because this model appeared to fit PFOA better (p = 0.002 vs. 0.006) and there was no uncertainty due to potential confounding in the BMDL.

For immunotoxicity related to tetanus associated with PFOA exposure measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 3.47 ng/mL.

E.1.1.1.2 Timmerman et al. (2021)

Timmerman et al. (2021) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOA against log10-transformed anti-tetanus antibody concentrations measured at the same time as PFOA, controlling for time since vaccine booster/estimated time since vaccine booster, and duration of being breastfed (< 6 months, 6–12 months, > 1 year) and area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq) and including children with known tetanus-diphtheria booster date only. Estimates from the linear regression models were subsequently backtransformed to express the percent difference in antibody concentrations at each ng/mL increase in serum PFOA concentrations in children, which was −8 (95% CI: −30, 21) (Table 4, Timmerman et al. (2021)). Using the equation provided below, EPA estimated the regression slope as −0.036 (95% CI: −0.155, 0.083).

$$Percent\ Difference = (10^{\beta} - 1) \times 100$$

Following the approach described previously for Budtz-Jørgensen and Grandjean (2018a), EPA derived BMDs and BMDLs for both a 1 SD change in the distribution of log10 (tetanus antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of log10 (tetanus antibody concentrations) (Table E-4). The SD of the log10 (tetanus antibody concentrations) was estimated from the median (25th, 75th percentiles) of 0.92 (0.25, 2.20) tetanus antibody concentrations in IU/mL (Table 1 in Timmerman (2021)). Log10-transforming these values provides the 25th and 75th percentiles in $\log_{10}$ (IU/mL) as (−0.60, 0.34). Assuming that these log10-transformed values are reasonably represented by a normal distribution, the IQR (which is the difference between the 75th and 25th percentiles) is approximately 1.35 SDs (Rosner, 2015). Thus, SD = IQR/1.35, and the SD of tetanus antibodies in log10 (IU/mL) is (0.34−(−0.60))/1.35 = 0.70 $\log_{10}$ (IU/mL).

Table E-4. BMDs and BMDLs for Effect of Serum PFOA in Children on Anti-Tetanus Antibody Concentrations Using a BMR of ½ SD Change in Log_{10}(Tetanus Antibodies

Concentration) and a BMR of 1 SD Change in Log_{10}(Tetanus Antibodies Concentration) Timmerman et al. (2021)

BMR	BMD (ng/mL) β = −0.036 per ng/mL	BMDL (ng/mL) β = −0.155 per ng/mL
½ SD	9.66	2.26
1 SD	19.3	4.52

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.

As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies in log_{10} (IU/mL), EPA calculated that 8.4% of those values would be below the cutoff value of 0.1 IU/mL. A BMR of ½ SD resulted in 19% of the values being below that cutoff, which is 10.6% extra risk. This suggest that in this case a BMR of ½ SD may not be a reasonably good estimate of 5% extra risk.

Note that this BMDL is based on a non-significant PFOA regression parameter (β) estimated as −0.013 (95% CI: −0.036, 0.013) (Timmermann et al., 2021), and thus this POD is identified with lower confidence.

For immunotoxicity related to tetanus associated with PFOA exposure measured at ages 5 to 10 years old, the POD estimated for comparison purposes was based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 2.26 ng/mL.

E.1.1.1.3 Summary of Modeling Results for Decreased Tetanus Antibody Concentrations

Table E-5 summarizes the PODs resulting from the modeling approaches for decreased tetanus antibody concentrations. The selected and comparison PODs were based on a BMR of ½ SD, resulting in BMDLs ranging from 2.3 to 12.1, with the selected POD of 3.47. The comparison POD of 2.26 ng/mL is considered lower confidence because it is based on a non-significant PFOA regression parameter.

Table E-5. BMDLs for Effect of PFOA on Anti-Tetanus Antibody Concentrations Using a BMR of ½ SD

Study	Effect	$BMDL_{½\ SD}$ (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018a)	PFOA at age 5 years and anti-tetanus antibody concentrations at age 7 years	**3.47**	1.05 log_2(IU/mL)
Budtz-Jørgensen and Grandjean (2018a)	PFOA perinatally and anti-tetanus antibody concentrations at age 5 years	3.31	0.78 log_2(IU/mL)
Timmerman et al. (2021)	PFOA and anti-tetanus antibody concentrations at ages 7–12 years	2.26	0.35 log_{10}(IU/mL)

Notes: BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.

E.1.1.1.4 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Tetanus Antibody Concentrations at 5 Years of Age and PFOA Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOA measured perinatally in maternal serum, against $\log_2$-transformed anti-tetanus antibody concentrations measured at the 5-year-old examination controlling for sex, and exact age at the 5-year-old examination, cohort, and interaction terms between cohort and sex, and between cohort and age. Models were evaluated with additional control for PFOS (as $\log_2$(PFOS)), and without PFOS. Three model shapes of PFOA were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions Budtz-Jørgensen and Grandjean (2018a). Compared to the linear model, the piecewise-linear model did not fit better than the linear model for either the PFOA exposure without adjustment for PFOS using a likelihood ratio test ($p = 0.25$; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS ($\log_2$(PFOS)) ($p = 0.26$).

Table E-6 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for tetanus in this exposure window. These β and their SE were obtained by EPA from the study authors (Budtz-Jørgensen and Grandjean, 2022, 2018a).

Table E-6. Results of the Linear Analyses of PFOA Measured Perinatally and Tetanus Antibodies Measured at Age 5 Years From Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOS Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope (β_{LB}) ng/mL
Perinatal PFOA	Linear	No[a]	−0.135	0.0601	p = 0.03	−0.234
Perinatal PFOA	Linear	Yes[b]	−0.126	0.0685	p = 0.07	−0.239

Notes: SE = standard error.

[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOA), and sex, exact age at the 5-year-old examination, cohort, interaction terms between cohort and sex, and between cohort and age.

[b] Multi-PFAS model: adjusted for PFOA and PFOS, and sex, exact age at the 7-year-old examination cohort, and interaction terms between cohort and sex, and between cohort and age.

Interpretation of results in Table E-6:

- PFOA is a significant predictor in the single-PFAS model ($\beta = -0.135$; $p = 0.03$).
- Effects are attenuated when $\log_2$(PFOS) are included in the model ($\beta = -0.126$; $p = 0.07$).
- The point estimate results for PFOA are potentially confounded by PFOS since there was a 7% reduction in the effect size for PFOA from −0.135 to −0.126 when controlling for PFOS.
- One explanation is that PFOS was a confounder of the PFOA effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.

- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which estimate is the best representation of any effect of PFOA.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. This is because there is only 7% difference in the BMD and a 3% difference in the BMDL when PFOS is included in the model.

E.1.1.1.4.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), 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 (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 SD change in the distribution of $\log_2$(tetanus antibody concentrations), and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(tetanus antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018a). Grandjean et al. (2012) reported on 587 5-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017b) reported on 349 5-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately by the authors. EPA then pooled the summary statistics to describe the common SD. The IQR of the tetanus antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.1, 0.51). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−3.32, −0.97). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (−0.97−(−3.32))/1.35 = 1.74 $\log_2$(IU/mL). The IQR of the tetanus antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−3.32, −1.74), and the SD of tetanus antibodies in $\log_2$(IU/mL) is (−1.74−(−3.32))/1.35 = 1.17 $\log_2$(IU/mL). The pooled variance is a weighted

sum of the independent SDs, and the pooled SD was estimated as 1.55 $\log_2$(IU/mL).[11] To show the impact of the BMR on these results, Table E-7 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-7. BMDs and BMDLs for Effect of PFOA Measured Perinatally and Anti-Tetanus Antibody Concentrations at Age 5 Years (Budtz-Jørgensen and Grandjean, 2018a)

BMR	Estimated Without Control of PFOS		Estimated With Control of PFOS	
	BMD (ng/mL) $\beta = -0.135$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.234$ per ng/mL	BMD (ng/mL) $\beta = -0.126$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.239$ per ng/mL
½ SD	5.76	3.31[a]	6.17	3.25
1 SD	11.5	6.62	12.3	6.49

Notes:
[a] Denotes the selected POD.

The lowest perinatal maternal serum PFOA concentration measured was 0.8 ng/mL, the 5th percentile was 1.7 ng/mL, and the 10th percentile was 2.0 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDLs for a BMR of ½ SD ($BMDL_{½\ SD}$ = 3.31 ng/mL) in the single-PFAS model is well within the observed range. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFOA well.

The $BMD_{½\ SD}$ estimate from the multi-PFAS models is 7% lower than the $BMD_{½\ SD}$ estimate from the models with just PFOA, and the $BMDL_{½\ SD}$ estimates is 3% lower. The change in BMD estimates may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which PFAS model provided the 'better' estimate of the point estimate of the effect of PFOA in light of potential confounding, the two $BMDL_{½\ SD}$ estimates are comparable (3.35 ng/mL vs. 3.25 ng/mL). EPA advanced the derivation based on results that did not control for PFOS because this model appeared to fit PFOA data better (p = 0.02 vs. 0.07) and there was little uncertainty due to potential confounding in the BMDL.

For immunotoxicity related to tetanus associated with PFOA exposure measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 3.31 ng/mL.

E.1.1.2 Modeling Results for Decreased Diphtheria Antibody Concentrations

E.1.1.2.1 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Diphtheria Antibody Concentrations at 7 Years of Age and PFOA Exposure Measured at 5 Years of Age

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOA measured at age 5 years, against $\log_2$-transformed anti-diphtheria antibody concentrations measured at the 7-year-old examination controlling for sex, exact age at the 7-year-old examination, and booster type at age 5 years. Models were evaluated with additional control for PFOS (as $\log_2$(PFOS)), and without PFOS. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a)

[11] Pooled variance for tetanus in 5-year-olds = $[(502-1)(1.74)^2+(298-1)(1.17)^2]/[502+298-2] = 2.41$. The pooled SD is the square root of 2.41, which is 1.55 $\log_2$(IU/mL).

using likelihood ratio tests: a linear model of PFOA, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). The piecewise-linear model did not fit better than the linear model for the PFOA exposure without adjustment for PFOS using a likelihood ratio test ($p = 0.86$; see Budtz-Jørgensen and Grandjean (2018a) Table 3), or for the model that did adjust for PFOS ($\log_2$(PFOS)) ($p = 0.92$). Table E-8 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These β and their SE were obtained by EPA from the study authors (Budtz-Jørgensen and Grandjean, 2022, 2018a).

Table E-8. Results Specific to the Slope From the Linear Analyses of PFOA Measured at Age 5 Years and Log_2(Diphtheria Antibodies) Measured at Age 7 Years From Table 1 in Budtz-Jørgensen and Grandjean (2018a) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOS Adjusted	Slope (β) per ng/mL	SE(β) ng/mL	Slope (β) Fit	Lower Bound Slope (β_{LB}) ng/mL
PFOA at Age 5	Linear	No[a]	−0.126	0.0588	p = 0.03	−0.223
PFOA at Age 5	Linear	Yes[b]	−0.0867	0.0649	p = 0.18	−0.194

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOA), and sex, exact age at the 7-year-old examination, and booster type at age 5 years.
[b] Multi-PFAS model: adjusted for PFOA and PFOS, and sex, exact age at the 7-year-old examination, and booster type at age 5 years

Interpretation of results in Table E-8:

- PFOA is a significant predictor in the single-PFAS model ($\beta = -0.126$; $p = 0.03$).
- Effects are attenuated when $\log_2$(PFOS) are included in the model ($\beta = -0.0867$; $p = 0.18$).
- The point estimate results for PFOA are potentially confounded by PFOS since there was a 30% reduction in the effect size for PFOA from −0.126 to −0.0867 when controlling for PFOS.
- One explanation is that PFOS was a confounder of the PFOA effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.
- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which estimate is the best representation of any effect of PFOA.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in

the estimate. This is because there is only 30% difference in the BMD and 15% difference in the BMDL when PFOS is included in the model.

E.1.1.2.1.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), 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 (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 SD change in the distribution of $\log_2$(diphtheria antibody concentrations), and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). A blood concentration for diphtheria antibodies of 0.1 IU/mL is sometimes cited in the diphtheria literature as a 'protective level.' Grandjean et al. (2017b) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL 'cutoff' level; and Galazka (1993) mentions the same concentration, but Galazka et al. (1993) argues:

> *"However, it has also been shown that there is no sharply defined level of antitoxin that gives complete protection from diphtheria (Ipsen, 1946). A certain range of variation must be accepted; the same degree of antitoxin may give an unequal degree of protection in different persons. Other factors may influence the vulnerability to diphtheria including the dose and virulence of the diphtheria bacilli and the general immune status of the person infected (Christenson and Böttiger, 1986). Thus, an antibody concentration between 0.01 and 0.09 IU/ml may be regarded as giving basic immunity, whereas a higher titer may be needed for full protection. In some studies that used in vitro techniques, a level of 0.1 IU/ml was considered protective (Cellesi et al., 1989; Galazka and Kardymowicz, 1989)."*

Statistically, the Technical Guidance suggests that studies of developmental effects can support lower BMRs. Biologically, a BMR of ½ SD is a reasonable choice as anti-diphtheria antibody concentrations prevent against diphtheria, which is very rare in the United States, but can cause life-threatening airway obstruction, or cardiac failure (Collier, 1975). Among 13 cases reported in the United States during 1996–2016, no deaths were mentioned (Liang et al., 2018). However, diphtheria remains a potentially fatal disease in other parts of the world (Galazka et al. (1993) mentions a case-fatality rate of 5%–10%) and PFOA-related changes in anti-diphtheria antibody concentrations cannot be considered 'minimally adverse' given the historic lethality of diphtheria in the absence of vaccination. Selgrade (2007) suggests 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.

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 SD change in the distribution of $\log_2$(diphtheria antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(diphtheria antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 7 years was estimated from the distributional data presented in Grandjean et al. (2012) as follows: the interquartile range (IQR) of the diphtheria antibody concentrations at age 7 years in IU/mL was (0.4, 1.6). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−1.32, 0.68). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of tetanus antibodies in $\log_2$(IU/mL) is (0.68−(−1.32))/1.35 = 1.48 $\log_2$(IU/mL). To show the impact of the BMR on these results, Table E-9 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-9. BMDs and BMDLs for Effect of PFOA at Age 5 Years on Anti-Diphtheria Antibody Concentrations at Age 7 Years (Budtz-Jørgensen and Grandjean, 2018a) Using a BMR of ½ SD Change in Log_2(Diphtheria Antibodies Concentration) and a BMR of 1 SD Log_2(Diphtheria Antibodies Concentration)

BMR	Estimated Without Control of PFOS		Estimated With Control of PFOS	
	BMD (ng/mL) $\beta = -0.126$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.223$ per ng/mL	BMD (ng/mL) $\beta = -0.0867$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.194$ per ng/mL
½ SD	5.88	3.32[a]	8.53	3.82
1 SD	11.8	6.64	17.1	7.64

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOA concentration measured at age 5 years was 0.8 ng/mL, the 5th percentile was 2.4 ng/mL, and the 10th percentile was 2.8 ng/mL (Grandjean and Bateson, 2021) so the estimated BMDL for a BMR of ½ SD ($\text{BMDL}_{½\text{ SD}}$ = 3.32 ng/mL) in the single-PFAS model is well within the observed range. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFOA well (p = 0.03).

The $\text{BMD}_{½\text{ SD}}$ estimate from the multi-PFAS models is 44% higher than the $\text{BMD}_{½\text{ SD}}$ estimate from the model with just PFOA, and the $\text{BMDL}_{½\text{ SD}}$ is 15% higher. This may, or may not, reflect control for any potential confounding of the regression effect estimates. While it is not clear which PFAS model provided the 'better' estimate of the point estimate of the effect of PFOA in light of potential confounding, the two $\text{BMDL}_{½\text{ SD}}$ estimates which serve as the PODs are comparable (3.30 ng/mL vs. 3.80 ng/mL). EPA advanced the POD based on results that did not control for PFOS because this model appeared to fit PFOA data better (p = 0.04 vs. 0.18) and there was low uncertainty due to potential confounding in the BMDL. However, confidence was diminished by potential confounding in the main effect – even though there was low confounding of the BMDL.

For immunotoxicity related to diphtheria, associated with PFOA measured at age 5 years, the POD is based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 3.32 ng/mL.

E.1.1.2.2 Budtz-Jørgensen and Grandjean (2018a) Results for Decreased Diphtheria Antibody Concentrations at 5 Years of Age and PFOA Exposure Measured Perinatally

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFOA measured perinatally, against log_2-transformed anti-diphtheria antibody concentrations measured at the 5-year-old examination controlling for sex and age. Models were evaluated with additional control for PFOS (as log_2(PFOS)), and without PFOS. Three model shapes were evaluated by Budtz-Jørgensen and Grandjean (2018a) using likelihood ratio tests: a linear model of PFOA, a piecewise-linear model with a knot at the median, and a logarithmic function. The logarithmic functions did not fit better than the piecewise-linear functions (Budtz-Jørgensen and Grandjean, 2018a). There was evidence that the piecewise-linear model fit better than the linear model for the PFOA exposure without adjustment for PFOS ($p = 0.012$; see in Budtz-Jørgensen and Grandjean (2018a), Table 3), and for the model that adjusted for PFOS (log_2(PFOS)) ($p = 0.05$). Table E-10 summarizes the results from Budtz-Jørgensen and Grandjean (2018a) for diphtheria in this exposure window. These β and their SE were computed by EPA from the published BMDs and BMDL based on a BMR of 5% change in diphtheria antibody concentrations in Table 2 of Budtz-Jørgensen and Grandjean (2018a).[12]

Table E-10. Results of the Analyses of PFOA Measured Perinatally and Diphtheria Antibodies Measured at Age 5 Years From Budtz-Jørgensen and Grandjean (2018b) in a Single-PFAS Model[a] and in a Multi-PFAS Model[b]

Exposure	Model Shape	PFOS Adjusted	Slope (β) per ng/mL	SE(β)	Slope (β) Fit	Lower Bound Slope (β_{LB})
Perinatal PFOA	Piecewise	No[a]	−0.495	0.163	p = 0.003	−0.764
Perinatal PFOA	Piecewise	Yes[b]	−0.347	0.180	p = 0.05	−0.644

Notes: SE = standard error.
[a] Single-PFAS model: adjusted for a single PFAS (i.e., PFOA), and sex, and exact age at the 5-year-old examination.
[b] Multi-PFAS model: adjusted for PFOA and PFOS, and sex, and exact age at the 5-year-old examination.

Interpretation of results in Table E-10:

- PFOA is a significant predictor in the single-PFAS model (β = −0.495; p = 0.003).
- Effects of PFOA are attenuated when PFOS is in the model (β = −0.347; p = 0.05).
- Results for PFOA are potentially confounded by PFOS since there was a 30% change in the effect size for PFOA from −0.495 to −0.347 when controlling for PFOS
- One explanation is that PFOS was a confounder of the PFOA effect.
- Another possibility is physiological confounding, which can arise when biomarkers measured from the same blood test are more highly correlated due to individual's

[12] Budtz-Jørgensen and Grandjean (2018a) computed BMDs and BMDLs using a BMR of 5% decrease in the antibody concentrations. Their formula, BMD = $log_2(1-BMR)/\beta$, can simply be reversed to solve for $\beta = log_2(1-BMR)/BMD$. For a negative dose-response when more exposure results in lower antibody concentration, the BMDL is based on the lower bound of β, (β_{LB}). Thus, the $\beta_{LB} = log_2(1-BMR)/BMDL$. The $SE(\beta) = (\beta-\beta_{LB})/1.645$. The p-value is the two-sided probability that $Z <= SE(\beta)/\beta$.

physiological processes. Physiological confounding can therefore induce confounding bias by the inclusion of co-measured co-exposures in regression models.

- The reasons for the change in main effect size are not known and remain an uncertainty because it is not known whether the change in estimate was induced by physiologic confounding or was the result of controlling for classical confounding. For this reason, there is uncertainty in knowing which estimate is the best representation of any effect of PFOA.
- The uncertainty from potential confounding does not have much impact on the RfD, which is defined as allowing for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. This is because there is only 30% difference in the BMD and 16% difference in the BMDL when PFOS is included in the model.

E.1.1.2.2.1 Selection of the Benchmark Response

In the 2021 *Proposed Approaches* draft (U.S. EPA, 2021c) reviewed by the SAB PFAS Review Panel, EPA relied on the BMDL modeling approach published in Budtz-Jørgensen and Grandjean (2018a), described above. During validation of the modeling, EPA reevaluated the approach chosen by Budtz-Jørgensen and Grandjean (2018a) and determined that a different approach should be used to be consistent with EPA guidance (U.S. EPA, 2012), which recommends the use of a 1 or ½ SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome. Additionally, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), 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 (2018a) assessed antibody response after PFAS exposure during childhood, this is considered a developmental study (U.S. EPA, 1991) based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which states that a developmental effect "may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation" and can be "detected at any point in the lifespan of the organism."

Following the technical guidance (U.S. EPA, 2012), EPA derived BMDs and BMDLs associated with a 1 SD change in the distribution of $\log_2$(tetanus antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_2$(tetanus antibody concentrations). The SD of the $\log_2$(diphtheria antibody concentrations) at age 5 years was estimated from two sets of distributional data presented from two different birth cohorts of 5-year-olds that were pooled in Budtz-Jørgensen and Grandjean (2018a). Grandjean et al. (2012) reported on 587 five-year-olds from the cohort of children born during 1997–2000 and Grandjean et al. (2017b) reported on 349 five-year-olds from the cohort of children born during 2007–2009. The means and SDs were computed separately by the authors. EPA then pooled the summary statistics to describe the common SD. The IQR of the diphtheria antibody concentrations in the earlier birth cohort at age 5 years in IU/mL was (0.05, 0.4). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−4.32, −1.32). Assuming that these $\log_2$-transformed values are similar to the normal distribution, the width of the IQR is approximately 1.35 SDs, thus SD = IQR/1.35, and the SD of diphtheria antibodies in $\log_2$(IU/mL) is (−1.32−(−4.32))/1.35 = 2.22 $\log_2$(IU/mL). The IQR of the diphtheria antibody concentrations in the later birth cohort at age 5 years in IU/mL was (0.1, 0.3). Log_2-tranforming these values provides the IQR in $\log_2$(IU/mL) as (−3.32, −1.74), and the SD of diphtheria antibodies in $\log_2$(IU/mL) is (−1.74−(−3.32))/1.35 = 1.17 $\log_2$(IU/mL).

The pooled variance is a weighted sum of the independent SDs, and the pooled SD was estimated as 1.90 $\log_2$(IU/mL).[13] To show the impact of the BMR on these results, Table E-11 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD.

Table E-11. BMDs and BMDLs for Effect of PFOA Measured Perinatally and Anti-Diphtheria Antibody Concentrations at Age 5 Years (Budtz-Jørgensen and Grandjean, 2018a)

BMR	Estimated Without Control of PFOS		Estimated With Control of PFOS	
	BMD (ng/mL) $\beta = -0.495$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.764$ per ng/mL	BMD (ng/mL) $\beta = -0.347$ per ng/mL	BMDL (ng/mL) $\beta_{LB} = -0.644$ per ng/mL
½ SD	1.92	1.24[a]	2.74	1.47
1 SD	3.84	2.49	5.47	2.95

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.
[a] Denotes the selected POD.

The lowest serum PFOA concentration measured perinatally was 0.8 ng/mL, the 5th percentile was 1.7 ng/mL, and the 10th percentile was 2.0 ng/mL (Grandjean and Bateson, 2021) so the estimated BMD for a BMR of ½ SD ($BMDL_{½\ SD}$ = 1.24 ng/mL) in the single-PFAS model is well within the observed range. No information was available to judge the fit of the model in the range of the BMDLs, but the BMD and BMDL were both within the range of observed values and the model fit PFOA well.

The $BMD_{½\ SD}$ estimate from the multi-PFAS model is 43% higher than the $BMD_{½\ SD}$ estimated from the model with just PFOA, and the $BMDL_{½\ SD}$ is 19% higher. This may, or may not, reflect control for any potential confounding of the regression effect estimates. The BMDLs which serve as the PODs are comparable (1.24 ng/mL vs. 1.47 ng/mL) and EPA advanced the derivation based on results that did not control for PFOS because this model appeared to fit PFOA well (p = 0.003 vs. 0.05) and there was moderate uncertainty due to potential confounding in the BMD and low uncertainty in the BMDL.

For immunotoxicity related to diphtheria, associated with PFOA measured at age 5 years, the POD is based on a BMR of ½ SD and a BMDL½ SD of 1.24 ng/mL.

E.1.1.2.3 Timmerman et al. (2021)

Timmerman et al. (2021) analyzed data from Greenlandic children ages 7–12 and fit multivariate models of PFOA against log10-transformed anti-diphtheria antibody concentrations measured at the same time as PFOA, controlling for time since vaccine booster/estimated time since vaccine booster, and duration of being breastfed (< 6 months, 6–12 months, > 1 year) and area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq) and including children with known tetanus-diphtheria booster date only. Estimates from the linear regression models were subsequently backtransformed to express the percent difference in antibody concentrations at each ng/mL increase in serum PFOA concentrations in children, which was −22 (95% CI: −48, 16) (Table 4, Timmerman et al. (2021)). Using the equation provided below, EPA estimated the regression slope as −0.11 (95% CI: −0.28, 0.06).

[13] Pooled variance for diphtheria in 5-year-olds = [(502−1)(2.22)^2+(298−1)(1.17)^2]/[502+298−2] = 3.60. The pooled SD is the square root of 3.60, which is 1.90 $\log_2$(IU/mL).

$$Percent\ Difference = (10^{\beta} - 1) \times 100$$

Following the approach provided for Budtz-Jørgensen and Grandjean (2018a), EPA derived BMDs and BMDLs for both a 1 SD change in the distribution of $\log_{10}$ (diphtheria antibody concentrations) as a standard reporting level, and ½ SD change in the distribution of $\log_{10}$ (diphtheria antibody concentrations) (Table E-12). The SD of the $\log_{10}$ (diphtheria antibody concentrations) was estimated from the median (25th, 75th percentiles) of 0.07 (0.02 and 0.28) diphtheria antibody concentrations in IU/mL (Table 1 in Timmerman (2021)). Log_{10}-transforming these values provides the 25th and 75th percentiles in $\log_{10}$ (IU/mL) as (−1.70, −0.55). Assuming that these $\log_{10}$-transformed values are reasonably represented by a normal distribution, the IQR (which is the difference between the 75th and 25th percentiles) is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of tetanus antibodies in $\log_{10}$ (IU/mL) is (−0.55 – (−1.70))/1.35 = 0.85 $\log_{10}$ (IU/mL).

Table E-12. BMDs and BMDLs for Effect of PFOA on Anti- Diphtheria Antibody Concentrations (Timmermann et al., 2021) Using a BMR of ½ SD Change in $\log_{10}$(Tetanus Antibodies Concentration) and a BMR of 1 SD Change in Log_{10}(Diphtheria Antibodies Concentration)

BMR	BMD (ng/mL) β = −0.11 per ng/mL	BMDL (ng/mL) β = −0.28 per ng/mL
½ SD	3.93	**1.49**
1 SD	7.87	2.99

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; SD = standard deviation.

As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of diphtheria antibodies in $\log_{10}$ (IU/mL), EPA calculated that 57% of those values would be below the cutoff value of 0.1 IU/mL. A BMR of ½ SD resulted in 75% of the values being below that cutoff, which is 18% extra risk. This suggests that in this case the BMR of ½ SD may not be a reasonably good estimate of 5% extra risk. This POD is considered lower confidence because it is based on a non-significant PFOA regression parameter.

For immunotoxicity related to tetanus associated with PFOA exposure measured at ages 5 to 10 years old, the POD estimated for comparison purposes were based on a BMR of ½ SD and a $\text{BMDL}_{½\ SD}$ of 1.49 ng/mL.

E.1.1.2.4 Summary of Modeling Results for Decreased Diphtheria Antibody Concentrations

Table E-13 summarizes the PODs resulting from the modeling approaches for decreased diphtheria antibody concentrations. The selected and comparison PODs were based on a BMR of ½ SD, resulting in BMDLs ranging from 1.24 to 14.5 ng/mL, with the selected POD of 3.32. The comparison POD of 1.49 is considered lower confidence because it is based on a non-significant PFOA regression parameter.

Table E-13. BMDLs for Effect of PFOA on Anti-Diphtheria Antibody Concentrations Using a BMR of ½ SD

Study Name	Effect	BMDL (ng/mL)	½ SD
Budtz-Jørgensen and Grandjean (2018a)	PFOA at age 5 years on anti-diphtheria antibody concentrations at age 7 years	**3.32**	0.74 $\log_2$(IU/mL)
Budtz-Jørgensen and Grandjean (2018a)	PFOA perinatally on anti-diphtheria antibody concentrations at age 5 years	1.24	0.95 $\log_2$(IU/mL)
Timmerman et al. (2021)	PFOA and anti-diphtheria antibody concentrations at ages 7–12 years	1.49	0.48 $\log_{10}$(IU/mL)

Notes: BMDL = benchmark dose lower limit; SD = standard deviation.

E.1.1.3 Modeling Results for Decreased Rubella Antibody Concentrations

Granum et al. (2013) investigated the association between prenatal exposure to perfluorinated compounds and vaccination responses and clinical health outcomes in early childhood in the BraMat subcohort of the Norwegian Mother and Child Cohort Study. A total of 56 mother-child pairs with maternal blood samples at delivery and blood samples from the children at 3 years of age were evaluated. Antibody titers specific to rubella were measured in 50 serum samples. Prenatal exposure to PFOA (mean = 1.1 ng/mL) was inversely associated with Rubella antibody levels at age 3. Granum et al. (2013) fit multivariate linear regression models of maternal PFOA against antibody concentrations in units of optical density (OD) adjusted for maternal allergy, paternal allergy, maternal education, child's gender, and/or age at 3-year follow-up. The estimated regression coefficient and 95% confidence interval was −0.40, 95% CI: −0.64, −0.17 (Table 4, Granum et al., 2013). The summary statistics for rubella antibody levels at the age of 3 in units of OD were median = 1.9; 25th, 75th percentiles: 1.5, 2.1. Study authors were contacted to provide these summary statistics in units of IU/mL (median = 60.6; 25th, 75th percentiles: 41.8, 80.2), and the corresponding regression coefficient and 95% confidence interval: −5.1, 95% CI: −9.0, −1.1 (Table E-14).

Table E-14. Levels of Rubella Vaccine-Induced Antibodies at the Age of 3 Years (Adapted From Table 3 in Granum et al. (2013))

Parameter	Optical Density (OD)	IU/mL[a]
25th percentile	1.5	41.8
Median	1.9	60.595
75th percentile	2.1	80.12
Min–Max	0.8–2.4	15.0–120.0
Mean	1.7	61.6
0.5 SD	0.22	14.3
1 SD	0.44	28.6
β (95% CI) for PFOA	−0.40 (−0.64, −0.17)	−23.33 (−39.36, −7.31)

Notes: IU = international units; OD = optical density; SD = standard deviation.
[a]Authors were contacted to provide summary statistics for Rubella antibody levels in IU/mL (n = 50).

Following the technical guidance (U.S. EPA, 2012) and the approach described previously for Budtz-Jørgensen and Grandjean (2018, 5083631; see Section E.1.1.1.1) and accounting for the

fact that here the outcome variable is not log-transformed, EPA derived BMDs and BMDLs for both a 1 and ½ SD change from the control mean in the distribution of Rubella antibody concentrations. However, Rubella differs from diphtheria and tetanus in that several levels for Rubella antibody have been cited in the literature as "protective levels," representing a clinically significant cutoff for an adverse response. These levels vary depending on geography and study, ranging from 4 IU/mL in Finland (Davidkin et al., 2008), to 11 IU/mL in Iran (Honarvar et al., 2013), or 15 IU/mL in the United States (Tosh et al., 2009). However, 10 IU/mL appears to be the most widely accepted standard for Rubella immunity. For example, Skenzdel et al. (1996) noted:

> *"…The Rubella Subcommittee of the National Committee for Clinical Laboratory Standards has proposed lowering the breakpoint to define rubella immunity from 15 to 10 IU/mL. This recommendation stems from epidemiologic studies on vaccinated persons with low levels of antibody and anecdotal reports. Additional support comes from Centers for Disease Control and Prevention studies and reports. The effectiveness of rubella vaccination is well documented and the 10 IU/mL antibody level is protective in the vast majority of persons… The Subcommittee, recognizing that sporadic and conflicting reports may suggest a relationship between antibody levels and protection against the rubella virus, did not advocate lowering the breakpoint < 10 IU/mL"*

Charlton et al. (2016), provides further context:

> *"…the level of rubella IgG antibody is used as a surrogate marker for protection. In 1985, the Rubella Subcommittee of the National Committee on Clinical Laboratory Standards (NCCLS) set a level of >15 IU/ml for rubella IgG antibodies as the indicator of immunity. In light of further epidemiological investigations, and additional studies indicating that individuals with low levels of antibody (<15 IU/ml) produced a secondary immune response upon vaccine challenge rather than a primary immune response, these cut offs were revised by the Subcommittee from 15 IU/ml to 10 IU/ml in 1992. However, since 1992, the rubella cutoffs have not been assessed."*

As noted by Charlton et al. (2016) and the other literature cited above, the geographical variability, lack of consensus, and relatively dated assessment of this cutoff precludes its use as the basis of the BMR.

In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of 1 or ½ SD change from the control mean may be selected (U.S. EPA, 2012). The SD of the Rubella antibody concentrations in OD units was estimated from the distributional data provided in Table 3 in Granum et al. (2013): the 25th and 75th percentiles of the Rubella antibody concentrations in OD units were 1.5 and 2.1, respectively. Assuming that these values are reasonably represented by a normal distribution, the IQR is approximately 1.35 SDs. Thus, SD = IQR/1.35, and the SD of Rubella antibodies in OD is 0.44. The SD of Rubella antibodies in IU/mL units was provided by study authors and was 28.6. Table E-15 presents the BMDs and BMDLs at BMRs of ½ SD and 1 SD. Note that the estimated BMD/BMDLs were almost the same regardless of the units (OD or IU/mL) used in the analysis.

As an additional check, EPA evaluated how much extra risk would have been associated with a BMR set at a 10 IU/mL cutoff value for Rubella seropositivity, given the uncertainty in

definitive cutoffs for Rubella in OD or IU/mL units discussed above. Because Rubella antibody levels were reported in OD units and IU/mL units, EPA investigated the extra risk using both units.

First, the extra risk was investigated using the distributional data in OD units and the BMR cutoff value of 0.990 or 0.927 OD, which were used to determine Rubella seropositivity in Granum et al. (2013). Communications with the study authors confirmed that in Granum et al. (2013), two different OD cutoffs were used for Rubella seropositivity in two different runs: >0.990 OD or >0.927 OD (Stølevik, 2012). Of the 50 samples, 47 samples were seropositive. The remaining three samples were equivocal (i.e., between 0.590–0.990 or 0.553–0.927 OD). None of the 50 samples were considered seronegative (i.e., <0.590 or <0.553 OD) for Rubella. All participants were vaccinated for Rubella, and Granum et al. (2013) noted that "[c]hildren not following the Norwegian Childhood Vaccination Program (n = 4) were excluded from the statistical analyses regarding vaccination responses."

Using these BMR cutoffs and the distribution of Rubella antibodies in OD, EPA calculated that 1.4–2.0% of the values would be below the cutoffs. A BMR of ½ SD resulted in 4.6% or 6.1% of the values being below the cutoffs of 0.927 or 0.990 OD, respectively, which is ~4% extra risk. A BMR of 1 SD resulted in 12% or 15% of the values being below the cutoffs of 0.927 or 0.990 OD, respectively, which is ~12.7% extra risk. This suggests that in this case, BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk.

Then, using the distributional data of Rubella antibodies in IU/mL and a cutoff of 10 IU/mL, which was considered as the protective antibody level for Rubella, EPA calculated that 3.8% of the values would be below the cutoff. A BMR of ½ SD resulted in 10% of the values being below the cutoff, which is ~6.3% extra risk. A BMR of 1 SD resulted in 21.8% of the values being below the cutoff, which is ~18% extra risk. This further suggests that in this case, BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk.

Table E-15. BMDs and BMDLs for Effect of Maternal Serum PFOA on Anti-Rubella Antibody Concentrations in Children Using a BMR of ½ SD Change in Rubella Antibodies Concentration and a BMR of 1 SD Change in Rubella Antibodies Concentration (Granum et al., 2013)

BMR	BMD (ng/mL) β = −0.80 per ng/mL (For Units of OD) β = −23.33 per ng/mL (For Units of IU/mL)	BMDL (ng/mL) β = −0.64 per ng/mL (For Units of OD) β = −39.36 per ng/mL (For Units of IU/mL)
½ SD	0.6	0.3 (0.4)
1 SD	1.1 (1.2)	0.7

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response; OD = optical density; SD = standard deviation. Values in parentheses represent estimates corresponding to anti-Rubella antibodies in IU/mL units.

For immunotoxicity related to Rubella associated with PFOA exposure measured at age 3 years old, the POD estimated for comparison purposes were based on a BMR of ½ SD and a $BMDL_{½\ SD}$ of 0.3 ng/mL.

E.1.2 Modeling Results for Decreased Birthweight

Five *high* confidence studies (Chu et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Govarts et al., 2016) reported decreased birth weight in infants whose mothers were exposed to PFOA. These candidate studies offer a variety of PFOA exposure measures across the fetal and neonatal window. 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. The logarithmic transformation of exposure yields a negative value for small numbers, which can result in implausible results from dose-response modeling (i.e., estimated risks are negative and unable to determine the responses at zero exposure). EPA first re-expressed the reported β coefficients in terms of per ng/mL, if necessary, according to Dzierlenga (2020). Then EPA used the re-expressed β and lower limit on the confidence interval to estimate BMD and BMDL values using the general equation $y = mx + b$, where y is birth weight and x is exposure, substituting the re-expressed β values from these studies for m. The intercept b represents the baseline value of birth weight in an unexposed population and it can be estimated through $\bar{y} = m\,\bar{x} + b$ using an average birth weight from an external population as $\bar{y}$, an average exposure as $\bar{x}$ and re-expressed β from the studies as m.

The CDC Wonder site (https://wonder.cdc.gov/natality.html) provides vital statistics for babies born in the United States. There were 3,791,712 all live births in the United States in 2018 according to final natality data. The mean and standard deviation of birth weight were 3,261.6 ± 590.7 g (7.19 ± 1.30 lb.), with 8.27% of live births falling below the public health definition of low birth weight (i.e., 2500 g, or 5.5 lb.). The full natality data for the U.S. data on birth weight was used as it is more relevant for deriving toxicity values for the U.S. general public than the study-specific birthweight data. Also, the CDC Wonder database may be queried to find the exact percentage of the population falling below the cutoff value for clinical adversity. EPA's America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals report (https://www.epa.gov/americaschildrenenvironment/data-tables-biomonitoring-perfluorochemicals-pfcs) provides in Table B6b the median blood serum levels of PFOA of 1.1 ng/mL in 2015–2016 in woman ages 16 to 49, using National Health and Nutrition Examination Survey (NHANES) as data source. These values are assumed to be representative of women of reproductive age and are subsequently used in the estimation of BMD and BMDL values from the available five epidemiological studies.

E.1.2.1 Chu et al. (2020)

Chu et al. (2020) reported a β coefficient of −73.6 g (95% CI: −126.4, 20.9) per ln(ng/mL) increase for the association between birth weight and maternal PFOA serum concentrations (collected within 3 days of delivery) in a China cohort. The reported β coefficient can be re-expressed in terms of per ng/mL according to Dzierlenga et al. (2020). Given the reported study-specific median (1.5 ng/mL) and the 25th and 75th percentiles (1.0 and 2.6 ng/mL) of the exposure from Chu et al. (2020), EPA estimated the distribution of exposure by assuming the exposure follows a lognormal distribution with mean and standard deviation as:

$$\mu = ln(q_{50}) = ln(1.5) = 0.43 \qquad (1)$$

$$\sigma = ln(q_{75}/q_{25})/1.349 = ln(2.6/1.0)/1.349 = 0.75 \qquad (2)$$

Then, EPA estimated the 25th–75th percentiles at 10 percentile intervals of the exposure distribution and corresponding responses of reported β coefficient. The re-expressed β coefficient is determined by minimizing the sum of squared differences between the curves generated by the re-expressed β and the reported β. Doing so results in a re-expressed β coefficient of −45.2 g (95% CI: −77.6, −12.8) per ng/mL.

Typically, for continuous data, the preferred definition of the BMR is to have a basis for what constitutes a minimal level of change in the endpoint that is biologically significant. For birth weight, there is no accepted percent change that is considered adverse. However, there is a clinical measure for what constitutes an adverse response. Babies born weighing less than 2,500 g are considered to have low birth weight, and further, low birth weight is associated with a wide range of health conditions throughout life (Tian et al., 2019a; Reyes and Mañalich, 2005; Hack et al., 1995). Given this clinical cutoff for adversity and that 8.27% of all live births in the United States in 2018 fell below this cutoff, the hybrid approach can be used to define the BMR. The hybrid approach harmonizes the definition of the BMR for continuous data with that for dichotomous data, and therefore is an advantageous approach[14]. Essentially, the hybrid approach involves the estimation of the dose that increases the percentile of responses falling below (or above) some cutoff for adversity in the tail of the response distribution. Application of the hybrid approach requires the selection of an extra risk value for BMD estimation. In the case of birth weight, an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from animal toxicology studies, and that low birth weight confers increased risk for adverse health effects throughout life, thus supporting a BMR lower than the standard BMR of 10% extra risk.

Therefore, given a background response and a BMR = 5% extra risk, the BMD would be the dose that results in 12.86% of the responses falling below the 2,500 g cutoff value:

$$Extra\ Risk(ER) = (P(d) - P(0)) \ / \ (1 - P(0))$$

$$P(d) = ER(1 - P(0)) + P(0) = 0.05(1 - 0.0827) + 0.0827 = 0.1286$$

Using the mean birth weight for all birth in the United States in 2018 of 3,261.6 g with a standard deviation of 590.7 g, EPA calculated the mean response that would be associated with the 12.86th percentile of the distribution falling below 2,500 g. In this case, the mean birth weight would be 3,169.2 g. Given the median exposure of 1.1 ng/mL from ACE Biomonitoring on Perfluorochemicals as $\overline{x}$, the mean birth weight in the United States as $\overline{y}$ and the re-expressed β as *m* term, the intercept *b* can be estimated as:

$$b = \overline{y} - m\overline{x} = 3261.6\ g - \left(-45.2\ g(\tfrac{ng}{mL})^{-1}\right) 1.1\tfrac{ng}{mL} = 3311.4\ g \quad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x, using 3,311.4 g for the *b* term and −45.2 for the *m* term. Doing so results in a value of 3.1 ng/mL:

[14] While the explicit application of the hybrid approach is not commonly used in IRIS dose/concentration/exposure-response analyses, the more commonly used SD-definition of the BMR for continuous data is simply one specific application of the hybrid approach. The SD-definition of the BMR assumes that the cutoff for adversity is the 1.4th percentile of a normally distributed response and that shifting the mean of that distribution by one standard deviation approximates an extra risk of 10%.

$$x = (y - b)/m = (3169.2\ g - 3311.4\ g)/(-45.2\ g(\frac{ng}{mL})^{-1}) = 3.1\ ng/mL$$

To calculate the BMDL, the method is essentially the same except that the lower limit (LL) on the β coefficient (−77.6) is used for the *m* term. However, Chu et al. (2020) reported a two-sided 95% confidence interval for the β coefficient, meaning that the LL of that confidence interval corresponds to a 97.5% one-sided LL. The BMDL is defined as the 95% LL of the BMD (i.e., corresponds to a two-sided 90% confidence interval), so the corresponding LL on the β coefficient needs to be calculated before calculating the BMDL. First, the standard error of the β coefficient can be calculated as:

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{-12.8\ g(\frac{ng}{mL})^{-1} - \left(-77.6\ g(\frac{ng}{mL})^{-1}\right)}{3.92} = 16.5\ g(\frac{ng}{mL})^{-1}$$

Then the corresponding 95% one-sided lower bound on the β coefficient can be calculated as:

$$95\%\ one - sided\ LL = \beta - 1.645(SE(\beta)) = -45.2\ g(\frac{ng}{mL})^{-1} - 1.645\left(16.5\ g(\frac{ng}{mL})^{-1}\right)$$
$$= -72.4 g(\frac{ng}{mL})^{-1}$$

Using this value for the *m* term results in a BMDL value of 2.0 ng/mL maternal serum concentration.

E.1.2.2 Wikström et al. (2020)

Wikström et al. (2020) reported a β coefficient of −68.0 g (95% CI: −112.0, −24.0) per ln(ng/mL) for the association between birth weight and maternal PFOA serum concentrations (collected during 9 weeks to 10 weeks of pregnancy with a median of 10 weeks) in a Swedish cohort. Given the reported study-specific median (1.6 ng/mL) and the 25th and 75th percentiles (1.1 and 2.3 ng/mL) of the exposure, EPA estimated the mean (0.48) and standard deviation (0.54) of the log normally distributed exposure. The re-expressed β coefficient is −41.0 g (95% CI: −67.5, −14.5) per ng/mL and the intercept *b* is 3,306.7 g. The 95% one-sided LL for the re-expressed β coefficient is −63.3 g per ng/mL. The values of the BMD and BMDL are 3.4 ng/mL and 2.2 ng/mL, respectively.

E.1.2.3 Govarts et al. (2016)

Govarts et al. (2016) reported a β coefficient of −34.5 g (95% CI: −129.0, 60.0) per IQR increase in z-score of ln(ng/mL) PFOA exposures, corresponding to a β coefficient of −53.4 g (95% CI: −199.5, 92.8) per ln(ng/mL) increase, for the association between birth weight and PFOA concentrations in umbilical cords plasma samples in a U.S. cohort. Given the reported study-specific median (1.5 ng/mL) and the 25th and 75th percentiles (1.1 and 2.1 ng/mL) of the exposure, EPA estimated the mean (0.42) and standard deviation (0.48) of the log normally distributed exposure. The re-expressed β coefficient is −34.3 g (95% CI: −128.2, 59.7) per ng/mL, and the intercept *b* is 3,299.4 g. A BMD of 3.8 ng/mL is calculated from Govarts et al. (2016) using the same approach as above with the same values for the mean birth weight in the United States.

To calculate the BMDL, the same procedure as above is used to calculate the corresponding 95% one-sided LL for the re-expressed β coefficient from the re-expressed LL on the 95% two-sided confidence interval of −128.2 g per ng/mL. Using the LL value (−113.1 g per ng/mL), a BMDL of 1.2 ng/mL is calculated.

E.1.2.4 Sagiv et al. (2018)

Sagiv et al. (2018) reported a β coefficient of −18.5 g (95% CI: −45.4, 8.3) per IQR increase in PFOA (ng/mL), corresponding to a β coefficient of −4.9 g (95% CI: −11.9, 2.2) per ng/mL increase, for the association between birth weight and maternal PFOA serum concentrations (collected during 5 weeks to 19 weeks of pregnancy with a median of 9 weeks) in a U.S. cohort. The intercept *b* is 3,267.0 g based on the β coefficient of −4.9 g per ng/mL and the corresponding 95% one-sided lower limits for the β coefficient is −10.8 g per ng/mL. A BMD of 20.1 ng/mL and a BMDL of 9.1 ng/mL are calculated using the same approach as above.

E.1.2.5 Starling et al. (2017)

Starling et al. (2017) reported a β coefficient of −51.4 g (95% CI: −97.2, −5.7) per ln(ng/mL) for the association between birth weight and maternal PFOA serum concentrations (collected during 20 to 34 weeks of pregnancy with a median of 27 weeks) in a U.S. cohort. Given the reported study-specific median (1.1 ng/mL) and the 25th and 75th percentiles (0.7 and 1.6 ng/mL) of the exposure, EPA estimated the mean (0.10) and standard deviation (0.61) of the log normally distributed exposure. The re-expressed β coefficient is −45.0 g (95% CI: −85.1, −5.0) per ng/mL and the intercept *b* is 3,311.1 g. The 95% one-sided LL for the re-expressed β coefficient is −78.6 g per ng/mL. The values of the BMD and BMDL are 3.2 ng/mL and 1.8 ng/mL, respectively.

E.1.2.6 Summary of Modeling Results for Decreased Birthweight

For all of the above calculations, EPA used the exact percentage (8.27%) of live births in the United States in 2018 that fell below the cutoff of 2,500 g as the tail probability to represent the probability of extreme ("adverse") response at zero dose ($P(0)$). However, this exact percentage of 8.27% was calculated without accounting for the existence of background PFOA exposure in the U.S. population (i.e., 8.27% is not the tail probability of response at zero dose). Thus, EPA considers an alternative control-group response distribution ($N(\mu_c, \sigma_c)$), using the study-specific intercept *b* obtained through equation (3) (representing the baseline value of birth weight in an unexposed population) as μ_c and the standard deviation of U.S. population as σ_c , to estimate the tail probability that fells below the cutoff of 2,500 g. EPA estimated the study-specific tail probability of live births falling below the public health definition of low birth weight (2,500 g) as:

$$P(0) = \frac{1}{\sigma_c\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2\sigma_c^2}\right)}\,dx = \frac{1}{590.7\sqrt{2\pi}}\int_{-\infty}^{2500} e^{\left(-\frac{(x-b)^2}{2*590.7^2}\right)}\,dx$$

$$b = \overline{y} - m\overline{x} = 3261.6 - (\beta_{re-expressed} * 1.0\frac{ng}{mL})$$

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\overline{x} = 0$). By using the median of serum PFOA concentrations (1.1 ng/mL) from ACE Biomonitoring on

Perfluorochemicals as background exposure ($\bar{x}$), the tail probability using this alternative approach was study-specific and ranged from 8.48% to 9.84%. As such, the results from this alternative approach, presented under the column of "Alternative Tail Probability" in Table E-16, are very similar to the main results, presented under the column of "Exact Percentage" in Table E-16, when background exposure was not accounted for while estimating the tail probability.

Table E-16 presents the BMDs and BMDLs for all studies considered for POD derivation, with and without accounting for background exposure while estimating the percentage of the population falling below the cutoff value. The BMDLs across the studies ranged from 1.2 ng/mL to 90.6 ng/mL.

Table E-16. BMDs and BMDLs for Effect of PFOA on Decreased Birth Weight, by Using Percentage (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight, or Alternative Study-Specific Tail Probability

Study	Exposure Median (IQR)	Exposure Distribution (μ, σ)	Reported β (95% CI)	Re-Expressed β (95% CI)	Intercept *b*	SE of β	95% One-Sided LL of β	Exact Percentage (P(0) = 8.27%)		Alternative Tail Probability[a]		
								BMD (ng/mL)	BMDL (ng/mL)	P(0)	BMD (ng/mL)	BMDL (ng/mL)
Wikström et al. (2020)	**1.6 (1.1–2.3)**	**(0.48, 0.54)**	**−68.0 (−112.0, −24.0) g/ln(ng/mL)**	**−41.0 (−67.5, −14.5) g/ng/mL**	**3,306.7**	**13.5**	**−63.3**	**3.4**	**2.2**	**8.60%**	**3.6**	**2.3**
Chu et al. (2020)	1.5 (1.0–2.6)	(0.43, 0.75)	−73.6 (−126.4, −20.9) g/ln(ng/mL)	−45.2 (−77.6, −12.8) g/ng/mL	3,311.4	16.5	−72.4	3.1	2.0	8.48%	3.3	2.0
Govarts et al. (2016)	1.5 (1.1–2.1)	(0.42, 0.48)	−34.5 (−129.0, 60.0) g/ln(ng/mL)	−34.3 (−128.2, 59.7) g/ng/mL	3,299.4	47.9	−113.1	3.8	1.2	8.80%	4.2	1.3
Sagiv et al. (2018)	5.8 (4.1–7.9)	(1.76, 0.49)	−18.5 (−45.4, 8.3) g/IQR (ng/mL)	−4.9 (−11.9, 2.2) g/ng/mL	3,267.0	3.6	−10.8	20.1	9.1	9.71%	27.7	12.5
Starling et al. (2017)	1.1 (0.7–1.6)	(0.1, 0.61)	−51.4 (−97.2, −5.7) g/ln(ng/mL)	−45.0 (−85.1, −5.0) g/ng/mL	3,311.1	20.4	−78.6	3.2	1.8	8.48%	3.3	1.9

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; CI = confidence interval; IQR = interquartile range; SE = standard error.

[a] The alternative study-specific tail probability of live births falling below the public health definition of low birth weight based on Normal distribution with intercept *b* as mean and standard deviation of 590.7 based on U.S. population.

ACE Biomonitoring on Perfluorochemicals also provides the median blood serum levels of PFOA among women ages 16 to 49 in 1999–2000 (4.6 ng/mL), in 2009–2010 (2.2 ng/mL), and in 2013–2014 (1.4 ng/mL). EPA performed a sensitivity analysis by estimating BMD and BMDL using these values as background exposures. The results for all studies considered for POD derivation, presented in Table E-17, demonstrate the robustness of EPA's approaches with alternative assumptions on background exposures.

Table E-17. BMDs and BMDLs for Effect of PFOA on Decreased Birth Weight by Background Exposure, Using the Exact Percentage of the Population (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight, or Alternative Tail Probability

Study	Background Exposure[a]	Intercept *b*	Exact Percentage (*P*(0) = 8.27%)		Alternative Tail Probability [b]		
			BMD (ng/mL)	BMDL (ng/mL)	*P*(0)	BMD (ng/mL)	BMDL (ng/mL)
Wikström et al. (2020)	1.1	3,306.7	3.4	2.2	8.60%	3.6	2.3
	1.4	3,319.0	3.7	2.4	8.28%	3.7	2.4
	2.2	3,351.8	4.5	2.9	7.46%	3.9	2.5
	4.6	3,450.2	6.9	4.4	5.38%	4.8	3.1
Chu et al. (2020)	1.1	3,311.4	3.1	2.0	8.48%	3.3	2.0
	1.4	3,325.0	3.4	2.2	8.13%	3.4	2.1
	2.2	3,361.1	4.2	2.7	7.24%	3.6	2.3
	4.6	3,469.7	6.6	4.2	5.03%	4.5	2.8
Govarts et al. (2016)	1.1	3,299.4	3.8	1.2	8.80%	4.2	1.3
	1.4	3,309.6	4.1	1.2	8.52%	4.3	1.3
	2.2	3,337.1	4.9	1.5	7.82%	4.5	1.4
	4.6	3,419.4	7.3	2.2	5.98%	5.4	1.6
Sagiv et al. (2018)	1.1	3,267.0	20.1	9.1	9.71%	27.7	12.5
	1.4	3,268.5	20.4	9.2	9.66%	27.8	12.5
	2.2	3,272.4	21.2	9.6	9.55%	28.0	12.6
	4.6	3,284.0	23.6	10.6	9.22%	28.7	12.9
Starling et al. (2017)	1.1	3,311.1	3.2	1.8	8.48%	3.3	1.9
	1.4	3,324.6	3.5	2.0	8.13%	3.4	1.9
	2.2	3,360.6	4.3	2.4	7.26%	3.6	2.1
	4.6	3,468.5	6.7	3.8	5.05%	4.6	2.6

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit.
[a] Assumptions on background exposure for the estimation of intercept using equation (3).
[b] The tail probability of live births falling below the public health definition of low birth weight based on Normal distribution.

For decreased birth weight associated with PFOA exposure, the POD selected from the available epidemiologic literature is 2.2 ng/mL maternal serum concentration, based on birth weight data from Wikström et al. (2020). Of the six individual studies, Sagiv et al. (2018) and Wikström et al. (2020) assessed maternal PFOA serum concentrations primarily or exclusively in the first

trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Therefore, the PODs from these two studies were considered further for POD selection. The POD from Wikström et al. (2020) was ultimately selected since the reported PFOA exposure concentrations were more representative of current U.S. exposure levels compared with the levels reported in Sagiv et al. (2018), and it was the lowest POD from these two studies.

E.1.3 Modeling Results for Liver Toxicity

This updated review indicated that PFOA is associated with increases in the liver enzyme ALT (see Toxicity Assessment, (U.S. EPA, 2024b)). Four *medium* confidence studies were selected as candidates for POD derivation. The two largest studies of PFOA and ALT in adults are Gallo et al. (2012) and Darrow et al. (2016), both conducted in over 30,000 adults from the C8 Study Project (for detailed descriptions of the study and findings, see Toxicity Assessment, (U.S. EPA, 2024b) and Table D-6). The main differences between the two studies are 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. One additional study (Nian et al., 2019) was considered by EPA for POD derivation because it reported significant associations in a high-exposed population in China, respectively. In an NHANES adult population, Lin et al. (2010) observed elevated ALT levels per log-unit increase in PFOA. The association between PFOA and liver enzymes was more evident in obese subjects, as well as subjects with insulin resistance and/or metabolic syndromes. When dividing the serum PFOA into quartiles in the fully adjusted models in subjects with a body mass index ≥30 kg/m^2, the ALT level trend across the serum PFOA quartiles was significant. 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.

Nian et al. (2019) examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) part of the Isomers of C8 Health Project and observed significant increases in ln-transformed ALT per each ln-unit increase in PFOA, as well significant increases in odds ratios of elevated ALT. Median serum PFOA concentrations in this study were 6.2 (ng/mL).

Both Gallo et al. (2012) and Darrow et al. (2016) studies evaluated the relationship between PFOA and ALT using two general types of analyses. In the first, subjects were divided into quantiles of PFOA exposure (quintiles in Darrow et al. (2016) and deciles in Gallo et al. (2012)), and linear regression models were used to compare mean ALT levels by each non-reference quantile versus mean ALT level in the lowest quantile. In the second type of analysis, a logistic regression evaluated ORs for having an ALT level above a certain cutoff for each non-reference quantile compared with the lowest (reference) quantile. The cutoff values used to define elevated ALT levels in both studies were 45 IU/L for men and 34 IU/L for women, clinically based value recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002), and were approximately the 90th percentile of all ALT values in these studies.

E.1.3.1 Nian et al. (2019)

E.1.3.1.1 Hybrid Method

The previously described hybrid method was implemented using data from Nian et al. (2019). The regression model adjusted for age, sex, career, income, education, drink, smoke, giblet and seafood consumption, exercise, and BMI. The percentage change in ln ALT for ln-ng/mL increase in PFOA was 7.4 (95% CI: 3.9, 11.0) (Table 3, Nian et al. (2019)). The reported regression coefficient β, which is also referred to as *m*, was calculated from the percent change expressed as $(e^{\beta}-1)*100$, resulting in a slope of 0.071 (95% CI: 0.038, 0.104) ln ALT (IU/L) per ln ng/mL PFOA. The estimated BMDs and BMDLs are presented in Table E-18.

Table E-18. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Nian et al. (2019)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
BMR = 5%, P(0) Empirical						
BMD	5.90	**4.61**	6.27	4.50	3.31	2.42
BMDL	4.88	**3.76**	5.08	3.68	2.72	2.03
BMR = 5%, P(0) Lognormal						
BMD	8.44	6.16	8.35	5.95	4.55	3.48
BMDL	6.31	4.63	6.24	4.50	3.43	2.63
BMR = 10%, P(0) Empirical						
BMD	15.57	11.65	16.38	11.23	8.55	6.13
BMDL	9.81	7.33	10.14	7.10	5.40	3.96
BMR = 10%, P(0) Lognormal						
BMD	21.06	14.81	20.87	14.16	11.22	8.29
BMDL	12.20	8.71	12.07	8.39	6.57	4.91

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.3.1.2 NOAEC/LOAEC Method

Categorical data, which can be used to develop NOAECs, were not available from the peer-reviewed publication.

E.1.3.2 Gallo et al. (2012) Gallo et al. (2012)

E.1.3.2.1 Elevated ALT

E.1.3.2.1.1 Hybrid Method

The hybrid method uses the regression slope from the linear regression model of ln-transformed ALT and ln PFOA concentrations, adjusted for age, sex, alcohol consumption, socioeconomic status, fasting status, race, month of blood sample collection, smoking status, body mass index, physical activity, and insulin resistance. The reported regression coefficient β, which is also referred to as *m*, was 0.022 (95% CI: 0.018, 0.025) ln ALT (IU/L) per ln ng/mL PFOA (Table 2, Gallo et al. (2012), model 3).

Using a normal approximation, the standard error of the regression coefficient is estimated as:

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{0.025 - 0.018}{3.92} = 0.0018$$

Elevated ALT is a biomarker of acute liver disease. For the following analyses, the adverse effect level of ALT for liver disease was chosen to be C = 42 IU/L for males and C = 30 IU/L for females, based on the most recent sex-specific upper reference limits reported in Valenti et al. (2021). These are slightly lower and more health protective than the cutoff values used in the original study (45 IU/L for men and 34 IU/L for women). These cutoff are also slightly higher than the American College of Gastroenterology (ACG) cutoffs, which considers that "true healthy normal ALT level ranges from 29 to 33 IU/L for males, 19 to 25 IU/L for females" (Kwo et al., 2017). They are the most updated clinical consensus cutoffs which update the American Association for the Study of Liver Diseases (AASLD) journal 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) determined the updated 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.

These analyses were for the periods 1999–2018, 2003–2018, and 2017–2018, separately for males and females ages 18 and over, assuming that the reported regression coefficient developed for the C8 Health Project data in Ohio starting in 2005 and 2006 can be applied to the alternative NHANES periods. These analyses used the NHANES-recommended regression model adjustment to correct the 2017–2018 ALT data to match the earlier laboratory method. EPA used the NHANES PFOA data for each NHANES cycle including data adjustments to stored biospecimen data collected in 1999–2000 and 2013–2014 that were publicly released in April 2022. NHANES survey weights were applied.

Using the NHANES data for each period and sex, EPA estimated the mean and standard deviation of ln ALT and the estimated mean ln PFOA (Table E-19). The unrounded values were used in the calculations:

Table E-19. NHANES Mean and Standard Deviation of Ln(ALT) (ln IU/L) and Mean PFOA (Ln ng/mL)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	Male	Female	Male	Female	Male	Female
Mean ln ALT (ln IU/L) ($\overline{y}$)	3.28	2.96	3.28	2.96	3.29	2.96
Standard Deviation ln ALT (ln IU/L) (S)	0.46	0.41	0.46	0.41	0.48	0.42
Mean ln PFOA (ln ng/mL) ($\overline{x}$)	1.10	0.80	1.08	0.78	0.50	0.25

Notes: ALT = alanine transaminase; IU = international units.

For the BMD analyses, the response of interest is elevated ALT, defined as ALT greater than or equal to an adverse effect threshold C IU/L defined as 42 IU/C for males and 30 IU/L for females. EPA estimated P(0), the prevalence of population with elevated ALT using two approaches. First, the empirical estimate of P(0), "P(0) Empirical," was calculated as the

proportion of the population with ALT greater than or equal to C, using the NHANES survey weights. Second, the lognormal estimate of P(0), "P(0) Lognormal," was calculated assuming that ALT is lognormally distributed using the equation:

$$P(0)\ Lognormal = 1 - \Phi\left\{\frac{ln(C) - mean(ln\ ALT)}{sd(ln\ ALT)}\right\}$$

where Φ is the normal cumulative distribution function.

The selected BMR is an extra risk of either 5% or 10%. The extra risk of high ALT is given by the equation

$$Extra\ Risk = \frac{P(d) - P(0)}{1 - P(0)}$$

where *P*(d) is the probability of ALT greater than or equal to C (IU/L) for a given PFOA dose d. Thus

$$P(d) = \{1 - P(0)\} \times Extra\ Risk + P(0)$$

The values of C, *P*(0) Empirical, *P*(d) Empirical, *P*(d) Lognormal for Extra Risk 5% or 10%, and *P*(d) Lognormal for Extra Risk 5% or 10% are shown in Table E-20.

Table E-20. Prevalence of Elevated ALT

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Adverse effect level C (IU/L)	42	30	42	30	42	30
P(0) Empirical	0.14	0.13	0.15	0.13	0.16	0.13
P(d) Empirical, Extra Risk 5%	0.19	0.17	0.19	0.17	0.20	0.17
P(d) Empirical, Extra Risk 10%	0.23	0.21	0.23	0.21	0.24	0.22
P(0) Lognormal	0.16	0.14	0.16	0.14	0.17	0.15
P(d) Lognormal, Extra Risk 5%	0.20	0.18	0.20	0.18	0.22	0.19
P(d) Lognormal, Extra Risk 10%	0.24	0.23	0.24	0.23	0.26	0.23

Notes: ALT = alanine transaminase; IU = international units.

The mean ln ALT *y* for a ln PFOA dose x is given by the equation

$$y = mx + b$$

where m is the slope, β, (from the Gallo regression model) and b is the intercept. The intercept b is the mean ln ALT for a population with a PFOA exposure of 1 ng/mL. For the U.S. population, the mean ln ALT is $\bar{y}$ (tabulated above) and the mean ln PFOA is $\bar{x}$ (tabulated above) so the intercept is given by the equation

$$b = \bar{y} - m\bar{x}$$

For a given group and dose, the probability of ALT greater than or equal to C is

$$P(d) = P(ALT \geq C) = P(\ln ALT \geq \ln C) = 1 - \Phi\left(\frac{\ln C - y}{S}\right)$$

where Φ is the normal cumulative distribution function. Thus, the mean ln ALT, y, is the solution of the last equation, i.e., $y = ln\ C - S \times \Phi^{-1}\{1 - P(d)\}$, where Φ^{-1} is the inverse of the normal cumulative distribution function.

The ln PFOA BMD is the corresponding dose x such that $y = mx + b$. Thus

$$ln\ BMD = \frac{y - b}{m}$$

This gives the PFOA BMD as exp(ln BMD).

For the BMDL, the lower bound of the dose is calculated, so that in the last equation, instead of m the 95th upper limit for β is used, which is given by

$$\beta 95 = 95th\ Upper\ limit\ for\ \beta = \beta + 1.645 \times se(\beta)$$

Thus

$$ln\ BMDL = \frac{y - b}{\beta 95}$$

This gives the PFOA BMDL as exp(ln BMDL).

Note that β95 is different from the upper bound of the 95% confidence interval, since that number is the 97.5th percentile. The values of the BMD and BMDL are presented in Table E-21.

Table E-21. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Gallo et al. (2012)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
BMR = 5%, P(0) Empirical						
BMD	27.10	**23.70**	34.05	22.93	15.67	10.17
BMDL	20.91	**17.93**	25.53	17.38	12.02	7.97
BMR = 5%, P(0) Lognormal						
BMD	86.46	60.64	86.48	56.74	43.97	32.74
BMDL	58.18	41.08	58.09	38.65	29.88	22.35
BMR = 10%, P(0) Empirical						
BMD	630.61	480.28	768.85	444.49	341.52	206.94
BMDL	335.77	254.96	399.24	237.61	182.28	113.68
BMR = 10%, P(0) Lognormal						
BMD	1,681.50	1,046.92	1,689.01	943.13	825.72	548.80
BMDL	797.64	507.03	799.39	461.42	397.19	268.75

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

For increased ALT associated with PFOA exposure, the POD is based on the data Gallo et al. (2012), a BMR of 5% and a $BMDL_5$ of 17.93 ng/mL.

E.1.3.2.1.2 Sensitivity Analyses

NOAEC/LOAEC Method

The results of the logistic regression analysis of elevated ALT across deciles of PFOA are presented in Table E-22. The mean, median and ranges of PFOA concentrations in each decile were not provided with the OR results in the publication. EPA obtained these from author correspondence, and they are illustrated in Table E-22. The NOAEC is bolded and is the mean PFOA serum concentration in the highest decile of PFOA that did not show a statistically significant OR of elevated ALT, which in this case is the 2nd decile, compared with the reference category (the lowest decile of PFOA). The NOAEC based on the elevated ALT data from Gallo et al. (2012) is 9.78 ng/mL.

BMDS Method

EPA used BMDS to calculate a BMD. In addition, EPA performed a sensitivity analysis using the generalized least squares for trend (glst) method (Greenland and Longnecker, 1992), which assumes a linear relationship between exposure and log-transformed ORs, and accounts for covariance between estimates. These analyses were performed in STATA v17.0 (StataCorp, 2021). Through author correspondence the number of participants with and without elevated ALT for each decile of PFOA were obtained (Table E-22).

Table E-22. Odds Ratios for Elevated ALT by Decile of PFOA Serum Concentrations (ng/mL) From Gallo et al. (2012)

Decile	Minimum (ng/mL)	Maximum (ng/mL)	Median (ng/mL)	Mean (ng/mL)	OR	95% CI	Participants Without Elevated ALT	Participants With Elevated ALT	Total (N)
0	0.25	7.9	5.8	5.46	1	ref	4,201	408	4,609
1	8.0	11.5	**9.7**	**9.76**	**1.09**	**0.94, 1.26**	4,123	450	4,573
2	11.6	15.5	13.5	13.5	1.19	1.03, 1.37	4,184	504	4,688
3	15.6	20.7	17.9	18.0	1.26	1.09, 1.45	4,137	541	4,678
4	20.8	27.9	24.0	24.1	1.4	1.22, 1.62	4,069	570	4,639
5	28.0	39.3	33.0	33.2	1.39	1.21, 1.60	4,126	555	4,681
6	39.4	57.0	47.2	47.5	1.31	1.14, 1.52	4,125	518	4,643
7	57.1	89.0	70.8	71.7	1.42	1.23, 1.64	4,100	542	4,642
8	89.1	189.3	118.1	124.9	1.4	1.21, 1.62	4,119	531	4,650
9	189.4	22,412	355.8	522.0	1.54	1.33, 1.78	4,074	575	4,649

Notes: ALT = alanine transaminase; CI = confidence interval; OR = odds ratio.
The NOAEC is bolded.

Applying BMDS v3.3rc10 using a BMR of 10% and 5% to the data for all 10 deciles did not result in any viable models. Applying BMDS v3.3rc10 to the data for the first five deciles did result in viable models. The data associated with the first five deciles was also run using a no intercept approach in which the lowest dose was subtracted out, subsequently referred to as an adjusted dose. The results of this modeling using both the mean and median PFOA levels are summarized in Table E-23, Table E-24, Table E-25, and Table E-26. The approaches provide similar BMDLs, with slightly higher values for unadjusted and adjusted models, using mean and

median concentration, ranging from 36.0 to 39.2 for a 10% BMR, and from 18.3 to 19.2 for a 5% BMR. The glst approach resulted in BMD (BMDL) values of 10.4 (9.0) ng/mL and 8.3 (7.5) ng/mL for BMRs of 10% and 5%, respectively.

Table E-23. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted Mean PFOA Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	-[b]	-	-	0.01	0.00	-	-	37.57	1.59
Gamma	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.02	24.27	19.00
Log-Logistic	**0.89**	**15,710.66**	**−0.45**	**−0.45**	**−0.33**	**50.88**	**39.15**	**24.10**	**18.73**
Multistage Degree 3	0.21	15,715.51	−0.73	−0.73	−1.08	40.01	32.72	27.39	17.39
Multistage Degree 2	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	38.03	24.27	19.00
Multistage Degree 1	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.01	24.27	19.00
Weibull	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.02	24.27	19.00
Logistic	0.75	15,711.31	−0.55	−0.55	−0.57	44.00	36.13	25.45	21.06
Log-Probit	0.95	15,712.09	−0.11	0.09	0.07	53.24	31.56	12.42	0.24
Probit	0.78	15,711.21	−0.54	−0.54	−0.54	44.85	36.58	25.30	20.79
Quantal Linear	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.02	24.27	19.00

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD computation failed.

Table E-24. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Mean PFOA Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	-[b]	-	-	0.00	0.00	-	-	44.13	19.91
Gamma	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	36.33	24.27	19.00
Log-Logistic	**0.89**	**15,710.66**	**−0.45**	**−0.45**	**−0.33**	**51.49**	**36.52**	**24.39**	**19.17**
Multistage Degree 3	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	30.23	24.27	18.99
Multistage Degree 2	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	33.44	24.27	19.00
Multistage Degree 1	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.02	24.27	19.00
Weibull	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	35.91	24.27	19.00
Logistic	0.75	15,711.31	−0.55	−0.55	−0.57	41.20	33.25	23.56	19.10
Log-Probit	0.94	15,712.10	−0.15	−0.15	0.04	80.42	42.50	26.88	19.41
Probit	0.78	15,711.21	−0.54	−0.54	−0.54	42.36	34.02	23.66	19.08
Quantal Linear	0.88	15,710.72	−0.47	−0.47	−0.36	49.86	39.02	24.27	19.00

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD computation failed.

Table E-25. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Unadjusted Median PFOA Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	-[b]	-	-	0.00	0.00	-	-	30.38	1.63
Gamma	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.18	23.77	18.59
Log-Logistic	**0.87**	**15,710.76**	**−0.48**	**−0.48**	**−0.39**	**49.77**	**38.59**	**23.57**	**18.29**
Multistage Degree 3	0.41	15,713.97	−0.69	−0.69	−0.70	41.72	34.14	25.48	17.84
Multistage Degree 2	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	37.41	23.77	18.59
Multistage Degree 1	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.18	23.77	18.59
Weibull	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.18	23.77	18.59
Logistic	0.72	15,711.45	−0.56	−0.56	−0.63	43.35	35.62	25.08	20.78
Log-Probit	0.96	15,712.05	−0.10	0.06	0.06	38.13	28.57	6.49	0.28
Probit	0.75	15,711.34	−0.55	−0.55	−0.60	44.15	36.03	24.92	20.49
Quantal Linear	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.18	23.77	18.59

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD computation failed.

Table E-26. Summary of Benchmark Dose Modeling Results for Elevated ALT in Gallo et al. (2012) Using the Adjusted, No Intercept Median PFOA Serum Concentration

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	-[b]	-	-	−0.01	0.00	-	-	39.27	19.50
Gamma	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	35.81	23.77	18.59
Log-Logistic	**0.87**	**15,710.76**	**−0.48**	**−0.48**	**−0.39**	**50.41**	**36.03**	**23.88**	**18.77**
Multistage Degree 3	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	29.64	23.77	18.59
Multistage Degree 2	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	32.80	23.77	18.59
Multistage Degree 1	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.17	23.77	18.58
Weibull	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	35.40	23.77	18.59
Logistic	0.72	15,711.45	−0.56	−0.56	−0.63	40.38	32.55	23.08	18.70
Log-Probit	0.95	15,712.07	−0.14	−0.14	0.04	82.86	42.57	26.64	18.96
Probit	0.75	15,711.34	−0.55	−0.55	−0.60	41.50	33.30	23.18	18.68
Quantal Linear	0.86	15,710.82	−0.49	−0.49	−0.42	48.82	38.18	23.77	18.59

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] Selected model in bold.

[b] BMD computation failed.

E.1.3.3 Darrow et al. (2016)

E.1.3.3.1 Hybrid Method

The previously described hybrid method was implemented using data from Darrow et al. (2016). The regression model adjusted for age, sex, BMI, alcohol consumption, regular exercise, smoking status, education, insulin resistance, fasting status, history of working at DuPont place, and race. The reported regression coefficient β, which is also referred to as *m*, 0.012 ln ALT (IU/L) per ln ng/mL PFOA (95% CI: 0.009, 0.016). The values of the BMD and BMDL are presented in Table E-27.

Table E-27. BMD and BMDL for Effect of PFOA (ng/mL) on Increased ALT in Darrow et al. (2016)

Time Period	1999–2018	1999–2018	2003–2018	2003–2018	2017–2018	2017–2018
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
BMR = 5%, P(0) Empirical						
BMD	170.00	**170.39**	261.56	162.90	102.05	57.26
BMDL	70.30	**65.99**	98.12	63.45	41.44	24.95
BMR = 5%, P(0) Lognormal						
BMD	1,425.99	953.63	1,444.21	857.24	676.42	487.93
BMDL	370.41	253.45	372.90	232.26	181.68	133.07
BMR = 10%, P(0) Empirical						
BMD	54,468.17	42,371.98	79,307.85	37,331.45	29,002.22	14,336.82
BMDL	6,380.83	4,913.47	8,530.54	4,432.63	3,425.39	1867.46
BMR = 10%, P(0) Lognormal						
BMD	328,872.91	176,813.58	335,682.39	148,268.33	146,339.24	85,701.65
BMDL	26,005.03	15,003.59	26,339.57	13,022.55	12,133.22	7,551.51

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.3.3.2 Sensitivity Analyses

E.1.3.3.2.1 BMDS Method

Darrow et al. (2016) the increased mean ALT concentration (Table E-28) was modeled using BMDS v3.3rc10. BMRs of a change in the mean equal to ½ and 1 SDs from the control mean were chosen (Table E-29). No viable models were identified.

Table E-28. Dose-Response Modeling Data for Increased Mean ALT Concentration in Darrow et al. (2016)

Dose (ng/mL)	N	Mean Response[a,b]
4.20	6,145	0.000 ± 0.48
8.60	6,145	0.001 ± 0.48
19.05	6,145	0.023 ± 0.47
54.10	6,145	0.036 ± 0.48

Notes: ALT = alanine transaminase.
[a] Data are presented as mean ± standard deviation.
[b] Linear regression coefficient for ln-transformed ALT

Table E-29. Summary of Benchmark Dose Modeling Results for Increased Mean ALT Concentrations in Darrow et al. (2016)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{1\ SD}$	$BMDL_{1\ SD}$	$BMD_{0.5\ SD}$	$BMDL_{0.5\ SD}$
	p-value	AIC	Dose Group Near $BMD_{1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group				
Exponential 3	<0.0001	35,008.88	−0.04	−0.04	−0.02	689.70	0.00	631.70	0.00
Exponential 5	–[b]	–	–	–	–	–	–	–	–
Hill	–	–	–	–	–	–	–	–	–
Polynomial Degree 3	<0.0001	34,974.38	−0.37	−0.37	−2.43	5,840.29	1,369.18	2,920.14	1,060.82
Polynomial Degree 2	<0.0001	34,974.38	−0.38	−0.38	−2.42	5,836.79	2,087.90	2,918.39	1,424.88
Power	<0.0001	34,974.38	−0.37	−0.37	−2.42	5,836.99	5,037.10	2,918.50	2,219.70
Linear	<0.0001	34,974.38	−0.37	−0.37	−2.42	5,836.99	4,554.37	2,918.50	2,277.24

Notes: AIC = Akaike information criterion; ALT = alanine transaminase; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to one standard deviation 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 equal to one standard deviation from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

[a] No viable models. No model was selected.

[b] BMD computation failed.

E.1.3.3.2.2 NOAEC/LOAEC Method

The results of the linear regression analysis of elevated ALT across quintiles of PFOA are presented in Table E-30. The PFOA dose levels in each quintile of exposure were calculated as the midpoint of the reported quintile ranges (Table 2 in Darrow et al. (2016)). The NOAEC is bolded and is the mean PFOA serum concentration in the highest quintile of PFOA that did not show a statistically significant change in ALT, which in this case is the 2nd quintile, compared with the reference category (the lowest quintile of PFOA). The NOAEC based on the elevated ALT data from Darrow et al. (2016) is 8.6 ng/mL.

Table E-30. Linear Regression Results for Ln(ALT) by Quintiles of Serum PFOA Concentration in Darrow et al. (2016)

Quintile	Dose (ng/mL)	N	Regression Coefficient[a]	95% CI
1	4.20	6,145	Ref	Ref
2	**8.60**	6,145	0.001	−0.016, 0.018
3	19.05	6,145	0.023	0.007, 0.040
4	54.10	6,145	0.036	0.019, 0.053
5`	1,8120.2	6,145	0.048	0.031, 0.066

Notes: ALT = alanine transaminase; CI = confidence interval.
[a] Linear regression coefficient for ln-transformed ALT.

E.1.3.4 Summary of Modeling Results for Liver Toxicity

Table E-31 summarizes the PODs resulting from the hybrid modeling approach for increased ALT. The selected PODs were based on a BMR of 5%, resulting in BMDLs ranging from 3.76 to 65.99 ng/mL.

Table E-31. BMDLs for Effect of PFOA on Serum ALT Using a BMR of 5%

Study Name	BMDL (ng/mL)
Gallo et al. (2012)	17.93
Darrow et al. (2016)	65.99
Nian et al. (2019)	3.76

E.1.4 Modeling Results for Increased Cholesterol

This updated review indicated that there was as association between increases in PFOA and increases in total cholesterol (TC) in adults. Three *medium* confidence studies were considered for POD derivation (Dong et al., 2019; Lin 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). Lin et al. (2019) collected data from prediabetic adults from the Diabetes Prevention Program (DPP) and DPP Outcomes Study at baseline (1996–1999).

E.1.4.1 Dong et al. (2019)

Using data from NHANES (2003–2014) on 8,948 adults, Dong et al. (2019) calculated a BMD for PFOA and TC using a hybrid model (Crump, 1995). The cutoff point for adverse response

(i.e., elevated TC) was set at the upper 5th percentile of TC values in the lowest PFOA exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 10.5 and 5.6 ng/mL, respectively. Key variables or other results such as the cutoff point used to define elevated TC or model fit parameters were not provided.

Although the hybrid approach has several advantages (Crump, 1995), few details were provided in Dong et al. (2019) on several important aspects of this approach or on other key issues, including the definition of the unexposed reference group, the distribution of PFOA or TC values in this group, model fit (e.g., the fit of linear vs. non-linear models), the impact of potential confounders, or the potential role of reverse causality.

EPA re-analyzed the data using the regression models from the Dong et al. (2019) study, together with updated NHANES data, applied to a modified hybrid model to develop BMD and BMDL estimates for various time periods and assumptions. The BMD values for a BMR of 5% ranged from 3.95 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 9.11 ng/mL for the period 2017–2018, for all adults. The BMDL values for a BMR of 5% ranged from 2.29 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 5.28 ng/mL for the period 2017–2018, for all adults. The BMD values for a BMR of 10% ranged from 8.79 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 13.85 ng/mL for the period 2017–2018, for all adults. The BMDL values for a BMR of 10% ranged from 5.10 ng/mL for the period 1999–2018, excluding adults taking cholesterol medications, up to 8.03 ng/mL for the period 2017–2018, for all adults.

An important caveat is that these calculations assume that Dong's regression model is still applicable, or at least a good approximation, for all the time periods, for all adults and for adults taking cholesterol medications, and for the recently updated NHANES data.

Dong et al. (2019) reported a regression coefficient β, which is also referred to as m, of 1.48 mg/dL TC per ng/mL PFOA (95% CI: 0.2, 2.8). After correspondence with the study author, EPA obtained an updated estimated coefficient of 1.44 mg/dL TC per ng/mL PFOA (95% CI: 0.2, 2.69), which EPA used for these analyses. The regression model applies to all adults 20 to 80 years old and was adjusted for age, gender, race, poverty income ratio, body mass index, waist circumference, physical activity level, diabetes status, smoking status, and number of alcoholic drinks per day. Using a normal approximation, the standard error of the regression coefficient is estimated as:

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{2.69 - 0.2}{3.92} = 0.635 \frac{mg}{dL} \left(\frac{ng}{mL}\right)^{-1}$$

These analyses were for the periods 1999–2008, 2003–2014, 2003–2018, and 2017–2018, assuming that the regression model coefficient developed for the period 2003–2014 in the Dong et al. (2019) study can be applied to the alternative NHANES periods. These analyses used the NHANES-recommended reference method data for TC. EPA used the NHANES PFOA data for each NHANES period including data adjustments to stored biospecimen data collected in 1999–2000 and 2013–2014 that were publicly released in April 2022. Alternative analyses were for all

adults ages 20 and over, and for adults ages 20 and over that reported not taking prescribed cholesterol medications. NHANES survey weights were applied.

EPA estimated the distribution of TC assuming a normal distribution and the estimated mean PFOA levels for each of the analysis periods (Table E-32).

Table E-32. NHANES Mean and Standard Deviation of TC (mg/dL) and Mean PFOA (ng/mL)

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?		No		No		No		No
Mean TC ($\bar{y}$)	196.17	197.89	196.36	198.01	194.86	196.96	189.01	192.12
Standard Deviation TC (S)	41.99	41.47	41.84	41.39	41.80	41.28	40.57	39.67
Mean PFOA ($\bar{x}$)	3.43	3.43	3.90	3.90	3.37	3.37	1.80	1.80

Notes: NHANES = National Health and Nutrition Examination Survey; TC = total cholesterol.

For the BMD analyses, the response of interest is having elevated serum cholesterol, defined as greater than or equal to 240 mg/dL, which is the cutoff that the American Heart Association recommends (www.heart.org). The baseline probability P(0) of such a response is estimated as 11.5%, for adults aged 20 and older in 2015–2018, as reported by the CDC Health, United States, 2019 Data Finder (NCHS, 2019).

The selected BMR is an extra risk of either 5% or 10%. The extra risk of high serum cholesterol is given by the equation

$$Extra\ Risk = \frac{P(d) - P(0)}{1 - P(0)}$$

where P(d) is the probability of serum cholesterol greater than or equal to 240 mg/dL for a given PFOA dose d. Thus

$$P(d) = \{1 - P(0)\} \times Extra\ Risk + P(0)$$

$$P(d) = \{1 - 0.115\} \times Extra\ Risk + 0.115$$

P(d) = 0.1593 for 5% extra risk and P(d) = 0.2035 for 10% extra risk.

The mean serum cholesterol y for a PFOA dose x is given by the equation

$$y = mx + b$$

where m is the slope, β, (from the Dong regression model) and b is the intercept. The intercept b is the mean serum cholesterol for an unexposed population. For the U.S. population, the mean TC is $\bar{y}$ (tabulated above) and the mean PFOA is $\bar{x}$ (tabulated above) so the intercept is given by the equation

$$b = \bar{y} - m\bar{x}$$

For a given group and dose, the probability of serum cholesterol greater than or equal to 240 mg/dL is

$$P(d) = P(TC \geq 240) = 1 - \Phi\left(\frac{240 - y}{S}\right)$$

where Φ is the normal cumulative distribution function. Thus, the mean serum cholesterol y is the solution of the last equation, i.e., $y = 240 - S \times \Phi^{-1}\{1 - P(d)\}$, where Φ^{-1} is the inverse of the normal cumulative distribution function.

The benchmark dose (BMD) is the corresponding dose x such that $y = mx + b$. Thus

$$BMD = \frac{y - b}{m}$$

For the BMDL, the lower bound of the dose is calculated, so that in the last equation, instead of m the 95th upper limit for β is used, which is given by

$$\beta 95 = 95th\ Upper\ limit\ for\ \beta = \beta + 1.645 \times se(\beta)$$

Thus

$$BMDL = \frac{y - b}{\beta 95}$$

Note that β95 is different from the upper bound of the 95% confidence interval, which is the 97.5th percentile. The estimated BMDs and BMDLs are presented in Table E-33:

Table E-33. BMDs and BMDLs for Effect of PFOA on Increased Cholesterol in Dong et al. (2019)

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?		No		No		No		No
BMR = 5%								
BMD (ng/mL)	4.78	**3.95**	5.23	4.39	5.76	4.66	9.11	7.57
BMDL (ng/mL)	2.77	**2.29**	3.03	2.54	3.34	2.70	5.28	4.39
BMR = 10%								
BMD (ng/mL)	9.69	8.79	10.12	9.23	10.65	9.49	13.85	12.21
BMDL (ng/mL)	5.62	5.10	5.86	5.35	6.17	5.50	8.03	7.07

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

Given the potential impact of taking cholesterol medication on the true association between PFOA and increased TC, the results based on the data excluding such possibility is considered higher confidence. As illustrated in Table E-23, there was a slight decline over time in PFOA levels based on NHANES data, suggesting that reliance on distributional data based on the most recent NHANES cycle available (2017–2018) might be more reflective of recent exposure levels. However, given the chronic nature of both exposure and increased TC development, a higher confidence might be the given to estimates based on the largest period available (1999–2018).

For increased cholesterol associated with PFOA exposure, the POD is based on the data Dong et al. (2019) excluding people taking cholesterol medication, the longest period available, a BMR of 5% and a $BMDL_5$ of 2.29 ng/mL. A comparison BMDL of 4.39 ng/mL based on the most period available is also considered.

E.1.4.2 Steenland et al. (2009)

E.1.4.2.1 Hybrid Approach – Mean Serum TC

The same hybrid approach described previously was also applied to Steenland et al. (2009) using natural log-transformed values. Steenland et al. (2009) reported in Table 4 linear regression coefficient for change in ln-transformed TC per ln(PFOA): 0.0112 with a standard deviation of 0.00076. The NHANES data used in this approached is summarized in Table E-34 and BMD/BMDL values are presented in Table E-35.

Table E-34. NHANES Mean and Standard Deviation of Ln(TC) (Ln(mg/dL)) and Mean Ln(PFOA) (Ln(ng/mL))

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?		No		No		No		No
Mean ln(TC) ($\overline{y}$)	5.26	5.27	5.26	5.27	5.25	5.26	5.22	5.24
Standard Deviation ln(TC) (S)	0.21	0.21	0.21	0.21	0.21	0.21	0.22	0.21
Mean ln(PFOA) ($\overline{x}$)	0.94	0.94	1.11	1.11	0.92	0.92	0.37	0.37

Notes: TC = total cholesterol.

Table E-35. BMD and BMDL for Effect of PFOA on Increased Cholesterol in Steenland et al. (2009)

Time Period	1999–2018	1999–2018	2003–2014	2003–2014	2003–2018	2003–2018	2017–2018	2017–2018
Taking prescribed cholesterol medication?		No		No		No		No
BMR = 5%								
BMD (ng/mL)	8.08	**4.99**	9.17	5.44	13.60	7.25	99.46	44.76
BMDL (ng/mL)	6.54	**4.25**	7.33	4.58	10.45	5.93	62.48	30.48
BMR = 10%								
BMD (ng/mL)	199.25	115.42	224.12	126.37	340.22	168.85	2,590.14	1,012.48
BMDL (ng/mL)	116.69	71.43	129.70	77.49	188.76	100.55	1,170.53	503.12

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.4.2.2 Sensitivity Analyses

E.1.4.2.2.1 BMDS for Mean Serum TC

EPA also conducted dose-response modeling using mean serum TC reported across PFOA deciles from Table 3 in Steenland et al. (2009). BMDS 3.3rc10 was used to fit the dose-response data using all deciles, no viable models were identified. To further investigate, BMDS 3.3rc10 was used to fit the dose-response data in the lowest five deciles and regression coefficients for the mean change in ln-transformed serum TC (Table 3 in Steenland et al. (2009) and summarized

in Table E-36. BMRs of a change in the mean equal to ½ and 1 SDs from the control mean were chosen. The BMD modeling results are summarized in Table E-37.

Table E-36. Regression Results for Serum Total Cholesterol by Deciles of Serum PFOA from Steenland et al. (2009)

Decile	Dose (ng/mL)	N	Regression Coefficient[a] (SD)
1	5.8	4,629	0.00 (0.192)
2	9.7	4,629	0.01 (0.192)
3	13.6	4,629	0.02 (0.192)
4	17.9	4,629	0.03 (0.192)
5	24.0	4,629	0.04 (0.192)

Notes: SD =standard deviation.
[a] Regression coefficient, change in the natural log of total cholesterol.

Table E-37. Summary of Benchmark Dose Modeling Results for Increased Mean Serum Total Cholesterol in Steenland et al. (2009)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{1\ SD}$ (ng/mL)	$BMDL_{1\ SD}$ (ng/mL)	$BMD_{0.5\ SD}$ (ng/mL)	$BMDL_{0.5\ SD}$ (ng/mL)
	p-value	AIC	Dose Group Near $BMD_{1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group				
Exponential 3	0.00	−10,692.03	−0.93	−0.93	−2.86	40.73	39.46	33.41	32.20
Exponential 5	-	-	-	-	-	-	-	-	-
Hill	-	-	-	-	-	-	-	-	-
Polynomial Degree 3	0.44	−10,703.20	−0.45	−0.45	−0.76	77.42	51.38	43.06	35.34
Polynomial Degree 2	0.30	−10,702.46	−0.46	−0.46	−0.98	72.03	56.71	42.14	36.23
Power	0.74	−10,705.63	−0.62	−0.62	−0.48	86.48	70.77	43.24	37.70
Linear	**0.74**	**−10,705.63**	**−0.62**	**−0.62**	**−0.48**	**86.48**	**75.27**	**43.24**	**37.64**

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to one standard deviation 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 equal to one standard deviation from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

E.1.4.2.2.2 BMDS for Elevated TC

In additional to modeling the regression coefficients, dichotomous models using BMDS 3.3rc10 were used to fit the ORs from Steenland et al. (2009) for having an elevated TC level are shown in Table E-38. Sample sizes, mean PFOA concentrations in each quartile and prevalence of elevated TC in each exposure group were obtained from Dr. Kyle Steenland. A BMR of 10 and 5% extra risk were both included. The BMD modeling results are summarized in Table E-39. Note that this approach did not generate any viable models.

Table E-38. Odds Ratios for Elevated Serum Total Cholesterol by Quartiles of Serum PFOA From Steenland et al. (2009)

Quartile	Dose (ng/mL)	N	Incidence	OR	95% CI
1	6.55	11,575	1,431	1	Ref
2	19.85	11,434	1,687	1.21	1.12, 1.31
3	46.75	11,478	1866	1.33	1.23, 1.43
4	441	11,477	2082	1.38	1.28, 1.50

Notes: CI = confidence interval; OR = odds ratio; Ref = reference value.

Table E-39. Summary of Benchmark Dose Modeling Results for Elevated Total Cholesterol in Steenland et al. (2009)

Model[a]	Goodness of Fit		Scaled Residual			BMD_{10} (ng/mL)	$BMDL_{10}$ (ng/mL)	BMD_5 (ng/mL)	$BMDL_5$ (ng/mL)
	p-value	AIC	Dose Group Near BMD_{10}	Dose Group Near BMD_5	Control Dose Group				
Dichotomous Hill	-[b]	-	-	0.15	-	-	-	15.63	4.74
Gamma	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	788.57	450.43	383.91
Log-Logistic	< 0.0001	39,352.05	−0.55	−0.55	−5.25	947.03	803.50	448.60	381.25
Multistage Degree 3	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	702.09	450.43	383.86
Multistage Degree 2	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	785.94	450.43	383.76
Multistage Degree 1	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	788.56	450.43	383.72
Weibull	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	788.57	450.44	383.91
Logistic	< 0.0001	39,353.79	−0.42	−0.42	−5.37	839.18	728.71	457.78	398.00
Log-Probit	0.00	39,305.25	−2.00	−2.00	−2.00	0.38	0.18	0.00	0.00
Probit	< 0.0001	39,353.58	−0.43	−0.43	−5.35	851.39	737.27	456.88	396.03
Quantal Linear	< 0.0001	39,352.35	−0.52	−0.52	−5.27	925.23	788.57	450.43	383.91

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level; BMD_5 = dose level corresponding to a 5% response level; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level.

[a] No viable models. No model was selected.

[b] BMD computation failed.

Given the potential impact of taking cholesterol medication on the true association between PFOA and increased TC, the results based on the data excluding such possibility is considered higher confidence. As illustrated in Table E-26 there was a slight decline over time in PFOA levels based on NHANES data, suggesting that reliance on distributional data based on the most recent NHANES cycle available (2017–2018) might be more reflective or current impacts. However, given the chronic nature of both exposure and increased TC development, a higher confidence might be the given to estimates based on the largest period available (1999–2018).

For increased cholesterol associated with PFOA exposure, the POD is based on the data fro**m Steenland et al. (2009) excluding people taking cholesterol medication, the longest period available, a BMR of 5% and a $BMDL_5$ of 4.25 ng/mL.**

E.1.4.3 Lin et al. (2019)

Lin et al. (2019) collected data from prediabetic adults from the DPP and DPP Outcomes Study at baseline (1996–1999). This study included 888 pre-diabetic adults who were recruited from 27 medical centers in the U.S. Median PFOA levels at baseline were comparable to those from NHANES 1999–2000, 4.9 (25th, 75th percentiles: 3.5, 6.7 ng/mL). The study presented both cross-sectional and prospective analyses. The cross-sectional analyses evaluated associations between baseline PFAS and baseline lipid levels. The prospective analysis evaluated whether baseline PFAS levels predicted higher risk of incident hypercholesterolemia and hypertriglyceridemia, but in the placebo and the lifestyle intervention groups, separately. Both analyses showed evidence of an association between PFOA and increased TC.

EPA conducted dose-response modeling using mean serum TC reported across PFOA quartiles using data from Table S5 in Lin et al. (2019). For its POD calculations, EPA used the results from the cross-sectional analysis because they were presented in a format that was more amendable to dose-response analysis. BMDS 3.3rc10 was used to fit the dose-response data for the adjusted mean difference in lipid levels (mg/dL) per quartile of baseline plasma PFAS concentrations (ng/mL), summarized in Table E-40. BMRs of a change in the mean equal to ½ and 1 SDs from the control mean were chosen. The BMD modeling results are summarized in Table E-41.

Table E-40. Adjusted Mean Differences in Serum Total Cholesterol by Quartiles of Serum PFOA (ng/mL) From Lin et al. (2019)

Dose (ng/mL)	N	Mean TC[a,b]
2.6	221	0.00 ± 35.85
4.2	222	2.00 ± 36.68
5.6	227	10.13 ± 35.47*
8.4	228	13.36 ± 36.40*

Notes: TC = total cholesterol.
[a] Data are presented as mean ± standard deviation.
[b] Adjusted mean difference in lipid levels (mg/dL) per quartile of baseline plasma PFOA concentration (ng/mL) ; *p < 0.05.

Table E-41. Summary of Benchmark Dose Modeling Results for Increase Mean Serum Total Cholesterol From Lin et al. (2019)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{1\ SD}$ (ng/mL)	$BMDL_{1\ SD}$ (ng/mL)	$BMD_{0.5\ SD}$ (ng/mL)	$BMDL_{0.5\ SD}$ (ng/mL)
	p-value	AIC	Dose Group Near $BMD_{1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group				
Exponential 3	0.09	8,983.84	−0.31	−0.31	−1.04	11.63	9.85	9.41	8.52
Exponential 5	-[b]	-	-	-	-	-	-	-	-
Hill	-	-	-	-	-	-	-	-	-
Polynomial Degree 3	0.10	8,983.81	−0.39	−0.39	−0.30	13.63	10.28	8.64	5.14
Polynomial Degree 2	0.33	8,981.29	−0.38	−0.38	0.03	14.57	10.53	7.29	5.27
Power	0.34	8,981.24	−0.38	0.01	0.01	14.53	10.63	−7.27	0.00
Linear	**0.34**	**8,981.24**	**−0.38**	**−0.38**	**0.01**	**14.53**	**10.56**	**7.27**	**5.28**

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to one standard deviation 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 equal to one standard deviation from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviations from the control mean; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] BMD computation failed.

E.1.4.4 Summary of Modeling Results for Increased Cholesterol

Table E-42 summarizes the PODs resulting from the modeling approaches for increased cholesterol. The selected and comparison PODs were based on a BMR of 5%, resulting in BMDLs ranging from 2.29 to 5.28 ng/mL with the selected POD of 2.29 ng/mL.

Table E-42. BMDLs for Effect of PFOA on Serum Total Cholesterol Using a BMR of 5%

Study Name	Effect	BMD (ng/mL)	BMDL (ng/mL)
Dong et al. (2019)	Exclude those prescribed cholesterol medication, 1999–2018	3.95	2.29
Steenland et al. (2009)	Exclude those prescribed cholesterol medication	4.99	4.25
Lin et al. (2019)		7.27	5.28

Notes: BMD = benchmark dose; BMDL = benchmark dose lower limit; BMR = benchmark response.

E.1.5 Modeling Results for Cancer

This updated review indicated that there is an increase in risk for kidney or renal cell carcinoma (RCC) and testicular cancers and PFOA exposure (Bartell and Vieira, 2021; Shearer et al., 2021; Chang et al., 2014). Although newer studies generally show no association, there is some evidence that PFOA may be related to breast cancer risk especially in participants with specific polymorphisms or specific types of tumors (Mancini et al., 2020; Ghisari et al., 2017). There is also new evidence from two recent general population studies in the United States and China that suggests an association between PFOA exposure and risk of liver cancer (Cao et al., 2022; Goodrich et al., 2022). One occupational study (Girardi and Merler, 2019) supported an increase in risk for liver cancer, malignant neoplasm of the lymphatic and hematopoietic tissue, and one occupational study (Steenland et al., 2015) reported an increasing trend in prostate cancer that did not reach statistical significance. No associations were found for colorectal cancer in either the general population or occupational studies, or for lung cancer in occupational studies.

Results are most consistent for kidney cancer in adults based on a new nested case-control study (Shearer et al., 2021), two C8 Health Project studies (Barry et al., 2013; Vieira et al., 2013) and an occupational mortality study (Steenland and Woskie, 2012) from the 2016 PFOA HESD.

For dose-response modeling, EPA also considered Shearer (2021) and the C8 Health Project study (Vieira et al., 2013). Considerations included study population (general population vs. occupational or high-exposed populations), statistical power and study quality.

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 (> 2,384 ppm-years). This study was limited by the small number of observed cancer cases (six kidney cancer deaths). This study was not used for dose-response analysis because information on a range of exposures more relevant to the general population were available from the Shearer et al. (2021) and Vieira et al. (2013). The study by Barry et al. (2013) was not used for dose-response analysis because it was performed in the same study area as the Vieira et al. (2013) study and these two studies likely involved a number of the same participants. In addition, Barry et al. (2013) could not be used in the sensitivity analysis because it lacked the necessary exposure measurements for Cancer Slope Factor (CSF)

calculation. In this study, estimated PFOA concentrations are provided in Table 2 for community level and worker level. However, combined exposure levels of each quartile of the overall study population were not reported. Without overall exposure data in each quartile, CSF calculations are not feasible.

The study by Raleigh et al. (2014) was not selected because of the concerns of exposure assessment methods and study quality. This study used modeled estimates of PFOA air concentrations in the workplace rather than biomonitoring measurements. This is a concern because the assessment lack of information about the degree to which inhaled PFOA is absorbed in humans and factors that may affect the absorption, as well as PFOA exposure data in non-production workers was not based on actual measurements. In addition, this study did not observe an association between PFOA and kidney cancer. The possible reasons of this study could have missed to identify the association between PFOA, and kidney cancer include relatively small numbers of cases, lack of information adjustment on risk factors of kidney cancer such as smoking status and BMI, and the methods for exposure assessment.

Shearer et al. (2021) is a multicenter case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO). The PLCO is a randomized clinical trial of the use of serum biomarkers for cancer screening. The cases in this study (Shearer et al., 2021) included all the participants of the screening arm of the PLCO trial who were newly diagnosed with RCC during the follow-up period (N = 326). All cases were histopathologically confirmed. Controls were selected from among participants of the PLCO trial screening arm who had never had RCC. Controls were individually matched to the RCC cases by age at enrollment, sex, race/ethnicity, study center, and year of blood draw. PFOA concentrations were measured in the baseline serum samples collected between 1993 and 2002. Median PFOA levels in controls was 5.0 ng/mL, comparable with 4.8 ng/mL in adults 60 and over from NHHANES 1999–2000. The analyses accounted for numerous confounders including BMI, smoking, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, calendar and study year of blood draw, sex, race and ethnicity, study center. Socioeconomic status was not explicitly considered in the analyses.

There was 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). Although non-significant elevated risks were observed in the second and third quartiles, there was 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 men and in women, separately.

EPA also considered the C8 Health Project study (Vieira et al., 2013). Shearer et al. (2021) and Viera et al. (2013) have considerable differences with respect to several study design aspects. These include the types of outcomes considered (RCC vs. any kidney cancer), the type of exposure assessment (serum biomarker vs. modeled exposure), source population (multicenter vs. Ohio and WV regions), study size (324 cases and 324 matched controls vs. 59 cases and 7,585 registry-based controls). Additionally, the dramatically different regression slopes resulting from the two studies (0.0981, 95% CI: 0.0025, 0.1937 vs. 0.0122, 95% CI: 0.006, 0.0238 per ng/mL PFOA, from Shearer et al. (2021) and Vieira et al. (2013), respectively), are an indication that the studies have considerable differences.

E.1.5.1 Cancer Slope Factor Calculations

E.1.5.1.1 Shearer et al. (2021)

The methods used to calculate CSFs based on data from Shearer et al. (2021) are based on those used by U.S. EPA for its CSF calculation for TCE (U.S. EPA, 2011c) and by OEHHA for its PHG for arsenic (OEHHA, 2004).

The underlying model involves a linear regression between PFOA exposure and cancer relative risk used to estimate the dose response between PFOA and RCC risk, of the form:

$$RR = 1 + bx$$

This was calculated using a weighted linear regression of the quartile specific RRs. The variable b is then the slope of the excess risk (RR-1) and PFOA dose (x_i) in each non-reference exposure group, and can be estimated as follows (U.S. EPA, 2011c; Rothman et al., 2008):

$$b = \frac{\sum w_i x_i RR_i - \sum w_i x_i}{\sum w_i {x_i}^2}$$

where (w_i) are the weights defined as the inverse of the variance of each RR_i. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age, the ORs represent a good approximation of the underlying RRs. Thus, the variance of the quartile specific ORs can be used to estimate the weights (w_i) as follows (U.S. EPA, 2011c):

$$w_i = \frac{1}{Var(OR_i)} = \frac{1}{{OR_i}^2 \times \left(\frac{lnUCL_i - lnLCL_i}{2 \times 1.96}\right)^2}$$

where UCL_i and LCL_i are the upper and lower 95% CIs of the quartile specific ORs (Table E-43). The PFOA dose levels (x_i) in each quartile of exposure were calculated as the midpoint of the reported range (Table E-43). Since the intercept of the regression is set at 1 for a dose of 0, the midpoint of the lowest quartile was subtracted from each of the midpoint of the upper quartiles.

The standard error and 95% CIs for the regression slope b can then be calculated as follows:

$$SE_b = \sqrt{\frac{1}{\sum w_i {x_i}^2}}$$

and

$$95\%\ CI_b = b \pm SE_b$$

Table E-43. ORs for the Association Between PFOA Serum Concentrations and RCC in Shearer et al. (2021) and Data Used for CSF Calculations

PFOA Range (ng/mL)	x_i	OR_i	LCI_i	UCI_i	$Var(OR_i)$	w_i	w_ix_i	$w_ix_i^2$	$w_ix_iOR_i$	Cases	Controls
< 4	0 (reference)	1	-	-						47	81
4.0−5.5	2.75	1.47	0.77	2.80	0.234	4.267	11.734	32.267	17.248	83	79
5.5−7.3	4.4	1.24	0.64	2.41	0.176	5.685	25.012	110.053	31.015	69	83
7.3–27.2	15.25	2.63	1.33	5.20	0.837	1.195	18.224	277.909	47.928	125	81

Notes: CSF = Cancer Slope Factor; RCC = renal cell carcinoma.

The CSF is then calculated as the excess cancer risk associated with each ng/mL increase in serum PFOA (CSF_{serum}). The CSF_{serum} 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. 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 × 90% = 0.0182. The CSF_{serum} was then calculated as the product of the upper 95% CL of the dose-response slope (*b*) and R_0. The estimated CSF_{serum} is 0.00352 $(ng/mL)^{-1}$ (Table E-44). The estimated The CSF_{serum} based on the estimated slope *b* is 0.00178 $(ng/mL)^{-1}$

Table E-44. Internal CSF Calculations for Shearer et al. (2021)

Calculations	Shearer et al. (2021)
$\Sigma(w_jx_jOR_j)$	96.19
$\Sigma(w_jx_j)$	54.97
$\Sigma(w_jx_j^2)$	420.23
SE_b	0.0488
B	0.0981
CI_b LCL	0.0025
CI_b UCL	0.1937
R_O	0.01818
$CSF_{serum\text{- central}}$	0.00178
$CSF_{serum\text{- UCL}}$	**0.00352**

One potential limitation of the weighted linear regression for estimating the dose-response relationship between PFOA and relative risk of RCC, is that it ignores the covariance between the estimated OR for each exposure quartile compared with the lowest quartile, since they come from the same study and share a reference group. To evaluate the potential impact of the lack of

independence, EPA performed a sensitivity analysis using the generalized least squares for trend (glst) method (Greenland and Longnecker, 1992), which assumes a linear relationship between exposure and log-transformed ORs, and accounts for covariance between estimates. This approach is compared with the regression coefficients obtained using the variance-weighted least squares (vwls) approach, which does not adjust for covariance between estimates. These analyses were performed in STATA v17.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

While these estimates obtained under the assumption of a linear relationship between the exposure and the logarithm of the OR cannot be directly compared with the weighted linear regression with fixed intercept used for deriving the CSF, the findings suggest that the lack of independence in the study-specific ORs as minor impact on the CSF calculations (Figure E-3).

```
. glst logor dose if studyname=="Shearer, 2021", se(se) cov(n cases) cc

Generalized least-squares regression                Number of obs   =       3
Goodness-of-fit chi2(2)     =    0.84               Model chi2(1)   =    8.39
Prob > chi2                 =  0.6570               Prob > chi2     =  0.0038
------------------------------------------------------------------------------
       logor | Coefficient  Std. err.      z    P>|z|     [95% conf. interval]
-------------+----------------------------------------------------------------
        dose |   .0582322   .0201097     2.90   0.004     .0188178    .0976465
------------------------------------------------------------------------------

. glst logor dose if studyname=="Shearer, 2021", se(se) cov(n cases) cc vwls

Variance-weighted least-squares regression          Number of obs   =       3
Goodness-of-fit chi2(2)     =    0.44               Model chi2(1)   =    9.06
Prob > chi2                 =  0.8018               Prob > chi2     =  0.0026
------------------------------------------------------------------------------
       logor | Coefficient  Std. err.      z    P>|z|     [95% conf. interval]
-------------+----------------------------------------------------------------
        dose |    .064746   .0215109     3.01   0.003     .0225854    .1069066
------------------------------------------------------------------------------
```

Figure E-3. Regression Coefficients and 95% CIs Between the Log of the RCC ORs and Serum PFOA Concentrations Using Data From Shearer et al. (2021): Adjusted (glst) and Unadjusted (vwls) for OR Dependence

EPA considered evaluating the dose response using the Benchmark Dose Software (BMDS). However, categorical data from case-control studies cannot be used in the U.S. EPA BMDS since these models are based on cancer risk, and the data needed to calculate risks (i.e., the denominators) are not available.

E.1.5.1.2 Vieira et al. (2013)

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). The cancers of interest included kidney, pancreatic, testicular, and liver cancers. The researchers selected these because they had been linked to PFOA in previous animal and human

studies. The controls were all other cancer types. Initially, all incident cancer cases diagnosed from 1996 through 2005 were obtained from the Ohio Cancer Incidence Surveillance System (OCISS) and the West Virginia Cancer Registry (WVCR), respectively. However, only the OCISS provided the participants addresses, which could be used to develop individual estimates of PFOA exposure at the time of diagnosis and 10 years before diagnosis. Those living in one of the included counties, but outside of an exposed water district, were assigned to the "unexposed" reference category. For participants residing in one of the exposed water districts, PFOA exposure was categorized into groups of "low," "medium," and "high" based on the tertile cutoff points in these participants. Cumulative exposure was assessed by summing the yearly serum PFOA exposure estimates for the 10 years prior to cancer diagnosis. Analyses were adjusted for 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. The corresponding ORs were similar in the high and very high categories of cumulative exposure (2.0 and 2.1, respectively) but were slightly lower (1.8 and 1.7, respectively) in analyses without the 10-year lag. P-values for trends or analyses using continuous estimates of PFOA exposure were not provided.

Using the data from Table E-45 and the same approach used for modeling the data from Shearer et al. (2021), the model fit was better when the highest exposure level was excluded (Table E-44). With a lifetime kidney cancer of R_0 of 0.0202, the CSF_{serum} was then calculated as the product of the upper 95% CL of the dose-response slope (*b*) and R_0. When the highest exposure group was excluded, the estimated CSF_{serum} is 0.00048 $(ng/mL)^{-1}$ (and 0.00025 $(ng/mL)^{-1}$ when based on the slope *b*) (Table E-44). When the highest exposure group was included, the estimated CSF_{serum} is 0.00014 $(ng/mL)^{-1}$ (and 0.00007 $(ng/mL)^{-1}$ when based on the slope *b*) (Table E-46).

Table E-45. ORs for the Association Between PFOA Serum Concentrations and RCC in Vieira et al. (2013) and Data Used for CSF Calculations

PFOA Range (ng/mL)	x_i	OR_i	LCI_i	UCI_i	$Var(OR_i)$	w_i	w_ix_i	$w_ix_i^2$	$w_ix_iOR_i$	Cases	Controls
0	0 (reference)	1.0	-	-						187	5,957
3.7–12.8	8.25	0.8	0.4	1.5	0.073	13.743	113.382	935.400	90.705	11	446
12.9–30.7	21.8	1.2	0.7	2.0	0.103	9.682	211.074	4,601.413	253.289	17	455
30.8–109	69.9	2.0	1.3	3.2	0.211	4.734	330.937	23,132.508	661.874	22	339
110–655	382.5	2.0	1.0	3.9	0.482	2.074	793.309	303,440.633	1,586.618	9	142

Notes: CSF = Cancer Slope Factor; OR = odds ratio; RCC = renal cell carcinoma.

Table E-46. Internal CSF Calculations for Vieira et al. (2013)

Calculations	Vieira et al. (2013)[a]	Vieira et al. (2013)[b]
$\Sigma(w_jx_jOR_j)$	1,005.87	2,592.49
$\Sigma(w_jx_j)$	655.39	1,448.70

Calculations	Vieira et al. (2013)[a]	Vieira et al. (2013)[b]
$\Sigma(w_j x_j^2)$	28,669.32	332,109.95
SE_b	0.0059	0.0017
B	0.0122	0.0034
CI_b LCL	0.0006	0.0000
CI_b UCL	0.0238	0.0068
R_O	0.0202	0.0202
$CSF_{serum\text{- central}}$	0.00025	0.00007
$CSF_{serum\text{- UCL}}$	**0.000481**	**0.000138**

Notes: CSF = Cancer Slope Factor.
[a] Highest exposure level excluded.
[b] Highest exposure level included.

E.1.5.2 Sensitivity Analyses

E.1.5.2.1 Integrating Shearer et al. (2021) and Vieira et al. (2013)

The Shearer et al. (2021) and Vieira et al. (2013) have considerable differences with respect to several aspects including outcomes considered (RCC vs. any kidney cancer), exposure assessment (serum biomarker vs. modeled exposure), source population (multicenter nationally vs. Ohio and WV), study size (324 cases and 324 matched controls vs. 59 cases and 7,585 registry-based controls). Additionally, the dramatically different slopes resulting from 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), are an indication that the studies have considerable differences.

EPA performed a sensitivity analysis to derive a CSF_{serum} based on the pooled data from the two studies. EPA pooled the study-specific slopes estimated as previously described using a random effects REML approach. A pooled lifetime kidney cancer R_0 was calculated as a weighted average of the outcome specific R_0, weighted by the inverse of the sample size, applied to the upper 95% CL of the pooled dose-response slope. When the highest exposure group from was excluded (Vieira et al., 2013), the estimated CSF_{serum} is 0.00242 $(ng/mL)^{-1}$ (Table E-47). When the highest exposure group from Vieira et al. (2013) was included, the estimated CSF_{serum} is 0.00257 $(ng/mL)^{-1}$ (Table E-47).

Table E-47. CSF Calculations Pooling Dose Response for Shearer et al. (2021) and Vieira et al. (2013) Studies

Calculations	Shearer et al. (2021), Vieira et al. (2013)[a]	Shearer et al. (2021), Vieira et al. (2013)[b]
Pooled b	0.041	0.038
CI_b LCL	−0.038	−0.051
CI_b UCL	0.121	0.128
R_O	0.02004	0.02004

Calculations	Shearer et al. (2021), Vieira et al. (2013)[a]	Shearer et al. (2021), Vieira et al. (2013)[b]
$CSF_{serum\text{- }central}$	0.00082	0.00076
$CSF_{serum\text{- }UCL}$	**0.00242**	**0.00257**

Notes: CSF = Cancer Slope Factor.
[a] Highest exposure level excluded.
[b] Highest exposure level excluded.

Another approach for deriving a combined CSF is to take the geometric mean of the study-specific CSF_{serum}, resulting in a combined CSF_{serum} of 0.00130 $(ng/mL)^{-1}$ and of 0.00070 $(ng/mL)^{-1}$ when the highest exposure group from Vieira et al. (2013) was excluded or included, respectively and the upper limits of the dose-response slopes were used.

However, in this particular situation, given that the two studies have considerable differences listed above, EPA believes that these studies should not be combined in this manner.

E.2 Toxicology Studies

E.2.1 Butenhoff et al. (2012)

EPA conducted dose-response modeling of the Butenhoff et al. (2012) study using the BMDS 3.2 program. This study addresses Leydig cell adenomas in the testes in male Sprague-Dawley Crl:COBS@CD(SD)BR rats.

E.2.1.1 Leydig Cell Adenomas in the Testes

Increased incidence of Leydig cell adenomas in the testes was observed in male Sprague-Dawley Crl:COBS@CD(SD)BR rats. Dichotomous models were used to fit dose-response data. BMRs of 4% and 10% change in the response were chosen. A BMR of 10% is the recommended standard reporting level for comparison across chemicals per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). However, a BMR of 10% results in a BMDL higher than the lowest dose tested. The 4% change was ultimately selected as the BMR because it is the low end of the observed response within the study (Figure E-4). The doses and response data used for the modeling are listed in Table E-48. The AUC averaged over the study duration (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, was selected as the dose metric for cancer endpoints from this study (see Section 4.2.2 of the Toxicity Assessment (U.S. EPA, 2024b)).

Table E-48. Dose-Response Modeling Data for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA (Butenhoff et al., 2012)

Administered Dose (mg/kg/day)	Internal Dose (mg/L/day)	Number per Group	Incidence
0	0	50	0
1.3	43,263.7	50	2
14.2	167,102.5	50	7

BMD modeling results for Leydig cell adenomas in the testes are summarized in Table E-49 and Figure E-4. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the AIC. The lower bound on the dose level corresponding to the 95% lower confidence limit for a 4% change in the response ($BMDL_4$) from the selected Multistage Degree 1 model is 27,089.3 mg/L/day.

Table E-49. Summary of Benchmark Dose Modeling Results for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA (Butenhoff et al., 2012)

Model[a]	Goodness of Fit		Scaled Residual			BMD_4 (mg/L/day)	$BMDL_4$ (mg/L/day)	BMD_{10} (mg/L/day)	$BMDL_{10}$ (mg/L/day)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD_4	Dose Group Near BMD_{10}	Control Dose Group					
Multistage Degree 2	0.956	61.3	0.05	−0.03	$-8.8 \times e^{-4}$	44,791.1	27,088.1	115,604.6	69,904.0	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest AIC.
Multistage Degree 1	**0.956**	**61.3**	**0.05**	−0.03	$\mathbf{-8.9 \times e^{-4}}$	**44,791.1**	**27,089.3**	115,604.6	69,901.5	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_4 = dose level corresponding to a 4% change in the response; $BMDL_4$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 4% change in the response; BMD_{10} = dose level corresponding to a 10% change in the response; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change in the response.

[a] Selected model in bold.

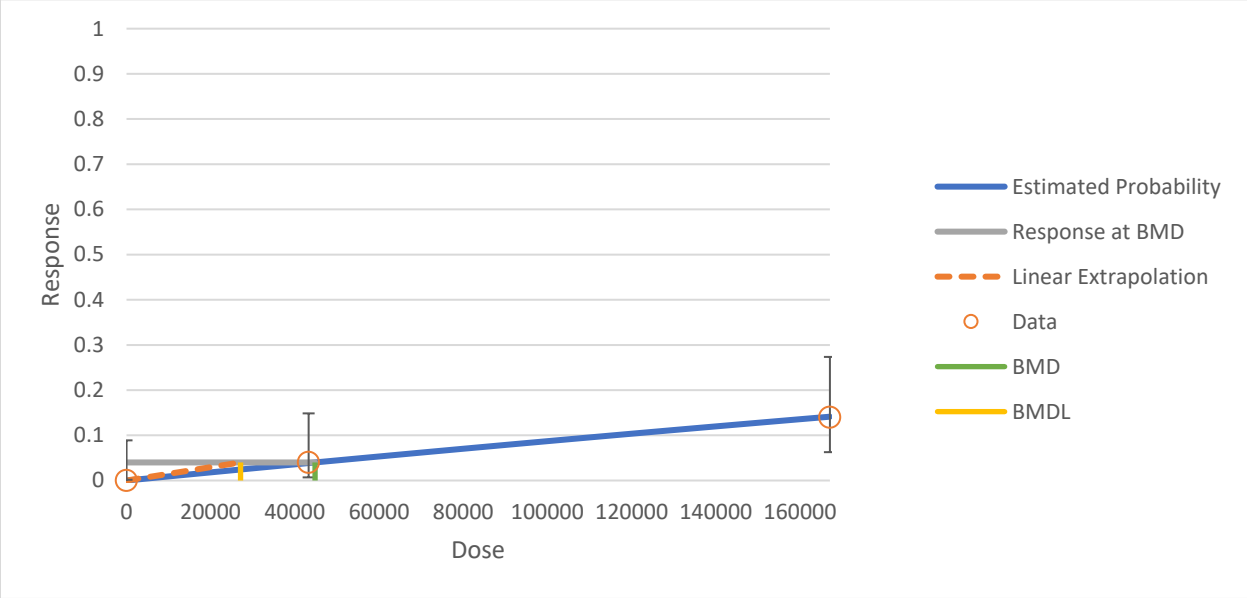


Figure E-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA with BMR 4% Extra Risk (Butenhoff et al., 2012)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.2 Dewitt et al. (2008)

EPA conducted dose-response modeling of the Dewitt et al. (2008) study using the BMDS 3.2 program. This study addresses serum sheep red blood cells (SRBC)-specific IgM antibody titers in female C57BL/6N mice (Study I) and SRBC-specific IgM antibody titers in female C57BL/6N mice (Study II).

E.2.2.1 Serum Sheep Red Blood Cells-Specific IgM Antibody Titers in Female C57BL/6N Mice (Study I)

Decreased mean response of SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Study I). Continuous models were used to fit dose-response data. A benchmark response (BMR) of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-50. As described in Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b), the average concentration over the final week of study $C_{last7,avg}$ was selected for all non-developmental studies 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.

Table E-50. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-Specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Dewitt et al., 2008)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (Log_2 to Reach 0.5 OD)[a]
0	0	8	8.0 ± 0.3[b]
3.75	73.0	8	7.1 ± 0.6
7.5	90.8	8	6.8 ± 0.3
15	103.7	8	6.1 ± 0.8
30	118.3	8	5.6 ± 0.8

Notes: OD = optical density.
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

BMD modeling results for serum SRBC-specific IgM antibody titers are summarized in Table E-51 and Figure E-5. The best fitting model was the Polynomial Degree 4 model based on adequate p-values (greater than 0.1), and the Polynomial Degree 4 model had the lowest AIC. The $BMDL_{1\ SD}$ from the selected Polynomial Degree 4 model is 18.2 mg/L.

Table E-51. Summary of Benchmark Dose Modeling Results for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Nonconstant Variance) (Dewitt et al., 2008)

Model[a]	Goodness of Fit		Scaled Residual		$BMD_{1\ SD}$ (mg/L)	$BMDL_{1\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Exponential 2	0.0183	77.4	−0.29	−0.29	15.5	10.9	EPA selected the Polynomial Degree 4 model. All models, except Exponential 2, Exponential 4, and Linear, had adequate fit (p-values greater than 0.1), and the Polynomial Degree 4 model had the lowest AIC.
Exponential 3	0.2736	72.0	−0.31	−0.02	47.5	28.8	
Exponential 4	0.0183	77.4	−0.30	−0.30	15.6	10.9	
Exponential 5	0.2736	72.0	−0.31	−0.02	47.4	28.8	
Hill	0.1148	73.9	−0.27	−0.03	45.8	27.7	
Polynomial Degree 4	**0.5269**	**69.6**	**−0.05**	**−0.05**	**31.9**	**18.2**	
Polynomial Degree 3	0.5127	69.7	−0.16	−0.05	38.4	20.2	
Polynomial Degree 2	0.4705	69.9	−0.13	−0.10	43.7	23.0	
Power	0.2888	71.9	−0.27	−0.03	45.7	27.0	
Linear	0.0323	76.2	−0.36	−0.36	17.2	12.1	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to 1 standard deviation 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 equal to 1 standard deviation from the control mean.
[a] Selected model in bold.

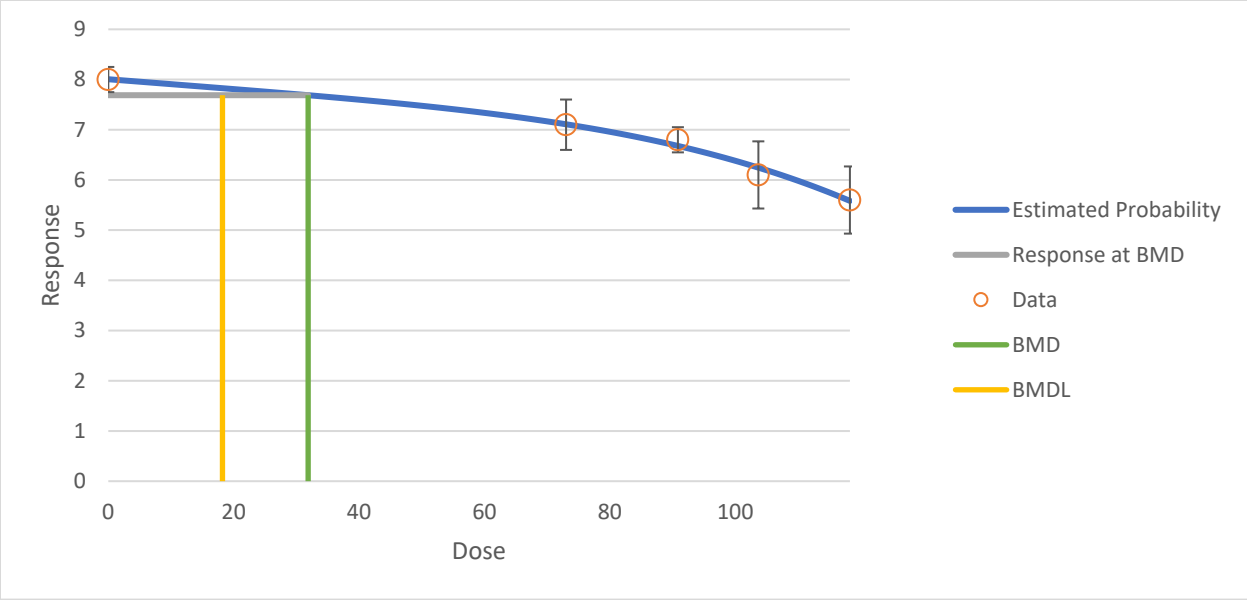


Figure E-5. Plot of Mean Response by Dose with Fitted Curve for the Polynomial Degree 4 Model for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (Dewitt et al., 2008)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.2.2 Serum Sheep Red Blood Cells-specific IgM antibody titers in Female C57BL/6N Mice (Study II)

Decreased mean response of serum SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Study II). Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-52. As described in Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b), the average concentration over the final week of study $C_{last7,avg}$ was selected for all non-developmental studies 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.

Table E-52. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA (Dewitt et al., 2008)

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (Log_2 to Reach 0.5 OD)[a]
0	0	8	7.9 ± 0.3[b]
0.94	24.2	8	8.0 ± 0.3
1.88	45.3	8	7.8 ± 0.3
3.75	73.1	8	7.4 ± 0.3
7.50	91.5	8	7.3 ± 0.3

Notes: OD = optical density.

[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.3 Lau et al. (2006)

EPA conducted dose-response modeling of the Lau et al. (2006) study using the BMDS 3.2 program. This study addresses time to eye opening, pup survival, and pup body weight in F_1 male and female CD-1 mice.

E.2.3.1 Time to Eye Opening

Decreased mean response of time to eye opening was observed in F_1 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 selected, and a BMR of a change in the mean equal to 1 standard deviations from the control mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-53. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For the time to eye opening endpoint, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed from GD1 to eye opening which occurs during the lactational period (PND 14–18).

Table E-53. Dose-Response Modeling Data for Time to Eye Opening in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (days)[a]
0	0	22	14.8 ± 0.5[b]
1	16.0	8	15.2 ± 0.6
3	27.5	8	15.5 ± 0.3
5	30.9	17	16.0 ± 0.8
10	35.1	13	17.2 ± 1.1
20	40.7	3	17.9 ± 1.4

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

For $C_{avg,pup,gest,lact}$, the benchmark dose (BMD) modeling results for time to eye opening are summarized in Table E-54 and Figure E-6. The best fitting model was the Polynomial Degree 5

model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 5 model had the lowest AIC. Therefore $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 5 model is 8.0 mg/L.

Table E-54. Summary of Benchmark Dose Modeling Results for Time to Eye Opening Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Nonconstant Variance) (Lau et al., 2006)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	$BMD_{1\ SD}$ (mg/L)	$BMDL_{1\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.5\ SD}$	Dose Group Near $BMD_{1\ SD}$	Control Dose Group					
Exponential 2	0.0002	170.5	0.5	−1.3	0.5	5.0	3.9	9.9	7.7	EPA selected the Polynomial Degree 5 model. The Polynomial 5, 4, 3, Power, Exponential 3, and Hill models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 5 model had the lowest AIC.
Exponential 3	0.105	156.3	1.3	−0.6	−0.5	20.4	18.1	24.4	23.6	
Exponential 4	<0.0001	173.4	0.5	−1.3	0.5	4.8	3.7	9.6	7.5	
Exponential 5	0.048	158.2	1.4	−0.5	−0.6	20.9	20.1	24.8	20.1	
Hill	0.122	156.3	0.7	0.7	−1.1	26.0	20.9	28.0	27.5	
Polynomial Degree 5	**0.291**	**153.1**	**0.8**	−0.7	**−0.2**	**16.3**	**8.0**	22.9	15.5	
Polynomial Degree 4	0.131	155.8	1.0	−0.8	−0.2	17.7	9.4	22.8	16.3	
Polynomial Degree 3	0.105	155.8	1.1	−1.1	−0.3	17.3	10.1	21.8	16.2	
Polynomial Degree 2	0.0199	159.8	0.2	0.2	0.3	12.3	8.9	17.4	14.2	
Power	0.109	156.2	1.4	−0.5	−0.6	20.9	18.7	24.8	19.9	
Linear	0.0001	171.3	0.5	−1.4	0.5	4.8	3.7	9.6	7.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation(s) from the control mean; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation(s) from the control mean; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to 1 standard deviation 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 equal to 1 standard deviation from the control mean.

[a] Selected model in bold.

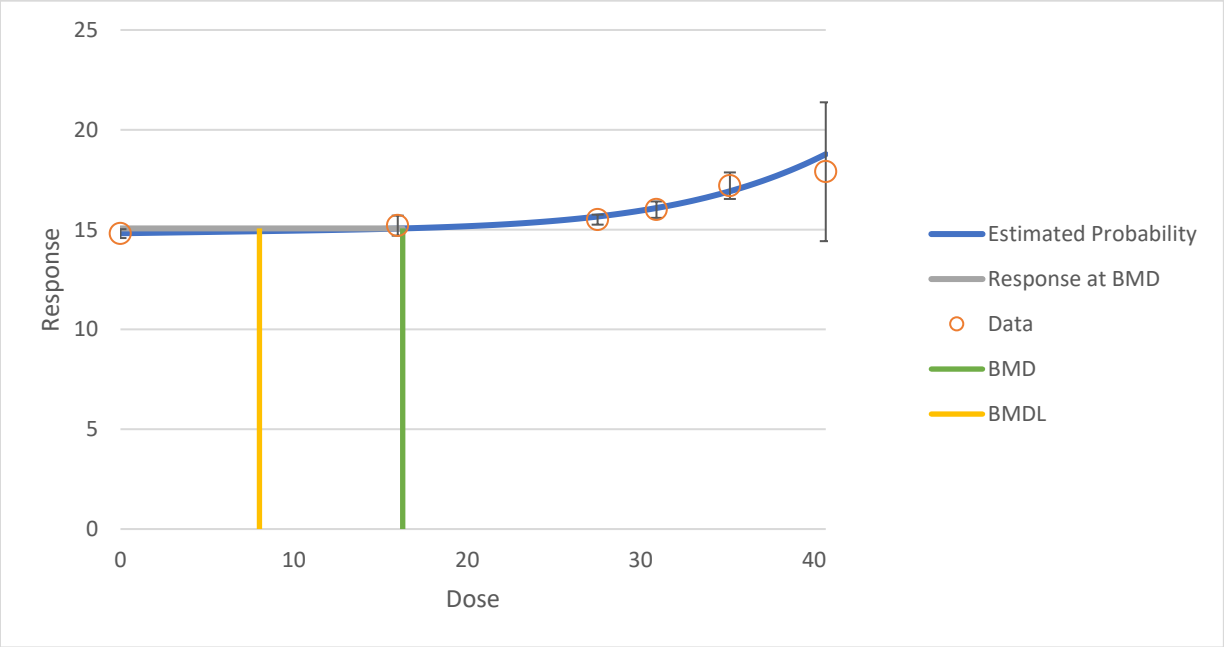


Figure E-6. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 5 Model for Time to Eye Opening in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.3.2 Pup Survival at PND 0

Decreased mean response of number of surviving offspring was observed in F_1 male and female CD-1 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 chosen. A BMR of 0.1 standard deviations was also shown for comparison purposes because this is a frank effect that could justify a lower BMR (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-55. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For decreased pup survival at PND 0, the $C_{avg,pup,gest}$ metric was selected because pups were exposed solely during gestation**.**

Table E-55. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest}$ (mg/L)	Number per Group	Mean Response (Percent Survival per Litter)[a]
0	0	24	92 ± 8.8[b]
1	8.8	8	95 ± 6.2
3	19.1	8	90 ± 22.3

5	23.2	30	80 ± 29.6
10	28.3	26	66 ± 31.1
20	35.1	8	70 ± 14.4

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.3.3 Pup Survival at PND 23

Decreased mean response of number of surviving offspring was observed in F_1 male and female CD-1 mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.1 and 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table E-56. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For decreased pup survival at PND 23, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation.

Table E-56. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (Percent Survival per Litter)[a]
0	0	24	86 ± 22.5[b]
1	15.8	8	94 ± 6.8
3	26.6	8	83 ± 18.4
5	29.6	30	68 ± 40.5
10	33.4	26	23 ± 28.0
20	38.3	8	14 ± 24.0

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

Tests for constant and nonconstant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for nonconstant models assumes that the model errors (or residuals) have nonconstant variance; if this assumption is violated the p-

values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and nonconstant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.3.4 Pup Body Weight at PND 23

Decreased mean response of pup body weight at weaning at PND 23 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 selected and a BMR of a 0.5 standard deviation change from the mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-57. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For decreased pup body weight at PND 23, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation.

Table E-57. Dose-Response Modeling Data for Pup Body Weight in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Lau et al., 2006)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (g)[a]
0	0	22	14.8 ± 9.7[b]
1	15.8	8	14.4 ± 5.0
3	26.6	8	12.2 ± 4.2
5	29.6	16	11.4 ± 8.0
10	33.4	13	10.7 ± 7.5
20	38.3	3	11.7 ± 0.6

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

The BMD modeling results for pup body weight at PND 23 are summarized in Table E-58. No models for $C_{avg,pup,gest,lact}$ provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table E-58. Summary of Benchmark Dose Modeling Results for Pup Weight Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Nonconstant Variance) (Lau et al., 2006)

Model[a]	Goodness of Fit		Scaled Residual			BMD_5 (mg/L)	$BMDL_5$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD_5	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.002	485.1	–0.1	0.3	–0.1	5.8	3.0	39.7	17.3	No models had adequate fit for constant and non-constant variance. Test for constant variance failed. For non-constant variance models, goodness of fit for all non-constant models was poor, with BMDs higher than the maximum tested dose, with BMDs or BMDLs being more than three times lower than the lowest tested dose, or with BMDLs not able to be estimated.
Exponential 3	0.002	485.1	–0.1	0.3	–0.1	5.8	3.1	39.9	17.3	
Exponential 4	0.0009	486.8	–0.1	–9,999	–0.1	1.8	–[a]	–9,999	–[a]	
Exponential 5	0.0008	487.1	–0.1	0.3	–0.1	5.8	–[a]	39.9	2.1	
Hill	0.0009	486.8	–0.1	–0.1	–0.1	1.3	–[a]	–28.3	–[a]	
Polynomial Degree 5	0.002	485.1	–0.1	0.4	–0.1	6.7	3.9	38.1	19.4	
Polynomial Degree 4	0.002	485.1	–0.1	0.3	–0.1	6.5	3.9	38.9	19.4	
Polynomial Degree 3	0.002	485.1	–0.1	0.3	–0.1	6.5	3.9	38.9	19.4	
Polynomial Degree 2	0.002	485.1	–0.1	0.3	–0.1	6.5	3.9	38.9	19.4	
Power	0.002	485.1	–0.1	0.3	–0.1	6.5	3.9	38.9	19.4	
Linear	0.002	485.1	–0.1	0.3	–0.1	6.5	3.9	38.9	19.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMD_5 = dose level corresponding to a 5% change; $BMDL_5$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change.

[a] Lower limit includes zero; BMDL not estimated.

E.2.4 Li et al. (2018a)

EPA conducted dose-response modeling of the Li et al. (2018a) study using the BMDS 3.2 program. This study addresses fetal body weight in F_1 male and female Kunming mice and maternal body weight in P_0 female Kunming mice.

E.2.4.1 Fetal Body Weight

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 a 5% change from the control mean was selected and a BMR of a 0.5 standard deviation change from the mean is provided for comparison purposes. The doses and response data used for the modeling are listed in Table E-59. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For decreased fetal body weight, the $C_{avg,pup,gest}$ metric was selected because pups were exposed solely during gestation.

Table E-59. Dose-Response Modeling Data for Fetal Body Weight in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Li et al., 2018a)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest}$ (mg/L)	Number per Group	Mean Response (g)[a]
0	0	10	1.5 ± 0.01
1	8.5	10	1.5 ± 0.01
5	22.9	10	1.3 ± 0.01
10	28.1	10	1.0 ± 0.10
20	34.9	10	0.9 ± 0.05

Notes:
[a] Data are presented as mean ± standard deviation.

Tests for constant and non-constant variance failed. In such cases, it is not recommended to model the dataset. Significance testing for constant variance models assumes that the model errors (or residuals) have constant variance; if this assumption is violated the p-values from the model are no longer reliable. Similarly, significance testing for non-constant models assumes that the model errors (or residuals) have non-constant variance; if this assumption is violated the p-values from the model are no longer reliable (Breusch and Pagan, 1979). For modeling endpoints where tests for constant and non-constant variance failed, it is thus not recommended to model the dataset, therefore, a NOAEL approach was taken for such endpoints.

E.2.5 Loveless et al. (2008)

EPA conducted dose-response modeling of the Loveless et al. (2008) study using the BMDS 3.2 program. This study addresses focal necrosis in male Crl:CD(SD)IGS BR rats and focal necrosis, individual cell necrosis, and IgM serum titer in male Crl:CD-1(ICR)BR mice.

E.2.5.1 Focal Necrosis in Male Crl:CD-1(ICR)BR Mice

Increased incidence of focal necrosis was observed in male Crl:CD-1(ICR)BR 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, 2012). The doses and response data used for the modeling are listed in Table E-60. As described in Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b), the average concentration over the final week of study $C_{last7,avg}$, was selected for all non-developmental studies 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.

Table E-60. Dose-Response Modeling Data for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Incidence
0	0	19	0
0.3	27.7	20	1
1	70.5	20	3
10	119.2	20	4
30	158.9	19	7

The BMD modeling results for focal necrosis are summarized in Table E-61 and Figure E-7. The best fitting model was the Dichotomous Hill model based on adequate p-values (greater than 0.1), and the Dichotomous Hill model had the lowest BMDL. The $BMDL_{10}$ from the selected Dichotomous Hill model is 10.0 mg/L.

Table E-61. Summary of Benchmark Dose Modeling Results for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	**0.809**	**76.3**	**0.10**	**−0.001**	**52.3**	**10.0**	EPA selected the Dichotomous Hill model as it had the lowest BMDL. All models had adequate fit (p-values greater than 0.1).
Gamma	0.824	76.3	0.12	−0.018	52.6	30.5	
Log-Logistic	0.936	74.3	0.10	−0.001	52.3	27.0	
Multistage Degree 4	0.972	74.1	0.28	−0.001	55.0	30.9	
Multistage Degree 3	0.870	76.2	0.26	−0.001	55.0	30.8	
Multistage Degree 2	0.847	76.2	0.20	−0.001	54.2	30.7	
Multistage Degree 1	0.906	74.4	−0.24	−0.001	45.0	30.2	
Weibull	0.829	76.3	0.14	−0.001	53.0	30.6	
Logistic	0.760	75.5	0.76	−0.735	83.8	65.2	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Log-Probit	0.781	76.4	0.02	−0.001	50.4	12.9	
Probit	0.798	75.3	0.69	−0.656	78.7	60.8	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

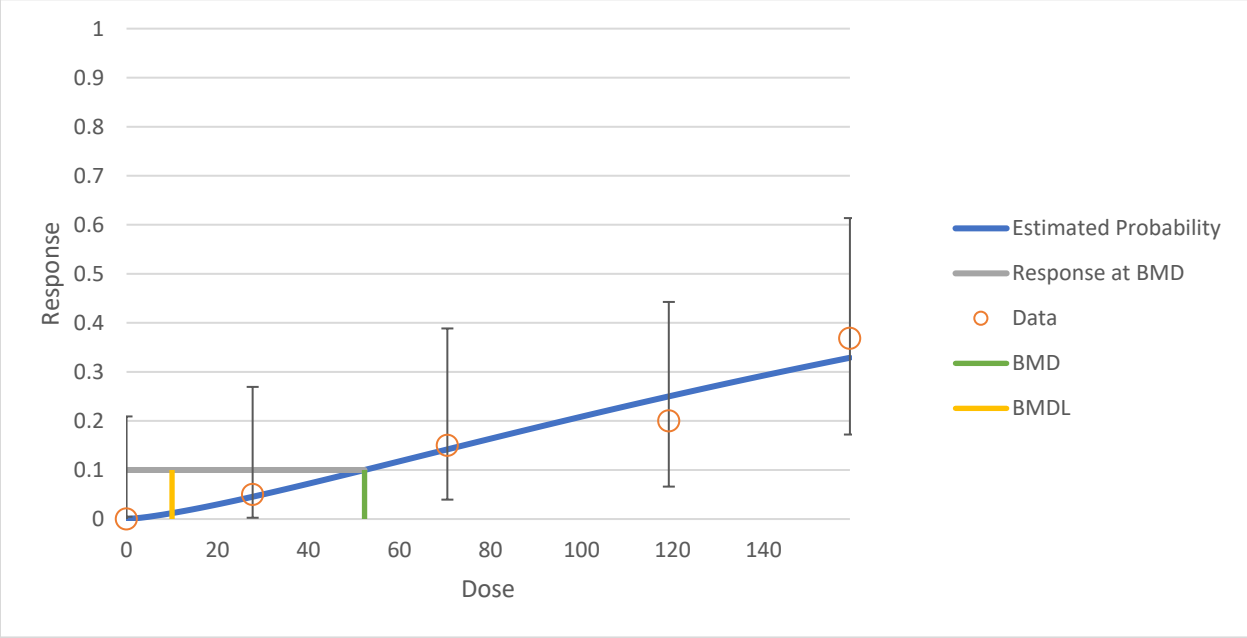


Figure E-7. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Dichotomous Hill Model for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.5.2 Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice

Increased incidence of individual cell necrosis was observed in male Crl:CD-1(ICR)BR 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, 2012). The doses and response data used for the modeling are listed in Table E-62. As described in Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b), the average concentration over the final week of study $C_{last7,avg}$, was selected for all non-developmental studies 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.

Table E-62. Dose-Response Modeling Data for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Incidence
0	0	19	0
0.3	27.7	20	0
1	70.5	20	11
10	119.2	20	20
30	158.9	19	19

The BMD modeling results for individual cell necrosis are summarized in Table E-63 and Figure E-8. The best fitting model was the Probit model based on adequate p-values (greater than 0.1), the benchmark dose lower limits (BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the Probit model had the lowest Akaike information criterion (AIC). The $BMDL_{10}$ from the selected Probit model is 36.0 mg/L.

Table E-63. Summary of Benchmark Dose Modeling Results for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	1.000	29.5	−0.001	−0.001	61.7	42.2	EPA selected the Probit model. All models, except Multistage Degree 1, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Probit model had the lowest AIC.
Gamma	0.990	31.7	−0.085	−0.001	49.4	36.7	
Log-Logistic	1.000	31.5	−0.001	−0.001	61.7	42.2	
Multistage Degree 4	0.981	30.3	−0.616	−0.001	42.7	31.5	
Multistage Degree 3	0.840	32.3	−1.020	−0.001	35.4	26.9	
Multistage Degree 2	0.283	38.8	−1.767	−0.001	23.6	18.2	
Multistage Degree 1	0.001	60.1	−0.001	−0.001	7.0	5.4	
Weibull	1.000	29.6	0.041	−0.001	50.3	50.1	
Logistic	1.000	29.5	<0.0001	−0.001	61.2	39.1	
Log-Probit	1.000	31.5	<0.0001	−0.001	61.6	39.1	
Probit	**1.000**	**29.5**	**<0.0001**	**<0.0001**	**58.1**	**36.0**	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

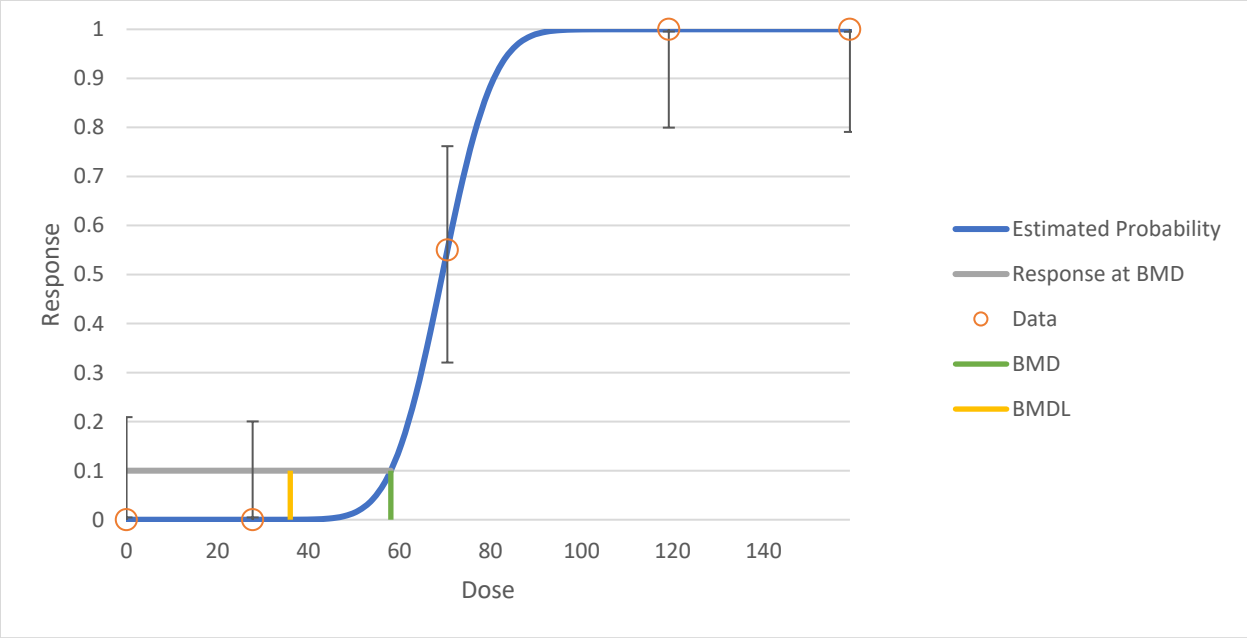


Figure E-8. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.5.3 IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice

Decreased mean response of IgM serum titer was observed in male Crl:CD-1(ICR)BR mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-64. As described in Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b), the average concentration over the final week of study $C_{last7,avg}$, was selected for all non-developmental studies 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.

Table E-64. Dose-Response Modeling Data for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Mean Response (mg/dL)[a]
0	0	20	8.9 ± 0.6
0.3	27.7	20	8.9 ± 0.8
1	70.5	20	8.4 ± 0.7
10	119.2	20	7.2 ± 0.8
30	158.9	20	6.4 ± 0.8

Notes:
[a] Data are presented as mean ± standard deviation.

The BMD modeling results for IgM serum titer are summarized in Table E-65 and Figure E-9. The best fitting model was the Exponential 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Exponential 3 model had the lowest AIC. The $BMDL_{1\ SD}$ from the selected Exponential 3 model is 57.6 mg/L.

Table E-65. Summary of Benchmark Dose Modeling Results for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Constant Variance) (Loveless et al., 2008)

Model[a]	Goodness of Fit		Scaled Residual		$BMD_{1\ SD}$ (mg/L)	$BMDL_{1\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Exponential 2	0.004	239.1	1.0	−1.9	42.4	35.4	EPA selected the Exponential 3 model. All models, except Exponential 2, Exponential 4 and Linear, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Exponential 3 model had the lowest AIC.
Exponential 3	**0.527**	**228.9**	**0.5**	**−0.4**	**75.0**	**57.6**	
Exponential 4	0.004	239.1	1.0	−1.9	42.4	35.4	
Exponential 5	0.261	230.9	0.5	−0.4	75.1	57.6	
Hill	0.901	229.6	0.0	−0.1	80.3	62.2	
Polynomial Degree 4	0.334	229.8	0.5	−0.5	75.3	54.4	
Polynomial Degree 3	0.334	229.8	0.5	−0.5	75.3	54.4	
Polynomial Degree 2	0.334	229.8	0.5	−0.5	75.3	54.4	
Power	0.422	229.3	0.6	−0.5	74.0	55.9	
Linear	0.018	235.7	0.9	−1.8	45.8	38.9	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{1\ SD}$ = dose level corresponding to a change in the mean equal to 1 standard deviation 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 equal to 1 standard deviation from the control mean.

[a] Selected model in bold.

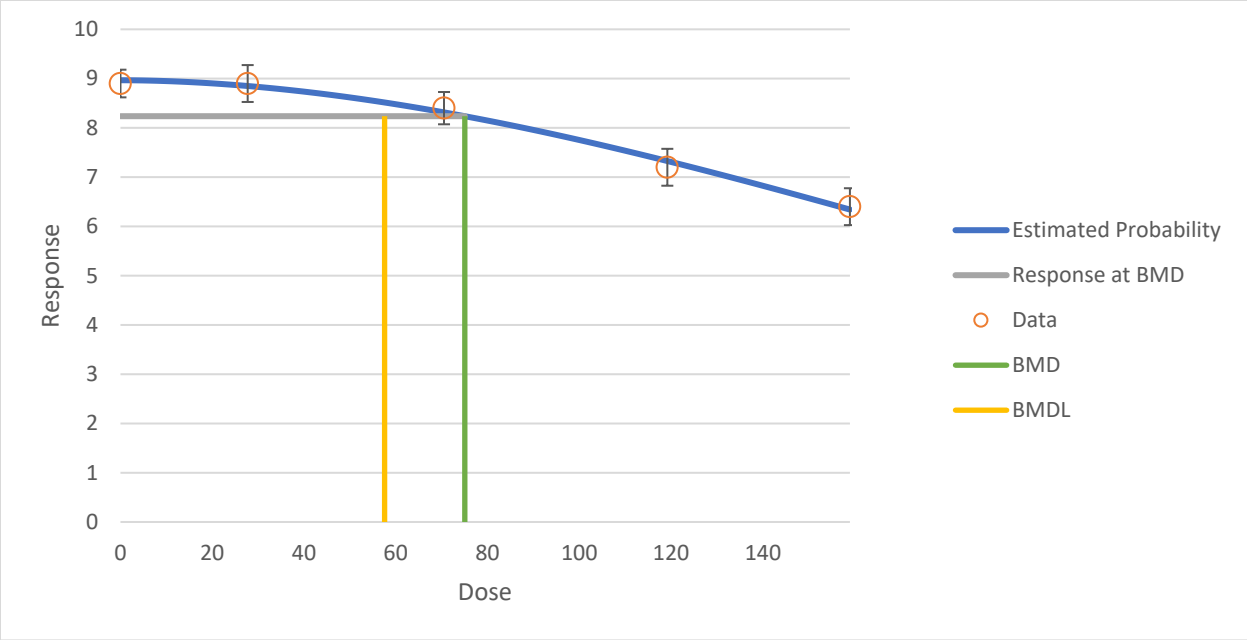


Figure E-9. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 3 Model for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (Loveless et al., 2008)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6 NTP (2020)

EPA conducted dose-response modeling of the NTP (2020) study using the BMDS 3.2 program. This study addresses hepatocyte single cell death, necrosis in the liver, relative kidney weight (right), hepatocellular adenomas, hepatocellular adenoma or carcinoma, and pancreatic acinar cell adenoma in F_1 male Sprague-Dawley rats and uterine adenocarcinoma in F_1 female Sprague-Dawley rats.

E.2.6.1 Hepatocyte Single Cell Death

Increased incidence of hepatocyte single cell death was observed in F_1 male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A benchmark response (BMR) of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). The doses and response data used for the modeling are listed in Table E-66. For NTP (2020), an additional dose metric was derived which averages 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 (preweaning) to the area under the curve from diet (postweaning) and then divides by 2 years.

Table E-66. Dose-Response Modeling Data for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	1

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
300 / 0	0.4	50	1
0 / 20	72.6	50	1
300 / 20	73.6	50	3
0 / 40	113.5	50	11
300 / 40	115.2	50	5
0 / 80	161.7	50	24
300 / 80	161.8	50	29

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Hepatocyte single cell death was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for POD_{HED} derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The benchmark dose (BMD) modeling results for hepatocyte single cell death following perinatal and postweaning exposure to PFOA are summarized in Table E-67 and Figure E-10. The best fitting model was the Gamma model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Gamma model had the lowest AIC. The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level $BMDL_{10}$ from the selected Gamma model is 100.1 mg/L.

Table E-67. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	–[b]	142.1	−0.07	−0.68	121.2	101.4	EPA selected the Gamma model. The Gamma, Log-Logistic, Weibull, and Log-Probit had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold
Gamma	**0.427**	**138.7**	**−0.69**	**−0.57**	**114.8**	**100.1**	
Log-Logistic	0.320	140.1	−0.07	−0.68	121.1	101.4	
Multistage Degree 3	0.043	144.1	−0.31	0.40	88.7	77.5	
Multistage Degree 2	0.002	151.1	−1.20	0.52	72.5	61.5	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 1	< 0.0001	163.2	−2.14	0.43	43.0	32.7	difference), and the Gamma model had the lowest AIC.
Weibull	0.330	140.0	−0.12	−0.64	121.2	98.3	
Logistic	0.044	141.9	−1.46	1.86	97.1	82.4	
Log-Probit	0.308	140.1	–0.01	−0.72	121.1	105.2	
Probit	0.004	145.0	–0.04	2.53	89.4	74.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.
[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

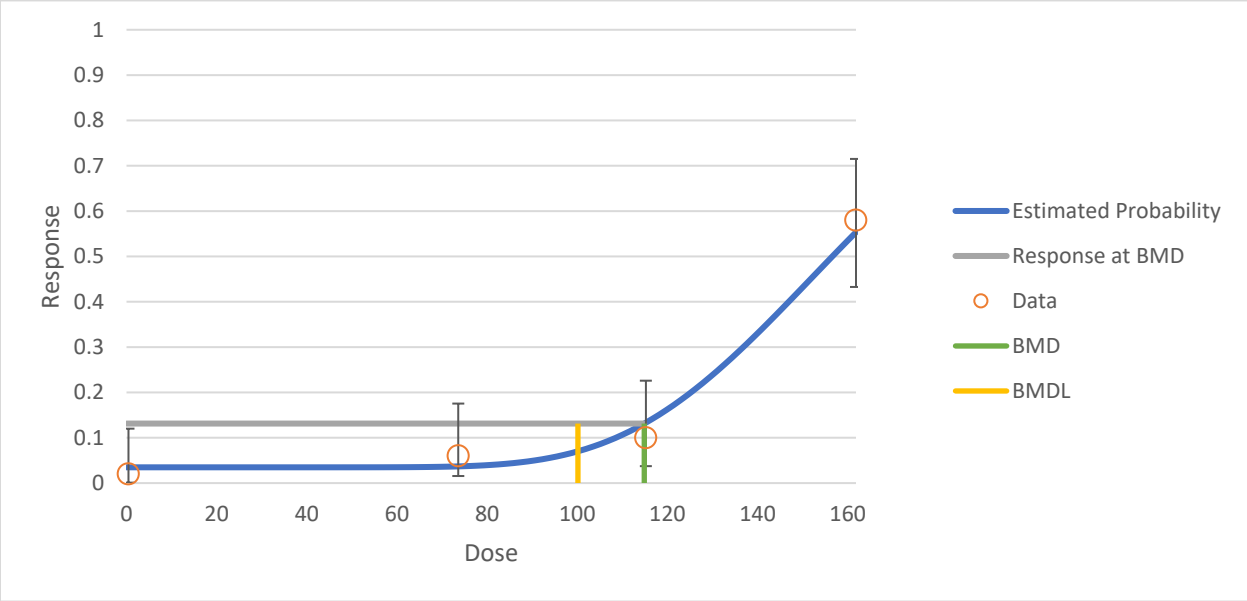


Figure E-10. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Gamma Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.1.1 Sensitivity Analyses

The BMD modeling results for hepatocyte single cell death following postweaning exposure to PFOA are summarized in Table E-68 and Figure E-11. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 3 model had the lowest AIC. The lower bound on the dose level corresponding to the 95% lower

confidence limit for a 10% response level $BMDL_{10}$ from the selected Multistage Degree 3 model is 77.1 mg/L.

Table E-68. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	–[b]	149.5	$9.4 \times e^{-4}$	0.03	104.5	85.9	EPA selected the Multistage Degree 3 model. All models, except Dichotomous Hill, Multistage Degree 2, Multistage Degree 1, and Probit, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Gamma	0.308	148.6	0.64	0.29	98.8	82.2	
Log-Logistic	0.262	148.9	0.67	0.32	98.5	81.5	
Multistage Degree 3	**0.354**	**148.2**	**−1.31**	**0.46**	**89.9**	**77.1**	
Multistage Degree 2	0.064	152.8	−2.00	0.52	73.8	61.9	
Multistage Degree 1	0.001	162.8	−2.81	0.42	44.6	33.8	
Weibull	0.200	149.3	0.80	0.32	98.4	80.1	
Logistic	0.222	148.7	−1.24	1.02	92.3	77.8	
Log-Probit	0.389	148.3	0.52	0.27	98.5	82.8	
Probit	0.090	149.7	−1.37	1.67	86.8	72.1	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

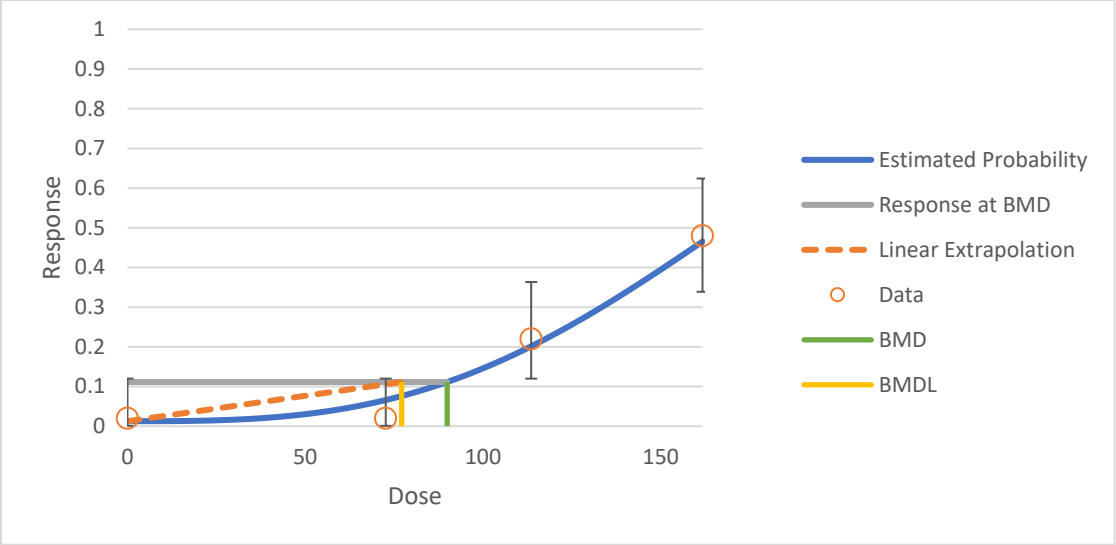


Figure E-11. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The benchmark dose (BMD) modeling results for hepatocyte single cell death using a pooled method are summarized in Table E-69 and Figure E-12. The best fitting model was the Multistage Degree 4 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 4 model had the lowest AIC. The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level $BMDL_{10}$ from the selected Multistage Degree 4 model is 90.9 mg/L.

Table E-69. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	0.273	287.8	1.16	−0.07	105.3	92.5	EPA selected the Multistage Degree 4 model. All models, except Multistage Degree 1 and 2, and Probit, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the
Gamma	0.380	286.0	1.07	−0.14	105.2	92.1	
Log-Logistic	0.399	285.8	1.16	−0.07	105.3	92.5	
Multistage Degree 7	0.170	289.7	1.25	0.02	104.9	91.1	
Multistage Degree 6	0.170	289.7	1.25	0.02	104.9	91.2	
Multistage Degree 5	0.285	287.7	1.25	0.01	104.9	91.5	
Multistage Degree 4	**0.536**	**284.1**	**0.90**	**0.17**	**100.5**	**90.9**	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.209	288.3	−0.27	0.43	89.3	82.6	Multistage Degree 4 model had the lowest AIC.
Multistage Degree 2	0.005	299.9	−1.17	0.52	73.1	66.1	
Multistage Degree 1	< 0.0001	322.0	−2.84	0.47	43.8	35.9	
Weibull	0.413	285.7	1.23	0.01	104.9	91.8	
Logistic	0.160	287.0	0.78	1.40	94.6	84.3	
Log-Probit	0.350	286.1	1.07	−0.23	106.0	92.4	
Probit	0.015	290.9	−0.18	2.08	88.0	77.6	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

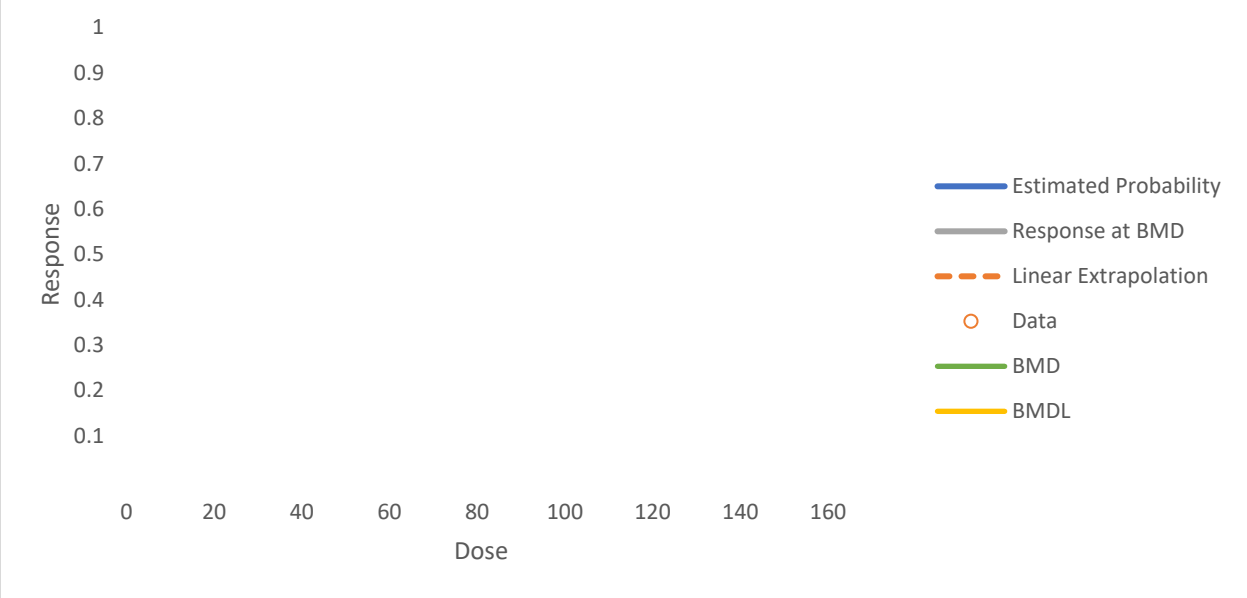


Figure E-12. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocyte Single Cell Death in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.2 Necrosis in the Liver

Increased incidence of necrosis was observed in F_1 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, 2012). The doses and response data used for the modeling are listed in Table E-70. For NTP (2020), an additional dose metric was derived which averages 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.

Table E-70. Dose-Response Modeling Data for Necrosis in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	2
300 / 0	0.4	50	1
0 / 20	72.6	50	17
300 / 20	73.6	50	11
0 / 40	113.5	50	23
300 / 40	115.2	50	14
0 / 80	161.7	50	20
300 / 80	161.8	50	21

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Necrosis in the liver was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for POD_{HED} derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The BMD modeling results for necrosis in the liver following perinatal and postweaning exposure to PFOA are summarized in Table E-71 and Figure E-13. The Dichotomous Hill model was saturated and while the Log-Probit model had adequate fit, the BMD/BMDL ratio was larger than three. Of the remaining models, the selected model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1) and lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 1 model is 26.9 mg/L.

Table E-71. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	–[b]	198.1	0.225	−0.009	40.7	10.0	EPA selected the Multistage Degree 1 model. All models, except Dichotomous Hill, had adequate fit (p-
Gamma	0.611	196.1	0.212	−0.007	38.6	27.0	
Log-Logistic	0.585	196.1	0.225	−0.008	40.7	22.4	
Multistage Degree 3	0.645	196.0	0.267	−0.018	37.8	27.0	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 2	0.627	196.1	0.246	−0.014	38.1	27.0	values greater than 0.1). The Log-Probit model had a BMD/BMDL ratio greater than three. The Multistage Degree 1 model had the lowest AIC of the remaining models.
Multistage Degree 1	**0.869**	**194.1**	**0.013**	**0.013**	**34.8**	**26.9**	
Weibull	0.614	196.1	0.220	−0.007	38.6	27.0	
Logistic	0.267	196.7	1.149	−1.063	68.0	57.3	
Log-Probit	0.567	196.1	0.222	−0.007	43.0	3.7	
Probit	0.348	196.0	1.095	−0.863	63.8	53.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.
[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

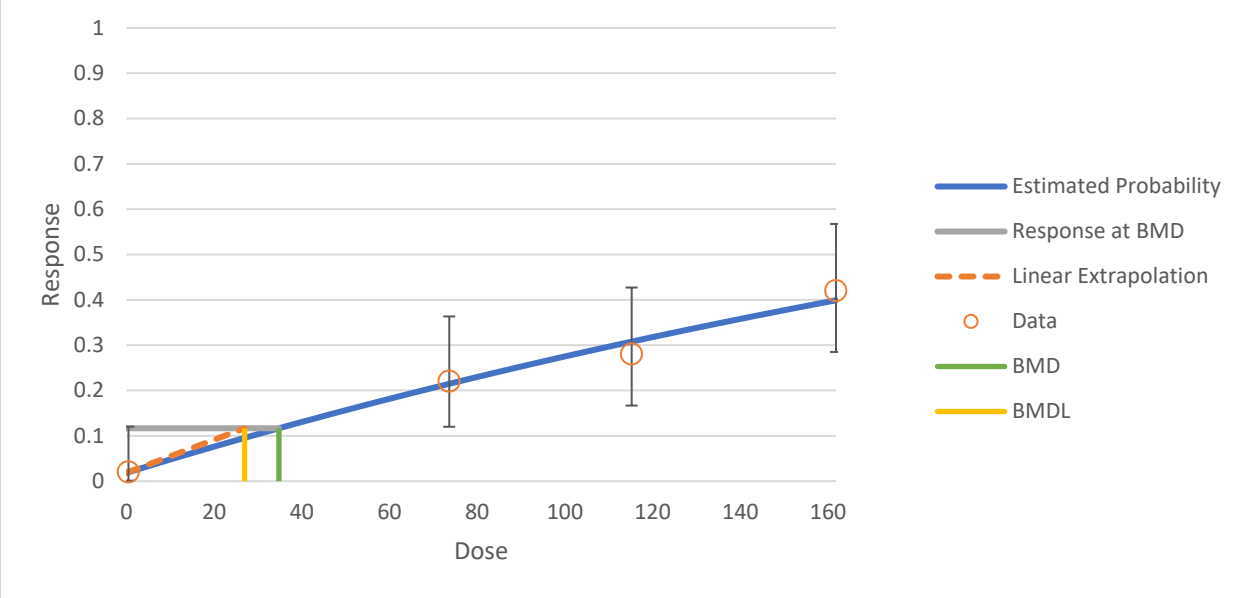


Figure E-13. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.2.1 Sensitivity Analyses

The BMD modeling results for necrosis in the liver following postweaning exposure to PFOA are summarized in Table E-72 and Figure E-14. The best fitting model was the Log-Logistic model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Log-Logistic model had the lowest AIC. The $BMDL_{10}$ from the selected Log-Logistic model is 15.3 mg/L.

Table E-72. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	–[b]	225.6	$-6.5 \times e^{-4}$	$-1.8 \times e^{-4}$	62.5	–[c]	EPA selected the Log-Logistic model. All models, except the Dichotomous Hill, Logistic and Probit, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Log-Logistic model had the lowest AIC.
Gamma	0.160	224.8	−0.2	−0.2	26.4	20.7	
Log-Logistic	**0.307**	**223.6**	**−0.1**	**−0.1**	**20.9**	**15.3**	
Multistage Degree 3	0.160	224.8	−0.2	−0.2	26.4	20.7	
Multistage Degree 2	0.160	224.8	−0.2	−0.2	26.4	20.7	
Multistage Degree 1	0.160	224.8	−0.2	−0.2	26.4	20.7	
Weibull	0.160	224.8	−0.2	−0.2	26.4	20.7	
Logistic	0.008	231.4	1.4	–1.7	52.4	43.9	
Log-Probit	0.307	224.2	$9.2 \times e^{-4}$	$9.2 \times e^{-4}$	1.7	–[c]	
Probit	0.011	230.6	1.4	–1.5	49.3	41.6	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

[c] Lower limit includes zero; BMDL not estimated.

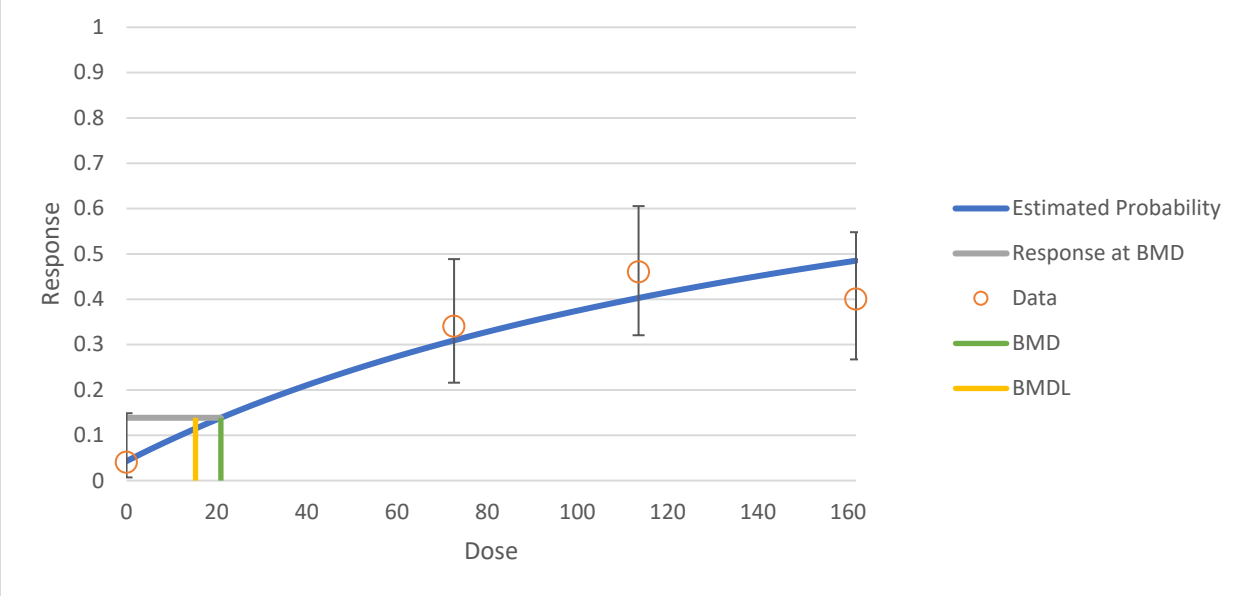


Figure E-14. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for necrosis using pooled methods are summarized in Table E-73 and Figure E-15. All models except the Logistic and Probit model had adequate fit with p-values (greater than 0.1). While the Dichotomous Hill and Log-Probit model had adequate fit, the BMD/BMDL ratio was larger than three. Of the remaining models, the selected model was the Log-Logistic model based on adequate p-values (greater than 0.1) and lowest AIC. The $BMDL_{10}$ from the selected Log-Logistic model is 20.1 mg/L.

Table E-73. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Dichotomous Hill	0.213	420.9	−0.4	0.4	35.5	4.9	EPA selected the Log-Logistic model. All models, except Logistic and Probit, had adequate fit (p-values greater than 0.1). The Log-Logistic model was selected based on the lowest AIC value for models with sufficiently close BMD and BMDL values (less than threefold difference).
Gamma	0.284	418.3	−0.5	0.3	30.1	25.2	
Log-Logistic	0.377	417.4	−0.5	0.4	25.1	20.1	
Multistage Degree 7	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 6	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 5	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 4	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 3	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 2	0.284	418.3	−0.5	0.3	30.1	25.2	
Multistage Degree 1	0.284	418.3	−0.5	0.3	30.1	25.2	
Weibull	0.284	418.3	−0.5	0.3	30.1	25.2	
Logistic	0.011	428.1	2.3	−1.1	59.1	52.3	
Log-Probit	**0.308**	**419.0**	**−0.4**	**0.4**	**19.2**	**3.4**	
Probit	0.017	426.6	2.3	−0.9	55.5	49.2	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

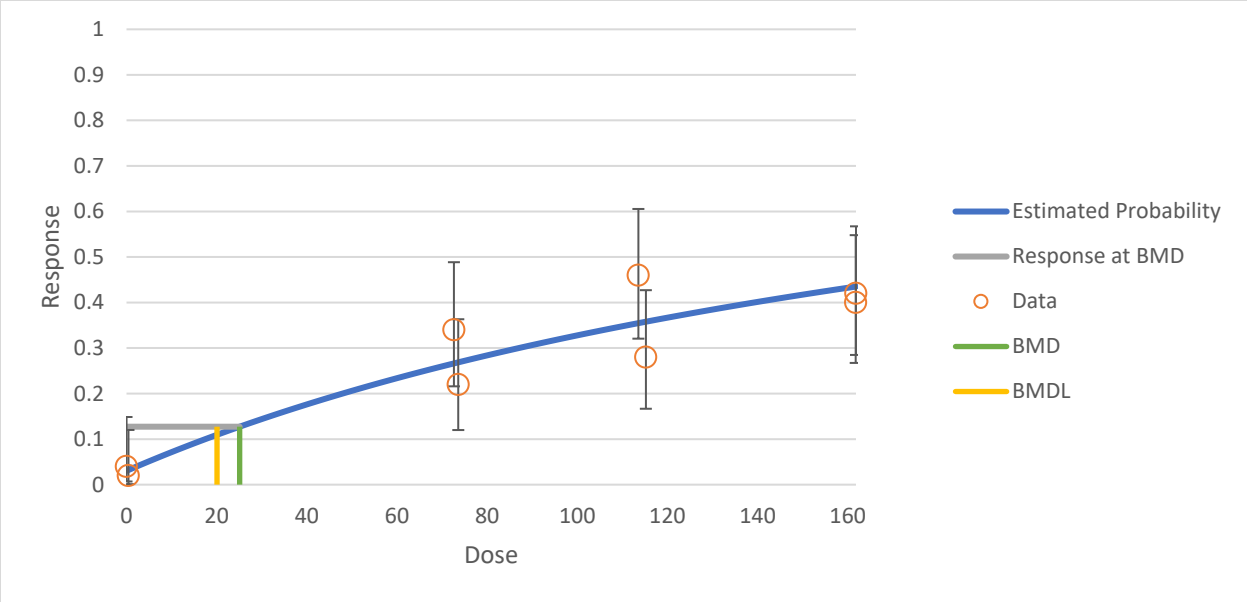


Figure E-15. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Necrosis in the Liver in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.3 Hepatocellular Adenomas

Increased incidence of hepatocellular adenomas was observed in F_1 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, 2012). The doses and response data used for the modeling are listed in Table E-74. For NTP (2020), an additional dose metric was derived which averages 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.

Table E-74. Dose-Response Modeling Data for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	0
300 / 0	0.4	50	0
0 / 20	72.6	50	0
300 / 20	73.6	50	1
0 / 40	113.5	50	7
300 / 40	115.2	50	5
0 / 80	161.7	50	11
300 / 80	161.8	50	10

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Hepatocellular adenomas were assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for CSF derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The BMD modeling results for hepatocellular adenomas following perinatal and postweaning exposure are summarized in Table E-75 and Figure E-16. The best fitting model was the Multistage Degree 2 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 2 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 2 model is 93.0 mg/L.

Table E-75. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.897	96.6	0.4	−0.004	122.1	96.7	EPA selected the Multistage Degree 2 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 2 model had the lowest AIC.
Multistage Degree 2	**0.883**	**95.1**	**0.1**	**−0.009**	**116.8**	**93.0**	
Multistage Degree 1	0.378	98.0	−0.1	−0.146	107.9	73.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

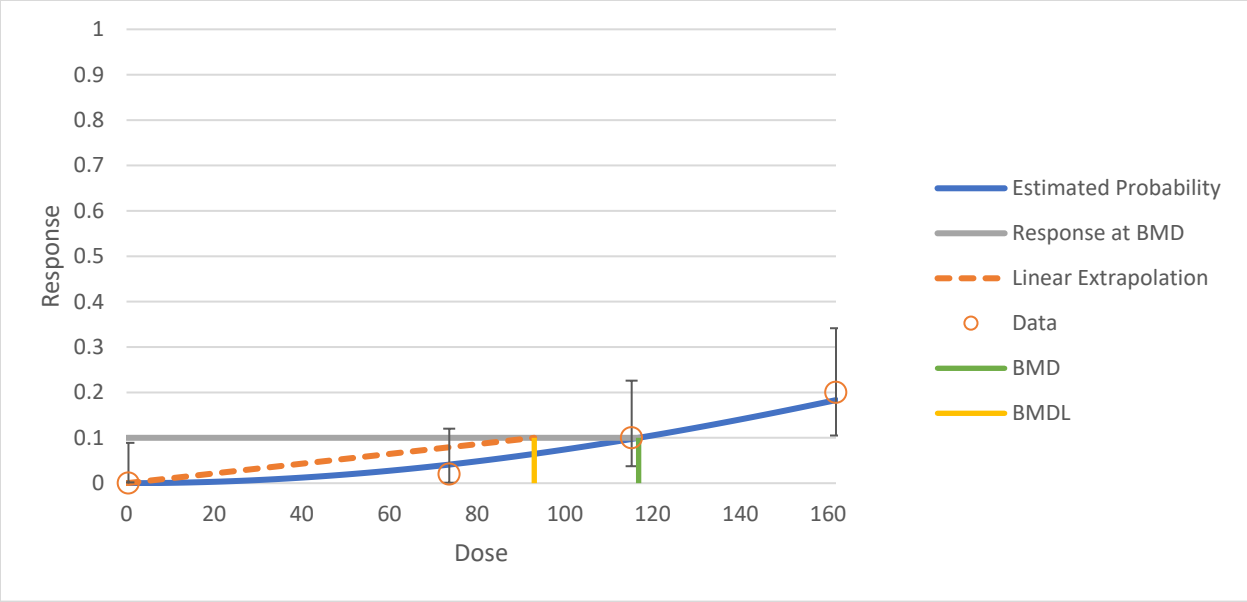


Figure E-16. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.3.1 Sensitivity Analyses

The BMD modeling results for hepatocellular adenomas following postweaning exposure are summarized in Table E-76 and Figure E-17. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 3 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 3 model is 95.3 mg/L.

Table E-76. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	**0.420**	**99.1**	**1.2**	**−0.001**	**117.1**	**95.3**	EPA selected the Multistage Degree 3 model. All models, except Multistage Degree 1, had adequate fit (p-values greater than 0.1), the BMDLs were
Multistage Degree 2	0.397	100.4	0.7	−0.001	108.8	88.7	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 1	0.064	106.5	0.4	−0.001	94.1	65.3	sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

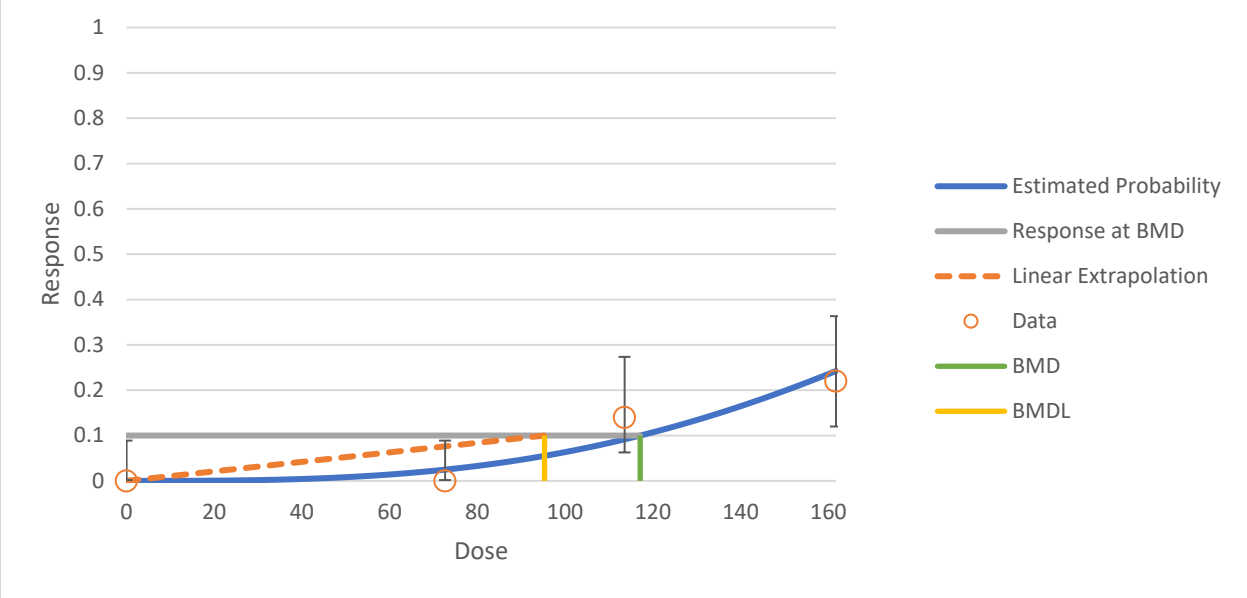


Figure E-17. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for hepatocellular adenomas using pooled methods are summarized in Table E-77 and Figure E-18. The best fitting model was the Multistage Degree 6 model based on adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models. Two models (Multistage Degree 6 and 7) had the same lowest AIC value. The $BMDL_{10}$ from the selected Multistage Degree 6 model is 104.2 mg/L.

Table E-77. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 7	0.843	191.9	0.3	−0.001	119.9	104.2	EPA selected the Multistage Degree 6 model. All models had adequate fit (p-values greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference). The Multistage Degree 6 had the lowest AIC value.
Multistage Degree 6	**0.834**	**191.9**	**0.3**	**−0.001**	**119.9**	**104.2**	
Multistage Degree 5	0.754	193.9	0.3	−0.001	119.9	104.2	
Multistage Degree 4	0.754	193.9	0.3	−0.001	119.9	104.2	
Multistage Degree 3	0.843	191.9	0.3	−0.001	119.9	104.2	
Multistage Degree 2	0.684	195.7	0.9	−0.001	112.6	98.4	
Multistage Degree 1	0.179	202.7	0.6	−0.001	100.6	76.8	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

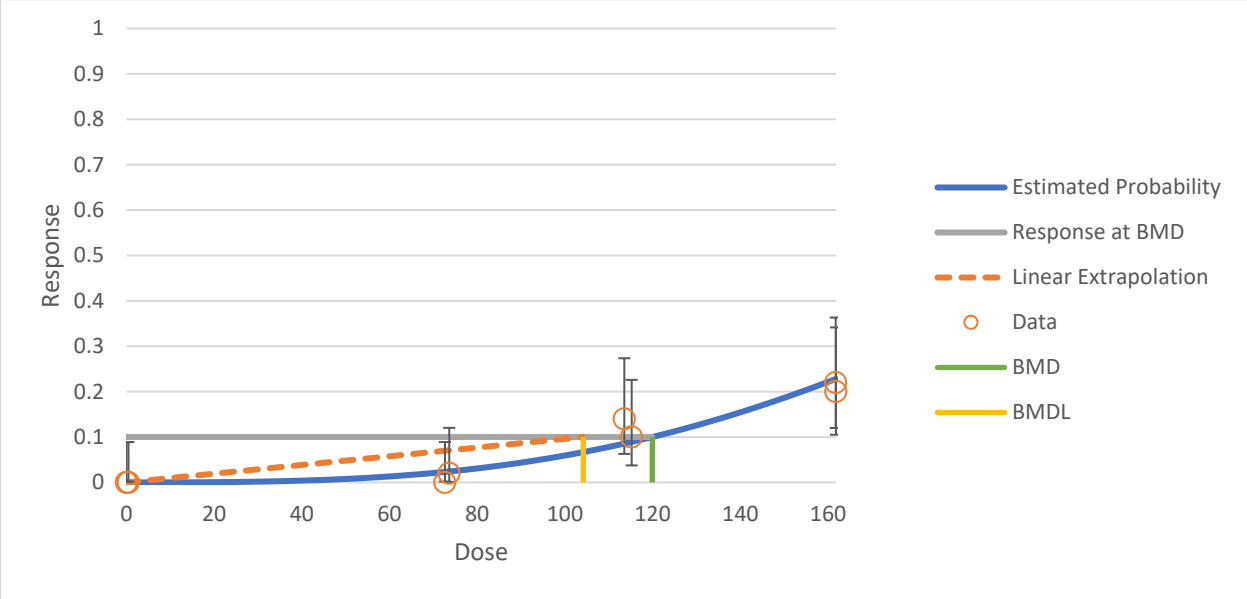


Figure E-18. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.4 Hepatocellular Adenoma or Carcinoma

Increased incidence of hepatocellular adenoma or carcinoma was observed in F_1 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, 2012). The doses and response data used for the modeling are listed in Table E-78. For NTP (2020), an additional dose metric was derived which averages 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.

Table E-78. Dose-Response Modeling Data for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	0
300 / 0	0.3	50	0
0 / 20	72.6	50	0
300 / 20	73.5	50	1
0 / 40	113.5	50	7
300 / 40	115.1	50	5
0 / 80	161.7	50	11
300 / 80	161.7	50	12

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Hepatocellular adenoma or carcinoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). The dose-response data (1) following postweaning exposure was the same between hepatocellular adenoma and hepatocellular adenoma or carcinoma therefore this modeling information can be found in Table E-76 and Figure E-17. Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for CSF derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The BMD modeling results for hepatocellular adenoma or carcinoma following perinatal and postweaning exposure to PFOA are summarized in Table E-79 and Figure E-19. The best fitting model was the Multistage Degree 2 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 2 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 2 model is 88.7 mg/L.

Table E-79. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.961	101.5	0.1	−0.001	117.5	95.8	EPA selected the Multistage Degree 2 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 2 model had the lowest AIC.
Multistage Degree 2	**0.752**	**100.8**	**–0.2**	**−0.009**	**109.4**	**88.7**	
Multistage Degree 1	0.199	104.8	–0.4	−0.155	94.9	65.9	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

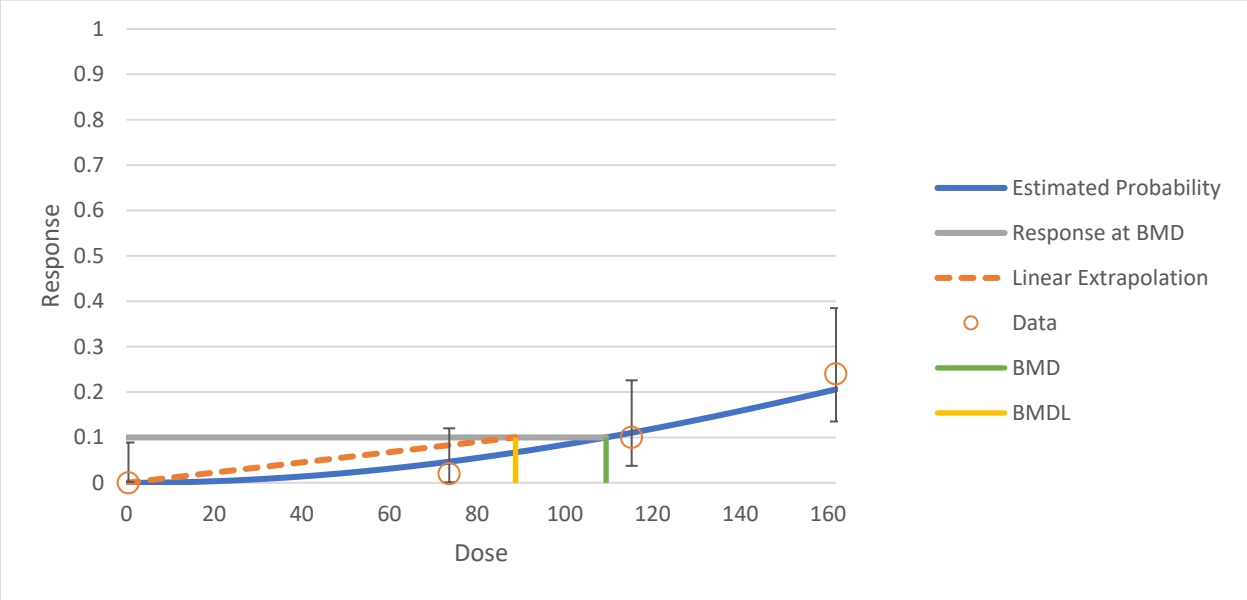


Figure E-19. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.4.1 Sensitivity Analyses

The BMD modeling results for hepatocellular adenoma or carcinoma following postweaning exposure to PFOA are summarized in Table E-80 and Figure E-20. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 3 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 3 model is 95.3 mg/L.

Table E-80. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	**0.420**	**99.1**	**1.2**	**−0.001**	**117.1**	**95.3**	EPA selected the Multistage Degree 3 model. Multistage Degree 2 and 3 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Multistage Degree 2	0.397	100.4	0.7	−0.001	108.8	88.7	
Multistage Degree 1	0.064	106.5	0.4	−0.001	94.1	65.3	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

[a] Selected model in bold.

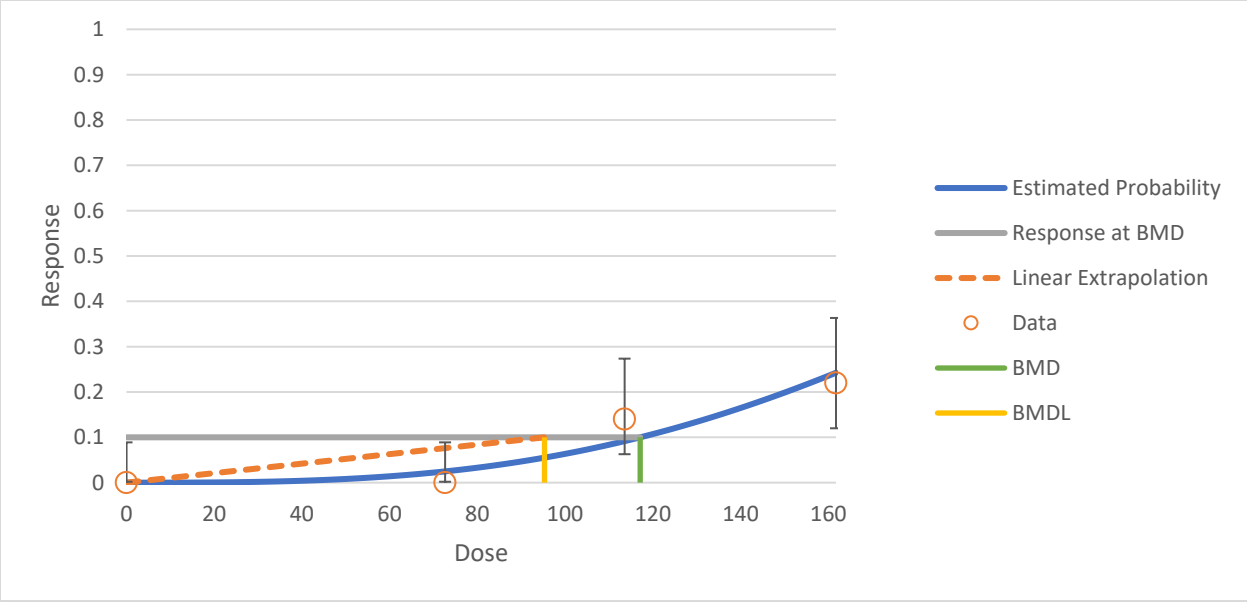


Figure E-20. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for hepatocellular adenoma or carcinoma using pooled methods are summarized in Table E-81 and Figure E-21. The best fitting model was the Multistage Degree 2 model based on adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models. The $BMDL_{10}$ from the selected Multistage Degree 3 model is 103.7 mg/L.

Table E-81. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 7	0.713	200.6	0.1	−0.001	117.3	103.7	EPA selected the Multistage Degree 3 model. All models had adequate fit (p-values greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference). The Multistage Degree
Multistage Degree 6	0.713	200.6	0.1	−0.001	117.3	103.7	
Multistage Degree 5	0.713	200.6	0.1	−0.001	117.3	103.8	
Multistage Degree 4	0.819	198.6	0.1	−0.001	117.3	103.7	
Multistage Degree 3	**0.893**	**196.6**	**0.1**	**−0.001**	**117.3**	**103.7**	
Multistage Degree 2	0.759	199.2	0.7	−0.001	109.3	95.7	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 1	0.179	207.3	0.5	−0.001	94.5	72.7	3 had the lowest AIC.

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

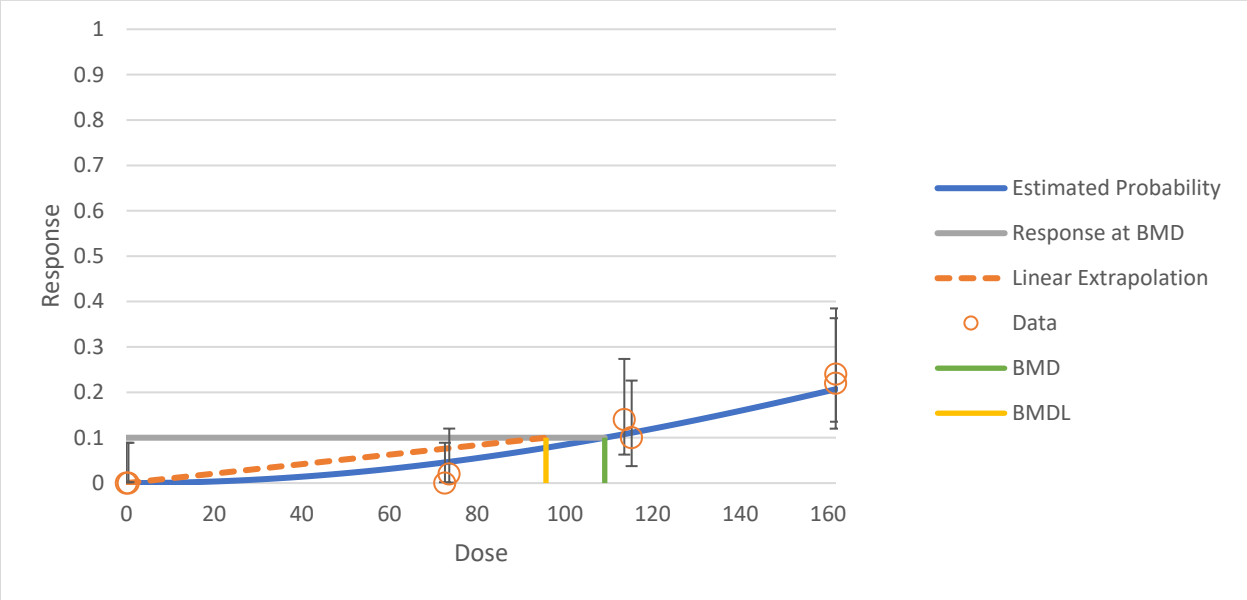


Figure E-21. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenoma or Carcinoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.5 Pancreatic Acinar Cell Adenoma

Increased incidence of pancreatic acinar cell adenoma was observed in F_1 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, 2012). The doses and response data used for the modeling are listed in Table E-82. For NTP (2020), an additional dose metric was derived which averages 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.

Table E-82. Dose-Response Modeling Data for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	3
300 / 0	0.4	50	7
0 / 20	72.6	50	28
300 / 20	73.6	50	18
0 / 40	113.5	50	26
300 / 40	115.2	50	30
0 / 80	161.7	50	32
300 / 80	161.8	50	30

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Pancreatic acinar cell adenoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for CSF derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The BMD modeling results for pancreatic acinar cell adenoma following perinatal and postweaning exposure are summarized in Table E-83 and Figure E-22. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 1 model is 15.7 mg/L.

Table E-83. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.178	248.3	0.1	0.1	20.6	15.7	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than
Multistage Degree 2	0.178	248.3	0.1	0.1	20.7	15.7	

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 1	**0.404**	**246.3**	**0.1**	**0.1**	**20.2**	**15.7**	threefold difference), and the Multistage Degree 1 model had the lowest AIC.

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

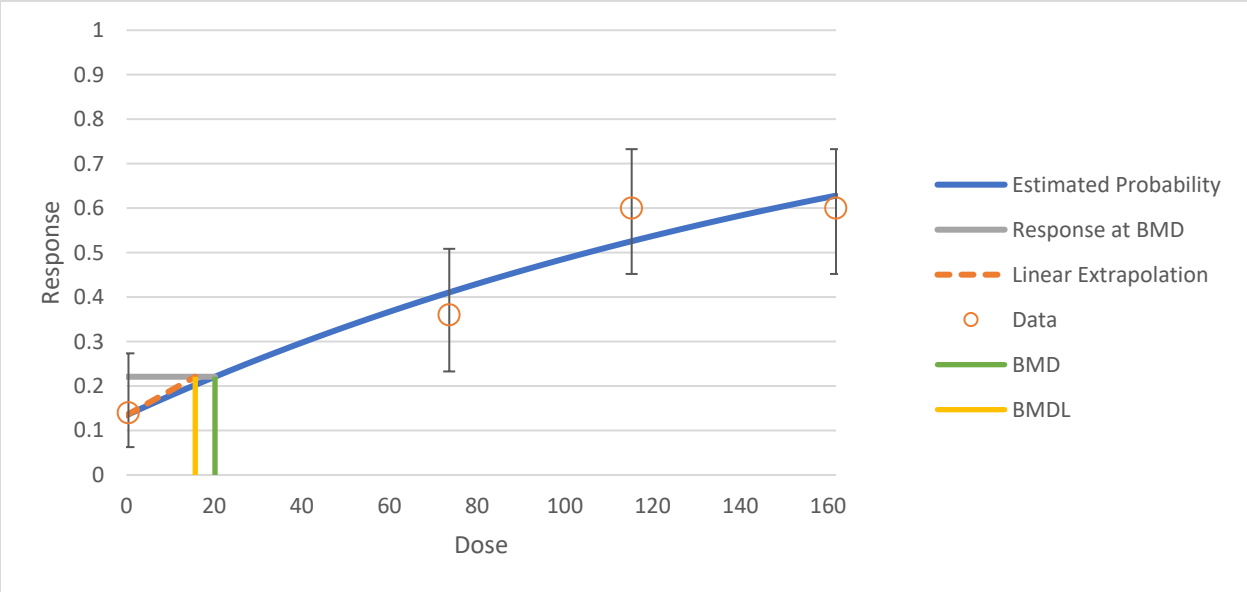


Figure E-22. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.5.1 Sensitivity Analyses

The BMD modeling results for pancreatic acinar cell adenoma following postweaning exposure to PFOA are summarized in Table E-84. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint.

Table E-84. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.105	234.3	−0.27	−0.3	15.5	12.6	No models had adequate fit. All Multistage models had identical fit (p-values greater than 0.1), but BMDLs were more than threefold lower than lowest tested dose.
Multistage Degree 2	0.105	234.3	−0.27	−0.3	15.5	12.6	
Multistage Degree 1	0.105	234.3	−0.27	−0.3	15.5	12.6	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

The BMD modeling results for pancreatic acinar cell adenoma using the pooled method are summarized in Table E-85. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint.

Table E-85. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 7	0.239	478.4	0.8	–1.0	17.7	15.0	No models had adequate fit. All Multistage models had identical fit (p-values greater than 0.1).
Multistage Degree 6	0.239	478.4	0.8	–1.0	17.7	15.0	
Multistage Degree 5	0.239	478.4	0.8	–1.0	17.7	15.0	
Multistage Degree 4	0.239	478.4	0.8	–1.0	17.7	15.0	
Multistage Degree 3	0.239	478.4	0.8	–1.0	17.7	15.0	
Multistage Degree 2	0.239	478.4	0.8	–1.0	17.7	15.0	
Multistage Degree 1	0.239	478.4	0.8	–1.0	17.7	15.0	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

E.2.6.6 Pancreatic Acinar Cell Adenoma or Adenocarcinoma

Increased incidence of pancreatic acinar cell combined adenoma or adenocarcinoma was observed in F_1 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, 2012). The doses and response data used for the modeling are listed in Table E-86. For NTP (2020), an additional dose metric was derived which averages 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.

Dichotomous multitumor models were initially selected as the most appropriate model type; however due to insufficient power, the run failed. In order to calculate a BMDL for this endpoint, a simple dichotomous model was used as it adequately reflects the data type.

Table E-86. Dose-Response Modeling Data for Pancreatic Acinar Cell Adenoma or Adenocarcinoma (Combined) in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (NTP, 2020)

Administered Dose (ppm)[a]	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	3
300 / 0	0.4	50	7
0 / 20	72.6	50	29
300 / 20	73.6	50	20
0 / 40	113.5	50	26
300 / 40	115.2	50	30
0 / 80	161.7	50	32
300 / 80	161.8	50	30

Notes:
[a] Doses are presented as perinatal exposure/postnatal exposure.

Pancreatic acinar cell adenoma or adenocarcinoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose-response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). Overall, EPA selected results from group 2 (perinatal and postweaning treatment groups) for CSF derivation because the exposure period encompassed the critical window of perinatal development and this group would likely be more sensitive to effects than the postweaning only (1) or pooled (3) groups. However, EPA provides modeling results for all three groups for comparison purposes.

The BMD modeling results for pancreatic acinar cell adenoma or adenocarcinoma (combined) following perinatal and postweaning exposure are summarized in Table E-87 and Figure E-23. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 3 model had the lowest AIC. The $BMDL_{10}$ from the selected Multistage Degree 3 model is 15.2 mg/L.

Table E-87. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	**0.541**	**247.6**	**–0.02**	**–0.02**	**19.6**	**15.2**	EPA selected the Multistage Degree 3 model. All models had adequate fit (p-values greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Multistage Degree 2	0.541	247.6	–0.02	–0.02	19.6	15.2	
Multistage Degree 1	0.541	247.6	–0.02	–0.02	19.6	15.2	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.
[a] Selected model in bold.

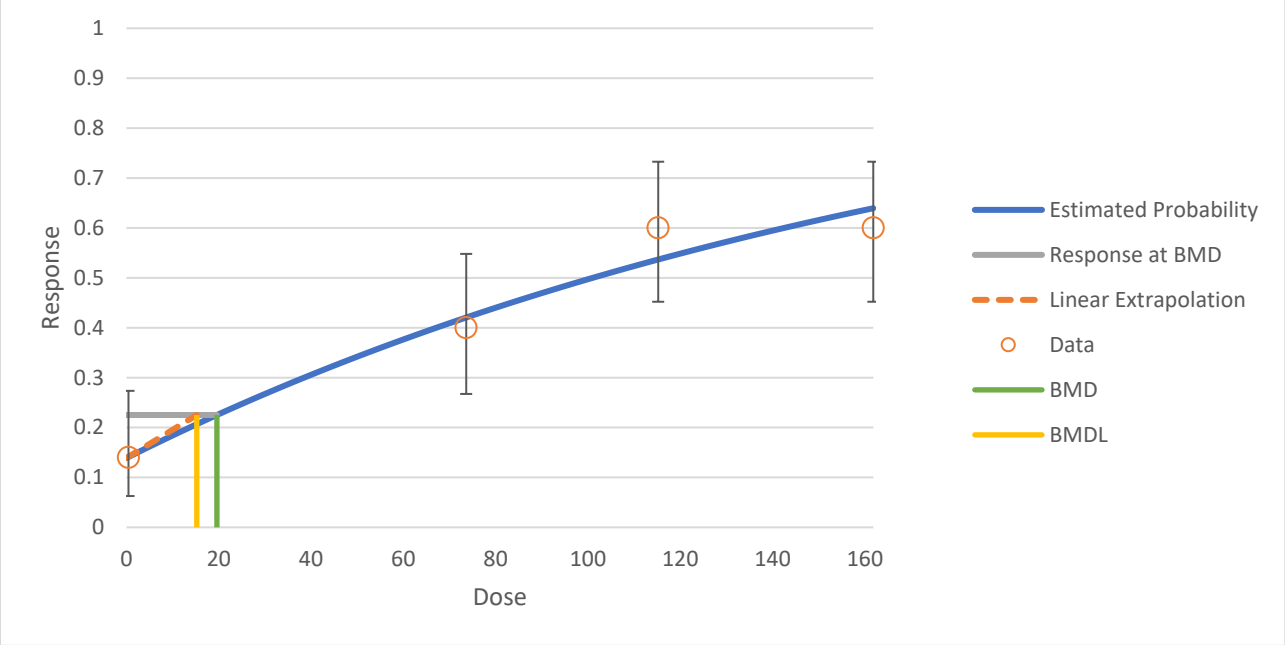


Figure E-23. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (NTP, 2020)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

E.2.6.6.1 Sensitivity Analyses

The BMD modeling results for pancreatic acinar cell adenoma or adenocarcinoma following postweaning exposure to PFOA are summarized in Table E-88. No models provided an adequate fit.

Table E-88. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma or Adenocarcinoma in F_1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (NTP, 2020)

Model[a]	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 3	0.061	234.8	–0.3	–0.3	15.3	12.4	No models had adequate fit. All Multistage models had equally poor fit, and BMDLs were more than threefold lower than lowest tested dose.
Multistage Degree 2	0.061	234.8	–0.3	–0.3	15.3	12.4	
Multistage Degree 1	0.061	234.8	–0.3	–0.3	15.3	12.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

The BMD modeling results for pancreatic acinar cell adenoma or adenocarcinoma (combined) using the pooled method are summarized in Table E-89. No models provided an adequate fit.

Table E-89. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F_1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (NTP, 2020)

Model	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 7	0.211	480.2	0.7	–1.1	17.3	14.6	No models had adequate fit. All Multistage models had identical fit (p-values greater than 0.1).
Multistage Degree 6	0.211	480.2	0.7	–1.1	17.3	14.6	
Multistage Degree 5	0.211	480.2	0.7	–1.1	17.3	14.6	
Multistage Degree 4	0.211	480.2	0.7	–1.1	17.3	14.6	
Multistage Degree 3	0.211	480.2	0.7	–1.1	17.3	14.6	
Multistage Degree 2	0.211	480.2	0.7	–1.1	17.3	14.6	

Model	Goodness of Fit		Scaled Residual		BMD_{10} (mg/L)	$BMDL_{10}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near BMD	Control Dose Group			
Multistage Degree 1	0.211	480.2	0.7	–1.1	17.3	14.6	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{10} = dose level corresponding to a 10% response level; $BMDL_{10}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level.

E.2.7 Song et al. (2018a)

EPA conducted dose-response modeling of the Song et al. (2018a) study using BMDS 3.2 program. This study addresses the offspring survival in F_1 male and female Kunming mice.

E.2.7.1 Pup Survival at PND 21

Decreased mean response of number of pup survival at weaning (PND 21) was observed in F_1 male and female Kunming mice. Continuous models were used to fit dose-response data. BMR of a change in the mean equal to 0.1 and 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table E-90. For developmental endpoints, a dose metric that represents the average concentration normalized per day (C_{avg}) during the relevant exposure window used for the study (i.e., gestation ($C_{avg,pup,gest}$), lactation ($C_{avg,pup,lact}$), or gestation and lactation ($C_{avg,pup,gest,lact}$)). See Section 4.1.3.1.3 of the Toxicity Assessment (U.S. EPA, 2024b) for additional details. For decreased pup survival at PND 21, the $C_{avg,pup,gest,lact}$ metric was selected because pups were exposed during gestation and lactation.

Table E-90. Dose-Response Modeling Data for Pup Survival in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Song et al., 2018a)

Administered Dose (mg/kg/day)	Internal Dose $C_{avg,pup,gest,lact}$ (mg/L)	Number per Group	Mean Response (Incidences)[a]
0	0	10	15.1 ± 7.6[b]
1	15.4	10	13.0 ± 14.5
2.5	25.3	10	12.0 ± 10.1
5	29.6	10	6.4 ± 17.1

Notes:
[a] Data are presented as mean ± standard deviation.
[b] Standard deviations were calculated from standard errors.

For $C_{avg,pup,gest,lact}$, the BMD modeling results for offspring survival are summarized in Table E-91 and Figure E-24. The best fitting model was the Polynomial Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 3 model had the lowest AIC. The $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 3 model is 12.3 mg/L.

Table E-91. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,gest,lact}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (constant variance) (Song et al., 2018a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.1\ SD}$ (mg/L)	$BMDL_{0.1\ SD}$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.637	320.6	−0.16	0.54	–0.16	4.5	1.5	27.2	8.8	EPA selected the Polynomial Degree 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.703	321.8	0.01	$-2.96 \times e^{-3}$	0.27	23.8	1.7	28.7	10.1	
Exponential 4	0.637	320.6	−0.16	0.54	−0.16	4.5	1.5	27.2	8.8	
Exponential 5	0.703	321.8	0.01	$-2.96 \times e^{-3}$	0.27	23.8	1.7	28.7	10.1	
Hill	0.701	321.8	$5.88 \times e^{-5}$	$2.09 \times e^{-6}$	0.27	24.4	–[b]	28.1	–[b]	
Polynomial Degree 3	**0.852**	**320.0**	−0.23	**−0.25**	**0.03**	16.1	2.5	**27.5**	**12.3**	
Polynomial Degree 2	0.801	320.1	−0.09	0.54	−0.08	11.9	2.4	26.7	12.2	
Power	0.706	321.8	0.02	$-4.97 \times e^{-3}$	0.26	23.4	2.5	28.8	12.6	
Linear	0.679	320.5	−0.19	0.57	−0.19	5.1	2.3	25.7	11.7	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.1\ SD}$ = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; $BMDL_{0.1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] Lower limit includes zero; BMDL not estimated.

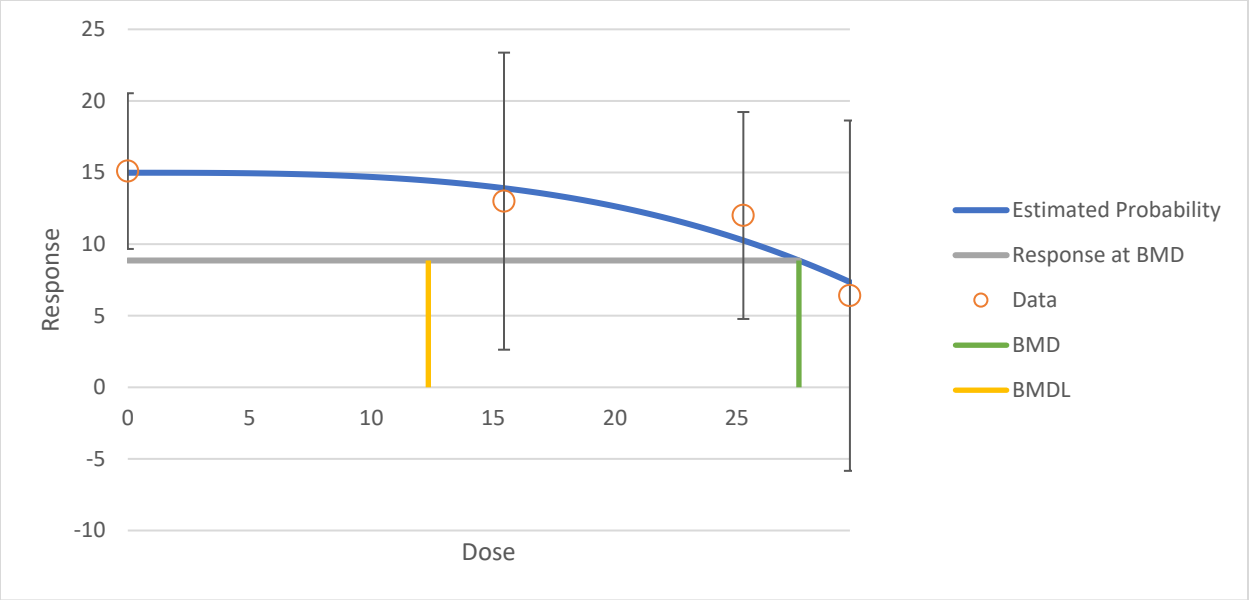


Figure E-24. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Pup Survival using $C_{avg,pup,gest,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{avg,pup,gest}$, the BMD modeling results for offspring survival are summarized in Table E-92 and Figure E-25. The best fitting model was the Polynomial Degree 2 model based on adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models. The $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 2 model is 8.8 mg/L.

Table E-92. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,gest}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.1\ SD}$ (mg/L)	$BMDL_{0.1\ SD}$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.736	320.3	−0.15	0.53	−0.15	3.0	1.1	18.5	6.1	EPA selected the Polynomial Degree 2 model. All models had adequate fit (p-values greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference).
Exponential 3	0.709	321.8	0.03	−0.01	0.25	15.0	1.1	21.5	6.6	
Exponential 4	0.736	320.3	−0.15	0.53	−0.15	3.0	1.1	18.5	6.1	
Exponential 5	0.709	321.8	0.03	−0.01	0.25	15.0	1.1	21.5	6.6	
Hill	0.701	321.8	$-1.2\times e^{-4}$	$-1.2\times e^{-4}$	0.27	16.4	9.3	19.4	–[b]	
Polynomial Degree 3	0.711	321.8	−0.25	−0.09	0.10	10.7	1.8	20.7	8.9	
Polynomial Degree 2	**0.898**	**319.9**	−0.23	**−0.17**	**0.03**	9.0	1.8	**20.1**	**8.8**	
Power	0.718	321.8	0.06	−0.01	0.23	14.2	1.8	21.5	8.9	
Linear	0.791	320.2	−0.16	0.53	−0.16	3.6	1.7	18.2	8.6	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.1\ SD}$ = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; $BMDL_{0.1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] Lower limit includes zero; BMDL not estimated.

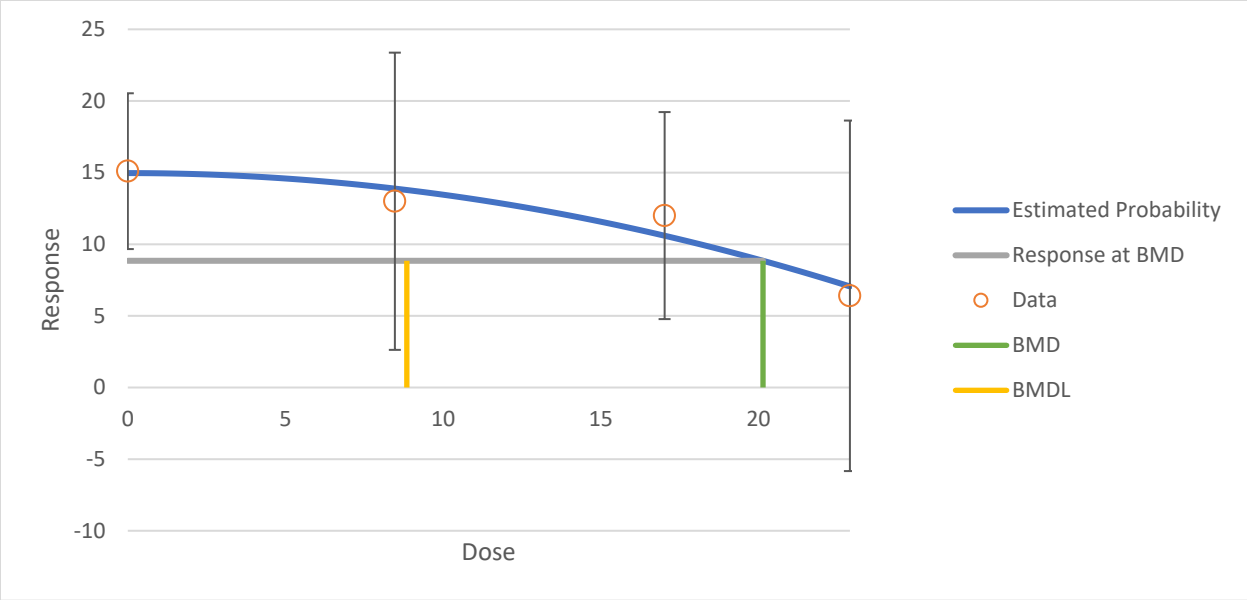


Figure E-25. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 2 Model for Pup Survival using $C_{avg,pup,gest}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{avg,pup,lact}$, the BMD modeling results for offspring survival are summarized in Table E-93 and Figure E-26. The best fitting model was the Polynomial Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 3 model had the lowest AIC. The $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 3 model is 15.2 mg/L.

Table E-93. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{avg,pup,lact}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.1\ SD}$ (mg/L)	$BMDL_{0.1\ SD}$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.586	320.8	−0.139	−0.782	−0.139	5.7	1.9	35.1	11.1	EPA selected the Polynomial Degree 3 model. All models, except Exponential 5 and Hill, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.701	321.8	0.001	$-4.583 \times e^{-4}$	0.271	30.9	2.3	34.4	13.1	
Exponential 4	0.586	320.8	−0.139	−0.782	−0.139	5.7	1.9	35.1	11.1	
Exponential 5	0.701	321.8	0.001	$-4.615 \times e^{-4}$	0.271	30.9	2.3	34.4	13.1	
Hill	–[b]	323.8	0.004	−0.001	0.269	30.7	–[c]	34.5	–[c]	
Polynomial Degree 3	**0.768**	**320.2**	−0.158	**0.581**	**−0.020**	19.5	3.0	**33.3**	**15.2**	
Polynomial Degree 2	0.721	320.3	0.011	0.612	−0.112	14.6	3.0	32.6	15.0	
Power	0.702	321.8	0.003	$-4.564 \times e^{-4}$	0.270	30.7	3.2	34.5	16.0	
Linear	0.617	320.7	−0.174	0.574	−0.174	6.6	2.9	32.8	14.5	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.1\ SD}$ = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; $BMDL_{0.1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

[c] Lower limit includes zero; BMDL not estimated.

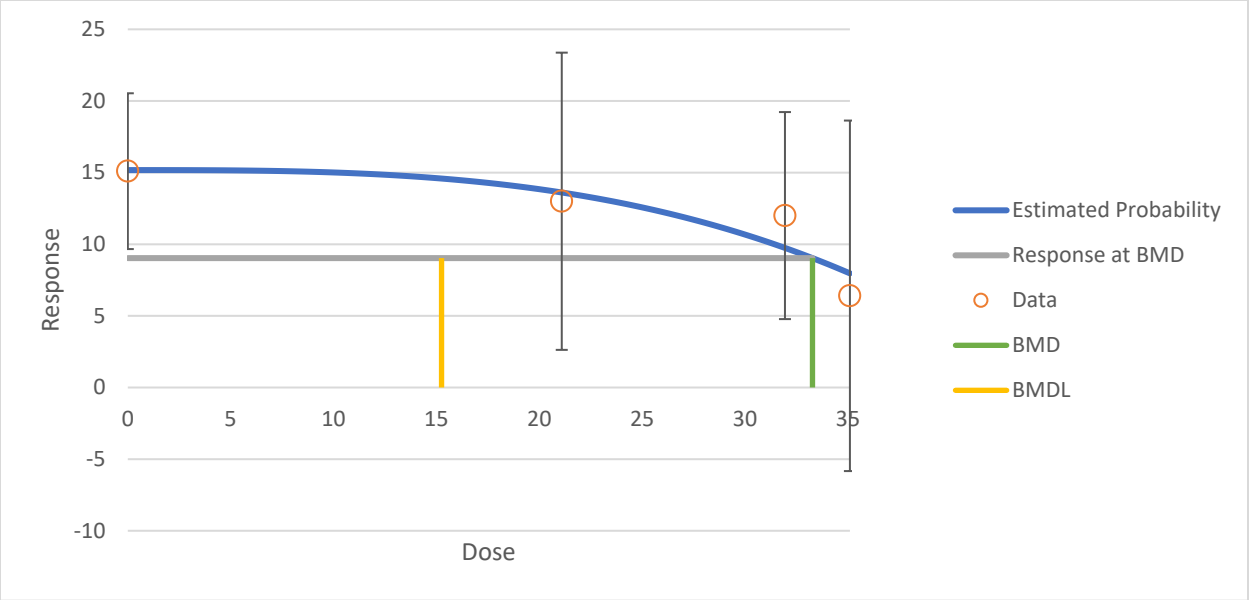


Figure E-26. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Pup Survival using $C_{avg,pup,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{max,pup,gest}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table E-94 and Figure E-27. The best fitting model was the Polynomial Degree 2 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 2 model had the lowest AIC. The $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 2 model is 13.4 mg/L.

Table E-94. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{max,pup,gest,}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.1\ SD}$ (mg/L)	$BMDL_{0.1\ SD}$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.686	320.4	−0.167	0.529	−0.167	4.8	1.7	29.1	9.5	EPA selected the Polynomial Degree 2 model. All models, except for the Hill model, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 2 model had the lowest AIC.
Exponential 3	0.708	321.8	0.031	−0.009	0.252	24.4	1.8	32.3	10.6	
Exponential 4	0.686	320.4	−0.167	0.529	−0.167	4.8	1.7	29.1	9.5	
Exponential 5	0.701	323.8	0.029	−0.008	0.253	24.5	1.8	32.3	10.6	
Hill	–[b]	323.8	$8.798 \times e^{-5}$	$-2.097 \times e^{-4}$	0.272	26.0	16.5	30.6	–[c]	
Polynomial Degree 3	0.667	321.9	−0.253	−0.132	0.073	17.8	2.7	31.1	13.6	
Polynomial Degree 2	**0.867**	**320.0**	−0.157	**0.445**	**−0.040**	13.4	2.7	**29.9**	**13.4**	
Power	0.717	321.8	0.058	−0.013	0.229	23.5	2.7	32.4	13.7	
Linear	0.738	320.3	−0.193	0.543	−0.193	5.6	2.6	27.8	13.0	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.1\ SD}$ = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; $BMDL_{0.1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

[c] Lower limit includes zero; BMDL not estimated.

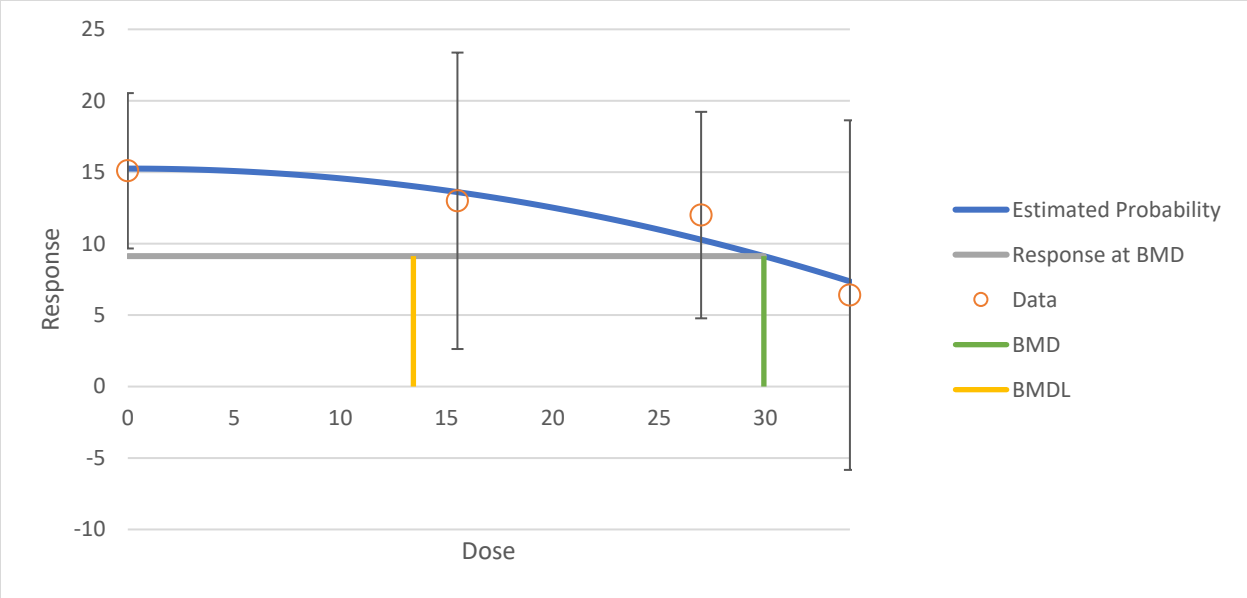


Figure E-27. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Pup Survival using $C_{max,pup,gest}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{max,pup,lact}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table E-95 and Figure E-28. The best fitting model was the Polynomial Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 3 model had the lowest AIC. The $BMDL_{0.5\ SD}$ from the selected Polynomial Degree 3 model is 20.3 mg/L.

Table E-95. Summary of Benchmark Dose Modeling Results for Pup Survival Using $C_{max,pup,lact,}$ in F_1 Male and Female Kunming Mice Following Exposure to PFOA (Constant Variance) (Song et al., 2018a)

Model[a]	Goodness of Fit		Scaled Residual			$BMD_{0.1\ SD}$ (mg/L)	$BMDL_{0.1\ SD}$ (mg/L)	$BMD_{0.5\ SD}$ (mg/L)	$BMDL_{0.5\ SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group Near $BMD_{0.1\ SD}$	Dose Group Near $BMD_{0.5\ SD}$	Control Dose Group					
Exponential 2	0.589	320.8	−0.140	–0.778	−0.140	7.6	2.6	46.6	14.8	EPA selected the Polynomial Degree 3 model. All models, except the Hill model, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.701	321.8	0.002	$–6.406 \times e^{-4}$	0.270	41.1	3.0	45.9	17.5	
Exponential 4	0.589	320.8	−0.140	–0.778	−0.140	7.6	2.6	46.6	14.8	
Exponential 5	–[b]	323.8	0.002	$–4.655 \times e^{-4}$	0.271	41.1	–[c]	45.9	2.6	
Hill	–[b]	323.8	0.005	–0.001	0.269	40.9	–[c]	46.1	–[c]	
Polynomial Degree 3	**0.772**	**320.2**	−0.163	**0.575**	**–0.017**	25.9	4.1	**44.4**	**20.3**	
Polynomial Degree 2	0.725	320.3	0.005	0.610	−0.111	19.4	4.0	43.4	20.0	
Power	0.702	321.8	0.004	$–8.674 \times e^{-4}$	0.269	40.9	4.3	46.1	21.3	
Linear	0.620	320.6	−0.175	0.574	−0.175	8.7	3.9	43.5	19.4	

Notes: AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; $BMD_{0.1\ SD}$ = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; $BMDL_{0.1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; $BMD_{0.5\ SD}$ = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean.

[a] Selected model in bold.

[b] Degrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

[c] Lower limit includes zero; BMDL not estimated.

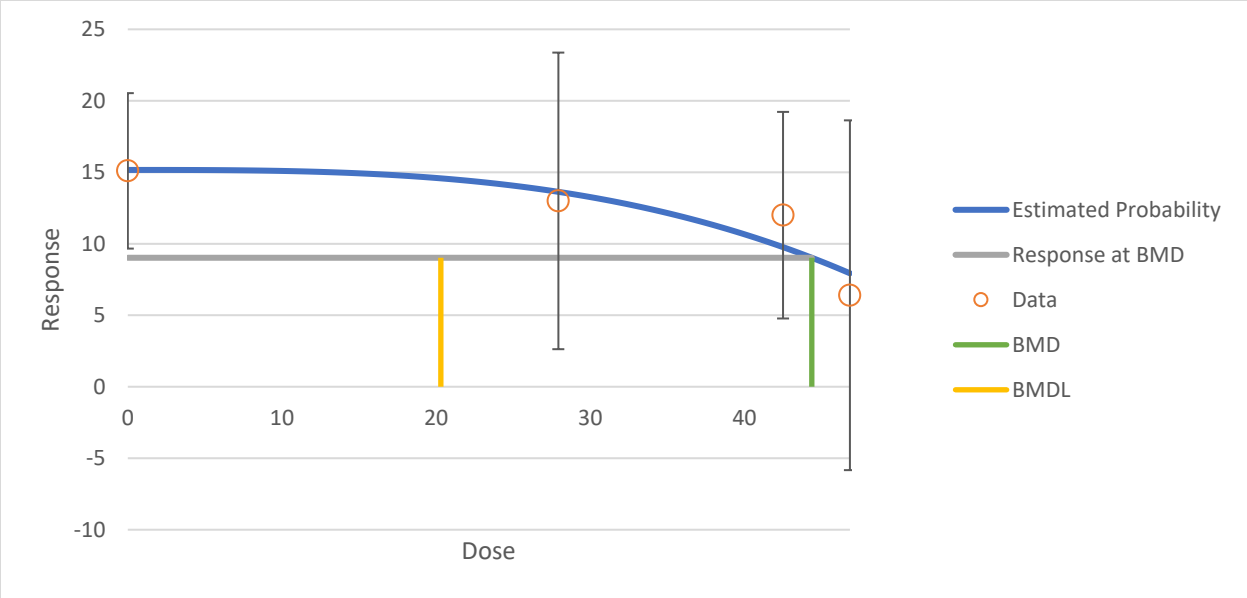


Figure E-28. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Offspring Survival using $C_{max,pup,lact}$ in F_1 Male and Female CD-1 Mice Following Exposure to PFOA (Song et al., 2018a)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

Appendix F. Pharmacokinetic Modeling

F.1 Animal Pharmacokinetic Model

For the animal pharmacokinetic model, model predictions from Wambaugh et al. (2013) were evaluated by comparing each predicted final serum concentration with the serum value in the supporting animal studies (training dataset) and with animal studies published since the publication of Wambaugh et al. (2013) (test dataset). The predictions from these two datasets were generally similar to the experimental values. There were no systematic differences between the experimental data and the model predictions across species, strain, or sex, and median model outputs uniformly appeared to be biologically plausible despite the uncertainty reflected in some of the 95th percentile confidence intervals (Cis). The application of the model outputs in the derivation of a human RfD can be found in the main text (see main perfluorooctanoic acid (PFOA) document).

F.1.1 Comparison of Fits to Training Datasets Used in Wambaugh et al. (2013)

The following figures show comparisons of the model-predicted serum concentrations with the data used for model training. Fits also presented in supplemental material of Wambaugh et al. (2013).

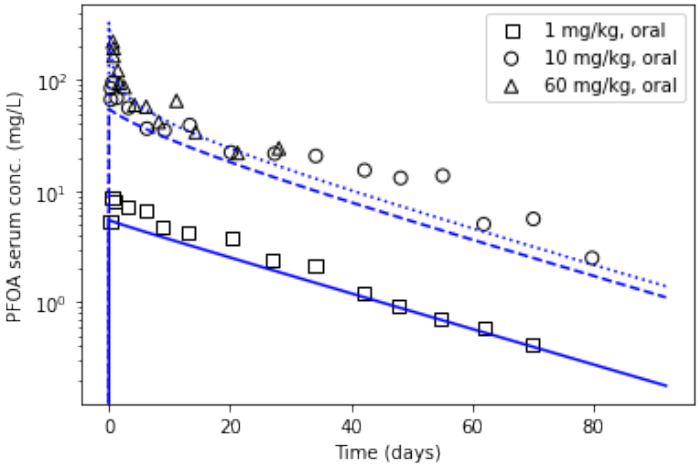


Figure F-1. Experimentally Observed Serum Concentrations (Lou et al., 2009) and Median Predictions for a Single Oral Dose of 1, 10, or 60 mg/kg PFOA to Female CD1 Mice

One mg/kg oral dose represented by the squares and solid line; 10 mg/kg oral dose represented by the circles and dashed line; 60 mg/kg oral dose represented by the upward triangle and dotted line.

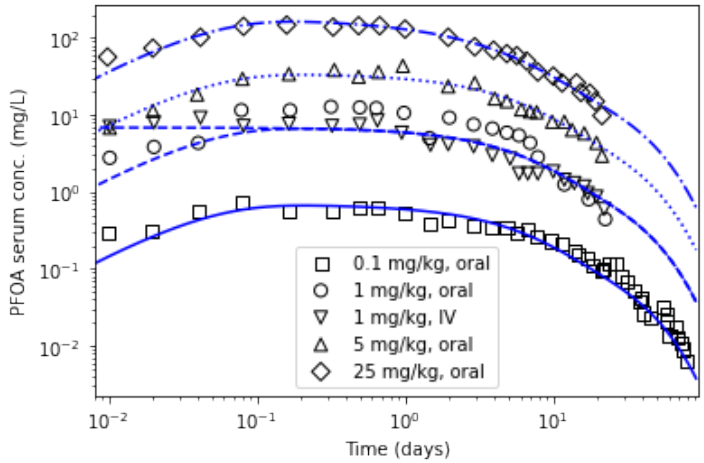


Figure F-2. Experimentally Observed Serum Concentrations (Kemper, 2003) and Median Prediction for a Single IV Dose of 1 mg/kg or an Oral Dose of 0.1, 1, 5, or 25 mg/kg PFOA to Male Sprague-Dawley Rats

One mg/kg intravenous (IV) dose represented by the downward triangles and dashed line; 0.1 mg/kg oral dose represented by the squares and solid line; 1 mg/kg oral dose represented by the circle and dashed line; 5 mg/kg oral dose represented by the upward triangles and dotted line; 25 mg/kg oral dose represented by the diamonds and dash-dot line.

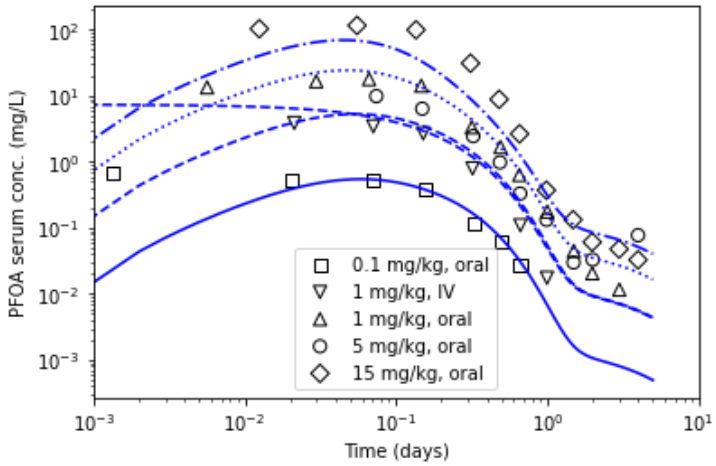


Figure F-3. Experimentally Observed Serum Concentrations (Kemper, 2003) and Median Prediction for a Single IV Dose of 1 mg/kg or a Single Oral Dose of 0.1, 1, 5, or 15 mg/kg PFOA to Female Sprague-Dawley Rats[a]

One mg/kg intravenous (IV) dose represented by the downward triangles and dashed line; 0.1 mg/kg oral dose represented by the squares and solid line; 1 mg/kg oral dose represented by the circle and dashed line; 5 mg/kg oral dose represented by the upward triangles and dotted line; 15 mg/kg oral dose represented by the diamonds and dash-dot line.

[a] Change in slope from 1 to 10 days represents a transition to a "beta-phase" elimination in female rats.

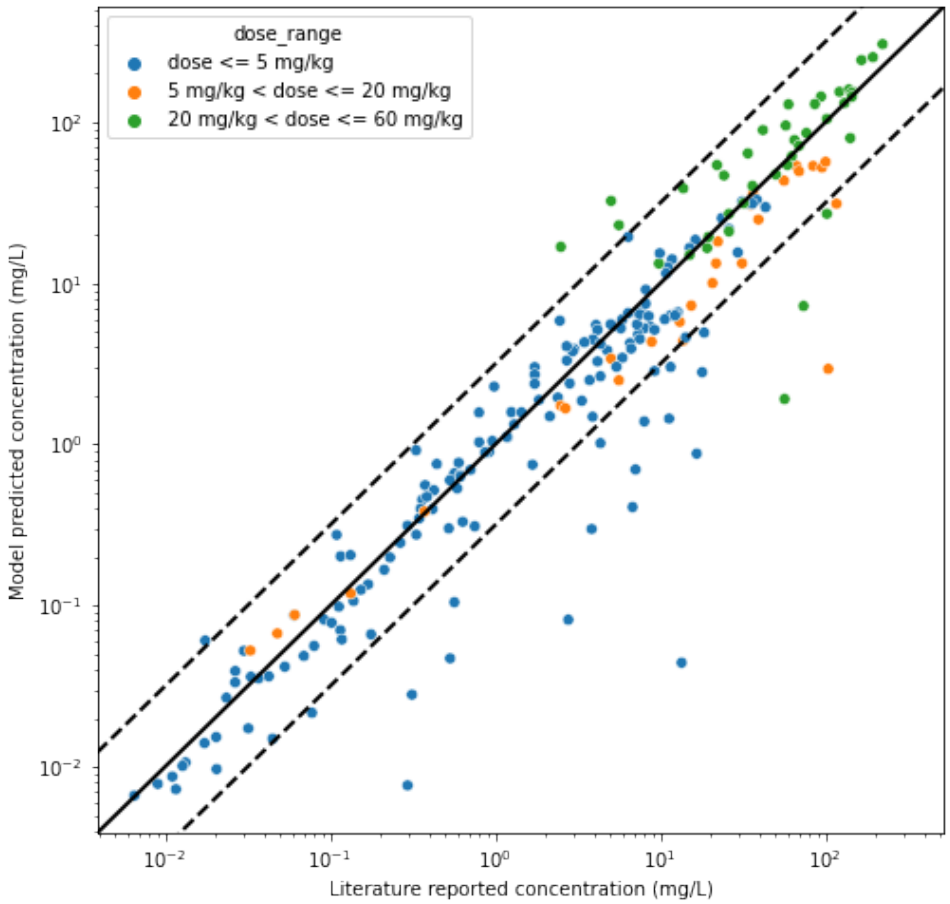


Figure F-4. Model Prediction Summary for PFOA Training Data

Model predictions on the training data result in a mean squared log error (MSLE) of 0.395. Dashed lines represent ± one-half $\log_{10}$.

EPA conducted a local, one-at-a-time sensitivity analysis to examine how parameter sensitivity varied across the adult and developmental models (Figure F-5). For each parameter/dose metric pair, sensitivity coefficients were calculated to describe the relative change in a dose metric relative to the proportional change in a parameter value. A sensitivity coefficient of 1 describes the situation where a 1% increase in a parameter resulted in a 1% increase in the dose metric.

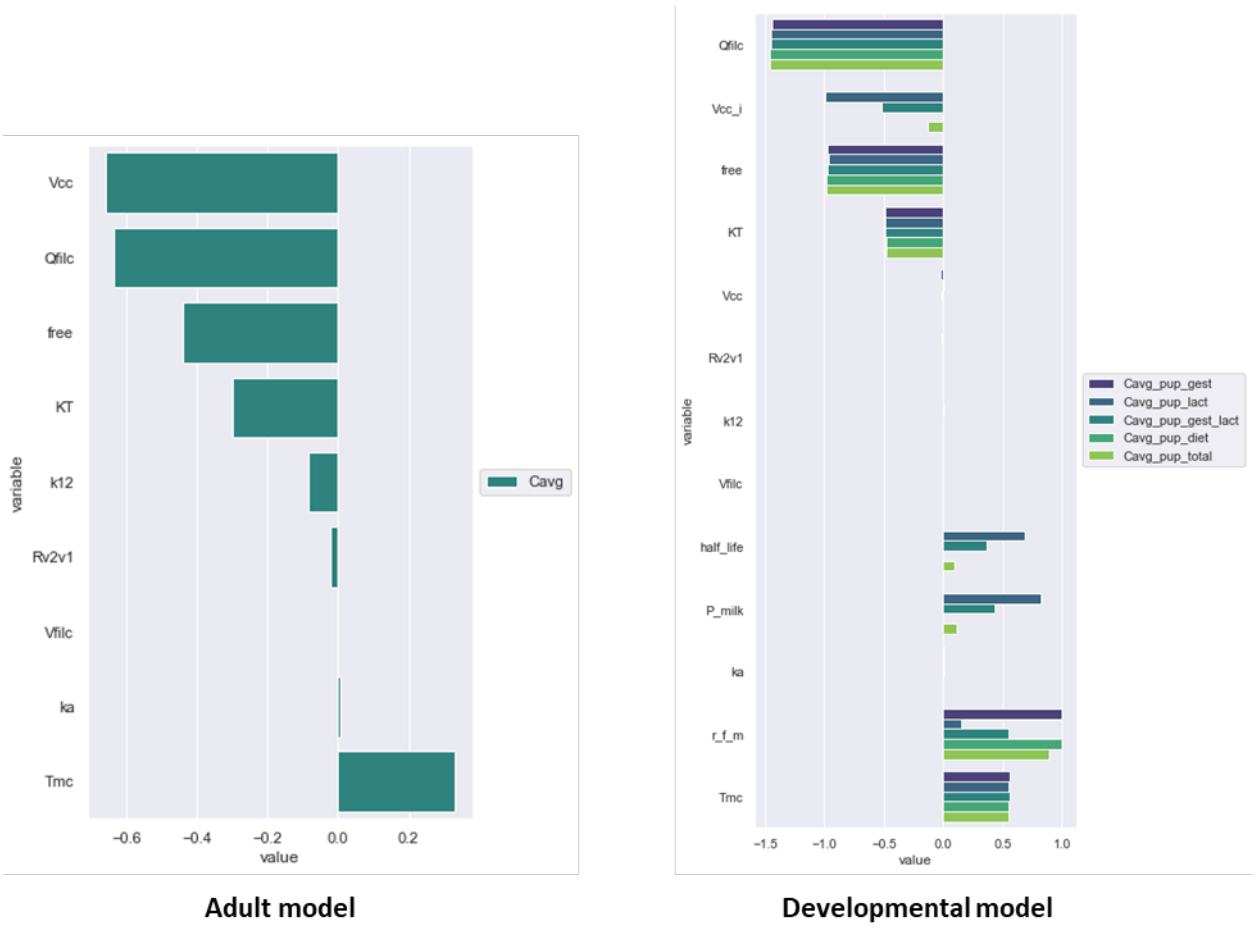


Figure F-5. PFOA Sensitivity Coefficients of the Adult Model and Developmental Model

As demonstrated in Figure F-5, the renal resorption mechanism (T_{mc} and KT) and the volume of distribution (V_{cc}) represent the most sensitive pathways for concentrations in the adult animal, which is to be expected because the renal resorption parameters govern the effective half-life of PFOA in the adult. Comparatively, the four one-compartment parameters for the infant (volume of distribution, half-life, serum:milk partition coefficient, and fetal:maternal ratio) are all sensitive to the gestational/lactational dose metrics. However, once the pup transitions to the adult model (Wambaugh model), PFOA transfer during gestation/lactation does not impact the average concentration during the post-weaning phase ($C_{avg\text{-}pup\text{-}diet}$). This is because the steady-state concentration for the pup exposed to PFOA in the diet during growth is much larger than the steady-state concentration during the 21 days of lactational exposure.

F.1.2 Visual Inspection of Test Datasets Not Used for Initial Fitting

The following figures show a comparison between model predictions and data from more recently published studies that were not part of the Wambaugh et al. (2013) parameterization.

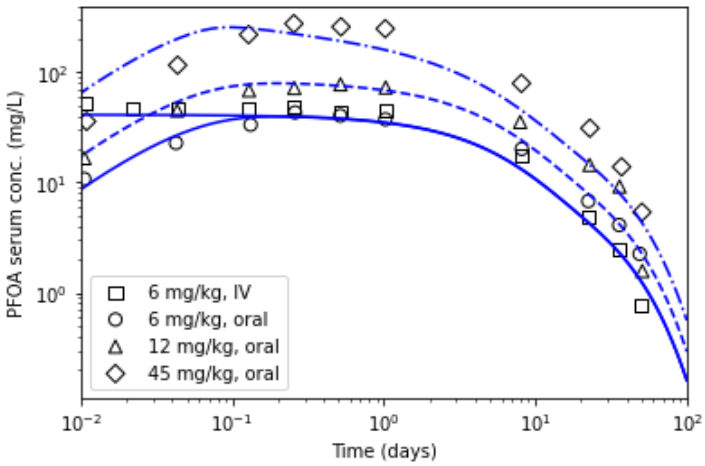


Figure F-6. Experimentally Observed Serum Concentrations (Dzierlenga et al., 2019) and Median Predictions for a Single IV Dose of 6 mg/kg or a Single Oral Dose of 6, 12, or 45 mg/kg PFOA to Male Sprague-Dawley Rats

Six mg/kg intravenous (IV) dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 12 mg/kg oral dose represented by the upward triangles and dashed line; 45 mg/kg oral dose represented by the diamonds and dash-dot line.

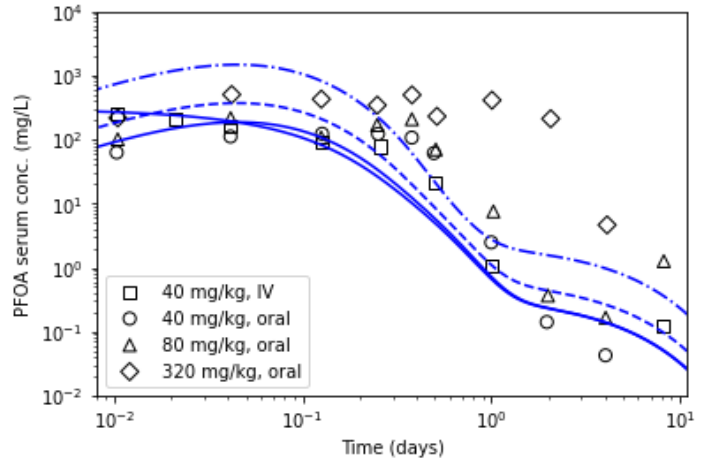


Figure F-7. Experimentally Observed Serum Concentrations (Dzierlenga et al., 2019) and Median Predictions for a Single IV Dose of 40 mg/kg or a Single Oral Dose of 40, 80, or 320 mg/kg PFOA to Female Sprague-Dawley Rats[a,b]

Forty mg/kg intravenous (IV) dose represented by the squares and solid line; 40 mg/kg oral dose represented by the circles and solid line; 80 mg/kg oral dose represented by the upward triangles and dashed line; 320 mg/kg oral dose represented by the diamonds and dash-dot line.

[a] Change in slope from 1 to 10 days represents a transition to a "beta-phase" elimination in female rats.

[b] The poor fit to 320 mg/kg reflects a dose that is outside the scope of the currently parametrized model.

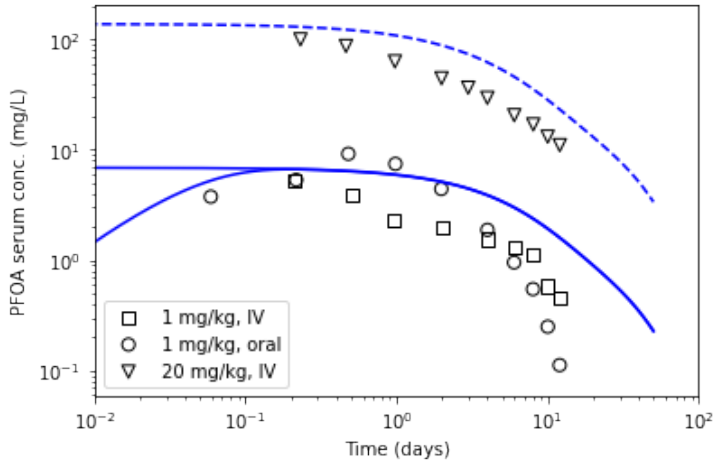


Figure F-8. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA (Kim et al., 2016b) or an IV Dose of 20 mg/kg PFOA (Kudo et al., 2002) to Male Sprague-Dawley Rats

One mg/kg intravenous (IV) dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 20 mg/kg IV dose represented by the downward triangles and dashed line.

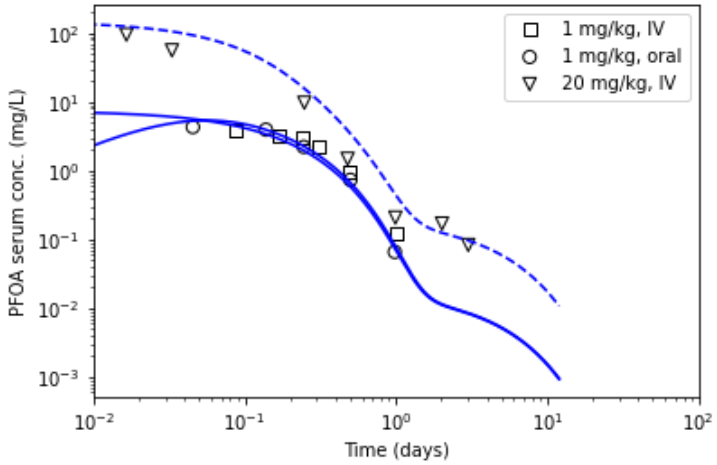


Figure F-9. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA (Kim et al., 2016b) or an IV Dose of 20 mg/kg PFOA (Kudo et al., 2002) to Female Sprague-Dawley Rats[a]

One mg/kg intravenous (IV) dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 20 mg/kg IV dose represented by the downward triangles and dashed line.

[a] Change in slope from 1 to 10 days represents a transition to a "beta-phase" elimination in female rats.

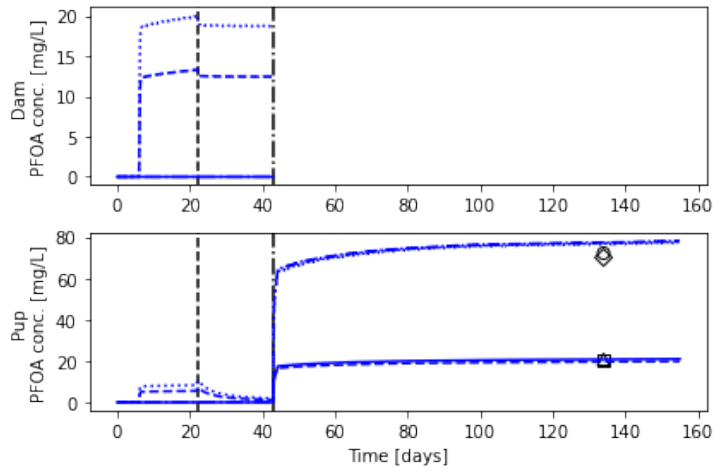


Figure F-10. Observed and Predicted PFOA Plasma Concentration in Female Sprague-Dawley Rats Following Perinatal, Lactational, and Post-weaning Exposure During Study 1 of NTP (2020)[a,b]

[a] Vertical black dashed and dash-dot lines represent the end of gestation and weaning, respectively.
[b] Top panel represents dam concentrations (mg/L) from conception (t = 0 days) to weaning (t = 43 days) while bottom panel represents fetal/pup concentrations from conception (t = 0 days) to postnatal week 16 (PNW 16) during interim evaluation. Each simulation represents a dam daily dietary exposure of 0, 150, or 300 ppm coupled with either 300 ppm or 1,000 ppm daily dietary exposure to the pup post-weaning. Using the "dam/pup ppm" nomenclature, four total dosing scenarios are modeled: 0/300 ppm (square, solid line), 0/1000 ppm (circle, dot-dash line), 150/300 ppm (triangle, dashed line), and 300/1000 ppm (diamond, dotted line) with corresponding PNW 16 pup plasma concentrations represented as color-matched circles. Dam concentrations only tracked through the end of weaning.

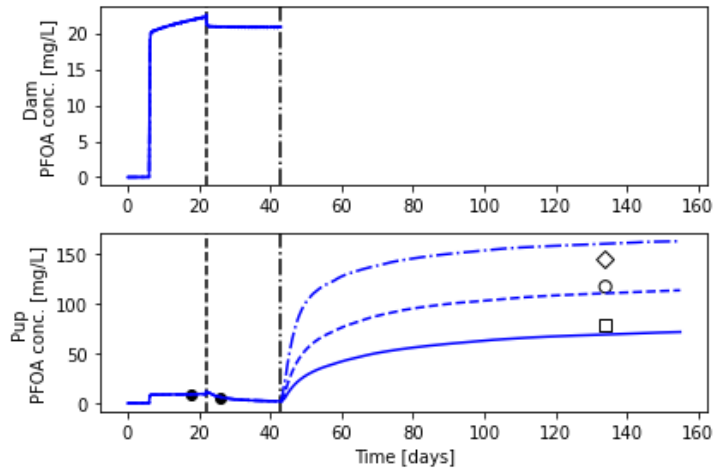
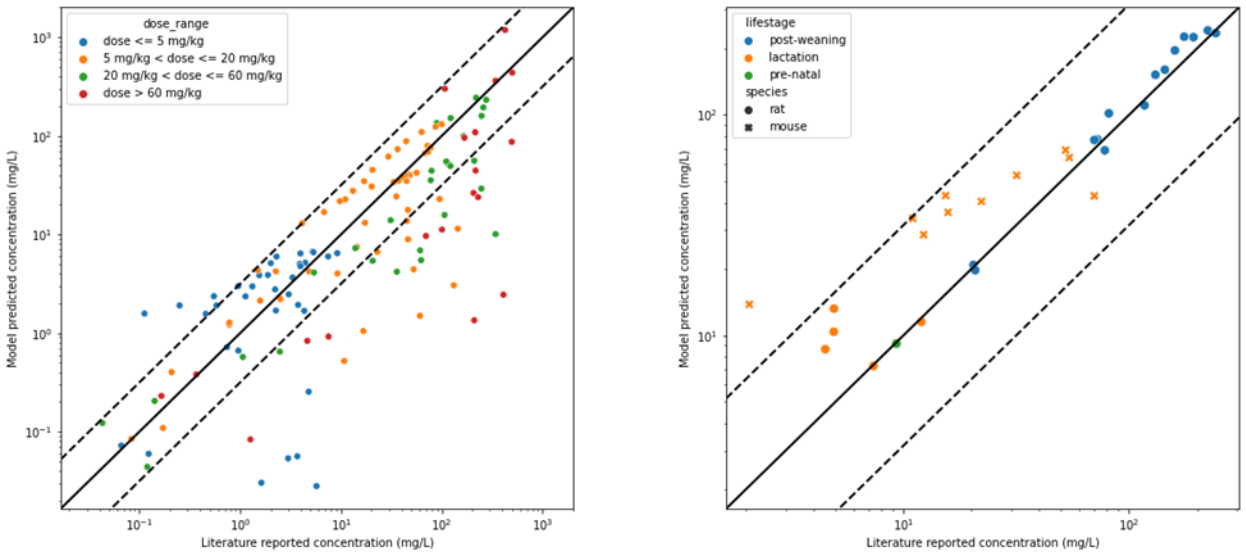


Figure F-11. Observed and Predicted PFOA Plasma Concentrations in Male Sprague-Dawley Rats Following Perinatal, Lactational, and Post-weaning Exposure During Study 2 of NTP (2020)[a,b]

[a] Vertical black dashed and dash-dot lines represent the end of gestation and weaning, respectively.
[b] Top panel represents dam concentrations (mg/L) from conception (t = 0 days) to weaning (t = 43 days) while bottom panel represents fetal/pup concentrations from conception (t = 0 days) to postnatal week 16 (PNW 16) during interim evaluation. Each simulation represents a dam daily dietary exposure of 300 ppm with 20 (solid line) 40 (dashed) and 80 (dot-dash) ppm daily dietary exposure to the pup post-weaning. Black circles represent fetal and pup concentrations at gestation day 18 and postnatal day 4 while the open square (20 ppm), open circle (40 ppm), and open diamond (80 ppm) represent the reported PFOA plasma concentrations in pup at PNW 16. Dam concentrations only tracked through the end of weaning.



Adult test data **Fetal/infant test data**

Figure F-12. Model Prediction Summary for PFOA Test Data

Left: Model predictions on the adult, single-dose test data result in a mean squared log error (MSLE) of 1.44. Right: Model predictions on fetal/infant pharmacokinetics during development broken out by lifestages (pre-natal – green, lactation – orange, post-weaning – blue) and species (rat – circle, mouse – ×) with an MSLE of 0.285. Dashed lines represent ± one-half $\log_{10}$

F.1.3 Consideration of Hinderliter et al. (2006b) in the Animal Model

On the basis of SAB's recommendation, the U.S. Environmental Protection Agency (EPA) examined Hinderliter et al. (2006b) and compared the reported pharmacokinetic data at 2-hours postdosing and at 24-hours postdosing for the 3-, 4-, and 5-week animals given a single oral gavage PFOA dose of 10 or 30 mg/kg to determine how the model predicts single-dose pharmacokinetics at this young age (Figure F-13). During the post-weaning phase, the modeling framework in the analysis of the Hindlerliter et al. (2006b) study uses the Wambaugh et al. (2013) model with reported juvenile body weights to simulate the post-weaning animals. Across all three age groups, this approach works reasonably well for juvenile male rats (blue and orange symbols in Figure F-13). As a result of investigating Hinderliter et al. (2006b), EPA found an age-dependent change in model predictions for the female juvenile rat (red symbols), where the Wambaugh et al. (2013) model dramatically underpredicts the 3-week-old female rats at 24 hours postdosing while slightly underpredicting the 5-week-old female rats at 24 hours postdosing. This is due to the rapid female rat-specific PFOA clearance in the Wambaugh et al. (2013) model which was parameterized on adult female rat pharmacokinetic data. One possibility is that this model underprediction for young animals could be due to a not yet modeled age-dependent change in PFOA urinary excretion as female pups mature to adult rats and could be attributed to changes in OAT1/OAT3 expression as the pup ages. However, as outlined in Figure F-12, the one-compartment model approach for breastfed pups successfully predicts the reported pup pre-natal and lactation lifestages. Additionally, Figure F-10, Figure F-11, and Figure F-12 demonstrate that the switch to the Wambaugh et al. (2013) for post-weaning and pup maturation successfully predicts steady-state PFOA concentrations in the post-

weaning male and female rats at postnatal week 19 when the endpoint of interest from NTP (2020) is measured. While it might be possible to use the reported PK data in post-weaning, juvenile, rats from Hinderliter et al. (2006b) to estimate an age-dependent clearance for these young rats, EPA's assessment of the study indicates that, due to the single-dose study design and age at which the measurements were reported (i.e., 3–5 weeks of age), incorporation of the results would not impact the current risk estimation of the endpoints used in the NTP study because those measurements were taken at 19 weeks of age with continuous dosing between 15 and 19 weeks.

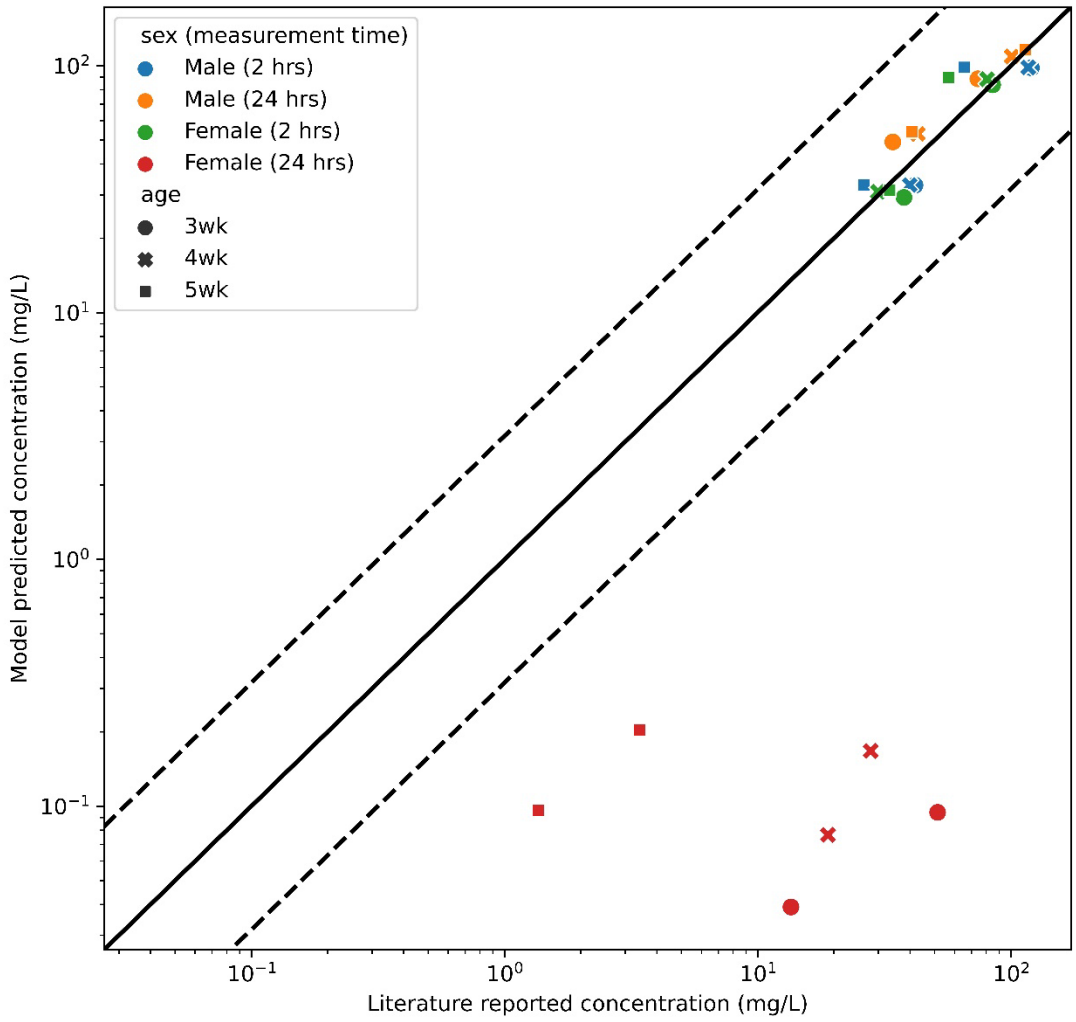


Figure F-13. Model Prediction Summary for PFOA Data from Hinderliter et al. (2006b)

Model predictions of juvenile rats dosed with PFOA from Hinderliter et al. (2006b) using the adult toxicokinetic parameters determined in Wambaugh et al. (2013). Symbol color reflects the sex of the rat at the given hours postdosing where blue and

orange represent male rats at 2 and 24 hours postdosing, respectively. Female rats are represented as green and red at 2 and 24 hours postdosing, respectively. Symbol types represent the rat age when dosing began and correspond to 3 weeks (circle), 4 weeks (×) and 5 weeks (square) of age. Dashed lines represent ± one-half log10. Female rats measured at 24 hours postdosing represent the predicted concentrations falling outside the ± one-half log10 bounds.

F.2 Human Model Validation

As mentioned in the Toxicity Assessment (U.S. EPA, 2024b), the human model was implemented in R/MCSim from the original AcslX model (Verner et al., 2016). Comparison with model output from the original model shows that, with the original parameters, the R model exactly replicates the original model (Figure F-14). The only difference remaining was that the start of pregnancy occurs at slightly different times in the two models, but this does not affect predictions outside of that very narrow time. Validation figures shown in this section include data for PFOS as well as PFOA. This is because model validation and decisions related to model structure were made for both chemicals together due to the preference for a similar model structure for the two chemicals.

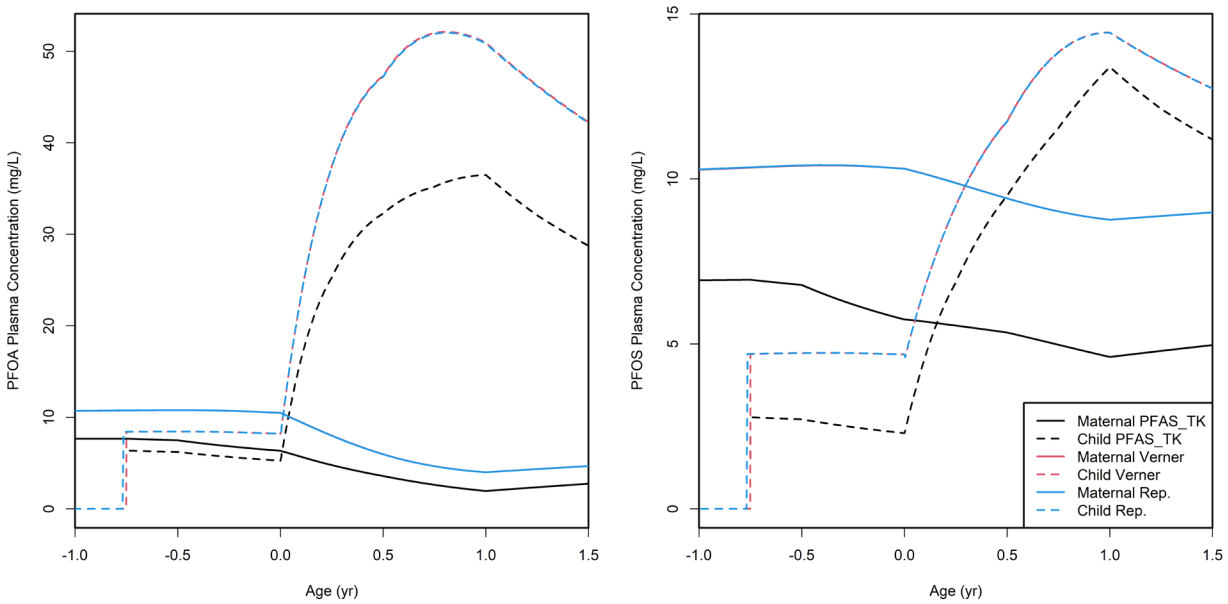


Figure F-14. Model Comparison

Comparison of the original AcslX model output (red, "Verner" label), the R model output with original model parameters (blue, "Rep." label), and the R model output with updated parameters (black, "PFAS_TK" label). Note that the red lines are almost entirely obscured by the blue lines.
The updated parameters result in lower serum concentrations for both the maternal and child. This is mainly due to lower half-lives selected during the parameter update.

Application of the updated parameters to predictions of serum levels in children showed good agreement between model predictions and reported values (Figure F-15; Figure F-16). This simulation was performed using mean breastmilk consumption estimates rather than the 95th percentile values from EPA's *Exposure Factors Handbook* (U.S. EPA, 2011b). Exposure in the validation scenario was assumed to be constant relative to body weight and was the same in the mother and child. This exposure was set such that predicted maternal serum level at delivery matched the reported value. Unlike the version of the model applied for human exposure

prediction, validation was performed using the age-dependent mean breastmilk consumption estimates. The main application of the model used the 95th quantile of breastmilk consumption to provide a health-protective estimate of exposure. Each validation scenario was customized based on information about the length of breastfeeding typical in that cohort. As a reminder, the default modeling scenario consisted of 1 year of breastfeeding, with an instantaneous transition to non-breastfeeding exposure (i.e., with exposure to other PFAS sources at weaning). One year is more typical of total (exclusive and partial) breastfeeding, as opposed to exclusive breastfeeding which typically lasts up to around 6 months of age.

For the simulation of the Fromme et al. (2010) cohort, information on breastfeeding status was only available 6 months after birth. At this point 37 of 50 participants were exclusively breastfed, 6 predominantly breastfed, 6 partially breastfed, and 1 received no breast milk. As in the analysis by Verner et al. (2016), EPA chose to model this scenario as exclusive breastfeeding to 6 months of age at which point the constant per bodyweight exposure starts equivalent to maternal exposure. For the cohort of the MOBA study (Granum et al., 2013), the average breastfeeding duration was 12.8 months. Because breastfeeding parameters were only developed in the model up to 1 year, and the information used to inform the model only extended to 1 year, the simulation for this scenario used the default 1 year of breastfeeding. In the Mogensen et al. (2015b) study, the median length of exclusive breastfeeding was 4.5 months, and the median length of partial breastfeeding was 4.0 months so 8.5 months was chosen as the breastfeeding duration for simulation of this study.

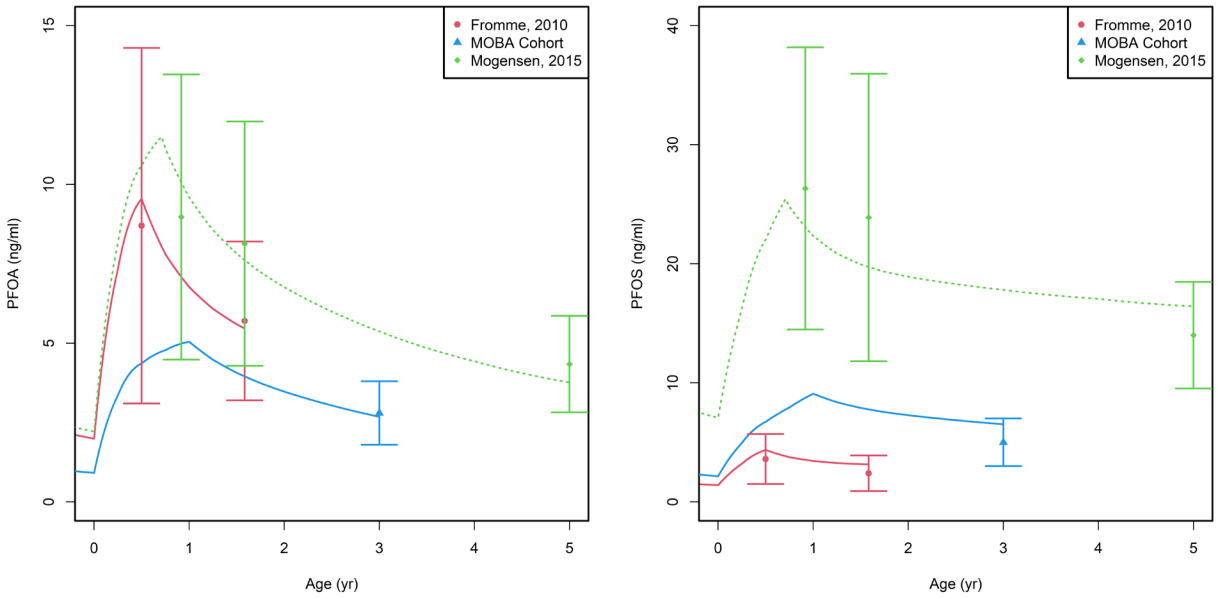


Figure F-15. Predicted Child Serum Levels Compared with Reported Values

These values were calculated using the updated parameters with constant V_d and exposure relative to body weight.
MOBA = Norwegian Mother and Child Cohort Study.

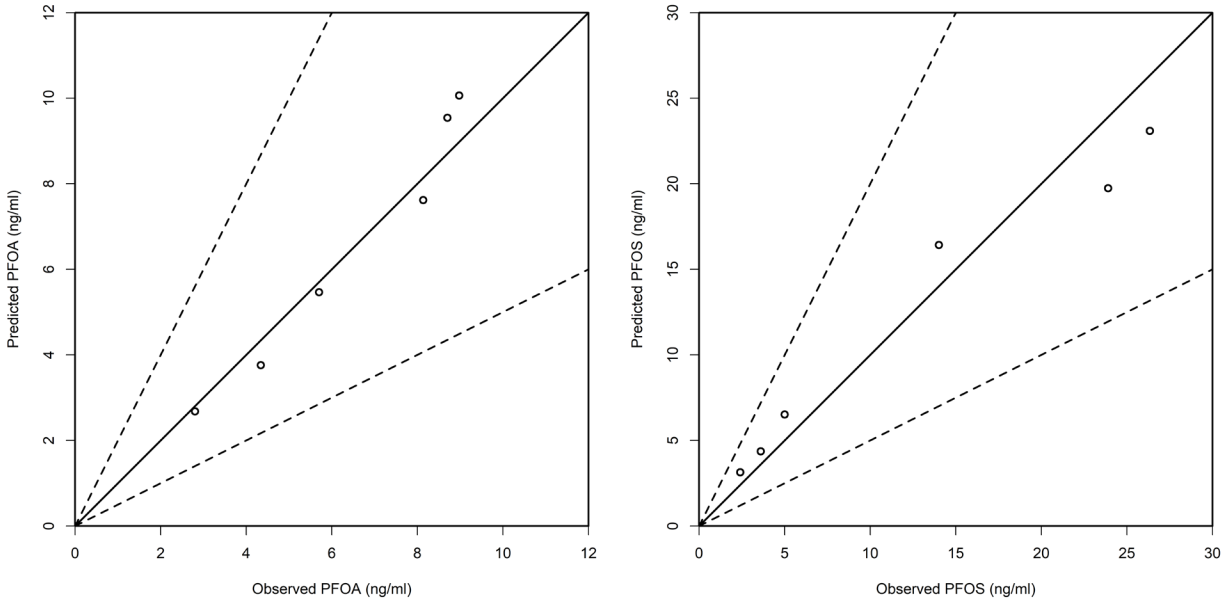


Figure F-16. Comparison of Predicted and Observed Child Serum Concentration

Dashed guidelines represent a twofold difference between observed and predicted concentration.

Local, one-at-a-time sensitivity analysis was performed to examine how parameter sensitivity varied across age and between maternal and child serum (Figure F-17). Sensitivity coefficients describe the change in a dose metric, in this case serum concentration, relative to the proportional change in a parameter value, in this case a 1% increase. A sensitivity coefficient of 1 describes the situation where a 1% increase in a parameter resulted in a 1% increase in serum concentration. Half-life and V_d were sensitive for every dose metric because they govern the distribution and excretion in all lifestages and have a synergistic effect on child levels because they influence the serum levels in children directly as well as the indirect exposure to the child early in life through maternal exposure.

For maternal serum at delivery, only the half-life and the V_d influenced the serum concentration. This was expected as the other parameters evaluated govern distribution of PFOA to the child and are not in play at this point. For cord blood, a similar effect is seen from V_d and half-life as in the maternal serum, because cord blood levels are based on maternal levels in the model, but a high sensitivity on the cord blood:maternal serum ratio parameter is also seen. This was not unexpected but emphasizes the importance of this parameter for this endpoint. The 1-year timepoint occurs at the peak serum concentration associated with the end of breastfeeding. Consistent with this, the parameters that govern lactational transfer of PFOA (i.e., breastmilk intake and the milk:maternal serum ratio) have high sensitivity coefficients. Additionally, sensitivity to V_d is high because that governs the relationship between exposure and serum levels by accounting for the amount of PFOA distributed to tissues. At the 5-year timepoint the sensitivity to parameters associated with lactational exposure has decreased. The sensitivity to V_d is somewhat lower compared with the value at 1 year, and the sensitivity to half-life has increased. This reflects the increased importance of excretion relative to the distribution of incoming PFOA during the period following lactational exposure.

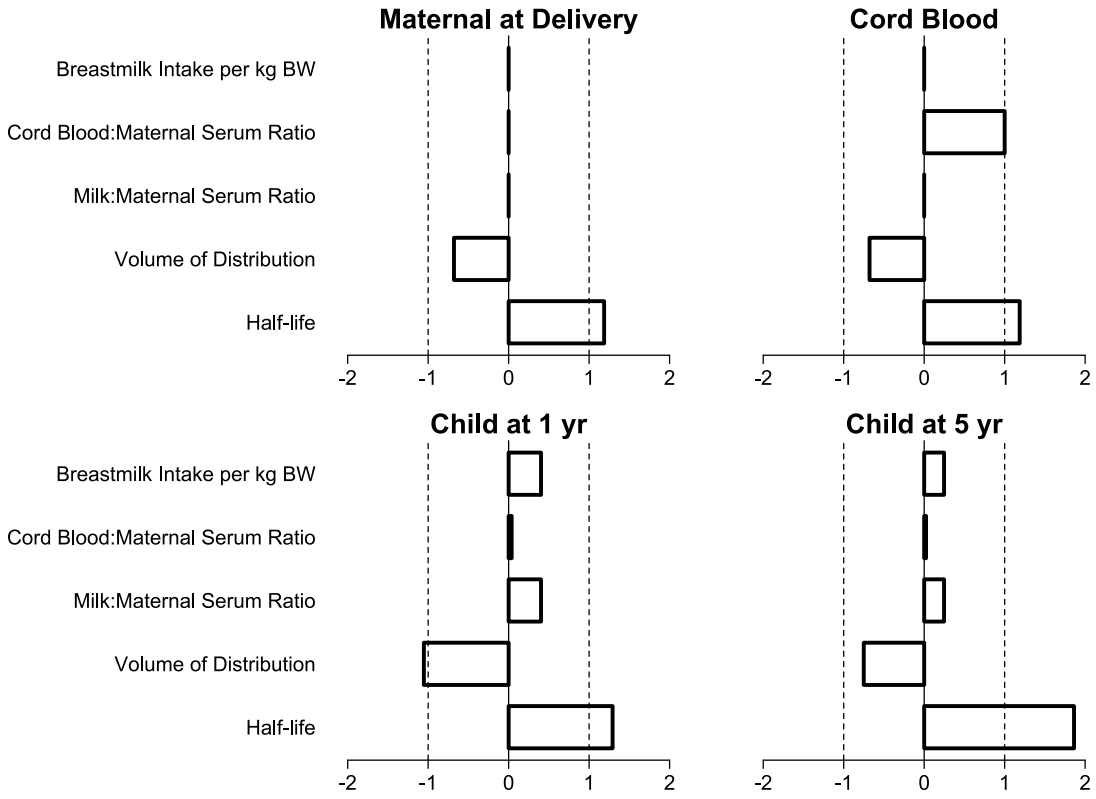


Figure F-17. Sensitivity Coefficients

Sensitivity coefficients from a local sensitivity analysis of maternal serum at delivery, cord blood at delivery, and child serum at 1 and 5 years old. The child was female. Results for a male child were similar (not shown).
BW = body weight.

A model developed by the Minnesota Department of Health (MDH model) (Goeden et al., 2019) was also considered for application to this assessment. This model has a similar model structure to the chosen model, with single compartments to represent the mother and child and excretion handled by first-order clearance.

To evaluate the effect of V_d in children, EPA integrated the V_d scaling in the MDH model into our model (Figure F-18). The main effect is to reduce the peak serum levels in children that occurs due to exposure through breastmilk. Using the root mean squared error, EPA determined that the model with constant V_d had better performance (Table F-1).

Table F-1. Root Mean Squared Error Comparison Between the Baseline Model (as Applied in the Main Risk Assessment) and Alternative Models with Features Inspired by the MDH Model.

Chemical	Root Mean Squared Error		
	Baseline Model	Model with Variable V_d	Model with Drinking Water Exposure
PFOA	0.65	1.59	1.27
PFOS	2.48	5.06	4.82

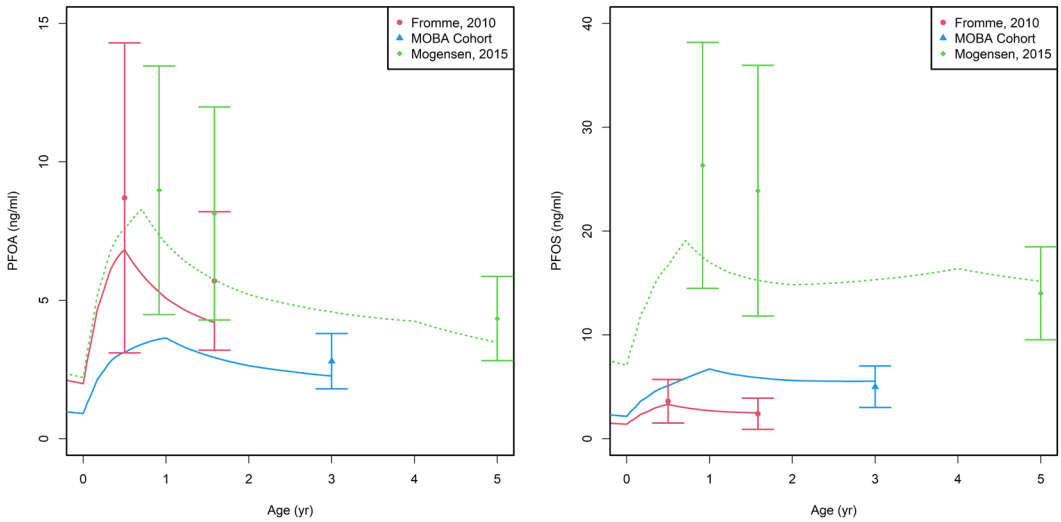


Figure F-18. Predicted Child Serum Levels Compared with Reported Values with Increased Volume of Distribution in Children as Implemented in the Minnesota Department of Health Model

MOBA = Norwegian Mother and Child Cohort Study.

EPA also implemented exposure based on drinking water consumption in the modified Verner model to examine the effect on model predictions and especially on the results of the risk assessment (Figure F-19). Using the root mean squared error, EPA determined that the model with constant exposure relative to bodyweight had better performance than a model that explicitly adjusts for drinking water consumption (Table F-1). Furthermore, a maximum contaminant level goal (MCLG) based on constant exposure does not greatly underestimate the risk to populations with greater water consumption per body weight (e.g., children and lactating women) because the method for calculating the MCLG from a RfD that assumes constant exposure accounts for the greater drinking water consumption in these populations.

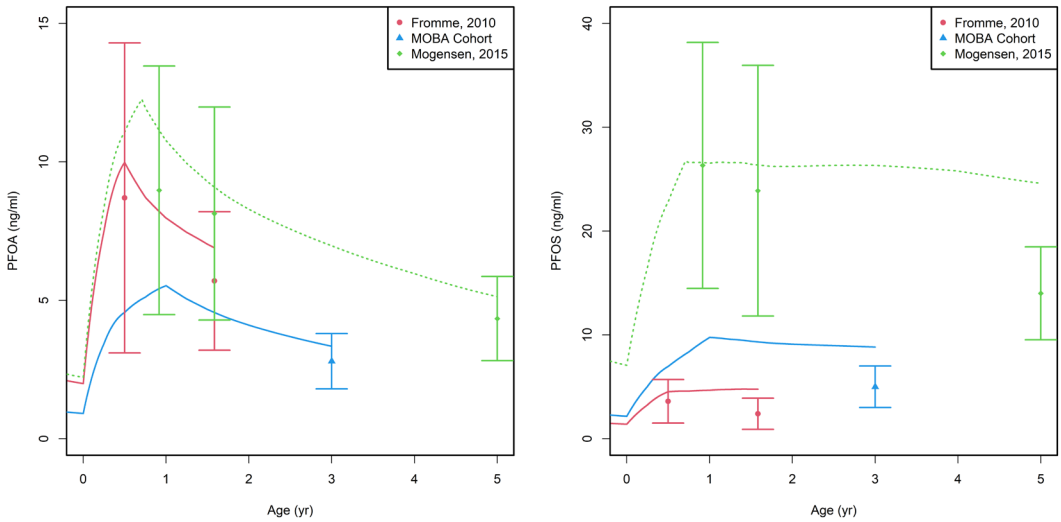


Figure F-19. Predicted Child Serum Levels Compared with Reported Values with Constant Volume of Distribution and Variable Exposure Based on Drinking Water Intake

MOBA = Norwegian Mother and Child Cohort Study.

Appendix G. Relative Source Contribution

G.1 Background

The U.S. Environmental Protection Agency (EPA) applies a relative source contribution (RSC) to the reference dose (RfD) when calculating a maximum contaminant level goal (MCLG) based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (U.S. EPA, 2000). EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (U.S. EPA, 2000). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (U.S. EPA, 2000). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (U.S. EPA, 2000). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies (U.S. EPA, 2000).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of 20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (U.S. EPA, 2000). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

G.2 Literature Review

In 2019, EPA's Office of Research and Development (ORD) conducted a literature search to evaluate evidence for pathways of human exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). This search was not date limited and spanned information collected across the Web of Science, PubMed, and ToxNet/ToxLine (now ProQuest) databases. An updated literature search was conducted and captured relevant literature published through March 2021. Literature captured by this search is housed in EPA's HERO database (https://hero.epa.gov/).

Results of this broad literature search were further distilled to address two questions. First, a systematic review was conducted to investigate evidence for important per- and polyfluoroalkyl substances (PFAS) exposure pathways from indoor environment media including consumer products, household articles, cleaning products, personal care products, and indoor air and dust (Deluca et al., 2022a). Literature that reported exposure measures from household media paired with occupant PFAS concentrations in blood serum was identified. Second, systematic evidence mapping was conducted for literature reporting measured occurrence of PFAS in exposure media (Holder et al., 2023). This review focused on real-world occurrences (measured concentrations) primarily in media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil).

G.2.1 Systematic Review

Deluca et al. (2022b) investigated evidence for important PFAS exposure pathways from indoor environment media including consumer products, household articles, cleaning products, personal care products, and indoor air and dust. The authors adapted existing systematic review methodologies and study evaluation tools to identify and screen exposure studies that presented concordant data on PFAS occurrence in indoor media and PFAS concentrations in blood or serum. Studies included in the systematic review report exposure measures from household media paired with occupant PFAS concentrations in blood serum, focusing on PFOA and seven other frequently measured PFAS (PFOS, perfluorobutanoic acid (PFBA), PFBS, PFDA, PFHxA, PFHxS, and PFNA). Machine learning approaches were used during the literature scoping and title/abstract screening to prioritize exposure pathways of interest by automated tagging and to select studies for inclusion using an iterative predictive screening model. Title/abstract screening according to the PECO criteria identified 486 studies that moved on to full-text screening; only 6 studies fully addressed the protocol requirements (Balk et al., 2019; Kim et al., 2019; Poothong

et al., 2019; Byrne et al., 2017; Makey et al., 2017; Wu et al., 2014). The extraction of exposure measurement data and study characteristics from each included study was performed using DistillerSR software. Exposure intake calculations were performed to estimate a percentage of participant serum concentrations that could be attributed to indoor exposure pathways other than drinking water and diet. The included studies were evaluated using an approach modified from the IRIS Handbook (U.S. EPA, 2022c). This systematic review provided evidence for an estimated range of indoor exposure media's contribution to serum PFAS concentrations and highlighted the limited availability of concordant measurement data from indoor exposure media and participant serum.

The Deluca and coworkers review (2022a) described above focused on indoor pathways and therefore excluded non-indoor pathways such as surface water or soil. Ninety-seven articles fell into this excluded group (i.e., PFOA was measured in a non-indoor environmental medium). These 97 papers were reviewed for this effort, though are not fully described in this appendix.

G.2.2 Evidence Mapping

Holder et al. (2023) investigated evidence for important pathways of exposure to PFAS by reviewing literature reporting measured occurrence of PFAS in exposure media. The review focused on eight PFAS (PFOA, PFOS, PFBA, PFBS, PFDA, PFHxA, PFHxS, and PFNA) and their real-world occurrences primarily in human matrices and media commonly related to human exposure (outdoor and indoor air, indoor dust, drinking water, food, food packaging, articles and products, and soil). The initial review identified 3,622 peer-reviewed papers matching these criteria that were published between 2003 and 2020. ICF's Litstream® software was used to conduct title-abstract (TiAb) and full-text screening, and to extract relevant primary data into a comprehensive evidence database. Parameters of interest included: sampling dates and locations (focused on locations in the United States, Canada, and Europe), numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics.

Detailed data on PFAS occurrence in high-priority household and environmental media from 210 studies were extracted, as well as limited data on human matrices from 422 additional papers. Published studies of PFAS occurrence became numerous after about 2005 and were most abundant for PFOA and PFOS. Co-measurements for PFAS occurrence in human matrices plus other media, while relatively infrequent, were typically for occurrence in food and drinking water. Most studies found detectable levels of PFAS, and half or more of the limited studies of indoor air and products detected PFAS in 50% or more of their samples. Levels of PFOA in these media ranged widely.

Literature search results were categorized into seven types of exposure pathway categories, including environmental media, home products/articles/building materials, cleaning products, food packaging, personal care products, clothing, and specialty products. The environmental media pathway category included the sub-categories of food, water, air, dust, soil, wastewater, and landfill. The identified studies were reviewed for this effort, though are not fully described in this appendix.

G.3 Summary of Potential PFOA Sources

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 (NLM, 2022). PFOA has been used in flame repellents, cosmetics, paints, polishes, and processing aids used in the manufacture of non-stick coatings on cookware. It is one of a large group of perfluoroalkyl substances that are used in consumer and industrial products to improve their resistance to stains, grease, and water. 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 PFNA and longer-chain perfluorinated carboxylic acids (PFCA), by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (USEPA, 2021c). Despite the United States phase out of production, there is widespread PFOA contamination in water, sediments, and soils in the reported literature. Exposure to PFOA can also occur through food, including fish and shellfish, house dust, air, and contact with consumer products.

G.3.1 Dietary Sources

Ingestion of food is a potentially significant source of exposure to PFOA and is often claimed to be the dominant source of exposure based on early studies that modeled the relative contributions of various sources among the general populations of North America and Europe (Fromme et al., 2009; Vestergren and Cousins, 2009; Trudel et al., 2008). The exposure among adults is typically estimated to be about 2–3 ng/kg/day (Gleason et al., 2017). The dominance of the food ingestion pathway is attributed to bioaccumulation in food from environmental emissions, relatively large amounts of foods being consumed, and high gastrointestinal uptake (Trudel et al., 2008). However, the estimates are highly uncertain because of analytical methods with poor sensitivity, relatively few food items with detectable levels, and levels that can vary greatly depending on sources or location (Gleason et al., 2017).

There is currently no comprehensive, nationwide total diet study (TDS) for PFOA that can be used to draw conclusions about the occurrence and potential risk of PFOA in the U.S. food supply for the general population. In 2021, the U.S. Food and Drug Administration (FDA) released PFAS testing results from their first survey of nationally distributed processed foods, including several baby foods, collected for the TDS. Results of the survey showed that 164 of the 167 foods tested had no detectable levels of the PFAS measured. Three food samples had detectable levels of PFAS, but not including PFOA: fish sticks (PFOS and PFNA), canned tuna (PFOS and PFDA), and protein powder (PFOS). PFOA was not detected in any of the food samples analyzed in FDA TDS samples of produce, meats, dairy and grain products in 2019 or 2021 (FDA, 2021). In a 2018 focused study near a PFAS production plant in the Fayetteville, North Carolina area, PFOA was detected in several produce samples (cabbage, collard greens, kale, mustard greens, Swiss chard, and lettuce) (FDA, 2018). The sample size in all of these studies was limited, and thus, the results cannot be used to draw definitive conclusions about the general levels of PFAS in the U.S. food supply (FDA, 2021). In a 2010 study, PFOA was detected in food samples collected from five grocery stores in Texas (Schecter et al., 2010); given the results from this study and on dietary intakes from the 2007 U.S. Department of

Agriculture food availability dataset, the estimated daily exposure to PFOA per capita was 60 ng/day (U.S. EPA, 2016a).

As a component of a scientific evaluation on the risks to human health related to PFAS in food, the European Food Safety Authority (EFSA) conducted an exposure assessment using consumption data from the EFSA Comprehensive Food Consumption Database and 69,433 analytical results for 26 PFAS in 1,528 samples of food and beverages obtained from 16 European countries (EFSA, 2020). Samples were collected between the years 2000 and 2016 (74% after 2008), mainly from Norway, Germany, and France. With 92% of the analytical results below the limit of detection (LOD) or limit of quantitation (LOQ), lower bound dietary exposure estimates were obtained by assigning zero to values below LOD/LOQ. Median chronic dietary exposures of PFOA for children and adults were estimated as 0.30 and 0.18 ng/kg body weight per day, respectively. The most important contributor was "Fish and other seafood," followed by "Eggs and egg products," "Meat and meat products," and "Fruit and fruit products." "Vegetables and vegetable products" were also found to be important contributors to dietary PFOA exposure. It is unclear whether or not the contribution from food contact material is reflected in the data. The authors determined diet to be the major source of PFAS exposure for most of the population but noted that dust ingestion and indoor air inhalation may provide substantial contributions for some individuals.

The 2020 EFSA report highlighted a recent study of aggregate exposure to PFAS from diet, house dust, indoor air, and dermal contact among Norwegian adults (Poothong et al., 2020). Dietary exposures were estimated for 61 study participants using food diaries and data on concentrations from an extensive Norwegian database of concentrations in 68 different food and drinks (including drinking water). For PFOA, the authors concluded that dietary intake was by far the greatest contributor to aggregate exposure (contributing 92% of total estimated PFOA intake), but intake from ingestion of house dust represented the dominant pathway for some of the top 20% most highly exposed individuals. On average, measured serum concentrations of PFOA were similar to modeled concentrations based on intakes. It is notable that while the authors reported significant positive correlations between PFOA concentrations in serum and estimated intakes based on surface dust and vacuum cleaner bag dust samples, correlations with estimated dietary intakes were not significant, which the authors attributed to temporal variations in dietary intakes over several years. While the authors did not separately quantify intake from food and drinking water, an earlier article from the same research group (Papadopoulou et al., 2017) reported measured concentrations in duplicate diets with median estimated intake of PFOA approximately three times higher from solid food than from liquids.

EPA's *Emerging Issues in Food Waste Management Persistent Chemical Contaminants* (U.S. EPA, 2021b) further describes global PFOA and other PFAS occurrence in food items, waste, and compost, as well as food contact materials, which are discussed below (Section G.3.1.2).

G.3.1.1 Fish and Shellfish

EPA collaborates with federal agencies, states, Tribes, and other partners to conduct freshwater fish contamination studies as part of a series of statistically based surveys to produce information on the condition of U.S. lakes, streams, rivers, and coastal waters. PFOA has been detected in freshwater fish fillet samples collected during several national studies in rivers and the Great

Lakes; however, PFOA is reported at a lower frequency and at lower levels compared with other PFAS, including PFOS (Table G-1).

Table G-1. Summary of EPA National Freshwater Fish Tissue Monitoring Results for PFOA

Reference	Most Commonly Sampled Species	Site Description	Results
U.S. EPA (2010)	Smallmouth bass Largemouth bass Channel catfish	162 urban river sites across the United States	No PFOA detections reported.
U.S. EPA (2015)	Largemouth bass Smallmouth bass Black crappie White crappie Walleye/sauger Yellow perch White bass Northern pike Lake trout Brown trout Rainbow trout Brook trout	349 urban and nonurban river sites across the United States	PFOA detected in 4% of samples. Maximum detected concentration 0.27 ng/g.
U.S. EPA (2011a)	Lake trout Smallmouth bass Walleye	157 nearshore sites along the U.S. shoreline of the Great Lakes	PFOA detected in 12% of samples. Maximum detected concentration 0.97 ng/g.
U.S. EPA (2016d)	Freshwater drum Longnose sucker White sucker Lake whitefish Northern pike Channel catfish Burbot Smallmouth bass White perch White bass Coho salmon Rainbow trout Chinook salmon Yellow perch Brown trout Lake trout Walleye	152 nearshore sites along the U.S. shoreline of the Great Lakes	PFOA detected in 14% of samples. Maximum detected concentration 1.93 ng/g.

Notes: U.S. = United States.

In addition, there are several available studies that assess PFAS concentrations in fish, shellfish, and other aquatic species. In 2015, Penland et al. (2020) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin – Pee Dee River in North Carolina and South Carolina. PFOA was detected in whole-body tissues of unionid mussels (7.41 ng/g wet weight) and aquatic insects (10.68 ng/g wet weight), but was not detected in Asian clam, snails, or crayfish. PFOA was measured in muscle tissue of 2/11 sampled fish species: the channel catfish (21.19 ng/g wet weight) and notchlip redhorse (45.66 ng/g wet weight).

Zafeiraki et al. (2019) analyzed about 250 samples of marine fish, farmed fish, crustaceans, bivalves and European eel, caught in Dutch waters or purchased at Dutch markets between 2012 and 2018. Samples were analyzed for 16 PFAS, including PFOA. Brown crab and shrimps had the highest average concentrations of PFOA (0.78 and 0.43 ng/g ww, respectively). PFOA was also detected in farmed fish including eel and trout, and marine fish species including cod, haddock, and sole. However, the PFAS with generally the highest percent detection and average concentration in all sample types was PFOS.

In seafood samples collected for FDA 2021–2022 seafood survey, Young et al. (2022), analyzed concentrations of 20 PFAS, including PFOA, in eight of the most highly consumed seafood products in the United States. PFOA was detected most frequently (100% of samples; n = 10) and at the highest average concentrations (8,334 ppt) in clams and was also detected in 100% of crab samples (n = 11; 300.9 ppt average concentration). The study reported detections in cod (20% of samples; n = 10; 103.5 ppt average concentration in samples with detections). PFOA was not detected above the method detection limit (90 or 68 ppt) in tuna, salmon, shrimp, tilapia, or pollock.

In summary, PFOA has been detected in fish and shellfish samples from freshwater and marine fish and shellfish, as well as in both farmed and wild-caught samples. While most of the data were collected from freshwater fish samples, recent studies suggest ingestion of many types of fish and shellfish can be a potential source of exposure to PFOA. However, PFOA concentrations in biotic media tend to be low, or below detection levels, highlighting the relatively low bioaccumulation potential for this chemical compared with other PFAS, such as PFOS. In addition, trophic biomagnification is rarely observed in aquatic food webs with PFOA.

G.3.1.2 Food Contact Materials

FDA has authorized the use of PFAS in food contact substances because of their non-stick and grease, oil, and water-resistant properties since the 1960's. There are four categories of products that may contain PFAS:

- "Non-stick cookware: PFAS may be used as a coating to make cookware non-stick.
- Gaskets, O-Rings, and other parts used in food processing equipment: PFAS may be used as a resin in forming certain parts used in food processing equipment that require chemical and physical durability.
- Processing aids: PFAS may be used as processing aids for manufacturing other food contact polymers to reduce buildup on manufacturing equipment.
- Paper/paperboard food packaging: PFAS may be used as grease-proofing agents in fast-food wrappers, microwave popcorn bags, take-out paperboard containers, and pet food bags to prevent oil and grease from foods from leaking through the packaging." (FDA, 2020)

Paper products used for food packaging are often treated with PFAS for water and grease resistance. In previous testing, sandwich wrappers, french-fry boxes, and bakery bags were all found to contain PFAS (Schreder and Dickman, 2018). Older generation PFAS (e.g., PFOA, PFOS) were manufactured and used in products for decades, and the bulk of the information available on PFAS toxicity relates to the older compounds. However, because newer-generation PFAS are more mobile than their predecessors, they migrate more readily into food. In 2016,

FDA deauthorized the remaining uses of long-chain "C8" PFAS in food packaging, which are therefore, no longer used in food contact applications sold in the United States (FDA, 2020).

Under FDA rules, there are dozens of PFAS chemicals still approved for food contact materials. In 2018, Safer Chemicals Healthy Families and Toxic-Free Future co-published a report of study in which 78 samples of food packaging, including take-out containers and deli or bakery paper, among others, were collected from 20 stores in 12 states (Schreder and Dickman, 2018). An independent laboratory tested the samples for fluorine. The utility of measuring fluorine content is limited because it does not allow for identification and quantification of individual PFAS; however, this method can be used to determine if a food-packaging material has been treated with PFAS. Over 10% of 78 samples tested contained PFAS. The sample size was not large enough to indicate how widespread the use of PFAS in food packaging is at this time. However, the study demonstrated that PFAS in food packaging is still a concern, especially for fiber bowls and trays.

Several other relatively recent studies found PFAS in fast-food packaging collected in the United States, China, or Europe (Zabaleta et al., 2020; Schaider et al., 2017; Yuan et al., 2016). The data from the cited and other publications likely contributed to the recent regulatory actions of FDA and several states to ban or restrict the presence of PFAS in food contact materials (Keller & Heckman LLP, 2021). Schaider et al. (2017) collected 407 samples of food contact papers, beverage containers, and paperboard boxes from locations throughout the United States. As was the case with the Schreder and Dickman (2018) report, inorganic fluoride was the analyte for the initial analysis. Fifty-six percent of the dessert and bread wrappers were positive for fluoride, 38% of the sandwich and burger wrappers, and 20% of the paperboard containers. None of the 30 (hot/cold) paper beverage cups tested positive in contrast to 16% of beverage containers (milk/juice) made from other materials. Generally, food contact papers had higher fluoride detection frequencies than food contact paperboard. Twenty fast-food packaging samples of the 407 total samples were selected for more extensive PFAS-specific analysis. PFOA, PFHxA, and PFBS were among the PFAS with the highest detection rates; PFOA was detected in 6/20 samples.

An analysis of popcorn bags, snack bags, and sandwich bags purchased in 2018 from international vendors and grocery stores in the United States found little evidence of PFOA, with only two popcorn bags with content above the limit of quantitation of 5.11 ng/g of paper (Monge Brenes et al., 2019). The authors presented these results as evidence of a reduction in PFOA concentrations in microwave packaging between 2005 and 2018. In an analysis of microwave popcorn bags from around the world, Zabaleta et al. (2017) reported no measurable concentrations of PFOA in the two bags from the United States, levels typically at about 4 ng/g in those from several European countries, and levels around 50 ng/g in bags from China.

Yuan et al. (2016) analyzed 25 food contact materials purchased in Columbus, Ohio, for PFAS as compared with 69 products purchased in China. The primary PFAS substances detected were consistently the C6 to C14 telomer alcohols. In food-packaging materials from China, of the 15 detected perfluorinated carboxylic acids, PFOA was the most frequently detected (90%) and was detected with the highest median concentration (1.72 ng/g). In contrast to the products from China, the primary analyte from U.S. paper food contact products other than popcorn bags was the 6:2 telomer alcohol. The authors also report a migration efficiency of PFOA from paper bowl packaging into food stimulants of 1.58%. This is a relatively low efficiency compared with

several of the fluorotelomer alcohols (FTOHs), which the authors reported to migrate with greater than 90% efficiency.

Zabaleta et al. (2020) looked at PFAS in 25 paper and paperboard packaging materials primarily collected in Spain. Except in the single microwave popcorn bag collected from China, none of the perfluorocarboxylic acids (C3, C6, C7, C8, C 9, C10), including PFOA, were above the level of detection. The packaging materials with the largest number of detectable analytes was a popcorn bag from China and the inside paper lining from three individual pet food products, which contained a spectrum of C3 to C10 perfluorinated carboxylates. Zabaleta et al. (2020) also monitored migration of the PFAS carboxylates (C6 to C10) from packaging materials into cereal, rice, or milk. For each PFAS studied, the percent migration to milk exceeded that to rice with the lowest percent migration being that to cereal. Percent migration to foods decreased as the carbon chain length increased (C6 to C10) after a 6-month period. The migration percentage of PFOA into cereal, rice, and milk powder products over 6 months ranged from 1.4% to 5.6%.

G.3.2 Consumer Product Uses

A targeted analysis of 29 U.S. and Canadian cosmetic products with high fluorine content (Whitehead et al., 2021) found high concentrations of FTOH, including 8:2 FTOH, commonly present in the formulations. A fraction of 8:2 FTOH is believed to undergo metabolic transformation into PFOA. In addition to direct contact with personal care products, products and articles (and the use of these) may be sources in the indoor environment that manifest as measured occurrence in house dust and indoor air. An earlier investigation of consumer exposure to PFOA by Trudel et al. (2008) used mechanistic modeling together with information on product-use habits to estimate oral and dermal exposures from clothes, carpet, upholstery, and food contact materials. Noting that PFOA may be contained as a contaminant in older and in new products, the authors estimated exposure via both mill-treated and home-treated carpets. The authors concluded that contact with consumer products is not a significant contributor to total exposure, but that since PFOA may be a contaminant in even new products, consumer exposure may continue to occur, particularly via both mill-treated and home-treated carpets. The authors also point out that carpet and other textiles are likely to be continuing sources of PFOA in house dust. In contrast, in an analysis of 116 articles of commerce from the United States, U.S. EPA (2009) identified carpets and related products as potentially the most significant source of PFCA out of 13 total product categories analyzed. PFOA was detected in all 13 product types. Other important indoor sources of PFCA include floor wax/sealant and home textiles, upholstery, and apparel. In a similar analysis of 52 European products collected between 2014 and 2016, Borg and Ivarrson (2017) reported that PFOA was the most commonly detected PFAS and was detected in all samples except those that did not contain any detectable levels of PFAS. Notably, the authors specifically targeted products that were known or suspected to contain PFAS in their analyses.

Liu et al. (2014) investigated trends in PFAS content of household goods between 2007 and 2011. They reported that while PFOA concentrations displayed an overall downward trend with significant reductions observed in nearly all product categories, PFOA was still detected in many products. Kotthoff et al. (2015) similarly reported broad detection of PFOA in a 2010 sampling effort that collected 115 European consumer products, including carpets, leather, outdoor materials, cooking materials, and others. PFOA was detected in all but one sample type, often at

the highest median concentration compared with other PFCA. FTOHs were frequently detected at the highest median concentration overall. The products with the highest concentrations of total PFAS included ski wax (median concentration of 15.5 µg/kg PFOA), leather products (median concentration of 12.4 µg/m^2), and outdoor materials (median concentration of 6 µg/m^2 PFOA). PFOA has also been detected in textile samples of outdoor apparel from Europe and Asia (van der Veen et al., 2020; Gremmel et al., 2016). PFOA was detected in jackets ranging from concentrations of 0.02–4.59 µg/m^2 (Gremmel et al., 2016). Interestingly, the level of almost all individual PFAS, including PFOA, and total PFAS increased when the textiles were subjected to weathering (i.e., increased ultraviolet (UV) radiation, temperature, and humidity for 300 hours to mimic the average lifespan of outdoor apparel) (van der Veen et al., 2020).

G.3.3 Indoor Dust

Several studies suggest that PFOA and its precursors in indoor air and/or house dust may be an important exposure source for some individuals (Poothong et al., 2020; Gebbink et al., 2015; Schlummer et al., 2013; Shoeib et al., 2011). PFOA is generally a dominant ionic PFAS constituent in indoor air and dust, frequently occurring above detection limits and at relatively high concentrations in all or most samples (Poothong et al., 2020; Kim et al., 2019; Byrne et al., 2017; Makey et al., 2017; Wu et al., 2014; Fraser et al., 2013; Shoeib et al., 2011).

PFOA was measured at the highest concentrations (geometric mean concentrations ranging from 41.4 to 45.0 ng/g) and frequencies (ranging from 89% to 91% detected) in dust sampled from Californian households (Wu et al., 2014). Similarly, PFOA was found at the second highest levels (mean concentration of 1.98 ng/g) of 15 PFAS measured in dust samples taken from households in Seoul, South Korea (Kim et al., 2019). PFOA was detected in all dust samples from that study. Makey et al. (2017) measured PFOA and PFOA precursors in dust and found weak correlations between concentrations in dust and serum PFOA concentrations in pregnant Canadian participants. One study in Alaska Natives found no correlation between dust and serum PFOA concentrations (Byrne et al., 2017).

G.3.4 Ambient Air

Perfluoroalkyl chemicals have been found in ambient air globally, with the highest concentrations observed or expected in urban areas and nearest to industrial facilities, areas where firefighting aqueous film-forming foams are used, wastewater treatment plants, waste incinerators, and landfills (Ahrens et al., 2011). Perfluorinated acids were measured in Albany, New York, air samples (gas mean concentration of 3.16 pg/m^3 and particulate phase mean concentration of 2.03 pg/m^3) (Kim and Kannan, 2007). In Minneapolis, Minnesota, PFOA in the particulate phase ranged from 1.6 to 5.1 pg/m^3 and from 1.7 to 16.1 pg/m^3 in the gas phase (MPCA, 2008). Even remote areas far from urban centers have previously reported PFOA concentrations in air samples: PFOA has been detected in Resolute Bay, Nunavut, Canada (Stock et al., 2007), as well as other Arctic environments (Butt et al., 2010).

PFOA is not listed as a hazardous air pollutant under the Clean Air Act. However, two states (New York and Michigan) have set enforceable air emissions limits. Ambient air is a possible source of exposure to PFOA for the general population; however, the contribution of air to total exposure is likely low. For example, De Silva et al. (2021) estimated that <1% of PFOA exposure to humans in the United States is from inhalation.

G.3.5 Other Exposure Considerations

PFOA has been detected in soils and dust from carpets and upholstered furniture in homes, offices, and vehicles. Incidental exposure from soils and dust is an important exposure route, particularly for small children because of their increase level of hand-to-mouth behaviors compared with adults. Also, the levels in soils and surface waters can affect the concentrations in local produce, meat/poultry, dairy products, fish, and particulates in the air.

G.4 Recommended Relative Source Contribution

EPA followed the Exposure Decision Tree approach to determine the RSC for PFOA, as outlined in Figure G-1 (U.S. EPA, 2000). EPA first identified several potential populations of concern (Box 1): pregnant women and their developing fetuses, infants, children, lactating women, and women of childbearing age. However, limited information was available regarding specific exposure of these populations to PFOA in different environmental media. EPA considered exposures in the general U.S. population as likely applying to the majority of these populations. Second, EPA identified several relevant PFOA exposures and pathways (Box 2), including dietary consumption, incidental oral, inhalation, or dermal exposure via dust, consumer products, and soil, and inhalation exposure via ambient air. Several of these may be potentially significant exposure sources. Third, EPA determined that there were inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, studies from the United States, Canada and Europe indicate that consumer products may be significant sources of exposure to PFOA. Although several studies report PFOA detections in consumer products, most examined very few samples (i.e., n = 1−5) of only a few types of media. Therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, the studies summarized in the sections above suggest there are significant known or potential uses/sources of PFOA other than drinking water (Box 6), although information is not available on each source to make a characterization of exposure (Box 8A). For example, there are several studies from the U.S., Canada, and Europe indicating that PFOA may occur in multiple food products (e.g., eggs, seafood, meats, vegetables, fruit). However, similar to studies on consumer products, the majority of studies examined very few samples (i.e., n = 1−5) of various food products and a nationally representative TDS does not exist. There are also uncertainties regarding how PFOA concentrations in all media, including food may decline with the implementation of the PFOA Stewardship Program. Therefore, it is not possible to determine whether food or other types of media can be considered a major or minor contributor to total PFOA exposure. Given these

considerations, following recommendations of the Exposure Decision Tree (U.S. EPA, 2000), EPA recommends an RSC of 20% (0.20) for PFOA.

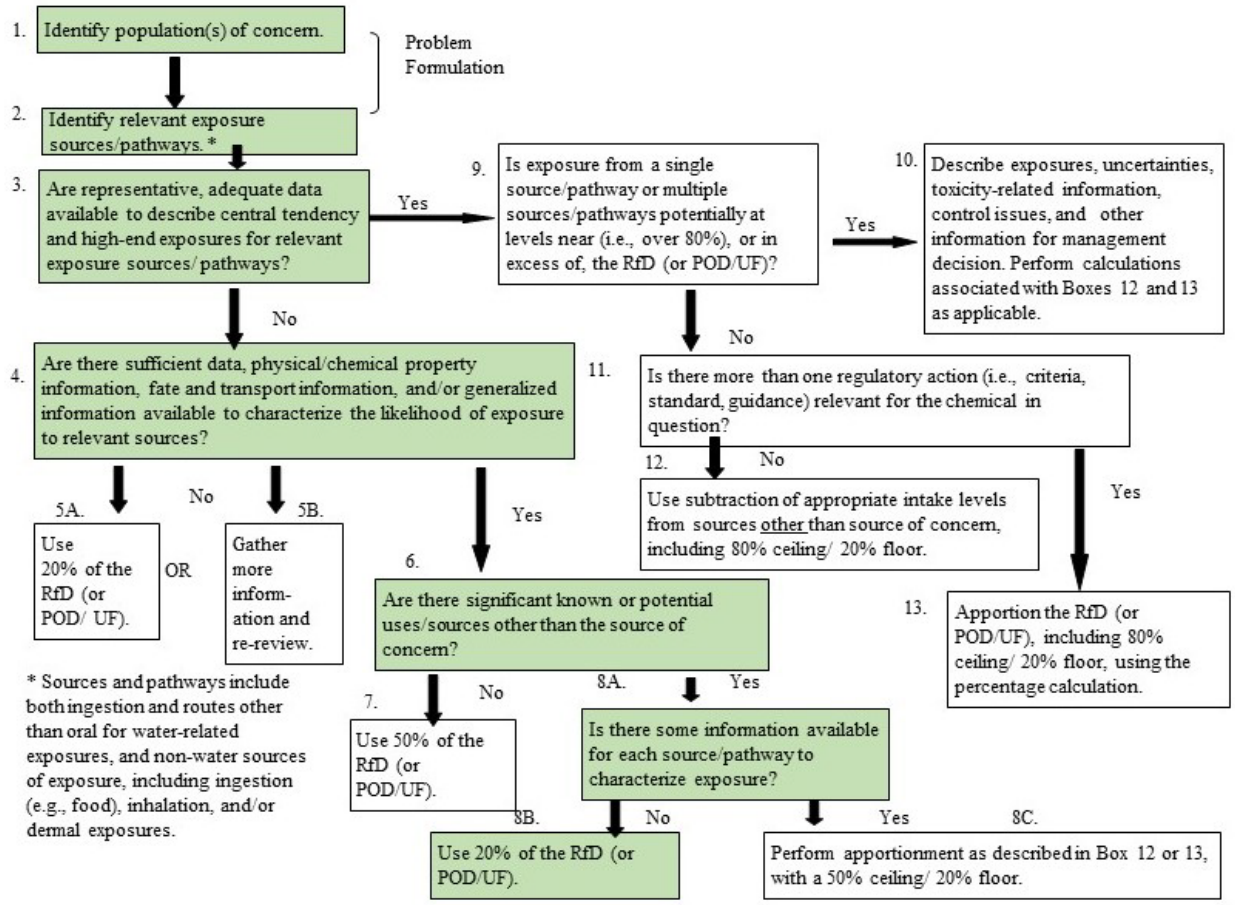


Figure G-1. Application of the Exposure Decision Tree (U.S. EPA, 2000) for PFOA

Green highlighted boxes indicate selections made at each branch of the Decision Tree.
POD = point of departure; RfD = reference dose; UF = uncertainty factor.

References

3M. (2000). Determination of serum half-lives of several fluorochemicals, Interim report #1, June 8, 2000 [TSCA Submission]. In TSCA 8(e) Supplemental Notice for Sulfonate-based and Carboxylic-based Fluorochemicals-DocketNumbers 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381- 0394; 8EHQ-0598-373. (8EHQ-80-373. 8EHQ-0302-00373. 89(811844Q). AR226-0611).

3M. (2002). Determination of serum half-lives of several fluorochemicals, Interim report #2, January 11, 2002 [TSCA Submission]. In TSCA 8(e) Supplemental Notice for Sulfonate-based and Carboxylic-based Fluorochemicals-DocketNumbers 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381- 0394; 8EHQ-0598-373. (8EHQ-80-373. 8EHQ-0302-00373. 89(811844Q). AR226-1086).

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

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.

Agier, L; Basagaña, X; Maitre, L; Granum, B; Bird, PK; Casas, M, et al. (2019). Early-life exposome and lung function in children in Europe: an analysis of data from the longitudinal, population-based HELIX cohort. The Lancet Planetary Health 3: e81-e92.

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

Ahrens, L; Shoeib, M; Harner, T, om; Lee, S; Guo, R, ui; Reiner, EJ. (2011). Wastewater Treatment Plant and Landfills as Sources of Polyfluoroalkyl Compounds to the Atmosphere. Environmental Science and Technology 45: 8098-8105. http://dx.doi.org/10.1021/es1036173

Aimuzi, R; Luo, K; Chen, Q; Wang, H; Feng, L; Ouyang, F; Zhang, J. (2019). Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environment International 130: 104929.

Aimuzi, R; Luo, K; Huang, R; Huo, X; Nian, M; Ouyang, F, et al. (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environmental Pollution 264: 114557.

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.

Alderete, TL; Jin, R; Walker, DI; Valvi, D; Chen, Z; Jones, DP, et al. (2019). Perfluoroalkyl substances, metabolomic profiling, and alterations in glucose homeostasis among overweight and obese Hispanic children: A proof-of-concept analysis. Environment International 126: 445-453.

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

Andersson, AG; Lundgren, A; Xu, Y; Nielsen, C; Lindh, CH; Pineda, D, et al. (2023). High Exposure to Perfluoroalkyl Substances and Antibody Responses to SARS-CoV-2 mRNA Vaccine-an Observational Study in Adults from Ronneby, Sweden. Environmental Health Perspectives 131: 87007. http://dx.doi.org/10.1289/EHP11847

Anzai, N; Kanai, Y; Endou, H. (2006). Organic anion transporter family: current knowledge. 100: 411-426. http://dx.doi.org/10.1254/jphs.crj06006x

Apelberg, BJ; Witter, FR; Herbstman, JB; Calafat, AM; Halden, RU; Needham, LL; Goldman, LR. (2007). 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, TE; Macpherson, S; Foster, WG; Sathyanarayana, S; Fisher, M; Monnier, P, et al. (2020). Prenatal Perfluoroalkyl Substances and Newborn Anogenital Distance in a Canadian Cohort. Reproductive Toxicology 94: 31-39.

Arrebola, JP; Ramos, JJ; Bartolomé, M; Esteban, M; Huetos, O; Cañas, AI, et al. (2019). Associations of multiple exposures to persistent toxic substances with the risk of hyperuricemia and subclinical uric acid levels in BIOAMBIENT.ES study. Environment International 123: 512-521.

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.

Ashley-Martin, J; Dodds, L; Arbuckle, TE; Morisset, AS; Fisher, M; Bouchard, MF, et al. (2016). Maternal and Neonatal Levels of Perfluoroalkyl Substances in Relation to Gestational Weight Gain. International Journal of Environmental Research and Public Health 13: 146.

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

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.

Attema, B; Janssen, AWF; Rijkers, D; van Schothorst, EM; Hooiveld, GJEJ; Kersten, S. (2022). Exposure to low-dose perfluorooctanoic acid promotes hepatic steatosis and disrupts the hepatic transcriptome in mice. Molecular metabolism 66: 101602. http://dx.doi.org/10.1016/j.molmet.2022.101602

Avanasi, R; Shin, HM; Vieira, VM; Bartell, SM. (2016a). Impacts of geocoding uncertainty on reconstructed PFOA exposures and their epidemiological association with preeclampsia. Environmental Research 151: 505-512.

Avanasi, R; Shin, HM; Vieira, VM; Bartell, SM. (2016b). 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.

Aylward, LL; Hays, SM; Kirman, CR; Marchitti, SA; Kenneke, JF; English, C, et al. (2014). Relationships of chemical concentrations in maternal and cord blood: a review of available data [Review]. Journal of Toxicology and Environmental Health, Part B: Critical Reviews 17: 175-203. http://dx.doi.org/10.1080/10937404.2014.884956

Bach, C; Matthiesen, B; Olsen; Henriksen, B. (2018). Conditioning on parity in studies of perfluoroalkyl acids and time to pregnancy: an example from the danish national birth cohort. Environmental Health Perspectives 126: 117003.

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.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bossi, R, et al. (2015). Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. Environmental Research 142: 535-541.

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; 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

Batzella, E; Girardi, P; Russo, F; Pitter, G; Da Re, F; Fletcher, T; Canova, C. (2022a). Perfluoroalkyl substance mixtures and cardio-metabolic outcomes in highly exposed

male workers in the Veneto Region: A mixture-based approach. Environmental Research 212: 113225. http://dx.doi.org/10.1016/j.envres.2022.113225

Batzella, E; Zare Jeddi, M; Pitter, G; Russo, F; Fletcher, T; Canova, C. (2022b). Associations between Mixture of Perfluoroalkyl Substances and Lipid Profile in a Highly Exposed Adult Community in the Veneto Region. International Journal of Environmental Research and Public Health 19. http://dx.doi.org/10.3390/ijerph191912421

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.

Beesoon, S; Webster, GM; Shoeib, M; Harner, T, om; Benskin, JP; Martin, JW. (2011). Isomer Profiles of Perfluorochemicals in Matched Maternal, Cord, and House Dust Samples: Manufacturing Sources and Transplacental Transfer. Environmental Health Perspectives 119: 1659-1664. http://dx.doi.org/10.1289/ehp.1003265

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

Berg, V; Nøst, TH; Hansen, S; Elverland, A; Veyhe, AS; Jorde, R, et al. (2015). Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environment International 77: 63-69. http://dx.doi.org/10.1016/j.envint.2015.01.007

Berk, M; Williams, LJ; Andreazza, A; Pasco, JA; Dodd, S; Jacka, FN, et al. (2014). Pop, heavy metal and the blues: secondary analysis of persistent organic pollutants (POP), heavy metals and depressive symptoms in the NHANES National Epidemiological Survey. British Medical Journal Open 4: e005142.

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

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.

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.

Blake, BE; Pinney, SM; Hines, EP; Fenton, SE; Ferguson, KK. (2018). Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. Environmental Pollution 242: 894-904.

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

Bloom, MS; Kannan, K; Spliethoff, HM; Tao, L; Aldous, KM; Vena, JE. (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. Physiology & Behavior 99: 240-245. http://dx.doi.org/10.1016/j.physbeh.2009.02.005

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-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

Borg, D; Ivarsson, J. (2017). Analysis of PFASs and TOF in Products. (TemaNord 2017:543). Nordic Council of Ministers. https://norden.diva-portal.org/smash/get/diva2:1118439/FULLTEXT01.pdf

Borghese, MM; Liang, CL; Owen, J; Fisher, M. (2022). Individual and mixture associations of perfluoroalkyl substances on liver function biomarkers in the Canadian Health Measures Survey. Environmental Health 21: 85. http://dx.doi.org/10.1186/s12940-022-00892-6

Borghese, MM; Walker, M; Helewa, ME; Fraser, WD; Arbuckle, TE. (2020). Association of perfluoroalkyl substances with gestational hypertension and preeclampsia in the MIREC study. Environment International 141: 105789.

Braun, JM; Chen, A; Romano, ME; Calafat, AM; Webster, GM; Yolton, K; Lanphear, BP. (2016). Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity 24: 231-237.

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.

Brede, E; Wilhelm, M; Göen, T; Müller, J; Rauchfuss, K; Kraft, M; Hölzer, J. (2010). Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. International Journal of Hygiene and Environmental Health 213: 217-223. http://dx.doi.org/10.1016/j.ijheh.2010.03.007

Breusch, TS; Pagan, AR. (1979). A simple test for heteroscedasticity and random coefficient variation. 47: 1287-1294. http://dx.doi.org/10.2307/1911963

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, CO; Eliot, MN; Kelsey, KT; Calafat, AM; Chen, A; Ehrlich, S, et al. (2018). Prenatal exposure to perfluoroalkyl substances and adipocytokines: the HOME Study. Pediatric Research 84: 854-860.

Buck Louis, GM; Chen, Z; Schisterman, EF; Kim, S; Sweeney, AM; Sundaram, R, et al. (2015). Perfluorochemicals and human semen quality: The LIFE Study. Environmental Health Perspectives 123: 57-63. http://dx.doi.org/10.1289/ehp.1307621

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

Buck, RC; Korzeniowski, SH; Laganis, E; Adamsky, F. (2021). Identification and classification of commercially relevant per- and poly-fluoroalkyl substances (PFAS). Integrated Environmental Assessment and Management 17: 1045–1055. http://dx.doi.org/10.1002/ieam.4450

Budtz-Jørgensen, E; Grandjean, P. (2018a). 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

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

Butt, CM; Berger, U; Bossi, R; Tomy, GT. (2010). Levels and trends of poly- and perfluorinated compounds in the arctic environment [Review]. Science of the Total Environment 408: 2936-2965. http://dx.doi.org/10.1016/j.scitotenv.2010.03.015

Byrne, S; Seguinot-Medina, S; Miller, P; Waghiyi, V; von Hippel, FA; Buck, CL; Carpenter, DO. (2017). Exposure to polybrominated diphenyl ethers and perfluoroalkyl substances in a remote population of Alaska Natives. Environmental Pollution 231: 387-395. http://dx.doi.org/10.1016/j.envpol.2017.08.020

Byrne, SC; Miller, P; Seguinot-Medina, S; Waghiyi, V; Buck, CL; von Hippel, FA; Carpenter, DO. (2018). Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska Natives. Environmental Research 166: 537-543.

C8 Science Panel. (2012). C8 study results - Status reports. Available online at http://www.c8sciencepanel.org/study_results.html

Cai, A; Portengen, L; Govarts, E; Martin, LR; Schoeters, G; Legler, J, et al. (2023). Prenatal exposure to persistent organic pollutants and changes in infant growth and childhood growth trajectories. Chemosphere 314: 137695. http://dx.doi.org/10.1016/j.chemosphere.2022.137695

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.

Cakmak, S; Lukina, A; Karthikeyan, S; Atlas, E; Dales, R. (2022). The association between blood PFAS concentrations and clinical biochemical measures of organ function and metabolism in participants of the Canadian Health Measures Survey (CHMS). Science of the Total Environment 827: 153900. http://dx.doi.org/10.1016/j.scitotenv.2022.153900

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, S; Raza, M; Pollack, AZ. (2016). Perfluoroalkyl substances and endometriosis in US women in NHANES 2003-2006. Reproductive Toxicology 65: 230-235.

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.

Cardenas, A; Gold, DR; Hauser, R; Kleinman, KP; Hivert, MF; Calafat, AM, et al. (2017). Plasma concentrations of per- and polyfluoroalkyl substances at baseline and associations with glycemic indicators and diabetes incidence among high-risk adults in the Diabetes Prevention Program trial. Environmental Health Perspectives 125: 107001.

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.

Caron-Beaudoin, É; Ayotte, P; Laouan Sidi, EA; Simon, CoL; Nation, CoWLPF; Nutashkuan, CTKo, et al. (2019). Exposure to perfluoroalkyl substances (PFAS) and associations with thyroid parameters in First Nation children and youth from Quebec. Environment International 128: 13-23.

Caserta, D; Ciardo, F; Bordi, G; Guerranti, C; Fanello, E; Perra, G, et al. (2013). Correlation of endocrine disrupting chemicals serum levels and white blood cells gene expression of nuclear receptors in a population of infertile women. International Journal of Endocrinology 2013: 510703.

Caserta, D; Pegoraro, S; Mallozzi, M; Di Benedetto, L; Colicino, E; Lionetto, L; Simmaco, M. (2018). Maternal exposure to endocrine disruptors and placental transmission: a pilot study. Gynecological Endocrinology 34: 1-4.

CDC. (2011). Tetanus surveillance --- United States, 2001-2008. MMWR Morbidity and Mortality Weekly Report 60: 365-369.

Cellesi, C; Michelangeli, C; Rossolini, GM; Giovannoni, F; Rossolini, A. (1989). Immunity to diphtheria, six to 15 years after a basic three-dose immunization schedule. Journal of Biological Standardization 17: 29-34. http://dx.doi.org/10.1016/0092-1157(89)90025-5

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

Chang, S; Parker, GA; Kleinschmidt, SE; Olsen, GW; Ley, CA; Taiwo, OA. (2020). A Pathology Review of the Lower Gastrointestinal Tract in Relation to Ulcerative Colitis in Rats and Cynomolgus Macaques Treated With Ammonium Perfluorooctanoate. Toxicologic Pathology192623320911606.

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

Chen, A; Jandarov, R; Zhou, L; Calafat, AM; Zhang, G; Urbina, EM, et al. (2019a). 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; Liao, HF; Jeng, SF; Su, YN; Wen, TW, et al. (2013). Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology 24: 800-808.

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, Q; Zhang, X; Zhao, Y; Lu, W; Wu, J; Zhao, S, et al. (2019b). Prenatal exposure to perfluorobutanesulfonic acid and childhood adiposity: A prospective birth cohort study in Shanghai, China. Chemosphere 226: 17-23.

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, J; Fujimura, M; Zhao, W; Wang, W. (2013). Neurobehavioral effects, c-Fos/Jun expression and tissue distribution in rat offspring prenatally co-exposed to MeHg and PFOA: PFOA impairs Hg retention. Chemosphere 91: 758-764. http://dx.doi.org/10.1016/j.chemosphere.2013.02.016

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. (2008). 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. (2009). Tissue distribution, ontogeny, and hormonal regulation of xenobiotic transporters in mouse kidneys. Drug Metabolism and Disposition 37: 2178-2185. http://dx.doi.org/10.1124/dmd.109.027177

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

Cheng, X; Wei, Y; Zhang, Z; Wang, F; He, J; Wang, R, et al. (2022). Plasma PFOA and PFOS Levels, DNA Methylation, and Blood Lipid Levels: A Pilot Study. Environmental Science & Technology 56: 17039-17051. http://dx.doi.org/10.1021/acs.est.2c04107

Christensen, KY; Maisonet, M; Rubin, C; Holmes, A; Calafat, AM; Kato, K, et al. (2011). Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. Environment International 37: 129-135. http://dx.doi.org/10.1016/j.envint.2010.08.007

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. (2016a). Perfluoroalkyl substances in older male anglers in Wisconsin. Environment International 91: 312-318.

Christensen, KY; Raymond, MR; Thompson, BA; Anderson, HA. (2016b). Fish consumption, levels of nutrients and contaminants, and endocrine-related health outcomes among older male anglers in Wisconsin. Journal of Occupational and Environmental Medicine 58: 668-675. http://dx.doi.org/10.1097/JOM.0000000000000758

Christenson, B; Böttiger, M. (1986). Serological immunity to diphtheria in Sweden in 1978 and 1984. Scandinavian Journal of Infectious Diseases 18: 227-233. http://dx.doi.org/10.3109/00365548609032331

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.

Cluett, R; Seshasayee, SM; Rokoff, LB; Rifas-Shiman, SL; Ye, X; Calafat, AM, et al. (2019). Per- and Polyfluoroalkyl Substance Plasma Concentrations and Bone Mineral Density in Midchildhood: A Cross-Sectional Study (Project Viva, United States). Environmental Health Perspectives 127: 87006.

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.

Collier, RJ. (1975). Diphtheria toxin: mode of action and structure. 39: 54-85. http://dx.doi.org/10.1128/br.39.1.54-85.1975

Conley, JM; Lambright, CS; Evans, N; Medlock-Kakaley, E; Dixon, A; Hill, D, et al. (2022). Cumulative maternal and neonatal effects of combined exposure to a mixture of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) during pregnancy in the Sprague-Dawley rat. Environment International 170: 107631. http://dx.doi.org/10.1016/j.envint.2022.107631

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.

Conway, B; Innes, KE; Long, D. (2016). Perfluoroalkyl substances and beta cell deficient diabetes. Journal of Diabetes and its Complications 30: 993-998.

Conway, BN; Badders, AN; Costacou, T; Arthur, JM; Innes, KE. (2018). Perfluoroalkyl substances and kidney function in chronic kidney disease, anemia, and diabetes. Diabetes, Metabolic Syndrome and Obesity 11: 707-716.

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

Costa, G; Sartori, S; Consonni, D. (2009). Thirty years of medical surveillance in perfluooctanoic 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

Cox, GG; Haigh, D; Hindley, RM; Miller, DJ; Moody, CJ. (1994). COMPETING O-H INSERTION AND BETA-ELIMINATION IN RHODIUM CARBENOID REACTIONS - SYNTHESIS OF 2-ALKOXY-3-ARYLPROPANOATES. Tetrahedron Letters 35: 3139-3142. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1201708C3 - 18

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.

Crissman, JW; Goodman, DG; Hildebrandt, PK; Maronpot, RR; Prater, DA; Riley, JH, et al. (2004). Best practices guideline: Toxicologic histopathology. Toxicologic Pathology 32: 126-131. http://dx.doi.org/10.1080/01926230490268756

Cropp, C, .D.; Komori, T, .; Shima, J, .E.; Urban, T, .J.; Yee, S, .W.; More, S, .S.; Giacomini, K, .M. (2008). Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. 73: 1151-1158. http://dx.doi.org/10.1124/mol.107.043117

Crump, KS. (1995). Calculation of benchmark doses from continuous data. Risk Analysis 15: 79-89. http://dx.doi.org/10.1111/j.1539-6924.1995.tb00095.x

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.

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.

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

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.

Daston, GP; Kimmel, CA. (1998). An evaluation and interpretation of reproductive endpoints for human health risk assessment. In An evaluation and interpretation of reproductive endpoints for human health risk assessment. Washington, DC: ILSI Press.

Davidkin, I; Jokinen, S; Broman, M; Leinikki, P; Peltola, H. (2008). Persistence of measles, mumps, and rubella antibodies in an MMR-vaccinated cohort: a 20-year follow-up. Journal of Infectious Diseases 197: 950-956. http://dx.doi.org/10.1086/528993

de Cock, M; de Boer, MR; Lamoree, M; Legler, J; van De Bor, M. (2014a). 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. (2014b). Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study. Environmental Health: A Global Access Science Source 13: 106. http://dx.doi.org/10.1186/1476-069X-13-106

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 Silva, AO; Armitage, JM; Bruton, TA; Dassuncao, C; Heiger-Bernays, W; Hu, XC, et al. (2021). PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding [Review]. Environmental Toxicology and Chemistry 40: 631-657. http://dx.doi.org/10.1002/etc.4935

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

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

Deluca, NM; Angrish, M; Wilkins, A; Thayer, K; Cohen Hubal, EA. (2022a). Human exposure pathways to poly- and perfluoroalkyl substances (PFAS) from indoor media: A systematic review protocol. Environment International 146: 106308. http://dx.doi.org/10.1016/j.envint.2020.106308

Deluca, NM; Minucci, JM; Mullikin, A; Slover, R; Cohen Hubal, EA. (2022b). Human exposure pathways to poly- and perfluoroalkyl substances (PFAS) from indoor media: A systematic review. Environment International 162: 107149. http://dx.doi.org/10.1016/j.envint.2022.107149

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.

Dhingra, R; Darrow, LA; Klein, M; Winquist, A; Steenland, K. (2016a). Perfluorooctanoic acid exposure and natural menopause: A longitudinal study in a community cohort. Environmental Research 146: 323-330.

Dhingra, R; Lally, C; Darrow, LA; Klein, M; Winquist, A; Steenland, K. (2016b). Perfluorooctanoic acid and chronic kidney disease: Longitudinal analysis of a Mid-Ohio Valley community. Environmental Research 145: 85-92.

Dhingra, R; Winquist, A; Darrow, LA; Klein, M; Steenland, K. (2017). A study of reverse causation: Examining the associations of perfluorooctanoic acid serum levels with two outcomes. Environmental Health Perspectives 125: 416-421.

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.

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.

Ding, N; Karvonen-Gutierrez, CA; Mukherjee, B; Calafat, AM; Harlow, SD; Park, SK. (2022). Per- and Polyfluoroalkyl Substances and Incident Hypertension in Multi-Racial/Ethnic Women: The Study of Women's Health Across the Nation. Hypertension 79: 101161HYPERTENSIONAHA12118809. http://dx.doi.org/10.1161/HYPERTENSIONAHA.121.18809

Ding, N; Park, SK. (2020). Perfluoroalkyl substances exposure and hearing impairment in US adults. Environmental Research 187: 109686.

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

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.

Domazet, SL; Jensen, TK; Wedderkopp, N; Nielsen, F; Andersen, LB; Grøntved, A. (2020). Exposure to perfluoroalkylated substances (PFAS) in relation to fitness, physical activity, and adipokine levels in childhood: The European youth heart study. Environmental Research 191: 110110.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Kiviranta, H, et al. (2019a). Perfluoroalkyl substances and risk of type II diabetes: A prospective nested case-control study. Environment International 123: 390-398.

Donat-Vargas, C; Bergdahl, IA; Tornevi, A; Wennberg, M; Sommar, J; Koponen, J, et al. (2019b). 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, 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.

Dreyer, AF; Jensen, RC; Glintborg, D; Schmedes, AV; Brandslund, I; Nielsen, F, et al. (2020). Perfluoroalkyl substance exposure early in pregnancy was negatively associated with late pregnancy cortisone levels. Journal of Clinical Endocrinology and Metabolism 105: E2834-E2844.

Duan, Y; Sun, H; Yao, Y; Meng, Y; Li, Y. (2020). Distribution of novel and legacy per-/polyfluoroalkyl substances in serum and its associations with two glycemic biomarkers among Chinese adult men and women with normal blood glucose levels. Environment International 134: 105295.

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.

Dufour, P; Pirard, C; Seghaye, MC; Charlier, C. (2018). Association between organohalogenated pollutants in cord blood and thyroid function in newborns and mothers from Belgian population. Environmental Pollution 238: 389-396.

Dunder, L; Lind, PM; Salihovic, S; Stubleski, J; Kärrman, A; Lind, L. (2022). Changes in plasma levels of per- and polyfluoroalkyl substances (PFAS) are associated with changes in plasma lipids - A longitudinal study over 10 years. Environmental Research 211: 112903. http://dx.doi.org/10.1016/j.envres.2022.112903

Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA; Gibbs, ST, et al. (2019). 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. (2020). Birth weight and perfluorooctane sulfonic acid: a random-effects meta-regression analysis. Environmental Epidemiology 4: e095. http://dx.doi.org/10.1097/EE9.0000000000000095

E, L; Zhang, S; Jiang, X. (2023). Association between perfluoroalkyl substances exposure and the prevalence of nonalcoholic fatty liver disease in the different sexes: a study from the National Health and Nutrition Examination Survey 2005-2018. Environmental Science and Pollution Research International 30: 44292-44303. http://dx.doi.org/10.1007/s11356-023-25258-4

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.

EFSA. (2020). Risk to human health related to the presence ofperfluoroalkyl substances in food. EFSA Journal 18. http://dx.doi.org/https://doi.org/10.2903/j.efsa.2020.6223

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; 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

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

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

Etzel, TM; Braun, JM; Buckley, JP. (2019). Associations of serum perfluoroalkyl substance and vitamin D biomarker concentrations in NHANES, 2003-2010. International Journal of Hygiene and Environmental Health 222: 262-269.

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, M; Curry, S; Terreno, E; Galliano, M; Fanali, G; Narciso, P, et al. (2005a). The extraordinary ligand binding properties of human serum albumin [Review]. IUBMB Life 57: 787-796. http://dx.doi.org/10.1080/15216540500404093

Fasano, WJ; Kennedy, GL; Szostek, B; Farrar, DG; Ward, RJ; Haroun, L; Hinderliter, PM. (2005b). 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. (2018). Analytical Results for PFAS in 2018 Produce Sampling (Parts Per Trillion). Retrieved from https://www.fda.gov/media/127848/download

FDA. (2020). Authorized Uses of PFAS in Food contact Applications. https://www.fda.gov/food/chemical-contaminants-food/authorized-uses-pfas-food-contact-applications

FDA. (2021). Analytical Results of Testing Food for PFAS from Environmental Contamination. Retrieved from https://www.fda.gov/food/chemical-contaminants-food/analytical-results-testing-food-pfas-environmental-contamination

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. (2010). 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

Fei, C; Olsen, J. (2011). Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environmental Health Perspectives 119: 573-578. http://dx.doi.org/10.1289/ehp.1002026

Fei, CY; Mclaughlin, JK; Lipworth, L; Olsen, J. (2009). Maternal levels of perfluorinated chemicals and subfecundity. Human Reproduction 24: 1200-1205. http://dx.doi.org/10.1093/humrep/den490

Feng, X; Long, G; Zeng, G; Zhang, Q; Song, B; Wu, KH. (2022a). Association of increased risk of cardiovascular diseases with higher levels of perfluoroalkylated substances in the serum of adults. Environmental Science and Pollution Research 29: 89081-89092. http://dx.doi.org/10.1007/s11356-022-22021-z

Feng, Y; Bai, Y; Lu, Y; Chen, M; Fu, M; Guan, X, et al. (2022b). Plasma perfluoroalkyl substance exposure and incidence risk of breast cancer: A case-cohort study in the Dongfeng-Tongji cohort. Environmental Pollution 306: 119345. http://dx.doi.org/10.1016/j.envpol.2022.119345

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

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

Fleisch, AF; Rifas-Shiman, SL; Mora, AM; Calafat, AM; Ye, X; Luttmann-Gibson, H, et al. (2017). Early-life exposure to perfluoroalkyl substances and childhood metabolic function. Environmental Health Perspectives 125: 481-487.

Foley, GL. (2001). Overview of male reproductive pathology [Review]. Toxicologic Pathology 29: 49-63. http://dx.doi.org/10.1080/019262301301418856

Forns, J; Iszatt, N; White, RA; Mandal, S; Sabaredzovic, A; Lamoree, M, et al. (2015). Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study. Environment International 83: 176-182.

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

Fraser, AJ; Webster, TF; Watkins, DJ; Strynar, MJ; Kato, K; Calafat, AM, et al. (2013). Polyfluorinated compounds in dust from homes, offices, and vehicles as predictors of concentrations in office workers' serum. Environment International 60: 128-136. http://dx.doi.org/10.1016/j.envint.2013.08.012

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

Fromme, H; Mosch, C; Morovitz, M; Alba-Alejandre, I; Boehmer, S; Kiranoglu, M, et al. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environmental Science and Technology 44: 7123-7129. http://dx.doi.org/10.1021/es101184f

Fromme, H; Tittlemier, SA; Volkel, W; Wilhelm, M; Twardella, D. (2009). Perfluorinated compounds - Exposure assessment for the general population in western countries [Review]. International Journal of Hygiene and Environmental Health 212: 239-270. http://dx.doi.org/10.1016/j.ijheh.2008.04.007

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.

Gabriel, K. (1976). Primary eye irritation study in rabbits, Report 226-0422. Biosearch, Inc.

Gabrielsson, J; Weiner, D. (2000). Pharmacokinetic and pharmacodynamic data analysis: concepts and applications (3rd ed.). Stockholm: Swedish Pharmaceutical Press.

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; Brayne, C; Armstrong, B, en; Fletcher, T. (2013). Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study. British Medical Journal Open 3.

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

Gannon, SA; Fasano, WJ; Mawn, MP; Nabb, DL; Buck, RC; Buxton, LW, et al. (2016). Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. Toxicology 340: 1-9. http://dx.doi.org/10.1016/j.tox.2015.12.006

Gao, B; He, X; Liu, W; Zhang, H; Saito, N; Tsuda, S. (2015). 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, 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

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.

Gebbink, WA; Berger, U; Cousins, IT. (2015). Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. Environment International 74: 160-169. http://dx.doi.org/10.1016/j.envint.2014.10.013

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. (2013). Positive association between perfluoroalkyl chemicals and hyperuricemia in children. American Journal of Epidemiology 177: 1255-1262.

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

Genuis, SJ; Beesoon, S; Birkholz, D. (2013). Biomonitoring and Elimination of Perfluorinated Compounds and Polychlorinated Biphenyls through Perspiration: Blood, Urine, and Sweat Study. ISRN Toxicology 2013: 483832.

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.

Ghassabian, A; Bell, EM; Ma, WL; Sundaram, R; Kannan, K; Buck Louis, GM; Yeung, E. (2018). Concentrations of perfluoroalkyl substances and bisphenol A in newborn dried blood spots and the association with child behavior. Environmental Pollution 243: 1629-1636.

Ghisari, M; Eiberg, H; Long, M; Bonefeld-Jørgensen, EC. (2014). Polymorphisms in phase I and phase II genes and breast cancer risk and relations to persistent organic pollutant

exposure: a case-control study in Inuit women. Environmental Health: A Global Access Science Source 13: 19. http://dx.doi.org/10.1186/1476-069X-13-19

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.

Girardi, P; Lupo, A; Mastromatteo, LY; Scrimin, S. (2022). Mothers living with contamination of perfluoroalkyl substances: an assessment of the perceived health risk and self-reported diseases. Environmental Science and Pollution Research 29: 60491-60507. http://dx.doi.org/10.1007/s11356-022-20085-5

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.

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

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

Goecke, CM; Jarnot, BM; Reo, NV. (1992). A comparative toxicological investigation of perfluorocarboxylic acids in rats by fluorine-19 NMR spectroscopy. Chemical Research in Toxicology 5: 512-519. http://dx.doi.org/10.1021/tx00028a009

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

Goldenthal, E; Jessup, DC; Geil, RG; Mehring, JS. (1978). Ninety-day subacute rhesus monkey toxicity study: Fluorad ¨ Fluorochemical FC-143. (Study No. 137-090). St. Paul, MN: Report prepared for 3M by Institutional Research and Devlopment Corporation (Mattawan, MN).

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

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

Goudarzi, H; Araki, A; Itoh, S; Sasaki, S; Miyashita, C; Mitsui, T, et al. (2017a). The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: The Hokkaido study. Environmental Health Perspectives 125: 111-118.

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Chen, CJ; Ito, S, et al. (2017b). 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. (2016a). Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environment International 94: 124-132.

Goudarzi, H; Nakajima, S; Ikeno, T; Sasaki, S; Kobayashi, S; Miyashita, C, et al. (2016b). Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. Science of the Total Environment 541: 1002-1010.

Goulding, DR; White, SS; Mcbride, SJ; Fenton, SE; Harry, GJ. (2017). Gestational exposure to perfluorooctanoic acid (PFOA): Alterations in motor related behaviors. NeuroToxicology 58: 110-119.

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.

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; Bateson, T. (2021). RE: Benchmark analysis for PFAS immunotoxicity. Available online

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.

Gremmel, C; Frömel, T; Knepper, TP. (2016). Systematic determination of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in outdoor jackets. Chemosphere 160: 173-180. http://dx.doi.org/10.1016/j.chemosphere.2016.06.043

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

Gui, SY; Chen, YN; Wu, KJ; Liu, W; Wang, WJ; Liang, HR, et al. (2022a). Association Between Exposure to Per- and Polyfluoroalkyl Substances and Birth Outcomes: A Systematic Review and Meta-Analysis. Frontiers in Public Health 10: 855348. http://dx.doi.org/10.3389/fpubh.2022.855348

Gui, SY; Qiao, JC; Xu, KX; Li, ZL; Chen, YN; Wu, KJ, et al. (2022b). Association between per- and polyfluoroalkyl substances exposure and risk of diabetes: a systematic review and meta-analysis [Review]. Journal of Exposure Science & Environmental Epidemiology. http://dx.doi.org/10.1038/s41370-022-00464-3

Gump, BB; Hill, DT; Robinson, M; Kannan, K; Heffernan, K; Atallah-Yunes, NH, et al. (2023). Perfluoroalkyl substances (PFAS) and lead (Pb) as "cardiovascular disruptors" in 9-11-year-old children living in Syracuse, New York, United States. Environmental Research 236: 116758. http://dx.doi.org/10.1016/j.envres.2023.116758

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

Gutzkow, KB; Haug, LS; Thomsen, C; Sabaredzovic, A; Becher, G; Brunborg, G. (2012). Placental transfer of perfluorinated compounds is selective - A Norwegian Mother and

Child sub-cohort study. International Journal of Hygiene and Environmental Health 215: 216-219. http://dx.doi.org/10.1016/j.ijheh.2011.08.011

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; Benskin, JP; Sandblom, O; Berger, U; Ahrens, L; Lignell, S, et al. (2019). Perfluoroalkyl Acids (PFAAs) in Children's Serum and Contribution from PFAA-Contaminated Drinking Water. Environmental Science and Technology 53: 11447-11457.

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.

Hack, M; Klein, NK; Taylor, HG. (1995). Long-term developmental outcomes of low birth weight infants. The Future of Children 5: 176-196.

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

Hall, SM; Zhang, S; Hoffman, K; Miranda, ML; Stapleton, HM. (2022). Concentrations of per- and polyfluoroalkyl substances (PFAS) in human placental tissues and associations with birth outcomes. Chemosphere 295: 133873. http://dx.doi.org/10.1016/j.chemosphere.2022.133873

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

Hammer, T; Lophaven, SN; Nielsen, KR; Petersen, MS; Munkholm, P; Weihe, P, et al. (2019). Dietary risk factors for inflammatory bowel diseases in a high-risk population: Results from the Faroese IBD study. 7: 924-932. http://dx.doi.org/10.1177/2050640619852244

Han, X. (2003). Ammonium Perfluorooctanoate: Age Effect on the Plasma Concentration in Post-Weaning Rats Following Oral Gavage [EPA Report]. (Study No. Dupont-13267, December 15, 2003; US EPA Administrative Record 226-1553). Haskell Laboratory for Health and Environmental Sciences.

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

Hanhijarvi, H; Ophaug, RH; Singer, L. (1982). THE SEX-RELATED DIFFERENCE IN PERFLUOROOCTANOATE EXCRETION IN THE RAT. Proceedings of the Society

for Experimental Biology and Medicine 171: 50-55. http://dx.doi.org/10.3181/00379727-171-41476

Hanssen, L; Dudarev, AA; Huber, S; Odland, JØ; Nieboer, E; Sandanger, TM. (2013). Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Science of the Total Environment 447: 430-437.

Hanssen, L; Röllin, H; Odland, JØ; Moe, MK; Sandanger, TM. (2010). Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. Journal of Environmental Monitoring 12: 1355-1361. http://dx.doi.org/10.1039/b924420d

Harada, K; Inoue, K; Morikawa, A; Yoshinaga, T; Saito, N; Koizumi, A. (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environmental Research 99: 253-261. http://dx.doi.org/10.1016/j.envres.2004.12.003

Harkness, JE; Wagner, JE. (1983). The Biology and Medicine of Rabbits and Rodents (2nd ed.). Philadelphia, PA: Lea & Febiger.

Harris, MH; Oken, E; Rifas-Shiman, SL; Calafat, AM; Ye, X; Bellinger, DC, et al. (2018). Prenatal and childhood exposure to per- and polyfluoroalkyl substances (PFASs) and child cognition. Environment International 115: 358-369.

Hartman, TJ; Calafat, AM; Holmes, AK; Marcus, M; Northstone, K; Flanders, WD, et al. (2017). Prenatal exposure to perfluoroalkyl substances and body fatness in girls. Childhood Obesity 13: 222-230.

Haschek, WM; Rousseaux, CG; Wallig, MA. (2009). Fundamentals of toxicologic pathology

Male reproductive system (2nd ed.). Cambridge, MA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-370469-6.00018-0

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.

Heffernan, AL; Cunningham, TK; Drage, DS; Aylward, LL; Thompson, K; Vijayasarathy, S, et al. (2018). Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. International Journal of Hygiene and Environmental Health 221: 1068-1075.

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

Hines, EP; White, SS; Stanko, JP; Flournoy, EAG; Lau, C; Fenton, SE. (2009). Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated serum leptin and insulin, and overweight in mid-life. Molecular and Cellular Endocrinology 304: 97-105. http://dx.doi.org/10.1016/j.mce.2009.02.021

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.

Hoffman, K; Webster, TF; Weisskopf, MG; Weinberg, J; Vieira, VM. (2010). Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environmental Health Perspectives 118: 1762-1767. http://dx.doi.org/10.1289/ehp.1001898

Holder, C; Deluca, NM; Luh, J; Alexander, P; Minucci, JM; Vallero, D, et al. (2023). Systematic Evidence Mapping of Potential Exposure Pathways for Per- and Polyfluoroalkyl Substances Based on Measured Occurrence in Multiple Media. Environmental Science & Technology 57: 5107-5116. http://dx.doi.org/https://pubs.acs.org/doi/abs/10.1021/acs.est.2c07185

Honarvar, B; Moghadami, M; Moattari, A; Emami, A; Odoomi, N; Bagheri Lankarani, K. (2013). Seroprevalence of anti-rubella and anti-measles IgG antibodies in pregnant women in Shiraz, Southern Iran: outcomes of a nationwide measles-rubella mass vaccination campaign. PLoS ONE 8: e55043. http://dx.doi.org/10.1371/journal.pone.0055043

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.

Howard, BE; Phillips, J; Miller, K; Tandon, A; Mav, D; Shah, MR, et al. (2016). SWIFT-Review: a text-mining workbench for systematic review. Systematic Reviews 5: 87. http://dx.doi.org/10.1186/s13643-016-0263-z

Høyer, BB; Ramlau-Hansen, CH; Obel, C; Pedersen, HS; Hernik, A; Ogniev, V, et al. (2015). Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years--a prospective study. Environmental Health: A Global Access Science Source 14: 2. http://dx.doi.org/10.1186/1476-069X-14-2

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, Y; Liu, G; Rood, J; Liang, L; Bray, GA; de Jonge, L, et al. (2019). Perfluoroalkyl substances and changes in bone mineral density: A prospective analysis in the POUNDS-LOST study. Environmental Research 179: 108775.

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, 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, R; Chen, Q; Zhang, L; Luo, K; Chen, L; Zhao, S, et al. (2019). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy. Environmental Health: A Global Access Science Source 18: 5.

Huhtaniemi, I; Toppari, J. (1995). Endocrine, paracrine and autocrine regulation of testicular steroidogenesis. In AK Mukhopadhyay; MK Raizada (Eds.), Tissue renin-angiotensin systems: Current concepts of local regulators in reproductive and endocrine organs (pp. 33-54). New York, NY: Springer. http://dx.doi.org/10.1007/978-1-4899-0952-7_3

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.

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.

Inoue, K; Ritz, B; Andersen, SL; Ramlau-Hansen, CH; Høyer, BB; Bech, BH, et al. (2019). Perfluoroalkyl Substances and Maternal Thyroid Hormones in Early Pregnancy; Findings in the Danish National Birth Cohort. Environmental Health Perspectives 127: 117002.

Ipsen, J. (1946). Circulating antitoxin at the onset of diphtheria in 425 patients. Journal of Immunology 54: 325-347.

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

Itoh, S; Araki, A; Mitsui, T; Miyashita, C; Goudarzi, H; Sasaki, S, et al. (2016). Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environment International 94: 51-59.

Itoh, S; Araki, A; Miyashita, C; Yamazaki, K; Goudarzi, H; Minatoya, M, et al. (2019). Association between perfluoroalkyl substance exposure and thyroid hormone/thyroid antibody levels in maternal and cord blood: The Hokkaido Study. Environment International 133: 105139.

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.

Jaacks, LM; Boyd Barr, D; Sundaram, R; Grewal, J; Zhang, C; Buck Louis, GM. (2016). Pre-Pregnancy Maternal Exposure to Persistent Organic Pollutants and Gestational Weight Gain: A Prospective Cohort Study. International Journal of Environmental Research and Public Health 13.

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.

Jain, R. (2013). Association between thyroid profile and perfluoroalkyl acids: Data from NHNAES 2007-2008. Environmental Research 126: 51-59.

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). Associations between selected perfluoroalkyl acids in serum and hemoglobin in whole blood, a biomarker of anemia: Impact of deteriorating kidney function. Environmental Pollution 263: 114458.

Jain, RB. (2020b). 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; 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). Dynamics of associations between perfluoroalkyl substances and uric acid across the various stages of glomerular function. Environmental Science and Pollution Research 26: 12425-12434.

Jain, RB; Ducatman, A. (2019b). Perfluoroalkyl acids and thyroid hormones across stages of kidney function. Science of the Total Environment 696: 133994.

Jain, RB; Ducatman, A. (2019c). 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. (2019d). 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. (2019e). 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

James, K; Peters, RE; Laird, BD; Ma, WK; Wickstrom, M; Stephenson, GL; Siciliano, SD. (2011). Human exposure assessment: a case study of 8 PAH contaminated soils using in vitro digestors and the juvenile swine model. Environmental Science and Technology 45: 4586-4593. http://dx.doi.org/10.1021/es1039979

Janků, I. (1993). Physiological modelling of renal drug clearance. European Journal of Clinical Pharmacology 44: 513-519. http://dx.doi.org/10.1007%2FBF02440850

Jeddy, Z; Hartman, TJ; Taylor, EV; Poteete, C; Kordas, K. (2017). Prenatal concentrations of perfluoroalkyl substances and early communication development in British girls. Early Human Development 109: 15-20.

Jeddy, Z; Tobias, JH; Taylor, EV; Northstone, K; Flanders, WD; Hartman, TJ. (2018). Prenatal concentrations of perfluoroalkyl substances and bone health in British girls at age 17. Archives of Osteoporosis 13: 84.

Jensen, RC; Andersen, MS; Larsen, PV; Glintborg, D; Dalgård, C; Timmermann, CAG, et al. (2020a). 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, RC; Glintborg, D; Gade Timmermann, CA; Nielsen, F; Kyhl, HB; Frederiksen, H, et al. (2020b). Prenatal exposure to perfluorodecanoic acid is associated with lower circulating concentration of adrenal steroid metabolites during mini puberty in human female infants. The odense child cohort. Environmental Research 182: 109101.

Jensen, RC; Glintborg, D; Timmermann, CAG; Nielsen, F; Kyhl, HB; Andersen, HR, et al. (2018). Perfluoroalkyl substances and glycemic status in pregnant Danish women: The Odense Child Cohort. Environment International 116: 101-107.

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

Jia, J; Duan, L; Dong, B; Dong, Q; Liu, Y; Yu, W, et al. (2023). Perfluoroalkyl and polyfluoroalkyl substances in cord serum of newborns and their potential factors. Chemosphere 313: 137525. http://dx.doi.org/10.1016/j.chemosphere.2022.137525

Jiang, H; Liu, H; Liu, G; Yu, J; Liu, N; Jin, Y, et al. (2022). Associations between Polyfluoroalkyl Substances Exposure and Breast Cancer: A Meta-Analysis [Review]. Toxics 10. http://dx.doi.org/10.3390/toxics10060318

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.

Joensen, UN; Bossi, R; Leffers, H; Jensen, AA; Skakkebæk, NE; Jørgensen, N. (2009). Do perfluoroalkyl compounds impair human semen quality? Environmental Health Perspectives 117: 923-927. http://dx.doi.org/10.1289/ehp.0800517

Joensen, UN; Veyrand, B; Antignac, JP; Jensen, MB; Petersen, JH; Marchand, P, et al. (2014). PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Human Reproduction 29: 1600-1600. http://dx.doi.org/10.1093/humrep/deu104

Johansson, N; Eriksson, P; Viberg, H. (2009). Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicological Sciences 108: 412-418. http://dx.doi.org/10.1093/toxsci/kfp029

Johansson, N; Fredriksson, A; Eriksson, P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. NeuroToxicology 29: 160-169. http://dx.doi.org/10.1016/j.neuro.2007.10.008

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

Jones, LE; Ghassabian, A; Lawrence, DA; Sundaram, R; Yeung, E; Kannan, K; Bell, EM. (2022). Exposure to perfluoroalkyl substances and neonatal immunoglobulin profiles in the upstate KIDS study (2008-2010). Environmental Pollution 308: 119656. http://dx.doi.org/10.1016/j.envpol.2022.119656

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; Choi, K; Lee, HS; Kim, DH; Park, NY; Kim, S; Kho, Y. (2016). 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; Ding, N; Karvonen-Gutierrez, CA; Mukherjee, B; Calafat, AM; Park, SK. (2023). Per- and Polyfluoroalkyl Substances (PFAS) and Lipid Trajectories in Women 45-56 Years of Age: The Study of Women's Health Across the Nation. Environmental Health Perspectives 131: 87004. http://dx.doi.org/10.1289/EHP12351

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.

Karlsen, M; Grandjean, P; Weihe, P; Steuerwald, U; Oulhote, Y; Valvi, D. (2017). Early-life exposures to persistent organic pollutants in relation to overweight in preschool children. Reproductive Toxicology 68: 145-153.

Kärrman, A; Domingo, JL; Llebaria, X; Nadal, M; Bigas, E; van Bavel, B; Lindstrom, G. (2010). Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. Environmental Science and Pollution Research 17: 750-758. http://dx.doi.org/10.1007/s11356-009-0178-5

Kärrman, A; van Bavel, B; Järnberg, U; Hardell, L; Lindström, G. (2006). Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 64: 1582-1591. http://dx.doi.org/10.1016/j.chemosphere.2005.11.040

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.

Katakura, M; Kudo, N; Tsuda, T; Hibino, Y; Mitsumoto, A; Kawashima, Y. (2007). Rat organic anion transporter 3 and organic anion transporting polypeptide 1 mediate perfluorooctanoic acid transport. Journal of Health Science 53: 77-83. http://dx.doi.org/10.1248/jhs.53.77

Kataria, A; Trachtman, H; Malaga-Dieguez, L; Trasande, L. (2015). Association between perfluoroalkyl acids and kidney function in a cross-sectional study of adolescents. Environmental Health: A Global Access Science Source 14: 89.

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, S; Itoh, S; Yuasa, M; Baba, T; Miyashita, C; Sasaki, S, et al. (2016). Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environmental Health and Preventive Medicine 21: 334-344.

Kaur, K; Lesseur, C; Chen, L; Andra, SS; Narasimhan, S; Pulivarthi, D, et al. (2023). Cross-sectional associations of maternal PFAS exposure on SARS-CoV-2 IgG antibody levels during pregnancy. Environmental Research 219: 115067. http://dx.doi.org/10.1016/j.envres.2022.115067

Kawabata, K; Matsuzaki, H; Nukui, S; Okazaki, M; Sakai, A; Kawashima, Y; Kudo, N. (2017). Perfluorododecanoic acid induces cognitive deficit in adult rats. Toxicological Sciences 157: 421-428.

Keller & Heckman LLP. (2021). Attack on PFAS extends to food packaging. National Law Review X.

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

Kennedy, GL; Jr; Butenhoff, JL; Olsen, GW; Connor, JCO; Seacat, AM, et al. (2004). The toxicology of perfluorooctanoate [Review]. Critical Reviews in Toxicology 34: 351-384. http://dx.doi.org/10.1080/10408440490464705

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; Chen, A; Lee, M; Czerwinski, SA; Ebert, JR; Dewitt, JC; Kannan, K. (2016). Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the US Population in NHANES 2009-2010. Environmental Health Perspectives 124: 81-87.

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.

Kim, DH; Kim, UJ; Kim, HY; Choi, SD; Oh, JE. (2016a). Perfluoroalkyl substances in serum from South Korean infants with congenital hypothyroidism and healthy infants - Its relationship with thyroid hormones. Environmental Research 147: 399-404. http://dx.doi.org/10.1016/j.envres.2016.02.037

Kim, DH; Lee, JH; Oh, JE. (2019). Assessment of individual-based perfluoroalkly substances exposure by multiple human exposure sources. Journal of Hazardous Materials 365: 26-33. http://dx.doi.org/10.1016/j.jhazmat.2018.10.066

Kim, HY; Kim, KN; Shin, CH; Lim, YH; Kim, JI; Kim, BN, et al. (2020a). The relationship between perfluoroalkyl substances concentrations and thyroid function in early childhood: A prospective cohort study. Thyroid 30: 1556-1565.

Kim, MJ; Moon, S; Oh, BC; Jung, D; Ji, K; Choi, K; Park, YJ. (2018). Association between perfluoroalkyl substances exposure and thyroid function in adults: A meta-analysis. PLoS ONE 13: e0197244. http://dx.doi.org/10.1371/journal.pone.0197244

Kim, OJ; Kim, S; Park, EY; Oh, JK; Jung, SK; Park, S, et al. (2023). Exposure to serum perfluoroalkyl substances and biomarkers of liver function: The Korean national environmental health survey 2015-2017. Chemosphere 322: 138208. http://dx.doi.org/10.1016/j.chemosphere.2023.138208

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. (2011). 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. (2016b). Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. Food and Chemical Toxicology 97: 243-255.

Kim, SK; Kannan, K. (2007). Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. Environmental Science and Technology 41: 8328-8334. http://dx.doi.org/10.1021/es072107t

Kim, YR; White, N; Bräunig, J; Vijayasarathy, S; Mueller, JF; Knox, CL, et al. (2020b). 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.

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/

Klaassen, CD; Lu, H. (2008). Xenobiotic transporters: ascribing function from gene knockout and mutation studies. Toxicological Sciences 101: 186-196. http://dx.doi.org/10.1093/toxsci/kfm214

Klamt, A; Huniar, U; Spycher, S; Keldenich, J. (2008). COSMOmic: a mechanistic approach to the calculation of membrane-water partition coefficients and internal distributions within membranes and micelles. Journal of Physical Chemistry B 112: 12148-12157. http://dx.doi.org/10.1021/jp801736k

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; 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

Koponen, J; Winkens, K; Airaksinen, R; Berger, U; Vestergren, R; Cousins, IT, et al. (2018). Longitudinal trends of per- and polyfluoroalkyl substances in children's serum. Environment International 121: 591-599. http://dx.doi.org/10.1016/j.envint.2018.09.006

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; Koponen, J; Lehenkari, P; Viluksela, M; Korkalainen, M; Tuukkanen, J. (2017). Perfluoroalkyl substances in human bone: concentrations in bones and effects on bone cell differentiation. Scientific Reports 7: 6841.

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.

Kotthoff, M; Müller, J; Jürling, H; Schlummer, M; Fiedler, D. (2015). Perfluoroalkyl and polyfluoroalkyl substances in consumer products. Environmental Science and Pollution Research 22: 14546-14559. http://dx.doi.org/10.1007/s11356-015-4202-7

Kristensen, SL; Ramlau-Hansen, CH; Ernst, E; Olsen, SF; Bonde, JP; Vested, A, et al. (2013). Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Human Reproduction 28: 3337-3348. http://dx.doi.org/10.1093/humrep/det382

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

Kudo, N; Sakai, A; Mitsumoto, A; Hibino, Y; Tsuda, T; Kawashima, Y. (2007). Tissue distribution and hepatic subcellular distribution of perfluorooctanoic acid at low dose are different from those at high dose in rats. Biological and Pharmaceutical Bulletin 30: 1535-1540. http://dx.doi.org/10.1248/bpb.30.1535

Kullak-Ublick, G, .A.; Hagenbuch, B, .; Stieger, B, .; Schteingart, C, .D.; Hofmann, A, .F.; Wolkoff, A, .W.; Meier, P, .J. (1995). Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. 109: 1274-1282. http://dx.doi.org/10.1016/0016-5085(95)90588-x

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.

Kvist, L; Giwercman, YL; Jönsson, BA; Lindh, CH; Bonde, JP; Toft, G, et al. (2012). Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland. Reproductive Toxicology 34: 644-650.

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.

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.

Lauritzen, HB; Larose, TL; Øien, T; Sandanger, TM; Odland, JØ; van de Bor, M; Jacobsen, GW. (2018). Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. Environmental Health: A Global Access Science Source 17: 9.

Laws, SC; Stoker, TE; Ferrell, JM; Hotchkiss, MG; Cooper, RL. (2007). Effects of altered food intake during pubertal development in male and female wistar rats. Toxicological Sciences 100: 194-202. http://dx.doi.org/10.1093/toxsci/kfm219

Lebeaux, RM; Doherty, BT; Gallagher, LG; Zoeller, RT; Hoofnagle, AN; Calafat, AM, et al. (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Environmental Research 185: 109395.

Lee, J; Oh, S; Kang, H; Kim, S; Lee, G; Li, L, et al. (2020). Environment-wide association study of CKD. Clinical Journal of the American Society of Nephrology 15: 766-775.

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, 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.

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.

Leter, G; Consales, C; Eleuteri, P; Uccelli, R; Specht, IO; Toft, G, et al. (2014). Exposure to perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations. Environmental and Molecular Mutagenesis 55: 591-600.

Lewis, RC; Johns, LE; Meeker, JD. (2015). Serum Biomarkers of Exposure to Perfluoroalkyl Substances in Relation to Serum Testosterone and Measures of Thyroid Function among Adults and Adolescents from NHANES 2011-2012. International Journal of Environmental Research and Public Health 12: 6098-6114.

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, H; Chen, J; Jingchao, L; Yang, J; Tan, Z; Li, L, et al. (2023). Association of exposure to perfluoroalkyl substances and risk of the acute coronary syndrome: A case-control study in Shijiazhuang Hebei Province. Chemosphere 313: 137464. http://dx.doi.org/10.1016/j.chemosphere.2022.137464

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; 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. (2017a). Molecular Mechanisms of Perfluorooctanoate-Induced Hepatocyte Apoptosis in Mice Using Proteomic Techniques. Environmental Science and Technology 51: 11380-11389.

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. (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; Song, F; Liu, X; Shan, A; Huang, Y; Yang, Z, et al. (2022). Perfluoroalkyl substances (PFASs) as risk factors for breast cancer: a case-control study in Chinese population. Environmental Health 21: 83. http://dx.doi.org/10.1186/s12940-022-00895-3

Li, Y; Cheng, Y; Xie, Z; Zeng, F. (2017b). Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. Scientific Reports 7: 43380.

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; 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

Li, Y; Yu, N; Du, L; Shi, W; Yu, H; Song, M; Wei, S. (2020b). Transplacental Transfer of Per- and Polyfluoroalkyl Substances Identified in Paired Maternal and Cord Sera Using Suspect and Nontarget Screening. Environmental Science and Technology 54: 3407-3416.

Liang, JL; Tiwari, T; Moro, P; Messonnier, NE; Reingold, A; Sawyer, M; Clark, TA. (2018). Prevention of pertussis, tetanus, and diphtheria with vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP). US Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report Recommendations and Report 67: 1-44. http://dx.doi.org/10.15585/mmwr.rr6702a1

Liao, Q; Tang, P; Song, Y; Liu, B; Huang, H; Liang, J, et al. (2022). Association of single and multiple prefluoroalkyl substances exposure with preterm birth: Results from a Chinese birth cohort study. Chemosphere 307: 135741. http://dx.doi.org/10.1016/j.chemosphere.2022.135741

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.

Lien, GW; Huang, CC; Shiu, JS; Chen, MH; Hsieh, WS; Guo, YL; Chen, PC. (2016). Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere 156: 118-127.

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.

Liew, Z; Ritz, B; Bach, CC; Asarnow, RF; Bech, BH; Nohr, EA, et al. (2018). Prenatal exposure to perfluoroalkyl substances and iq scores at age 5; a study in the danish national birth cohort. Environmental Health Perspectives 126: 067004.

Liew, Z; Ritz, B; Bonefeld-Jørgensen, EC; Henriksen, TB; Nohr, EA; Bech, BH, et al. (2014). Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. American Journal of Epidemiology 180: 574-581. http://dx.doi.org/10.1093/aje/kwu179

Liew, Z; Ritz, B; von Ehrenstein, OS; Bech, BH; Nohr, EA; Fei, C, et al. (2015). Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: A nested case-control study in the Danish National Birth Cohort. Environmental Health Perspectives 123: 367-373.

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; 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. (2013a). 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, CY; Wen, LL; Lin, LY; Wen, TW; Lien, GW; Hsu, SH, et al. (2013b). The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. Journal of Hazardous Materials 244-245: 637-644. http://dx.doi.org/10.1016/j.jhazmat.2012.10.049

Lin, HW; Feng, HX; Chen, L; Yuan, XJ; Tan, Z. (2020a). Maternal exposure to environmental endocrine disruptors during pregnancy is associated with pediatric germ cell tumors. Nagoya Journal of Medical Science 82: 323-333.

Lin, LY; Wen, LL; Su, TC; Chen, PC; Lin, CY. (2014). Negative association between serum perfluorooctane sulfate concentration and bone mineral density in US premenopausal women: NHANES, 2005-2008. Journal of Clinical Endocrinology and Metabolism 99: 2173-2180.

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. (2020b). 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. (2020c). 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

Linakis, MW; Gustafson, P; Allen, BC; Bachand, AM; Van Landingham, C; Keast, DR; Longnecker, MP. (2022). Is the cholesterol-perfluoroalkyl substance association confounded by dietary fiber intake?: a Bayesian analysis of NHANES data with adjustment for measurement error in fiber intake. Environmental Health 21: 114. http://dx.doi.org/10.1186/s12940-022-00923-2

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, L; Zethelius, B; Salihovic, S; van Bavel, B; Lind, PM. (2014). Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 57: 473-479.

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

Liu, G; Dhana, K; Furtado, JD; Rood, J; Zong, G; Liang, L, et al. (2018a). 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; Pan, Y; Jin, S; Li, Y; Zhao, L; Sun, X, et al. (2020b). Associations of per-/polyfluoroalkyl substances with glucocorticoids and progestogens in newborns. Environment International 140: 105636.

Liu, HS; Wen, LL; Chu, PL; Lin, CY. (2018b). 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, JJ; Cui, XX; Tan, YW; Dong, PX; Ou, YQ; Li, QQ, et al. (2022). Per- and perfluoroalkyl substances alternatives, mixtures and liver function in adults: A community-based population study in China. Environment International 163: 107179. http://dx.doi.org/10.1016/j.envint.2022.107179

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, P; Yang, F; Wang, Y; Yuan, Z. (2018c). Perfluorooctanoic acid (PFOA) exposure in early life increases risk of childhood adiposity: a meta-analysis of prospective cohort studies. International Journal of Environmental Research and Public Health 15. http://dx.doi.org/10.3390/ijerph15102070

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, 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, X; Guo, Z; Krebs, KA; Pope, RH; Roache, NF. (2014). Concentrations and trends of perfluorinated chemicals in potential indoor sources from 2007 through 2011 in the US. Chemosphere 98: 51-57. http://dx.doi.org/10.1016/j.chemosphere.2013.10.001

Liu, X; Zhang, L; Chen, L; Li, J; Wang, Y; Wang, J, et al. (2019). Structure-based investigation on the association between perfluoroalkyl acids exposure and both gestational diabetes mellitus and glucose homeostasis in pregnant women. Environment International 127: 85-93.

Long, M; Ghisari, M; Kjeldsen, L; Wielsøe, M; Nørgaard-Pedersen, B; Mortensen, EL, et al. (2019). Autism spectrum disorders, endocrine disrupting compounds, and heavy metals in amniotic fluid: a case-control study. Molecular autism 10: 1.

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; Fletcher, T; Armstrong, B, en; Genser, B; Dhatariya, K; Mondal, D, et al. (2011). Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with Age of Puberty among Children Living near a Chemical Plant. Environmental Science and Technology 45: 8160-8166. http://dx.doi.org/10.1021/es1038694

Lopez-Espinosa, MJ; Mondal, D; Armstrong, B; Bloom, MS; Fletcher, T. (2012). Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environmental Health Perspectives 120: 1036-1041. http://dx.doi.org/10.1289/ehp.1104370

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

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

Louis, GMB; Peterson, CM; Chen, Z; Hediger, ML; Croughan, MS; Sundaram, R, et al. (2012). Perfluorochemicals and endometriosis: The ENDO study. Epidemiology 23: 799-805.

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, Y; Luo, B; Li, J; Dai, J. (2016a). Perfluorooctanoic acid disrupts the blood-testis barrier and activates the TNFα/p38 MAPK signaling pathway in vivo and in vitro. Archives of Toxicology 90: 971-983. http://dx.doi.org/10.1007/s00204-015-1492-y

Lu, Y; Pan, Y; Sheng, N; Zhao, AZ; Dai, J. (2016b). 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.

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

Luo, J; Ramlau-Hansen, CH; Kesmodel, US; Xiao, J; Vasiliou, V; Deziel, NC, et al. (2022). Prenatal Exposure to Per- and Polyfluoroalkyl Substances and Facial Features at 5 Years of Age: A Study from the Danish National Birth Cohort. Environmental Health Perspectives 130: 17006. http://dx.doi.org/10.1289/EHP9478

Lyall, K; Yau, VM; Hansen, R; Kharrazi, M; Yoshida, CK; Calafat, AM, et al. (2018). Prenatal maternal serum concentrations of per- and polyfluoroalkyl substances in association with autism spectrum disorder and intellectual disability. Environmental Health Perspectives 126: 017001.

Lyngsø, J; Ramlau-Hansen, CH; Høyer, BB; Støvring, H; Bonde, JP; Jönsson, BA, et al. (2014). Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study. Human Reproduction 29: 359-367.

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, X; Fisher, JA; Vopham, T; Vasiliou, V; Jones, RR. (2023). Associations between per- and polyfluoroalkyl substances, liver function, and daily alcohol consumption in a sample of U.S. adults. Environmental Research 235: 116651. http://dx.doi.org/10.1016/j.envres.2023.116651

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

Macneil, J; Steenland, NK; Shankar, A; Ducatman, A. (2009). A cross-sectional analysis of type II diabetes in a community with exposure to perfluorooctanoic acid (PFOA). Environmental Research 109: 997-1003. http://dx.doi.org/10.1016/j.envres.2009.08.002

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; Calafat, AM; Marcus, M; Jaakkola, JJ; Lashen, H. (2015a). Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 years of age in female ALSPAC study participants. Environmental Health Perspectives 123: 1325-1330.

Maisonet, M; Näyhä, S; Lawlor, DA; Marcus, M. (2015b). Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environment International 82: 49-60. http://dx.doi.org/10.1016/j.envint.2015.05.001

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

Makey, CM; Webster, TF; Martin, JW; Shoeib, M; Harner, T; Dix-Cooper, L; Webster, GM. (2017). Airborne precursors predict maternal serum perfluoroalkyl acid concentrations. Environmental Science and Technology 51: 7667-7675. http://dx.doi.org/10.1021/acs.est.7b00615

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.

Mancini, FR; Rajaobelina, K; Praud, D; Dow, C; Antignac, JP; Kvaskoff, M, et al. (2018). Nonlinear associations between dietary exposures to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS) and type 2 diabetes risk in women: Findings from the

E3N cohort study. International Journal of Hygiene and Environmental Health 221: 1054-1060.

Mann, PC; Frame, SR. (2004). FC-143: Two year oral toxicity-oncogenicity study in rats. Peer review of ovaries. (Project ID 15261). Newark, DE: E.I. du Pont de Nemours and Company.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Basterrechea, M; Grimalt, JO, et al. (2015). Transfer of perfluoroalkyl substances from mother to fetus in a Spanish birth cohort. Environmental Research 142: 471-478.

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.

Maranhao Neto, GA; Polcrova, AB; Pospisilova, A; Blaha, L; Klanova, J; Bobak, M; Gonzalez-Rivas, JP. (2022). Associations between Per- and Polyfluoroalkyl Substances (PFAS) and Cardiometabolic Biomarkers in Adults of Czechia: The Kardiovize Study. International Journal of Environmental Research and Public Health 19: 13898. http://dx.doi.org/10.3390/ijerph192113898

Marks, KJ; Jeddy, Z; Flanders, WD; Northstone, K; Fraser, A; Calafat, AM, et al. (2019). Maternal serum concentrations of perfluoroalkyl substances during pregnancy and gestational weight gain: The Avon Longitudinal Study of Parents and Children. Reproductive Toxicology 90: 8-14.

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

Martinsson, M; Nielsen, C; Björk, J; Rylander, L; Malmqvist, E; Lindh, C; Rignell-Hydbom, A. (2020). Intrauterine exposure to perfluorinated compounds and overweight at age 4: A case-control study. PLoS ONE 15: e0230137.

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.

Mccoy, JA; Bangma, JT; Reiner, JL; Bowden, JA; Schnorr, J; Slowey, M, et al. (2017). Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. Science of the Total Environment 605-606: 9-17.

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.

Messmer, MF; Salloway, J; Shara, N; Locwin, B; Harvey, MW; Traviss, N. (2022). Risk of Cancer in a Community Exposed to Per- and Poly-Fluoroalkyl Substances. Environmental Health Insights 16: 11786302221076707. http://dx.doi.org/10.1177/11786302221076707

Mi, X; Lin, SQ; Zhang, XF; Li, JJ; Pei, LJ; Jin, F, et al. (2022). Maternal perfluorinated compound exposure and risk of early pregnancy loss: A nested case-control study [Letter]. Biomedical and Environmental Sciences 35: 174-179. http://dx.doi.org/10.3967/bes2022.026

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.

Midasch, O; Drexler, H; Hart, N; Beckmann, MW; Angerer, J. (2007). Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. International Archives of Occupational and Environmental Health 80: 643-648. http://dx.doi.org/10.1007/s00420-006-0165-9

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.

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.

Monge Brenes, AL; Curtzwiler, G; Dixon, P; Harrata, K; Talbert, J; Vorst, K. (2019). PFOA and PFOS levels in microwave paper packaging between 2005 and 2018. Food Additives and Contaminants: Part B: Surveillance 12: 191-198. http://dx.doi.org/10.1080/19393210.2019.1592238

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, AM; Oken, E; Rifas-Shiman, SL; Webster, TF; Gillman, MW; Calafat, AM, et al. (2017). Prenatal exposure to perfluoroalkyl substances and adiposity in early and mid-childhood. Environmental Health Perspectives 125: 467-473.

MPCA. (2008). PFCs in Minnesota's Ambient Environment: 2008 Progress Report. https://www.pca.state.mn.us/sites/default/files/c-pfc1-02.pdf

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

NCHS. (2019). Health, United States – Data Finder. 2019: Table 23. Hyattsville, MD. https://www.cdc.gov/nchs/hus/contents2019.htm

Needham, LL; Grandjean, P; Heinzow, B; Jørgensen, PJ; Nielsen, F; Patterson, DG, et al. (2011). Partition of environmental chemicals between maternal and fetal blood and tissues. Environmental Science and Technology 45: 1121-1126. http://dx.doi.org/10.1021/es1019614

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

Nian, M; Huo, X; Zhang, J; Mao, Y; Jin, F; Shi, Y; Zhang, J. (2022). Association of emerging and legacy per- and polyfluoroalkyl substances with unexplained recurrent spontaneous abortion. Ecotoxicology and Environmental Safety 239: 113691. http://dx.doi.org/10.1016/j.ecoenv.2022.113691

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.

Nielsen, C; Andersson Hall, U; Lindh, C; Ekström, U; Xu, Y; Li, Y, et al. (2020). Pregnancy-induced changes in serum concentrations of perfluoroalkyl substances and the influence of kidney function. Environmental Health: A Global Access Science Source 19: 80.

Nilsson, S; Smurthwaite, K; Aylward, LL; Kay, M; Toms, LM; King, L, et al. (2022). Associations between serum perfluoroalkyl acid (PFAA) concentrations and health related biomarkers in firefighters. Environmental Research 215: 114370. http://dx.doi.org/10.1016/j.envres.2022.114370

Niu, J; Liang, H; Tian, Y; Yuan, W; Xiao, H; Hu, H, et al. (2019). Prenatal plasma concentrations of Perfluoroalkyl and polyfluoroalkyl substances and neuropsychological development in children at four years of age. Environmental Health: A Global Access Science Source 18: 53.

NLM. (2022). 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. (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

NRC. (2008). Phthalates and cumulative risk assessment: The task ahead. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/12528

NTP. (2019a). 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. (2019b). 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. (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.

Ode, A; Källén, K; Gustafsson, P; Rylander, L; Jönsson, BA; Olofsson, P, et al. (2014). Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS ONE 9: e95891.

OECD. (2001). Test no. 416: Two-generation reproduction toxicity. In OECD guidelines for the testing of chemicals, Section 4: Health effects. Paris, France. http://dx.doi.org/10.1787/9789264070868-en

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.

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

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. (2001). 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, G; Ehresman, D; Froehlich, J; Burris, J; Butenhoff, J. (2005). Evaluation of the Half-life (T1/2) of Elimination of Perfluorooctanesulfonate (PFOS), Perfluorohexanesulfonate (PFHS) and Perfluorooctanoate (PFOA) from Human Serum. St. Paul, MN: 3M Company. https://www.researchgate.net/profile/Varun-Ahuja-2/post/Is_the_relatively_long_half-life_of_PFOA_in_humans_mainly_due_to_its_affinity_for_plasma_protein_binding/attachment/5aebf5e6b53d2f63c3c9baf6/AS%3A622442858967040%401525413350348/download/TOX017Olsen.pdf

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; Gilliland, FD; Burlew, MM; Burris, JM; Mandel, JS; Mandel, JH. (1998). An epidemiologic investigation of reproductive hormones in men with occupational exposure

to perfluorooctanoic acid. Journal of Occupational and Environmental Medicine 40: 614-622. http://dx.doi.org/10.1097/00043764-199807000-00006

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

Onishchenko, N; Fischer, C; Wan Ibrahim, WN; Negri, S; Spulber, S; Cottica, D; Ceccatelli, S. (2011). Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. Neurotoxicity Research 19: 452-461. http://dx.doi.org/10.1007/s12640-010-9200-4

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

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

Oulhote, Y; Coull, B; Bind, MA; Debes, F; Nielsen, F; Tamayo, I, et al. (2019). Joint and independent neurotoxic effects of early life exposures to a chemical mixture: A multi-pollutant approach combining ensemble learning and g-computation. Environmental Epidemiology 3: e063.

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.

Padula, AM; Ning, X; Bakre, S; Barrett, ES; Bastain, T; Bennett, DH, et al. (2023). Birth outcomes in relation to prenatal exposure to per-and polyfluoroalkyl substances and stress in the environmental influences on child health outcomes (echo) program [Supplemental Data]. Environmental Health Perspectives 131: (037006) 037001-037011. http://dx.doi.org/10.1289/EHP10723

Pan, K; Xu, J; Long, X; Yang, L; Huang, Z; Yu, J. (2023). The relationship between perfluoroalkyl substances and hypertension: A systematic review and meta-analysis [Review]. Environmental Research 232: 116362. http://dx.doi.org/10.1016/j.envres.2023.116362

Pan, Y; Cui, Q; Wang, J; Sheng, N; Jing, J; Yao, B; Dai, J. (2019). Profiles of Emerging and Legacy Per-/Polyfluoroalkyl Substances in Matched Serum and Semen Samples: New Implications for Human Semen Quality. Environmental Health Perspectives 127: 127005.

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; Nicolescu, A; Haug, LS; Husøy, T; Deleanu, C; Dirven, H; Lindeman, B. (2022). Lipoprotein profiles associated with exposure to poly- and perfluoroalkyl substances (PFASs) in the EuroMix human biomonitoring study. Environmental Pollution 308: 119664. http://dx.doi.org/10.1016/j.envpol.2022.119664

Papadopoulou, E; Poothong, S; Koekkoek, J; Lucattini, L; Padilla-Sánchez, JA; Haugen, M, et al. (2017). Estimating human exposure to perfluoroalkyl acids via solid food and drinks: Implementation and comparison of different dietary assessment methods. Environmental Research 158: 269-276. http://dx.doi.org/10.1016/j.envres.2017.06.011

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

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

Patel, JC; Mehta, BC. (1999). Tetanus: Study of 8,697 cases. Indian Journal of Medical Sciences 53: 393-401.

Penland, TN; Cope, WG; Kwak, TJ; Strynar, MJ; Grieshaber, CA; Heise, RJ; Sessions, FW. (2020). Trophodynamics of Per- and Polyfluoroalkyl Substances in the Food Web of a Large Atlantic Slope River. Environmental Science and Technology 54: 6800-6811. http://dx.doi.org/10.1021/acs.est.9b05007

Pennati, G; Corno, C; Costantino, ML; Bellotti, M. (2003). Umbilical flow distribution to the liver and the ductus venosus in human fetuses during gestation: an anatomy-based mathematical modeling. Medical Engineering & Physics 25: 229-238. http://dx.doi.org/10.1016/s1350-4533(02)00192-3

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.

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.

Peterson, AK; Eckel, SP; Habre, R; Yang, T; Faham, D; Amin, M, et al. (2022). Detected prenatal perfluorooctanoic acid (PFOA) exposure is associated with decreased fetal head biometric parameters in participants experiencing higher perceived stress during pregnancy in the MADRES cohort. 9. http://dx.doi.org/10.1016/j.envadv.2022.100286

Petro, EM; D'Hollander, W; Covaci, A; Bervoets, L; Fransen, E; De Neubourg, D, et al. (2014). Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence. Science of the Total Environment 496: 282-288.

Pilkerton, CS; Hobbs, GR; Lilly, C; Knox, SS. (2018). Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. PLoS ONE 13: e0203330.

Pinney, SM; Windham, GC; Xie, C; Herrick, RL; Calafat, AM; Mcwhorter, K, et al. (2019). Perfluorooctanoate and changes in anthropometric parameters with age in young girls in the Greater Cincinnati and San Francisco Bay Area. International Journal of Hygiene and Environmental Health 222: 1038-1046.

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

Poothong, S; Padilla-Sánchez, JA; Papadopoulou, E; Giovanoulis, G; Thomsen, C; Haug, LS. (2019). Hand Wipes: A Useful Tool for Assessing Human Exposure to Poly- and Perfluoroalkyl Substances (PFASs) through Hand-to-Mouth and Dermal Contacts. Environmental Science and Technology 53: 1985-1993. http://dx.doi.org/10.1021/acs.est.8b05303

Poothong, S; Papadopoulou, E; Padilla-Sánchez, JA; Thomsen, C; Haug, LS. (2020). Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. Environment International 134: 105244. http://dx.doi.org/10.1016/j.envint.2019.105244

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.

Porter, AK; Kleinschmidt, SE; Andres, KL; Reusch, CN; Krisko, RM; Taiwo, OA, et al. (2022). Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances. Environment International 169: 107537. http://dx.doi.org/10.1016/j.envint.2022.107537

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.

Preston, EV; Rifas-Shiman, SL; Hivert, MF; Zota, AR; Sagiv, SK; Calafat, AM, et al. (2020). Associations of per- and polyfluoroalkyl substances (PFAS) with glucose tolerance

during pregnancy in project viva. Journal of Clinical Endocrinology and Metabolism 105: E2864-E2876.

Preston, EV; Webster, TF; Oken, E; Claus Henn, B; Mcclean, MD; Rifas-Shiman, SL, et al. (2018). Maternal plasma per- and polyfluoroalkyl substance concentrations in early pregnancy and maternal and neonatal thyroid function in a prospective birth cohort: Project Viva (USA). Environmental Health Perspectives 126: 027013.

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

Pumarega, J; Gasull, M; Koponen, J; Campi, L; Rantakokko, P; Henríquez-Hernández, LA, et al. (2023). Prepandemic personal concentrations of per- and polyfluoroalkyl substances (PFAS) and other pollutants: Specific and combined effects on the incidence of COVID-19 disease and SARS-CoV-2 infection. Environmental Research 237: 116965. http://dx.doi.org/10.1016/j.envres.2023.116965

Purdue, MP; Rhee, J; Denic-Roberts, H; McGlynn, KA; Byrne, C; Sampson, J, et al. (2023). A Nested Case-Control Study of Serum Per- and Polyfluoroalkyl Substances and Testicular Germ Cell Tumors among U.S. Air Force Servicemen. Environmental Health Perspectives 131: 77007. http://dx.doi.org/10.1289/EHP12603

Puttige Ramesh, N; Arora, M; Braun, JM. (2019). Cross-sectional study of the association between serum perfluorinated alkyl acid concentrations and dental caries among US adolescents (NHANES 1999-2012). British Medical Journal Open 9: e024189.

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

Qin, XD; Qian, Z; Vaughn, MG; Huang, J; Ward, P; Zeng, XW, et al. (2016). Positive associations of serum perfluoroalkyl substances with uric acid and hyperuricemia in children from Taiwan. Environmental Pollution 212: 519-524.

Qin, XD; Qian, ZM; Dharmage, SC; Perret, J; Geiger, SD; Rigdon, SE, et al. (2017). Association of perfluoroalkyl substances exposure with impaired lung function in children. Environmental Research 155: 15-21.

Qu, A; Cao, T; Li, Z; Wang, W; Liu, R; Wang, X, et al. (2021). The association between maternal perfluoroalkyl substances exposure and early attention deficit hyperactivity disorder in children: a systematic review and meta-analysis. Environmental Science and Pollution Research 28: 67066-67081. http://dx.doi.org/10.1007/s11356-021-15136-2

Qu, J; Zhao, Y; Zhang, L; Hu, S; Liao, K; Zhao, M, et al. (2022). Evaluated serum perfluoroalkyl acids and their relationships with the incidence of rheumatoid arthritis in the general population in Hangzhou, China. Environmental Pollution 307: 119505. http://dx.doi.org/10.1016/j.envpol.2022.119505

Quaak, I; de Cock, M; de Boer, M; Lamoree, M; Leonards, P; van de Bor, M. (2016). Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children. International Journal of Environmental Research and Public Health 13.

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

Radke, EG; Glenn, B; Galizia, A; Persad, A; Nachman, R; Bateson, T, et al. (2019). Development of outcome-specific criteria for study evaluation in systematic reviews of epidemiology studies. Environment International 130: 104884. http://dx.doi.org/10.1016/j.envint.2019.05.078

Rahman, ML; Zhang, C; Smarr, MM; Lee, S; Honda, M; Kannan, K, et al. (2019). Persistent organic pollutants and gestational diabetes: A multi-center prospective cohort study of healthy US women. Environment International 124: 249-258.

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; Ahmad, S; Irudayaraj, JMK. (2020). Effect of Perfluorooctanoic Acid on the Epigenetic and Tight Junction Genes of the Mouse Intestine. Toxics 8: 64.

Reardon, AJF; Khodayari Moez, E; Dinu, I; Goruk, S; Field, CJ; Kinniburgh, DW, et al. (2019). Longitudinal analysis reveals early-pregnancy associations between perfluoroalkyl sulfonates and thyroid hormone status in a Canadian prospective birth cohort. Environment International 129: 389-399.

Reece, PA; Stafford, I; Russell, J; Gill, PG. (1985). Nonlinear renal clearance of ultrafilterable platinum in patients treated with cis-dichlorodiammineplatinum (II). 15: 295-299. http://dx.doi.org/10.1007/BF00263904

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

Ren, Y; Jin, L; Yang, F; Liang, H; Zhang, Z; Du, J, et al. (2020). Concentrations of perfluoroalkyl and polyfluoroalkyl substances and blood glucose in pregnant women. Environmental Health: A Global Access Science Source 19: 88.

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

Rhee, J; Chang, VC; Cheng, I; Calafat, AM; Botelho, JC; Shearer, JJ, et al. (2023). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma in the Multiethnic Cohort Study. Environment International 180: 108197. http://dx.doi.org/10.1016/j.envint.2023.108197

Rigden, M; Pelletier, G; Poon, R; Zhu, J; al, e. (2015a). Assessment of Urinary Metabolite Excretion After Rat Acute Exposure to Perfluorooctanoic Acid and Other Peroxisomal Proliferators. Archives of Environmental Contamination and Toxicology 68: 148.

Rigden, M; Pelletier, G; Poon, R; Zhu, J; Auray-Blais, C; Gagnon, R, et al. (2015b). Assessment of urinary metabolite excretion after rat acute exposure to perfluorooctanoic acid and other peroxisomal proliferators. Archives of Environmental Contamination and Toxicology 68: 148-158.

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; Heggeseth, BC; Gallagher, LG; Botelho, JC; Calafat, AM; Gilbert-Diamond, D; Karagas, MR. (2022). Gestational per- and polyfluoroalkyl substances exposure and infant body mass index trajectory in the New Hampshire Birth Cohort Study. Environmental Research 215: 114418. http://dx.doi.org/10.1016/j.envres.2022.114418

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.

Rosen, EM; Kotlarz, N; Knappe, DRU; Lea, CS; Collier, DN; Richardson, DB; Hoppin, JA. (2022). Drinking water-associated PFAS and fluoroethers and lipid outcomes in the GenX exposure study. Environmental Health Perspectives 130: 97002. http://dx.doi.org/10.1289/EHP11033

Rosner, B. (2015). Fundamentals of biostatistics (8th ed.). Boston, MA: Brooks/Cole, Cengage Learning.

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.

Rothman, K; Greenland, S; Lash, T. (2008). Modern epidemiology. In Modern Epidemiology (3 ed.). Philadelphia, PA: Lippincott, Williams & Wilkins. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1260377C3 - 44,2302,2489

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.

Rylander, L; Lindh, CH; Hansson, S. R.; Broberg, K; Källén, K. (2020). Per- and polyfluoroalkyl substances in early pregnancy and risk for preeclampsia: a case-control study in Southern Sweden. Toxics 8: 43.

Šabović, I; Cosci, I; De Toni, L; Ferramosca, A; Stornaiuolo, M; Di Nisio, A, et al. (2020). Perfluoro-octanoic acid impairs sperm motility through the alteration of plasma membrane. Journal of Endocrinological Investigation 43: 641-652.

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.

Sakolish, C; Chen, Z; Dalaijamts, C; Mitra, K; Liu, Y; Fulton, T, et al. (2020). Predicting tubular reabsorption with a human kidney proximal tubule tissue-on-a-chip and physiologically-based modeling. Toxicology In Vitro 63: 104752.

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

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.

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

Schaider, LA; Balan, SA; Blum, A; Andrews, DQ; Strynar, MJ; Dickinson, ME, et al. (2017). Fluorinated compounds in US fast food packaging. Environmental Science & Technology Letters 4: 105-111. http://dx.doi.org/10.1021/acs.estlett.6b00435

Schecter, A; Colacino, J; Haffner, D; Patel, K; Opel, M; Päpke, O; Birnbaum, L. (2010). Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. Environmental Health Perspectives 118: 796-802. http://dx.doi.org/10.1289/ehp.0901347

Schillemans, T; Donat-Vargas, C; Lindh, CH; de Faire, U; Wolk, A; Leander, K; Åkesson, A. (2022). Per- and Polyfluoroalkyl Substances and Risk of Myocardial Infarction and Stroke: A Nested Case-Control Study in Sweden. Environmental Health Perspectives 130: 37007. http://dx.doi.org/10.1289/EHP9791

Schlummer, M; Gruber, L; Fiedler, D; Kizlauskas, M; Müller, J. (2013). Detection of fluorotelomer alcohols in indoor environments and their relevance for human exposure. Environment International 57-58: 42-49. http://dx.doi.org/10.1016/j.envint.2013.03.010

Schreder, E; Dickman, J. (2018). Take Out Toxics: PFAS Chemicals in Food Packaging. Schreder, Erika Schreder; Dickman, Jennifer. https://48h57c2l31ua3c3fmq1ne58b-wpengine.netdna-ssl.com/wp-content/uploads/2019/05/Take-Out-Toxics-Full-Report.pdf

Schumann, G; Bonora, R; Ceriotti, F; Férard, G; Ferrero, CA; Franck, PFH, et al. (2002). IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 4. Reference Procedure for the Measurement of Catalytic Concentration of Alanine Aminotransferase. Clinical Chemistry and Laboratory Medicine 40: 718-724. https://doi.org/10.1515/CCLM.2002.124

Scinicariello, F; Buser, MC; Abadin, HG; Attanasio, R. (2020a). Perfluoroalkyl substances and anthropomorphic measures in children (ages 3-11 years), NHANES 2013-2014. Environmental Research 186: 109518.

Scinicariello, F; Buser, MC; Balluz, L; Gehle, K; Murray, HE; Abadin, HG; Attanasio, R. (2020b). Perfluoroalkyl acids, hyperuricemia and gout in adults: Analyses of NHANES 2009-2014. Chemosphere 259: 127446.

Scorer, CG. (1964). The descent of the testis. Archives of Disease in Childhood 39: 605-609.

Scott, HM; Mason, JI; Sharpe, RM. (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds [Review]. Endocrine Reviews 30: 883-925. http://dx.doi.org/10.1210/er.2009-0016

Seals, R; Bartell, SM; Steenland, K. (2011). Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. Environmental Health Perspectives 119: 119-124. http://dx.doi.org/10.1289/ehp.1002346

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.

Sevelsted, A; Gürdeniz, G; Rago, D; Pedersen, CT; Lasky-Su, JA; Checa, A, et al. (2022). Effect of perfluoroalkyl exposure in pregnancy and infancy on intrauterine and childhood growth and anthropometry. Sub study from COPSAC2010 birth cohort. EBioMedicine 83: 104236. http://dx.doi.org/10.1016/j.ebiom.2022.104236

Shah-Kulkarni, S; Kim, BM; Hong, YC; Kim, HS; Kwon, EJ; Park, H, et al. (2016). Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environment International 94: 607-613.

Shankar, A; Xiao, J; Ducatman, A. (2011). Perfluoroalkyl chemicals and chronic kidney disease in US adults. American Journal of Epidemiology 174: 893-900. http://dx.doi.org/10.1093/aje/kwr171

Shankar, A; Xiao, J; Ducatman, A. (2012). Perfluorooctanoic acid and cardiovascular disease in US adults. Archives of Internal Medicine 172: 1397-1403.

Shapiro, GD; Dodds, L; Arbuckle, TE; Ashley-Martin, J; Ettinger, AS; Fisher, M, et al. (2016). Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environmental Research 147: 71-81.

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, C; Ding, J; Xu, C; Zhang, L; Liu, S; Tian, Y. (2022). Perfluoroalkyl mixture exposure in relation to fetal growth: potential roles of maternal characteristics and associations with birth outcomes. Toxics 10: 650. http://dx.doi.org/10.3390/toxics10110650

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

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.

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

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.

Shoeib, M; Harner, T; M Webster, G; Lee, SC. (2011). Indoor sources of poly- and perfluorinated compounds (PFCS) in Vancouver, Canada: implications for human exposure. Environmental Science and Technology 45: 7999-8005. http://dx.doi.org/10.1021/es103562v

Shrestha, S; Bloom, MS; Yucel, R; Seegal, RF; Rej, R; Mccaffrey, RJ, et al. (2017). Perfluoroalkyl substances, thyroid hormones, and neuropsychological status in older adults. International Journal of Hygiene and Environmental Health 220: 679-685.

Shrestha, S; Bloom, MS; Yucel, R; Seegal, RF; Wu, Q; Kannan, K, et al. (2015). Perfluoroalkyl substances and thyroid function in older adults. Environment International 75: 206-214. http://dx.doi.org/10.1016/j.envint.2014.11.018

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

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

Skogheim, TS; Villanger, GD; Weyde, KVF; Engel, SM; Surén, P; Øie, MG, et al. (2019). Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. International Journal of Hygiene and Environmental Health 223: 80-92.

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, E; Weber, J; Rofe, A; Gancarz, D; Naidu, R; Juhasz, AL. (2012). Assessment of DDT Relative Bioavailability and Bioaccessibility in Historically Contaminated Soils Using an in Vivo Mouse Model and Fed and Unfed Batch in Vitro Assays. Environmental Science and Technology2928-2934. http://dx.doi.org/10.1021/es203030q

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

Sobolewski, M; Conrad, K; Allen, JL; Weston, H; Martin, K; Lawrence, BP; Cory-Slechta, DA. (2014). Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. NeuroToxicology 45: 121-130. http://dx.doi.org/10.1016/j.neuro.2014.09.008

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

Song, P; Li, D; Wang, X; Zhong, X. (2018a). Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice. Andrologia 50: e13059.

Song, X; Tang, S; Zhu, H; Chen, Z; Zang, Z; Zhang, Y, et al. (2018b). Biomonitoring PFAAs in blood and semen samples: Investigation of a potential link between PFAAs exposure and semen mobility in China. Environment International 113: 50-54.

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; Rauh, VA; Robinson, M; Kannan, K, et al. (2020a). The association between prenatal exposure to perfluoroalkyl substances and childhood neurodevelopment. Environmental Pollution 263: 114444.

Spratlen, MJ; Perera, FP; Lederman, SA; Robinson, M; Kannan, K; Herbstman, J; Trasande, L. (2020b). The association between perfluoroalkyl substances and lipids in cord blood. Journal of Clinical Endocrinology and Metabolism 105: 43-54.

Staples, RE; Burgess, BA; Kerns, WD. (1984). The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundamental and Applied Toxicology 4: 429-440. http://dx.doi.org/10.1093/toxsci/4.3part1.429

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; Richardson, DB; Baird, DD; Haug, LS; Stuebe, AM, et al. (2014a). Perfluoroalkyl Substances During Pregnancy and Validated Preeclampsia Among Nulliparous Women in the Norwegian Mother and Child Cohort Study. American Journal of Epidemiology 179: 824-833.

Starling, AP; Engel, SM; Whitworth, KW; Richardson, DB; Stuebe, AM; Daniels, JL, et al. (2014b). 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

StataCorp. (2021). Stata Statistical Software: Release 17 [Computer Program]. College Station, TX: StataCorp LLC.

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; 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; Tinker, S; Shankar, A; Ducatman, A. (2010). Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. Environmental Health Perspectives 118: 229-233. http://dx.doi.org/10.1289/ehp.0900940

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; Mcgovern, KJ; Pajak, AM; Maglione, PJ; Wolff, M. (2016). 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; Bellinger, DC. (2013). Perfluorooctanoate and neuropsychological outcomes in children. Epidemiology 24: 590-599.

Stein, CR; Savitz, DA; Bellinger, DC. (2014). Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behaviour in 6-12-year-old children. Paediatric and Perinatal Epidemiology 28: 146-156. http://dx.doi.org/10.1111/ppe.12097

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

Stock, NL; Furdui, VI; Muir, DC; Mabury, SA. (2007). Perfluoroalkyl contaminants in the Canadian Arctic: evidence of atmospheric transport and local contamination. Environmental Science and Technology 41: 3529-3536. http://dx.doi.org/10.1021/es062709x

Stølevik, S, .B. (2012) Immunotoxic effects of dietary toxicants: Focus on prenatal exposure to acrylamide, polychlorinated biphenyls and dioxins. (Doctoral Dissertation). University of Oslo, Oslo. Retrieved from https://www.duo.uio.no/bitstream/handle/10852/28057/dravhandling-stolevik.pdf?sequence=3&isAllowed=y

Stratakis, N; Rock, S; La Merrill, MA; Saez, M; Robinson, O; Fecht, D, et al. (2022). Prenatal exposure to persistent organic pollutants and childhood obesity: A systematic review and meta-analysis of human studies. Obesity Reviews 23(S1): e13383. http://dx.doi.org/10.1111/obr.13383

Strøm, M; Hansen, S; Olsen, SF; Haug, LS; Rantakokko, P; Kiviranta, H; Halldorsson, TI. (2014). Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up. Environment International 68: 41-48.

Su, TC; Kuo, CC; Hwang, JJ; Lien, GW; Chen, MF; Chen, PC. (2016). Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environment International 88: 15-22.

Sun, Q; Zong, G; Valvi, D; Nielsen, F; Coull, B; Grandjean, P. (2018a). 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; Wang, J; Lu, Y; Dai, J. (2018b). Corticosteroid-binding globulin, induced in testicular Leydig cells by perfluorooctanoic acid, promotes steroid hormone synthesis. Archives of Toxicology 92: 2013-2025.

Tan, Y; Taibl, KR; Dunlop, AL; Barr, DB; Panuwet, P; Yakimavets, V, et al. (2023). Association between a Mixture of Per- and Polyfluoroalkyl Substances (PFAS) and Inflammatory Biomarkers in the Atlanta African American Maternal-Child Cohort. Environmental Science & Technology. http://dx.doi.org/10.1021/acs.est.3c04688

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

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.

Thomford, PJ. (2001). 4-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. 159.

Thompson, J; Lorber, M; Toms, LM; Kato, K; Calafat, AM; Mueller, JF. (2010). 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

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

Tian, M; Reichetzeder, C; Li, J; Hocher, B. (2019a). 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; Liang, H; Miao, M; Yang, F; Ji, H; Cao, W, et al. (2019b). Maternal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances during pregnancy and anogenital distance in male infants. Human Reproduction 34: 1356-1368.

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

Tian, Y; Zhou, Q; Zhang, L; Li, W; Yin, S; Li, F; Xu, C. (2023). In utero exposure to per-/polyfluoroalkyl substances (PFASs): Preeclampsia in pregnancy and low birth weight for neonates. Chemosphere 313: 137490. http://dx.doi.org/10.1016/j.chemosphere.2022.137490

Tian, YP; Zeng, XW; Bloom, MS; Lin, S; Wang, SQ; Yim, SHL, et al. (2019c). Isomers of perfluoroalkyl substances and overweight status among Chinese by sex status: Isomers of C8 Health Project in China. Environment International 124: 130-138.

Tilston, EL; Gibson, GR; Collins, CD. (2011). Colon extended physiologically based extraction test (CE-PBET) increases bioaccessibility of soil-bound PAH. Environmental Science and Technology 45: 5301-5308. http://dx.doi.org/10.1021/es2004705

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, CA; Rossing, LI; Grøntved, A; Ried-Larsen, M; Dalgård, C; Andersen, LB, et al. (2014). Adiposity and glycemic control in children exposed to perfluorinated compounds. Journal of Clinical Endocrinology and Metabolism 99: E608-E614. http://dx.doi.org/10.1210/jc.2013-3460
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
Tosh, PK; Kennedy, RB; Vierkant, RA; Jacobson, RM; Poland, GA. (2009). Correlation between rubella antibody levels and cytokine measures of cell-mediated immunity. Viral Immunology 22: 451-456. http://dx.doi.org/10.1089/vim.2009.0068
Trudel, D; Horowitz, L; Wormuth, M; Scheringer, M; Cousins, IT; Hungerbuhler, K. (2008). Estimating consumer exposure to PFOS and PFOA.[erratum appears in Risk Anal. 2008 Jun;28(3):807]. Risk Analysis 28: 251-269. http://dx.doi.org/10.1111/j.1539-6924.2008.01017.x
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.
Tsai, MS; Lin, CC; Chen, MH; Hsieh, WS; Chen, PC. (2017). Perfluoroalkyl substances and thyroid hormones in cord blood. Environmental Pollution 222: 543-548.
Tsai, MS; Lin, CY; Lin, CC; Chen, MH; Hsu, SH; Chien, KL, et al. (2015). Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. International Journal of Hygiene and Environmental Health 218: 437-443.
U.S. EPA. (1991). Guidelines for developmental toxicity risk assessment. Federal Register 56: 63798-63826.
U.S. EPA. (1996). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf
U.S. EPA. (1998). Health effects test guidelines OPPTS 870.3800 reproduction and fertility effects [EPA Report]. (EPA 712-C-98-208). Washington D.C.: U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances.
U.S. EPA. (2000). Methodology for deriving ambient water quality criteria for the protection of human health (2000). (EPA/822/B-00/004). Washington, DC: U.S. Environmental Protection Agency, Office of Water. http://www.epa.gov/waterscience/criteria/humanhealth/method/complete.pdf

U.S. EPA. (2002). 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. (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). Perfluorocarboxylic acid content in 116 articles of commerce. (EPA/600/R-09/033). Research Triangle Park, NC: National Risk Management Research Laboratory, Office of Research and Development,U.S. Environmental Protection Agency.

U.S. EPA. (2010). 2008-2009 National Rivers and Streams Assessment Fish Tissue Study. Washington, DC. https://www.epa.gov/fish-tech/2008-2009-national-rivers-and-streams-assessment-fish-tissue-study

U.S. EPA. (2011a). 2010 Great Lakes Human Health Fish Tissue Study. Washington, DC: U.S. Environmental Protection Agency, National Coastal Condition Assessment. https://www.epa.gov/fish-tech/2010-great-lakes-human-health-fish-tissue-study

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. (2011c). Toxicological Review of Trichloroethylene (CASRN 79-01-6) in support of summary information on the Integrated Risk Information System (IRIS). Washington, DC.

U.S. EPA. (2012). 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. (2015). 2013-2014 National Rivers and Streams Assessment Fish Tissue Study. Washington, DC. https://www.epa.gov/fish-tech/2013-2014-national-rivers-and-streams-assessment-fish-tissue-study

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. (2016d). National Coastal Condition Assessment: 2015 Results. Washington, DC. https://www.epa.gov/national-aquatic-resource-surveys/national-coastal-condition-assessment-2015-results

U.S. EPA. (2017). Occurrence Data for the Unregulated Contaminant Monitoring Rule: UCMR 3 (2013–2015). Washington, D.C.: U.S. Environmental Protection Agency, Office of Water. Retrieved from https://www.epa.gov/monitoring-unregulated-drinking-water-contaminants/occurrence-data-unregulated-contaminant#3

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. Announcement of final regulatory determinations for contaminants on the Fourth Drinking Water Contaminant Candidate List, 86 FR 12272 (2021a). https://www.govinfo.gov/content/pkg/FR-2021-03-03/pdf/2021-04184.pdf

U.S. EPA. (2021b). Emerging issues in food waste management: Persistent chemical contaminants [EPA Report]. (EPA/600/R-21/115). U.S. Environmental Protection Agency, Office of Research and Development. https://www.epa.gov/system/files/documents/2021-08/emerging-issues-in-food-waste-management-persistent-chemical-contaminants.pdf

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 (U.S. Environmental Protection Agency). (2022a). 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. (2022b). 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. (2022c). 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). Technical support document - per- and polyfluoroalkyl substances (PFAS) occurrence & contaminant background [EPA Report]. (EPA-822-P-22-007).

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). Human health toxicity assessment for perfluorooctane sulfonic acid (PFOS) [EPA Report]. Washington, DC: U.S. Environmental Protection Agency, Office of Water.

U.S. EPA. (2024b). Human health toxicity assessment for perfluorooctanoic acid (PFOA) [EPA Report]. Washington, DC: U.S. Environmental Protection Agency, Office of Water.

Uhl, SA; James-Todd, T; Bell, ML. (2013). Association of Osteoarthritis with Perfluorooctanoate and Perfluorooctane Sulfonate in NHANES 2003-2008. Environmental Health Perspectives 121: 447-452, 452e441-443.

Vagi, SJ; Azziz-Baumgartner, E; Sjödin, A; Calafat, AM; Dumesic, D; Gonzalez, L, et al. (2014). Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol a in polycystic ovary syndrome: a case-control study. BMC Endocrine Disorders 14: 86.

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 den Dungen, MW; Murk, AJ; Kampman, E; Steegenga, WT; Kok, DE. (2017). Association between DNA methylation profiles in leukocytes and serum levels of persistent organic pollutants in Dutch men. Environmental Epigenetics 3: dvx001.

van der Veen, I; Hanning, AC; Stare, A; Leonards, PEG; de Boer, J; Weiss, JM. (2020). The effect of weathering on per- and polyfluoroalkyl substances (PFASs) from durable water repellent (DWR) clothing. Chemosphere 249: 126100. http://dx.doi.org/10.1016/j.chemosphere.2020.126100

van Gerwen, M; Colicino, E; Guan, H; Dolios, G; Nadkarni, GN; Vermeulen, RCH, et al. (2023). Per- and polyfluoroalkyl substances (PFAS) exposure and thyroid cancer risk. EBioMedicine 97: 104831. http://dx.doi.org/10.1016/j.ebiom.2023.104831

Vanden Heuvel, JP; Davis, JW, II; Sommers, R; Peterson, RE. (1992). Renal excretion of perfluorooctanoic acid in male-rats - inhibitory effect of testosterone. Journal of Biochemical Toxicology 7: 31-36. http://dx.doi.org/10.1002/jbt.2570070107

Vanden Heuvel, JP; Kuslikis, BI; Van Rafelghem, MJ; Peterson, RE. (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. Journal of Biochemical Toxicology 6: 83-92. http://dx.doi.org/10.1002/jbt.2570060202

Velarde, MC; Chan, AFO; Sajo, MEJ, V; Zakharevich, I; Melamed, J; Uy, GLB, et al. (2022). Elevated levels of perfluoroalkyl substances in breast cancer patients within the Greater Manila Area. Chemosphere 286 Pt 1: 131545. http://dx.doi.org/10.1016/j.chemosphere.2021.131545

Vélez, MP; Arbuckle, TE; Fraser, WD. (2015). Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Human Reproduction 30: 701-709. http://dx.doi.org/10.1093/humrep/deu350

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

Vested, A; Ramlau-Hansen, CH; Olsen, SF; Bonde, JP; Kristensen, SL; Halldorsson, TI, et al. (2013). Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environmental Health Perspectives 121: 453-458, 458e451-455. http://dx.doi.org/10.1289/ehp.1205118

Vestergren, R; Cousins, IT. (2009). Tracking the pathways of human exposure to perfluorocarboxylates [Review]. Environmental Science and Technology 43: 5565-5575. http://dx.doi.org/10.1021/es900228k

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

Völkel, W; Genzel-Boroviczény, O; Demmelmair, H; Gebauer, C; Koletzko, B; Twardella, D, et al. (2008). Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk: results of a pilot study. International Journal of Hygiene and Environmental Health 211: 440-446. http://dx.doi.org/10.1016/j.ijheh.2007.07.024

Vuong, A; Yolton, K; Webster, GM; Sjodin, A; Calafat, AM; Braun, JM, et al. (2016). Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environmental Research 147: 556-564.

Vuong, AM; Braun, JM; Yolton, K; Wang, Z; Xie, C; Webster, GM, et al. (2018a). Prenatal and childhood exposure to perfluoroalkyl substances (PFAS) and measures of attention, impulse control, and visual spatial abilities. Environment International 119: 413-420.

Vuong, AM; Xie, C; Jandarov, R; Dietrich, KN; Zhang, H; Sjödin, A, et al. (2020a). Prenatal exposure to a mixture of persistent organic pollutants (POPs) and child reading skills at school age. International Journal of Hygiene and Environmental Health 228: 113527.

Vuong, AM; Yolton, K; Braun, JM; Sjodin, A; Calafat, AM; Xu, Y, et al. (2020b). Polybrominated diphenyl ether (PBDE) and poly- and perfluoroalkyl substance (PFAS) exposures during pregnancy and maternal depression. Environment International 139: 105694.

Vuong, AM; Yolton, K; Wang, Z; Xie, C; Webster, GM; Ye, X, et al. (2018b). Childhood perfluoroalkyl substance exposure and executive function in children at 8 years. Environment International 119: 212-219.

Vuong, AM; Yolton, K; Xie, C; Dietrich, KN; Braun, JM; Webster, GM, et al. (2019). Prenatal and childhood exposure to poly- and perfluoroalkyl substances (PFAS) and cognitive development in children at age 8 years. Environmental Research 172: 242-248.

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

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. (2019a). PFOS, PFOA, estrogen homeostasis, and birth size in Chinese infants. Chemosphere 221: 349-355.

Wang, H; Li, W; Yang, J; Wang, Y; Du, H; Han, M, et al. (2023a). Gestational exposure to perfluoroalkyl substances is associated with placental DNA methylation and birth size. Science of the Total Environment 858: 159747. http://dx.doi.org/10.1016/j.scitotenv.2022.159747

Wang, H; Wei, K; Wu, Z; Liu, F; Wang, D; Peng, X, et al. (2022a). Association between per- and polyfluoroalkyl substances and semen quality. Environmental Science and Pollution Research 30: 27884-27894. http://dx.doi.org/10.1007/s11356-022-24182-3

Wang, H; Yang, J; Du, H; Xu, L; Liu, S; Yi, J, et al. (2018a). Perfluoroalkyl substances, glucose homeostasis, and gestational diabetes mellitus in Chinese pregnant women: A repeat measurement-based prospective study. Environment International 114: 12-20.

Wang, IJ; Hsieh, WS; Chen, CY; Fletcher, T; Lien, GW; Chiang, HL, et al. (2011). The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environmental Research 111: 785-791. http://dx.doi.org/10.1016/j.envres.2011.04.006

Wang, J; Pan, Y; Cui, Q; Yao, B; Wang, J; Dai, J. (2018b). Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. Environmental Science and Technology 52: 13553-13561.

Wang, J; Zeng, XW; Bloom, MS; Qian, Z; Hinyard, LJ; Belue, R, et al. (2019b). Renal function and isomers of perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS): Isomers of C8 Health Project in China. Chemosphere 218: 1042-1049.

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, W; Zhou, W; Wu, S; Liang, F; Li, Y; Zhang, J, et al. (2019c). Perfluoroalkyl substances exposure and risk of polycystic ovarian syndrome related infertility in Chinese women. Environmental Pollution 247: 824-831.

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; Han, W; Wang, C; Zhou, Y; Shi, R; Bonefeld-Jørgensen, EC, et al. (2019d). Efficiency of maternal-fetal transfer of perfluoroalkyl and polyfluoroalkyl substances. Environmental Science and Pollution Research 26: 2691-2698.

Wang, Y; Rogan, WJ; Chen, HY; Chen, PC; Su, PH; Chen, HY; Wang, SL. (2015). Prenatal exposure to perfluroalkyl substances and children's IQ: The Taiwan maternal and infant cohort study. International Journal of Hygiene and Environmental Health 218: 639-644.

Wang, Y; Rogan, WJ; Chen, PC; Lien, GW; Chen, HY; Tseng, YC, et al. (2014). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environmental Health Perspectives 122: 529-534.

Wang, Y; Zhang, L; Teng, Y; Zhang, J; Yang, L; Li, J, et al. (2018c). Association of serum levels of perfluoroalkyl substances with gestational diabetes mellitus and postpartum blood glucose. Journal of Environmental Sciences 69: 5-11.

Wang, Z; Luo, J; Zhang, Y; Li, J; Zhang, J; Tian, Y; Gao, Y. (2023b). High maternal glucose exacerbates the association between prenatal per- and polyfluoroalkyl substance exposure and reduced birth weight. Science of the Total Environment 858: 160130. http://dx.doi.org/10.1016/j.scitotenv.2022.160130

Wang, Z; Shi, R; Ding, G; Yao, Q; Pan, C; Gao, Y; Tian, Y. (2022b). 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

Wang, Z; Zhang, T; Wu, J; Wei, X; Xu, A; Wang, S; Wang, Z. (2021). Male reproductive toxicity of perfluorooctanoate (PFOA): Rodent studies [Review]. Chemosphere 270: 128608. http://dx.doi.org/10.1016/j.chemosphere.2020.128608

Ward-Caviness, CK; Moyer, J; Weaver, A; Devlin, R; Diaz-Sanchez, D. (2022). Associations between PFAS occurrence and multimorbidity as observed in an electronic health record cohort. Environmental Epidemiology 6: e217. http://dx.doi.org/10.1097/EE9.0000000000000217

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.

Watkins, DJ; Josson, J; Elston, B; Bartell, SM; Shin, HM; Vieira, VM, et al. (2013). Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environmental Health Perspectives 121: 625-630. http://dx.doi.org/10.1289/ehp.1205838

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

Webster, GM; Venners, SA; Mattman, A; Martin, JW. (2014). Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environmental Research 133: 338-347. http://dx.doi.org/10.1016/j.envres.2014.06.012

Weingand, K; Bloom, J; Carakostas, M; Hall, R; Helfrich, M; Latimer, K, et al. (1992). Clinical pathology testing recommendations for nonclinical toxicity and safety studies. Toxicologic Pathology 20: 539-543.

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

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, LL; Lin, LY; Su, TC; Chen, PC; Lin, CY. (2013). Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010. Journal of Clinical Endocrinology and Metabolism 98: E1456-E1464. http://dx.doi.org/10.1210/jc.2013-1282

Wen, X; Wang, M; Xu, X; Li, T. (2022). Exposure to Per- and Polyfluoroalkyl Substances and Mortality in U.S. Adults: A Population-Based Cohort Study. Environmental Health Perspectives 130: 67007. http://dx.doi.org/10.1289/EHP10393

Weng, J; Hong, C; Tasi, J; Shen, CY, u; Su, P; Wang, S. (2020). The association between prenatal endocrine-disrupting chemical exposure and altered resting-state brain fMRI in teenagers. Brain Structure and Function 225: 1669-1684.

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

Whitehead, HD; Venier, M; Wu, Y; Eastman, E; U, r, S.; Diamond, ML; al., e. (2021). Fluorinated Compounds in North American Cosmetics. Environmental Science & Technology Letters 8: 538–544. http://dx.doi.org/https://doi.org/10.1021/acs.estlett.1c00240

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. (2018). 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

Wiener, RC; Waters, C. (2019). Perfluoroalkyls/polyfluoroalkyl substances and dental caries experience in children, ages 3-11 years, National Health and Nutrition Examination Survey, 2013-2014. Journal of Public Health Dentistry 79: 307-319.

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.

Wikström, S; Lindh, CH; Shu, H; Bornehag, CG. (2019). Early pregnancy serum levels of perfluoroalkyl substances and risk of preeclampsia in Swedish women. Scientific Reports 9: 9179.

Winquist, A; Hodge, JM; Diver, WR; Rodriguez, JL; Troeschel, AN; Daniel, J; Teras, LR. (2023). Case-Cohort Study of the Association between PFAS and Selected Cancers among Participants in the American Cancer Society's Cancer Prevention Study II LifeLink Cohort. Environmental Health Perspectives 131: 127007. http://dx.doi.org/10.1289/EHP13174

Winquist, A; Steenland, K. (2014a). 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

Winquist, A; Steenland, K. (2014b). Perfluorooctanoic acid exposure and thyroid disease in community and worker cohorts. Epidemiology 25: 255-264. http://dx.doi.org/10.1097/EDE.0000000000000040

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.

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. (2017). Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. Environment International 106: 135-143.

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, XM; Bennett, DH; Calafat, AM; Kato, K; Strynar, M; Andersen, E, et al. (2014). Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and Adults in California. Environmental Research 136C: 264-273. http://dx.doi.org/10.1016/j.envres.2014.09.026

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, 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, H; Zhou, Q; Zhang, J; Chen, X; Zhao, H; Lu, H, et al. (2020b). Exposure to elevated per- and polyfluoroalkyl substances in early pregnancy is related to increased risk of gestational diabetes mellitus: A nested case-control study in Shanghai, China. Environment International 143: 105952.

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.

Xu, Z; Du, B; Wang, H; Li, Z; Wu, Y; Wang, Q, et al. (2023). Perfluoroalkyl substances in umbilical cord blood and blood pressure in offspring: a prospective cohort study. Environmental Health 22: 72. http://dx.doi.org/10.1186/s12940-023-01023-5

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

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. (2015). 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.

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, L; Ji, H; Liang, H; Yuan, W; Song, X; Li, X, et al. (2022a). Associations of perfluoroalkyl and polyfluoroalkyl substances with gestational hypertension and blood pressure during pregnancy: A cohort study. Environmental Research 215: 114284. http://dx.doi.org/10.1016/j.envres.2022.114284

Yang, L; Li, J; Lai, J; Luan, H; Cai, Z; Wang, Y, et al. (2016a). Placental transfer of perfluoroalkyl substances and associations with thyroid hormones: Beijing prenatal exposure study. Scientific Reports 6: 21699.

Yang, L; Wang, Z; Shi, Y; Li, J; Wang, Y; Zhao, Y, et al. (2016b). Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and cord serum. Chemosphere 144: 1631-1638.

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, Z; Liu, HY; Yang, QY; Chen, X; Li, W; Leng, J; Tang, NJ. (2022b). Associations between exposure to perfluoroalkyl substances and birth outcomes: A meta-analysis. Chemosphere 291: 132909. http://dx.doi.org/10.1016/j.chemosphere.2021.132909

Yao, PL; Ehresman, DJ; Rae, JM; Chang, SC; Frame, SR; Butenhoff, JL, et al. (2014). Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid. Toxicology 326: 62-73.

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, Q; Shi, R; Wang, C; Han, W; Gao, Y; Zhang, Y, et al. (2019). Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environment International 129: 573-582.

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

York, RG; Kennedy, GL; Olsen, GW; Butenhoff, JL. (2010). Male reproductive system parameters in a two-generation reproduction study of ammonium perfluorooctanoate in rats and human relevance. Toxicology 271: 64-72. http://dx.doi.org/10.1016/j.tox.2010.03.005

Young, W; Wiggins, S; Limm, W; Fisher, CM; Dejager, L; Genualdi, S. (2022). Analysis of Per- and Poly(fluoroalkyl) Substances (PFASs) in Highly Consumed Seafood Products from U.S. Markets. Journal of Agricultural and Food Chemistry 70: 13545-13553. http://dx.doi.org/10.1021/acs.jafc.2c04673

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, Y; Qin, XD; Bloom, MS; Chu, C; Dai, X; Li, QQ, et al. (2022). Associations of prenatal exposure to perfluoroalkyl substances with preterm birth: A family-based birth cohort study. Environmental Research 214: 113803. http://dx.doi.org/10.1016/j.envres.2022.113803

Yu, Y; Quan, X; Wang, H; Zhang, B; Hou, Y; Su, C. (2023). Assessing the health risk of hyperuricemia in participants with persistent organic pollutants exposure - A systematic review and meta-analysis [Review]. Ecotoxicology and Environmental Safety 251: 114525. http://dx.doi.org/10.1016/j.ecoenv.2023.114525

Yuan, G; Peng, H; Huang, C; Hu, J. (2016). Ubiquitous occurrence of fluorotelomer alcohols in eco-friendly paper-made food-contact materials and their implication for human exposure. Environmental Science and Technology 50: 942-950. http://dx.doi.org/10.1021/acs.est.5b03806

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.

Zabaleta, I; Blanco-Zubiaguirre, L; Baharli, EN; Olivares, M; Prieto, A; Zuloaga, O; Elizalde, MP. (2020). Occurrence of per- and polyfluorinated compounds in paper and board packaging materials and migration to food simulants and foodstuffs. Food Chemistry 321: 126746. http://dx.doi.org/10.1016/j.foodchem.2020.126746

Zabaleta, I; Negreira, N; Bizkarguenaga, E; Prieto, A; Covaci, A; Zuloaga, O. (2017). Screening and identification of per- and polyfluoroalkyl substances in microwave popcorn bags. Food Chemistry 230: 497-506. http://dx.doi.org/10.1016/j.foodchem.2017.03.074

Zafeiraki, E; Gebbink, WA; Hoogenboom, R; Kotterman, M; Kwadijk, C; Dassenakis, E; van Leeuwen, SPJ. (2019). Occurrence of perfluoroalkyl substances (PFASs) in a large number of wild and farmed aquatic animals collected in the Netherlands. Chemosphere 232: 415-423. http://dx.doi.org/10.1016/j.chemosphere.2019.05.200

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. (2021a). 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

Zare Jeddi, M; Soltanmohammadi, R; Barbieri, G; Fabricio, ASC; Pitter, G; Dalla Zuanna, T; Canova, C. (2021b). To which extent are per-and poly-fluorinated substances associated to metabolic syndrome? [Review]. Reviews on Environmental Health. http://dx.doi.org/10.1515/reveh-2020-0144

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

Zeeshan, M; Yang, Y; Zhou, Y; Huang, W; Wang, Z; Zeng, XY, et al. (2020). Incidence of ocular conditions associated with perfluoroalkyl substances exposure: Isomers of C8 Health Project in China. Environment International 137: 105555.

Zell-Baran, LM; Dabelea, D; Norris, JM; Glueck, DH; Adgate, JL; Brown, JM, et al. (2023). Prenatal Exposure to Poly- and Perfluoroalkyl Substances (2009-2014) and Vaccine Antibody Titers of Measles, Mumps, Rubella, and Varicella in Children Four to Eight Years Old from the Healthy Start Cohort. Environmental Health Perspectives 131: 127018. http://dx.doi.org/10.1289/EHP12863

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, X; Chen, T; Cui, Y; Zhao, J; Chen, Q; Yu, Z, et al. (2023). In utero exposure to perfluoroalkyl substances and early childhood BMI trajectories: A mediation analysis with neonatal metabolic profiles. Science of the Total Environment 867: 161504. http://dx.doi.org/10.1016/j.scitotenv.2023.161504

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; Lodge, CJ; Dharmage, SC; Bloom, MS; Yu, Y; Yang, M, et al. (2019c). Isomers of per- and polyfluoroalkyl substances and uric acid in adults: Isomers of C8 Health Project in China. Environment International 133: 105160.

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, C; Sundaram, R; Maisog, J; Calafat, AM; Barr, DB; Buck Louis, GM. (2015a). A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. Fertility and Sterility 103: 184-189. http://dx.doi.org/10.1016/j.fertnstert.2014.10.001

Zhang, H; Lu, Y; Luo, B; Yan, S; Guo, X; Dai, J. (2014). 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, H; Yolton, K; Webster, GM; Ye, X; Calafat, AM; Dietrich, KN, et al. (2018a). Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8 years. Environment International 111: 224-231.

Zhang, L; Liang, J; Gao, A. (2023a). Contact to perfluoroalkyl substances and thyroid health effects: A meta-analysis directing on pregnancy [Review]. Chemosphere 315: 137748. http://dx.doi.org/10.1016/j.chemosphere.2023.137748

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, P; Qi, C; Ma, Z; Wang, Y; Zhang, L; Hou, X. (2022a). Perfluorooctanoic acid exposure in vivo perturbs mitochondrial metabolic during oocyte maturation. Environmental Toxicology 37: 2965-2976. http://dx.doi.org/10.1002/tox.23652

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; Tan, R; Pan, R; Xiong, J; Tian, Y; Wu, J; Chen, L. (2018b). Association of perfluoroalkyl and polyfluoroalkyl substances with premature ovarian insufficiency in Chinese women. Journal of Clinical Endocrinology and Metabolism 103: 2543-2551.

Zhang, T; Fu, S; Yu, K; Albanes, D; Moore, SC; Purdue, MP; Stolzenberg-Solomon, RZ. (2023b). Nested Case-Control Studies Investigating Serum Perfluorooctanoate and Perfluorooctane Sulfonate Levels and Pancreatic Ductal Adenocarcinoma in Two Cohorts. Environmental Health Perspectives 131: 107702. http://dx.doi.org/10.1289/EHP13208

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. (2015b). 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; Xue, L; Deji, Z; Wang, X; Liu, P; Lu, J, et al. (2022b). Effects of exposure to per- and polyfluoroalkyl substances on vaccine antibodies: A systematic review and meta-analysis based on epidemiological studies [Review]. Environmental Pollution 306: 119442. http://dx.doi.org/10.1016/j.envpol.2022.119442

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; Mustieles, V; Sun, Y; Oulhote, Y; Wang, YX; Messerlian, C. (2022c). Association between serum per- and polyfluoroalkyl substances concentrations and common cold among children and adolescents in the United States. Environment International 164: 107239. http://dx.doi.org/10.1016/j.envint.2022.107239

Zhang, Y; Mustieles, V; Wang, YX; Sun, Q; Coull, B; Sun, Y, et al. (2023c). 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, Z; Wang, F; Zhang, Y; Yao, J; Bi, J; He, J, et al. (2022d). Associations of serum PFOA and PFOS levels with incident hypertension risk and change of blood pressure levels. Environmental Research 212: 113293. http://dx.doi.org/10.1016/j.envres.2022.113293

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.

Zhao, Y; Jin, H; Qu, J; Zhang, S; Hu, S; Xue, J; Zhao, M. (2022a). The influences of perfluoroalkyl substances on the rheumatoid arthritis clinic. BMC Immunology 23: 10. http://dx.doi.org/10.1186/s12865-022-00483-7

Zhao, Y; Liu, W; Qu, J; Hu, S; Zhang, L; Zhao, M, et al. (2022b). Per-/polyfluoroalkyl substance concentrations in human serum and their associations with immune markers of rheumatoid arthritis. Chemosphere 298: 134338. http://dx.doi.org/10.1016/j.chemosphere.2022.134338

Zheng, F; Sheng, N; Zhang, H; Yan, S; Zhang, J; Wang, J. (2017). Perfluorooctanoic acid exposure disturbs glucose metabolism in mouse liver. Toxicology and Applied Pharmacology 335: 41-48.

Zheng, T; Kelsey, K; Zhu, C; Pennell, KD; Yao, Q; Manz, KE, et al. (2023). Adverse birth outcomes related to concentrations of per- and polyfluoroalkyl substances (PFAS) in maternal blood collected from pregnant women in 1960-1966. Environmental Research117010. http://dx.doi.org/10.1016/j.envres.2023.117010

Zhou, W; Zhang, L; Tong, C; Fang, F; Zhao, S; Tian, Y, et al. (2017a). Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environmental Health Perspectives 125: 067012.

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; Chang, JJ; King, C; Paul, G, et al. (2016). Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environment International 94: 189-195.

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.

Zirkin, B, .R.; Papadopoulos, V, . (2018). Leydig cells: formation, function, and regulation. Biology of Reproduction 99: 101-111. http://dx.doi.org/10.1093/biolre/ioy059

Zong, G; Grandjean, P; Wang, X; Sun, Q. (2016). Lactation history, serum concentrations of persistent organic pollutants, and maternal risk of diabetes. Environmental Research 150: 282-288.