EPA/635/R-24/172Fa
www.epa.gov/iris

IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA) and Related Salts

CASRN 335-76-2

July 2024

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed by the U.S. Environmental Protection Agency, Office of Research and Development and approved for publication. Any mention of trade names, products, or services does not imply an endorsement by the U.S. government or the U.S. Environmental Protection Agency. EPA does not endorse any commercial products, services, or enterprises.

CONTENTS

AUTHORS | CONTRIBUTORS | REVIEWERS xiii
EXECUTIVE SUMMARY xvi
1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS 1-1
1.1. BACKGROUND INFORMATION ON PERFLUORODECANOIC ACID (PFDA) 1-1
1.1.1. Physical and Chemical Properties 1-1
1.1.2. Sources, Production, and Use 1-3
1.1.3. Environmental Fate and Transport 1-3
1.1.4. Potential for Human Exposure, Including Populations and Lifestages with Potentially Greater Exposure 1-4
1.2. SUMMARY OF ASSESSMENT METHODS 1-9
1.2.1. Literature Search and Screening 1-9
1.2.2. Evaluation of Individual Studies 1-12
1.2.3. Additional Epidemiology Considerations 1-13
1.2.4. Data Extraction 1-14
1.2.5. Evidence Synthesis and Integration 1-15
1.2.6. Dose-Response Analysis 1-16
2. LITERATURE SEARCH RESULTS 2-1
2.1. LITERATURE SEARCH AND SCREENING RESULTS 2-1
2.2. SUMMARY OF STUDIES MEETING PECO CRITERIA 2-3
3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND INTEGRATION 3-1
3.1. PHARMACOKINETICS 3-1
3.1.1. Absorption 3-2
3.1.2. Distribution 3-4
3.1.3. Metabolism 3-15
3.1.4. Excretion 3-16
3.1.5. Summary of Pharmacokinetic Parameters 3-26
3.1.6. Evaluation of Physiologically Based Pharmacokinetic (PBPK) and Pharmacokinetic PK Modeling 3-28
3.1.7. Approach for Pharmacokinetic Extrapolation of PFDA among Rats, Mice, and Humans 3-31

3.2. NONCANCER HEALTH EFFECTS 3-37
3.2.1. Hepatic Effects 3-37
3.2.2. Immune Effects 3-68
3.2.3. Developmental Effects 3-113
3.2.4. Male Reproductive Effects 3-179
3.2.5. Female Reproductive Effects 3-203
3.2.6. Cardiometabolic Effects 3-222
3.2.7. Neurodevelopmental Effects 3-253
3.2.8. Endocrine Effects 3-262
3.2.9. Urinary Effects 3-286
3.2.10. General Toxicity 3-299
3.2.11. Other Health Effects 3-304
3.3. CARCINOGENICITY 3-305
3.3.1. Cancer 3-305
4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS 4-1
4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS 4-1
4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY 4-5
4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES 4-5
5. DERIVATION OF TOXICITY VALUES 5-1
5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED 5-1
5.2. NONCANCER TOXICITY VALUES 5-1
5.2.1. Oral Reference Dose (RfD) Derivation 5-2
5.2.2. Selection of Lifetime Toxicity Value(s) 5-27
5.2.3. Subchronic toxicity values for oral exposure (subchronic oral reference dose [RfD]) derivation 5-32
5.2.4. Inhalation Reference Concentration (RfC) Derivation 5-46
5.3. CANCER TOXICITY VALUES 5-46
REFERENCES R-1
SUPPLEMENTAL INFORMATION ..... (see Volume 2)

TABLES

Table ES-1. Organ-specific RfDs for health effects with evidence available to synthesize and draw summary judgments for the derivation of toxicity values xviii
Table ES-2. Overall Lifetime and subchronic RfDs xx
Table 1-1. Physicochemical properties of PFDA and related salts 1-2
Table 1-2. Serum PFDA concentrations based on NHANES 2013–2014 data (µg/L) 1-5
Table 1-3. PFDA levels at 10 military installations 1-8
Table 1-4. Populations, exposures, comparators, and outcomes (PECO) criteria 1-10
Table 2-1. Animal toxicity studies examining health effects after PFDA administration 2-3
Table 3-1. Volume of distribution values reported for animal studies 3-7
Table 3-2. PFDA total clearance in rats and mice 3-17
Table 3-3. Rat, mouse, and human pharmacokinetic parameters 3-26
Table 3-4. Associations between PFDA and serum biomarkers of hepatic function in *medium* confidence epidemiological studies 3-41
Table 3-5. Incidence and severity of hepatocyte lesions in Sprague-Dawley rats exposed to PFDA in 28-day gavage studies 3-47
Table 3-6. Percent change relative to controls in hepatocellular serum markers in short-term animal studies after PFDA exposure 3-50
Table 3-7. Percent change relative to controls in hepatobiliary serum markers in a 28-day rat study after PFDA exposure (NTP, 2018) 3-50
Table 3-8. Percent change relative to controls in serum proteins in a 28-day rat study after PFDA exposure (NTP, 2018) 3-52
Table 3-9. Percent change relative to controls in liver weight (relative to body weight) due to PFDA exposure in short-term oral toxicity studies 3-55
Table 3-10. Evidence profile table for PFDA exposure and liver effects 3-64
Table 3-11. Summary of PFDA exposure and selected data on antibody response in humans 3-73
Table 3-12. Studies on PFDA and infectious disease in humans 3-77
Table 3-13. Studies on PFDA and hypersensitivity-related outcomes in humans 3-83
Table 3-14. Summary of PFDA and selected data on hypersensitivity in humans 3-84
Table 3-15. Percent change relative to controls in absolute spleen cell population counts in female B6C3F1/N mice exposed to PFDA exposure for 28-days 3-92
Table 3-16. Percent change relative to controls in blood leukocyte counts in female Sprague-Dawley rats exposed to PFDA exposure for 28-days (NTP, 2018) 3-95
Table 3-17. Percent change relative to controls in immune organ weights in short-term animal studies after exposure to PFDA 3-100
Table 3-18. Evidence profile table for PFDA exposure and immune effects 3-109
Table 3-19. Summary of 33 studies (from 35 publications) of PFDA exposure in relation to fetal and postnatal growth restriction measures sorted by overall confidence[a] 3-138
Table 3-20. Summary of 12 studies of PFDA exposure and gestational duration measures 3-160
Table 3-21. Associations between PFDA and spontaneous abortion in six epidemiological studies 3-164
Table 3-22. Percent changes relative to controls in fetal body weight in a developmental mouse study after PFDA exposure (Harris and Birnbaum, 1989) 3-167
Table 3-23. Evidence profile table for PFDA exposure and developmental effects 3-173
Table 3-24. Associations between serum PFDA and semen parameters in epidemiological studies 3-180
Table 3-25. Evidence profile table for PFDA exposure and male reproductive effects 3-199
Table 3-26. Associations between PFDA and time to pregnancy in epidemiological studies 3-207

Table 3-27. Associations between PFDA and breastfeeding duration in epidemiological studies 3-210
Table 3-28. Percent changes relative to controls in time spent in each estrous stage 3-213
Table 3-29. Evidence profile table for PFDA exposure and female reproductive effects 3-219
Table 3-30. Associations between PFDA and blood lipids in *medium* confidence epidemiological studies 3-226
Table 3-31. Associations between PFDA and hypertensive disorders of pregnancy in epidemiological studies 3-230
Table 3-32. Associations between PFDA and coronary heart disease in epidemiological studies 3-232
Table 3-33. Associations between PFDA and insulin resistance in epidemiological studies 3-237
Table 3-34. Associations between PFDA and adiposity in epidemiological studies 3-243
Table 3-35. Percent change relative to controls in serum lipids in a 28-day rat study after PFDA exposure (NTP, 2018) 3-246
Table 3-36. Percent change relative to controls in heart weights in a 28-day rat study after PFDA exposure (NTP, 2018) 3-247
Table 3-37. Evidence profile table for PFDA exposure and cardiometabolic effects 3-250
Table 3-38. Results for *medium* confidence epidemiological studies of PFDA exposure and behavioral and attention effects 3-257
Table 3-39. Evidence profile table for PFDA exposure and neurodevelopmental effects 3-261
Table 3-40. Associations between PFDA and blood lipids in medium confidence epidemiological studies 3-265
Table 3-41. Percent changes relative to controls in thyroid hormone levels in a 28-day rat study after PFDA exposure (NTP, 2018) 3-270
Table 3-42. Evidence profile table for PFDA exposure and endocrine effects 3-282
Table 3-43. Associations between serum PFDA and urinary effects in *low* confidence epidemiological studies 3-287
Table 3-44. Percent change relative to controls in serum biomarkers of kidney function in a 28-day rat study after PFDA exposure (NTP, 2018) 3-292
Table 3-45. Percent change relative to controls in kidney weights (absolute and relative to body weight) due to PFDA exposure in short-term oral toxicity studies 3-294
Table 3-46. Evidence profile table for PFDA exposure and urinary effects 3-297
Table 3-47. Test evaluating genotoxicity and mutagenicity 3-309
Table 4-1. Hazard conclusions across published EPA PFAS human health assessments 4-4
Table 5-1. Endpoints considered for dose-response modeling and derivation of points of departure for liver effects in animals 5-3
Table 5-2. Endpoints considered for dose-response modeling and derivation of points of departure for immune effects in humans 5-6
Table 5-3. Results from the analyses of PFDA measured in serum at age 5 years 5-7
Table 5-4. Results from the analyses of PFDA measured in serum at age 5 years 5-7
Table 5-5. Mean birth weight deficit studies considered for dose-response modeling and derivation of points of departure for developmental effects in humans 5-9
Table 5-6. Endpoints considered for dose-response modeling and derivation of points of departure for developmental effects in animals 5-11
Table 5-7. Endpoints considered for dose-response modeling and derivation of points of departure for male reproductive effects in animals 5-12
Table 5-8. Endpoints considered for dose-response modeling and derivation of points of departure for female reproductive effects in animals 5-14
Table 5-9. Benchmark response levels selected for BMD modeling of PFDA health outcomes 5-15
Table 5-10. PODs considered for the derivation of PFDA candidate values 5-17

Table 5-11. Uncertainty factors for the development of the candidate lifetime toxicity values for PFDA 5-22
Table 5-12. Candidate RfD values for PFDA 5-26
Table 5-13. Confidence in the organ/system-specific (osRfDs) for PFDA 5-27
Table 5-14. Organ/System-specific and overall lifetime RfDs for PFDA 5-30
Table 5-15. Uncertainty factors for the development of the candidate subchronic values for PFDA 5-33
Table 5-16. Candidate values for deriving the subchronic RfD for PFDA 5-36
Table 5-17. Confidence in the subchronic organ/system-specific RfDs (subchronic osRfDs) for PFDA 5-39
Table 5-18. Organ/system-specific and overall subchronic RfDs for PFDA 5-44

FIGURES

Figure 1-1. Chemical structure of PFDA and related salts. 1-2
Figure 2-1. Literature search for perfluorodecanoic acid and related salts 2-2
Figure 3-1. Ratio of extracellular water (% of body weight) in children versus adults. 3-13
Figure 3-2. Serum concentrations of PFDA and PFNA in U.S. males versus females as a function of age. 3-24
Figure 3-3. Comparison of PFDA PBPK model predictions 3-30
Figure 3-4. Evaluation results for epidemiological studies assessing effects of PFDA exposure on liver serum biomarkers. 3-39
Figure 3-5. Evaluation results for animal studies assessing effects of PFDA exposure on liver histopathology. 3-45
Figure 3-6. Effects on liver histopathology following exposure to PFDA in short-term oral studies in animals. 3-48
Figure 3-7. Evaluation results for animal studies assessing effects of PFDA exposure on liver serum biomarkers. 3-49
Figure 3-8. Effects on serum liver biomarkers following exposure to PFDA in short-term oral studies in animals 3-53
Figure 3-9. Evaluation results for animal studies assessing effects of PFDA exposure on liver weight. 3-54
Figure 3-10. Effects on relative liver weight following exposure to PFDA in short-term oral studies in animals 3-56
Figure 3-11. Evaluation results for epidemiological studies assessing effects of PFDA exposure on antibody response to vaccination 3-70
Figure 3-12. Evaluation results for epidemiological studies assessing of PFDA exposure on infectious disease 3-77
Figure 3-13. Evaluation results of epidemiological studies assessing effects of PFDA exposure on sensitization or allergic response 3-80
Figure 3-14. Evaluation results for an animal study assessing effects of PFDA exposure on host resistance. 3-87
Figure 3-15. Evaluation results for animal studies assessing effects of PFDA exposure on immune function assays 3-88
Figure 3-16. Effects on immune function assays following exposure to PFDA in short-term oral studies in animals 3-90

Figure 3-17. Evaluation results for animal studies assessing effects of PFDA exposure on general/observational immune assays. 3-91
Figure 3-18. Effects on general/observational immune assays following exposure to PFDA in short-term oral studies in animals. 3-93
Figure 3-19. Evaluation results for animal studies assessing effects of PFDA exposure on blood leukocyte counts. 3-94
Figure 3-20. Effects on blood leukocyte counts following exposure to PFDA in short-term oral studies in animals. 3-96
Figure 3-21. Evaluation results for animal studies assessing effects of PFDA exposure on immune histopathology and organ weights. 3-97
Figure 3-22. Effects on immune organ histopathology following exposure to PFDA in short-term oral studies in animals. 3-99
Figure 3-23. Effects on immune organ weights following exposure to PFDA in short-term oral studies in animals 3-102
Figure 3-24. Evaluation results for an animal study assessing effects of PFDA exposure on immune function assays for sensitization and allergic response. 3-103
Figure 3-25. Evaluation results for epidemiological studies assessing effects of PFDA exposure on birth weight 3-116
Figure 3-26. Twenty-eight perinatal studies of birth weight measures and subsets considered for different analyses. 3-117
Figure 3-27. PFDA and birth weight z-scores (overall population). 3-119
Figure 3-28. PFDA and birth weight z-score (sex-stratified).. 3-119
Figure 3-29. Overall study population mean birth weight results for 22 PFDA epidemiological studies. 3-123
Figure 3-30. Sex-specific male infants only mean birth weight results for 14 PFDA epidemiological studies. 3-124
Figure 3-31. Sex-specific female infants only mean birth weight results for 14 PFDA epidemiological studies. 3-125
Figure 3-32. Evaluation results for epidemiological studies assessing effects of PFDA exposure on low birth weight/small for gestational age. 3-126
Figure 3-33. Dichotomous fetal growth restriction (small for gestational age and low birth weight) results for 5 PFDA epidemiological studies. 3-127
Figure 3-34. Evaluation results for epidemiological studies assessing the effects of PFDA exposure on birth length 3-128
Figure 3-35. Overall study population mean birth length results for 15 PFDA epidemiological studies. 3-131
Figure 3-36. Sex-stratified birth length results for 10 PFDA epidemiological studies. 3-132
Figure 3-37.Evaluation results for epidemiological studies assessing the effects of PFDA exposure on head circumference. 3-133
Figure 3-38. Overall population head circumference results in 11 epidemiological studies 3-134
Figure 3-39. Sex-stratified head circumference results in eight PFDA epidemiological studies. 3-135
Figure 3-40. Evaluation results for epidemiological studies assessing the effects of PFDA exposure on postnatal growth 3-142
Figure 3-41. PFDA and postnatal growth-standardized weight measures in the overall population. 3-144
Figure 3-42. PFDA and postnatal growth – mean weight (in grams) results from two epidemiological studies 3-144

Figure 3-43. PFDA and postnatal growth – standardized weight (sex-stratified; boys above dashed line, girls below) 3-145
Figure 3-44. PFDA and postnatal growth – standardized height measures in the overall population 3-146
Figure 3-45. PFDA and postnatal growth – mean height (in centimeters). 3-146
Figure 3-46. PFDA and postnatal growth – standardized height measures (sex-stratified; boys above reference line, girls below) 3-147
Figure 3-47. PFDA and postnatal growth-standardized head circumference in the overall population. 3-148
Figure 3-48. PFDA and postnatal growth head circumference (sex-stratified; boys above reference line, girls below). 3-148
Figure 3-49. PFDA and postnatal growth measures-body mass index, adiposity, ponderal index, waist circumference (overall population). 3-149
Figure 3-50. PFDA and postnatal growth rapid growth (overall population) and sex-specific (in grams). 3-151
Figure 3-51. Evaluation results for epidemiological studies assessing effects of PFDA exposure on anogenital distance. 3-153
Figure 3-52. Evaluation results for epidemiological studies assessing effects of PFDA exposure on gestational duration. 3-154
Figure 3-53. Preterm birth forest plot-six studies based on the overall population 3-156
Figure 3-54. Overall population results for 10 gestational age studies. 3-158
Figure 3-55. Sex-stratified results for five gestational age studies. 3-159
Figure 3-56. Evaluation results for epidemiological studies assessing effects of PFDA exposure on spontaneous abortion. 3-162
Figure 3-57. Evaluation results for an animal study assessing effects of PFDA exposure on development. 3-165
Figure 3-58. PFDA fetal body weight after gestational exposure. 3-166
Figure 3-59. PFDA developmental effects. 3-169
Figure 3-60. Evaluation results for epidemiological studies assessing effects of PFDA exposure on semen parameters.. 3-180
Figure 3-61. Evaluation results for epidemiological studies assessing effects of PFDA exposure on male reproductive hormones 3-182
Figure 3-62. Evaluation results for epidemiological studies assessing effects of PFDA exposure on male pubertal development.. 3-184
Figure 3-63. Evaluation results for an animal study assessing effects of PFDA exposure on male reproduction. 3-185
Figure 3-64. Effects on sperm evaluations following exposure to PFDA in short-term oral studies in animals. 3-187
Figure 3-65. Effects on male reproductive organ histopathology following exposure to PFDA in short-term oral studies in animals. 3-189
Figure 3-66. Effects on serum testosterone levels following exposure to PFDA in short-term oral studies in animals 3-191
Figure 3-67. Effects on male reproductive organ weights following exposure to PFDA in short-term oral studies in animals. 3-193
Figure 3-68. Evaluation results for epidemiological studies assessing effects of PFDA exposure on female reproduction. 3-204
Figure 3-69. Evaluation results for an animal study assessing effects of PFDA on female reproduction.. 3-212

Figure 3-70. PFDA female reproductive effects. 3-215
Figure 3-71. Evaluation results for epidemiological studies assessing effects of PFDA exposure on serum lipids. 3-224
Figure 3-72. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cardiovascular risk factors other than serum lipids. 3-229
Figure 3-73. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cardiovascular disease.. 3-232
Figure 3-74. Evaluation results for epidemiological studies assessing effects of PFDA exposure on diabetes and insulin resistance. 3-234
Figure 3-75. Evaluation results for epidemiological studies assessing effects of PFDA exposure on adiposity. 3-241
Figure 3-76. Evaluation results for an animal study assessing effects of PFDA exposure on cardiometabolic effects.. 3-245
Figure 3-77. Cardiometabolic effects following exposure to PFDA in short-term oral studies in animals. 3-248
Figure 3-78. Evaluation results for epidemiological studies of PFDA and neurodevelopmental effects. 3-254
Figure 3-79. Study evaluation results for epidemiological studies assessing effects of PFDA exposure on thyroid effects. 3-263
Figure 3-80. Evaluation results for an animal study assessing effects of PFDA exposure on thyroid hormone levels. 3-269
Figure 3-81. PFDA thyroid hormone levels after short-term oral exposure. 3-271
Figure 3-82. Evaluation results for animal studies assessing effects of PFDA exposure on endocrine histopathology. 3-272
Figure 3-83. PFDA endocrine histopathology. 3-273
Figure 3-84. Evaluation results for animal studies assessing effects of PFDA exposure on endocrine organ weights. 3-274
Figure 3-85. PFDA endocrine organ weight. 3-276
Figure 3-86. Evaluation results for human studies assessing effects of PFDA exposure on urinary effects. 3-287
Figure 3-87. Evaluation results for animal studies assessing effects of PFDA exposure on urinary effects. 3-290
Figure 3-88. Kidney histopathology effects following exposure to PFDA in 28-day rat study. 3-291
Figure 3-89. Urinary effects following exposure to PFDA in short-term oral studies in animals. 3-295
Figure 3-90. Evaluation results for animal studies assessing effects of PFDA exposure on general toxicity 3-300
Figure 3-91. PFDA general toxicity effects. 3-302
Figure 3-92. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cancer 3-306

ABBREVIATIONS AND ACRONYMS

ADHD	attention deficient hyperactivity disorder
ADME	absorption, distribution, metabolism, excretion
AGD	anogenital distance
AIC	Akaike's information criterion
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AR	androgen
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMI	body max index
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
BWT	birth weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CDR	Chemical Reporting Data
CCTE	Center for Computational Toxicology and Exposure
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
CL	confidence limit
CL_A	clearance in animals
CL_H	clearance in humans
Cmax	peak concentration
CNS	central nervous system
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CS	cross-sectional study
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DDEFs	data-derived extrapolation factors
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DSB	double-strand breaks
DTH	delayed-type-hypersensitivity
EPA	Environmental Protection Agency
ER	estrogen
F_{abs}	fraction absorbed
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GA	gestational age
GD	gestation day
GDH	glutamate dehydrogenase
GFR	glomerular filtration rate
GGT	γ-glutamyl transferase
GLP	good laboratory practices
GSH	glutathione
GST	glutathione-S-transferase
HAWC	Health Assessment Workplace Collaborative
HBCD	hexabromocyclododecane
HCC	hepatocellular carcinoma
HFMD	hand, food, and mouth disease
Hb/g-A	animal blood: gas partition coefficient
Hb/g-H	human blood: gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HR	hazard ratio
HTS	high-throughput screening
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IQR	interquartile range
IRIS	Integrated Risk Information System
i.v.	intravenous
LBW	low birth weight
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDL	low-density lipoprotein
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LPS	lipopolysaccharide
MCH	mean corpuscular hemoglobin
MMR	measles, mumps, and rubella
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MPS	mononuclear phagocyte system
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level

NR	not reported
NTP	National Toxicology Program
NZW	New Zealand white (rabbit breed)
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PECO	population, exposure, comparator, outcome
PFAAs	perfluoroalkyl acids
PFAS	per and polyfluoroalkyl substances
PFBS	perfluorobutane sulfonic acid
PFCA	perfluoroalkylcarboxylic acids
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
POD_{HED}	human equivalent dose POD
PPARα	peroxisome proliferator-activator receptor alpha
PTB	preterm birth
QSAR	quantitative structure-activity relationship
RBC	red blood cells
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SDS	standard deviation scores
SE	standard error
SGA	small for gestational age
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SHBG	sex hormone binding globulin
SRBC	sheep red blood cell
Tmax	time to peak concentration
TNFα	tumor necrosis factor alpha
TRH	thyroid releasing hormone
TSCATS	Toxic Substances Control Act Test Submission
TSH	thyroid-stimulating hormone
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
VLBW	very low birth weight
WHO	World Health Organization
WOS	Web of Science

AUTHORS | CONTRIBUTORS | REVIEWERS

Assessment Managers (Lead Authors)

J. Phillip Kaiser, Ph.D.	EPA/ORD/CPHEA
Lucina Lizarraga, Ph.D.	

Authors

Xabier Arzuaga, Ph.D.	EPA/ORD/CPHEA
Thomas F. Bateson, Sc.D., M.P.H.	
J. Allen Davis, M.S.P.H.	
Andrew Kraft, Ph.D.	
Elizabeth Radke, Ph.D.	
Hongyu Ru, Ph.D.	
Paul Schlosser, Ph.D.	
J. Michael Wright, Sc.D.	

Contributors

Michelle M. Angrish, Ph.D.	EPA/ORD/CPHEA
Krista Christensen, M.P.H., Ph.D.	
Michael Dzierlenga, Ph.D.	
Ingrid Druwe, Ph.D.	
Barbara Glenn, Ph.D. (retired)	
Andrew Hotchkiss, Ph.D.	
Stephanie Kim, Ph.D.	
Christopher Lau, Ph.D.	
Geniece M. Lehman, Ph.D.	
Susan Makris, M.S. (retired)	
Anuradha Mudipalli, Ph.D.	
Kathleen Newhouse, M.S.	
Kristen Rappazzo, Ph.D.	
Tammy Stoker, Ph.D.	
Andre Weaver, Ph.D.	
Erin Yost, Ph.D.	
Jay Zhao, Ph.D.	
Chris Corton, Ph.D.	EPA/ORD/CCTE
Jason C. Lambert, Ph.D.	
April Luke, M.S.	EPA/OLEM
Andrew A Rooney, Ph.D.	DTT/NIEHS
Kyla Taylor, Ph.D.	
Dori Germolec, Ph.D.	
Nora Abdel-Gawad (former)	Oak Ridge Associated Universities (ORAU) Contractor
Alexis Agbai	

Angela Scafidi (former)	
Rebecca Schaefer	
Robyn B. Blain, Ph.D.	ICF
Alexandra E. Goldstone, M.P.H.	
Alexander J. Lindahl, M.P.H.	
Christopher A. Sibrizzi, M.P.H.	

Production Team

Ryan Jones (HERO Director)	EPA/ORD/CPHEA
Jack Rehmann (CPHEA Webmaster)	
Dahnish Shams (Project Management Team)	
Avanti Shirke (Project Management Team)	
Jessica Soto-Hernandez (Project Management Team)	
Samuel Thacker (HERO Team)	
Garland Waleko (Project Management Team)	

Executive Direction

Wayne Cascio, M.D. (CPHEA Director)	EPA/ORD/CPHEA
V. Kay Holt, M.S. (CPHEA Deputy Director)	
Samantha Jones, Ph.D. (CPHEA Associate Director)	
Jon Jeffery Sutton, MBA (CPHEA POS Director)	
Kristina Thayer, Ph.D. (CPAD Director)	
Andrew Kraft, Ph.D. (IRIS PFAS Team Lead and CPAD Associate Director)	
Paul White, Ph.D. (CPAD Senior Science Advisor)	
Ravi Subramanian, Ph.D. (CPAD Senior Science Advisor)	
Elizabeth Radke, Ph.D. (Branch Chief)	
Janice Lee, Ph.D. (Branch Chief)	
Viktor Morozov, Ph.D. (Branch Chief)	
Shannon Hanna, Ph.D. (Branch Chief)	
Glenn Rice, Ph.D. (Branch Chief)	
Vicki Soto, B.S. (Branch Chief)	

Reviewers

CPAD Executive Review Committee	
Kristina Thayer	CPAD Division Director
Paul White	CPAD Senior Science Advisor
Glenn Rice	CPHEA/CPAD/TEAB-C Branch Chief
Karen Hogan	CPHEA/CPAD/Emeritus
Alan Stern	NJDEP (retired), Contractor

Agency Reviewers

This assessment was provided for review to scientists in EPA's program and regional offices. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection

Office of Air and Radiation/Office of Air Quality Planning and Standards

Office of Chemical Safety and Pollution Prevention

Office of Land and Emergency Management

Office of Water

Region 2, New York City, NY

Region 8, Denver, CO

Interagency Reviewers

This assessment was provided for review to other federal agencies and the Executive Office of the President (EOP). Comments were submitted by:

The White House

- Office of Science and Technology Policy

Office of Management and Budget

Department of Defense

Department of Agriculture

Department of Health and Human Services

Agency for Toxic Substances and Disease Registry

National Institute of Environmental Health Sciences

- National Institute of Occupational Safety and Health

EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Perfluorodecanoic acid (PFDA, CASRN 335-76-2),[1] and its related salts are members of the group per- and polyfluoroalkyl substances (PFAS). This Toxicological Review applies to PFDA as well as salts (including nonmetal or alkali metal salts) of PFDA that would be expected to fully dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body). Thus, while this Toxicological Review would not necessarily apply to nonalkali metal salts of PFDA because of the possibility of PFDA-independent contributions of toxicity, it does apply to PFDA salts including ammonium perfluorodecanoate (PFDA NH4, CASRN 3108-42-7) and sodium perfluorodecanoate (PFDA-Na, CASRN 3830-45-3), and other nonmetal or alkali metal salts of PFDA. The synthesis of evidence and toxicity value derivation presented in this Toxicological Review focuses on the free acid of PFDA, given the currently available toxicity data.[2]

Concerns about PFDA and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment. PFAS are not naturally occurring in the environment; they are synthetic compounds that have been used widely over the past several decades in industrial applications and consumer products because of their resistance to heat, oil, stains, grease, and water. PFAS in the environment are linked to industrial sites, military fire training areas, wastewater treatment plants, and commercial products (see Section 1.1.3. for information specific to PFDA).

The Integrated Risk Information System (IRIS) Program is developing a series of five PFAS assessments (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexanesulfonic acid [PFHxS], perfluorononanoic acid [PFNA], PFDA, and their associated salts) (see December 2018 IRIS Program Outlook) at the request of EPA National Programs. Specifically, the development of human health toxicity assessments for exposure to these PFAS represents only one component of the broader PFAS strategic roadmap at EPA that is aimed at characterizing potential health effects of individual PFAS and groups of PFAS

[1]The CASRN given here is for linear PFDA; the source PFDA used in the animal toxicity study NTP (2018) was reported to be >97% pure, giving this CASRN. For the human studies [e.g., Valvi et al. (2017)] the purity of the PFDA source was not provided by the study authors. None of the available studies explicitly state that only the linear form was used. Therefore, there is the possibility that some proportion of the PFDA used in the studies were branched isomers and thus observed health effects may apply to the total linear and branched isomers in a given exposure source.

[2]Candidate values for different salts of PFDA were also calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

(https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024). For example, the EPA Office of Water (OW) has finalized a National Drinking Water Regulation (NPDWR) to establish Maximum Contaminant Levels (MCLs) for individual PFAS (PFOS, PFOA, PFNA, PFHxS, and hexafluoropropylene oxide dimer acid [HFPO-DA]) and mixtures of two or more PFAS (involving PFHxS, PFNA, PFBS, and HFPO-DA) (https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas) and has finalized a framework for estimating noncancer health effects from PFAS mixtures (U.S. EPA, 2024c). Additionally, the EPA Center for Computational Toxicology and Exposure (CCTE) has developed a tiered toxicity testing strategy for evaluating PFAS using new approach methods (NAMs) that will inform future category grouping and read-across efforts to fill data gaps for PFAS with limited or no toxicity data (https://www.epa.gov/chemical-research/pfas-chemical-lists-and-tiered-testing-methods-descriptions).

The systematic review protocol (see Appendix A) for these five PFAS assessments outlines the related scoping and problem formulation efforts, including a summary of other federal and state assessments of PFDA. The protocol also lays out the systematic review and dose-response methods used to conduct this review (see also Section 1.2). The systematic review protocol was released for public comment in November 2019 and was updated based on those public comments. Appendix A links to the updated version of the protocol, which summarizes the history of the revisions.

Human epidemiological studies have examined possible associations between PFDA exposure and health outcomes, in particular liver serum biomarkers, antibody responses, sensitization and allergic responses, fetal growth restrictions, semen parameters, reproductive hormones, pubertal development, neurodevelopment, thyroid hormones, urinary effects, serum lipids, adiposity, cardiovascular disease, atherosclerosis, and cancer. With the exception of immune (i.e., decreased antibody responses) and developmental (i.e., decreased birth weight) outcomes, the ability to draw judgments regarding these associations based on the available human evidence is limited by the overall quality of the epidemiological studies (studies were generally *low* confidence), the small number of studies per health outcome, and, in some studies, the lack of a quantifiable measure of exposure.

Animal studies of PFDA exposure exclusively examined the oral exposure route; therefore, an inhalation assessment was not conducted and an RfC was not derived (see Section 5.2.3). The available animal studies of oral PFDA exposure examined a variety of noncancer endpoints, including those relevant to liver, immune, developmental, male, and female reproductive, endocrine, urinary, cardiometabolic, and other health effects. Limited evidence was identified evaluating PFDA-induced carcinogenicity in animals.

Overall, the available ***evidence indicates*** that PFDA exposure is likely to cause liver, immune, developmental, and male and female reproductive effects in humans, given sufficient

exposure conditions.[3] Specifically, for liver effects, the primary support for this hazard conclusion included evidence of increased relative liver weights, altered serum biomarkers of liver injury (e.g., serum enzymes) and histopathology (including necrosis) in rats. For immune effects, the primary supporting evidence included decreased antibody responses in children. Developmental effects were identified as a hazard based primarily on consistent findings of dose-dependent decreases in fetal weight in mice supported by evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy. The primary basis for the hazard judgment on male reproductive effects involved coherent responses across sperm counts, testosterone levels, and male reproductive histopathology and organ weights in adult male rats. For female reproductive effects, the primary hazard judgment was based on decreased uterus weight and estrous cycle effects in adult female rats. Selected quantitative data from these identified hazards were used to derive lifetime and subchronic organ-specific reference doses (osRfDs) (see Table ES-1) and the overall lifetime and subchronic RfDs (see Table ES-2).

The available ***evidence suggests*** that PFDA exposure might have the potential to cause cardiometabolic and neurodevelopmental effects in humans under sufficient exposure conditions[4] based on findings from human studies; however, because of inconsistency issues, imprecision, and/or sensitivity, these health hazards were not used in the derivation of toxicity values. Likewise, some human and animal evidence was also identified for endocrine, urinary, and other health effects (e.g., hematological), but the ***evidence is inadequate*** to assess whether PFDA may cause these health effects in humans and was not advanced for the derivation of toxicity values.

Table ES-1. Organ-specific RfDs for health effects with evidence available to synthesize and draw summary judgments for the derivation of toxicity values

Organ/system	Integration judgment	Toxicity value	Value (mg/kg-d)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Immune (developmental immune effects)	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	2×10^{-9}	*Medium*	1	10	1	1	3	30	Decreased serum antibody concentrations for both tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr Grandjean et al. (2012); (Budtz-Jørgensen and Grandjean, 2018a)

[3]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

[4]Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

Organ/system	Integration judgment	Toxicity value	Value (mg/kg-d)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Developmental	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	2×10^{-9}	*Medium-low*	1	10	1	1	3	30	Decreased birth weight in male and female children (Wikström et al., 2020)
Liver	Evidence indicates (likely)	Lifetime osRfD	ND[a]								
		Subchronic osRfD	6×10^{-7}	*Medium*	3	10	10	1	3	1,000	Increased relative liver weight in SD female rats (NTP, 2018)
Male Reproductive	Evidence indicates (likely)	Lifetime osRfD	ND[a]								
		Subchronic osRfD	3×10^{-6}	*Medium-Low*	3	10	10	1	3	1,000	Decreased absolute whole epididymis weight in SD rats (NTP, 2018)
Female Reproductive	Evidence indicates (likely)	Lifetime osRfD	ND[a]								
		Subchronic osRfD	1×10^{-6}	*Medium-Low*	3	10	10	1	3	1,000	Increased number of days spent in diestrus in SD rats (NTP, 2018)

ND = not determined; RfD = reference dose (in mg/kg-day) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposure; osRfD = organ- or system-specific reference dose (in mg/kg-d); UF_A = animal to human uncertainty factor; UF_C = composite uncertainty factor; UFD = evidence base deficiencies uncertainty factor; UF_H = human variation uncertainty factor; UF_L = LOAEL to NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

[a]For hepatic, male reproductive, and female reproductive effects, derivation of candidate lifetime values was not attempted given the high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting.

Table ES-2. Overall Lifetime and subchronic RfDs

Organ/system	Integration judgment	Toxicity value	Value (mg/kg-d)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Immune/ developmental	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	2×10^{-9}	*Medium*	1	10	1	1	3	30	Decreased serum antibody concentrations for tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr Grandjean et al. (2012); (Budtz-Jørgensen and Grandjean, 2018a) Decreased birth weight in male and female children (Wikström et al., 2020)

ND = not determined; RfD = reference dose (in mg/kg-day) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-day) for less-than-lifetime exposure; osRfD = organ- or system-specific reference dose (in mg/kg-day); UF_A = animal to human uncertainty factor; UF_C = composite uncertainty factor; UF_D = evidence base deficiencies uncertainty factor; UF_H = human variation uncertainty factor; UF_L = LOAEL to NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Lifetime and Subchronic Oral Reference Dose (RfD) for Noncancer Effects

Both of the identified hazards with quantitative information to support the derivation of candidate lifetime values (i.e., immune, and developmental) were selected as the basis for the RfD of 2×10^{-9} mg/kg-day. [5,6] The specific effects were decreased serum antibody concentrations in children (male and female) (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and decreased birth weight (male and female) (Wikström et al., 2020). The PODs for these two osRfDs were similar (i.e., 6.04×10^{-8} and 5.44×10^{-8}, respectively). Identical UFs were applied resulting in the same RfD for both effects. $BMDL_{1/2SD(HED)}$ values for decreased antibody concentrations for both tetanus and diphtheria at age 7 years and PFDA measured at age 5 years were nearly identical (6.04×10^{-8} and 5.98×10^{-8} mg/kg-day, respectively) and were used as the point of departure (POD) for this endpoint. For decreased birth weight in males and females (Wikström et al., 2020), a $BMDL_{5RD(HED)}$ of 5.44×10^{-8} mg/kg-day was identified for this endpoint and was used as the POD. The osRfDs for both outcomes were calculated by dividing the POD_{HED} by an identical composite

[5]The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

[6]Note that the RfD for the free acid presented in this document and an RfD for the anion of PFDA (perfluorodecanoate, $C_{10}F_{19}O_2^-$, CASRN 73829-36-4) would be practically identical given the molecular weights between the two compounds differ by less than 0.5% (i.e., by the weight of a single hydrogen atom).

uncertainty factor of 30 to account for interindividual differences in human susceptibility (UF_H = 10), and deficiencies in the toxicity evidence base (UF_D = 3). It is important to emphasize that both critical effects supporting this RfD are observed during the developmental period.

The same approach was selected as the basis for the subchronic RfD of 2×10^{-9} mg/kg-day. The subchronic and lifetime RfDs are identical given that the duration extrapolation uncertainty factor (UF_S) is 1 for both values. A UF_S of 1 was selected since the immune and developmental osRfDs are based on effects observed during the developmental period after exposure during gestation, which is recognized as a susceptible lifestage; therefore, exposure during this time window can be considered more relevant to the induction of sensitive effects on these outcomes than chronic and subchronic exposures (see Sections 5.2.1 and 5.2.2 for more details).

Confidence in the Oral Reference Dose (RfD) and Subchronic RfD

The overall confidence in the RfD and subchronic RfD is **medium** and is driven by *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental (Wikström et al., 2020) endpoints using BMD modeling (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012).

Noncancer Effects Following Inhalation Exposure

No studies that examine toxicity in humans or experimental animals following inhalation exposure were available and no acceptable physiologically based pharmacokinetic (PBPK) models are available to support route-to-route extrapolation; therefore, no RfC was derived.

Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded there is *inadequate information to assess carcinogenic potential* for PFDA by either oral or inhalation routes of exposure. Therefore, the lack of adequate data on the carcinogenicity of PFDA precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS

1.1. BACKGROUND INFORMATION ON PERFLUORODECANOIC ACID (PFDA)

Section 1.1 provides a brief overview of aspects of the physicochemical properties, human exposure, and environmental fate characteristics of perfluorodecanoic acid (PFDA; CASRN 335-76-2), and its related salts that might provide useful context for this Toxicological Review. This overview is not intended to provide a comprehensive description of the available information on these topics. The reader is encouraged to refer to source materials cited below, more recent publications on these topics, and the assessment systematic review protocol (see Appendix A).

1.1.1. Physical and Chemical Properties

PFDA and its related salts are members of the group of per- and polyfluoroalkyl substances (PFAS). Buck et al. (2011) define PFAS as fluorinated substances that "contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1-})." More specifically, PFDA is classified as a perfluoroalkyl carboxylic acid (PFCA) (OECD, 2018). PFCAs containing seven or more perfluorinated carbon groups are considered long-chain PFAS (ATSDR, 2021). Thus, PFDA is a long-chain PFAS. The chemical structures of PFDA and some of its related salts are presented in Figure 1-1.[7] The physicochemical properties of PFDA and these related salts are provided in Table 1-1.

[7]This figure shows the linear structures, but the assessment may also apply to other nonlinear isomers of PFDA and related salts as described in the Executive Summary.

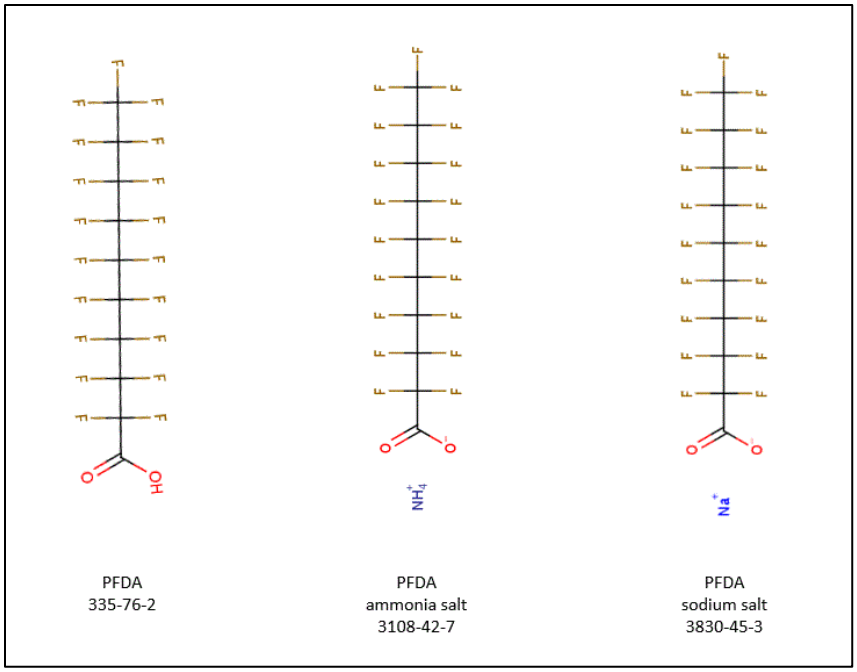


Figure 1-1. Chemical structure of PFDA and related salts.

Table 1-1. Physicochemical properties of PFDA and related salts

Property (unit)	Value		
	PFDA 335-76-2	**PFDA NH_4^+ salt 3108-42-7**	**PFDA Na^+ salt 3830-45-3**
Molecular weight (g/mol)	514[a]	531[a]	536[d]
Melting point (°C)	82.0[a]	83.0[a*]	84.0[a*]
Boiling point (°C)	198[a]	212[a*]	212[a*]
Density (g/cm^3)	1.79[a*]	1.76[a*]	1.76[a*]
Vapor pressure (mm Hg)	1.53e–3[a]	2.39e–02[a*]	2.39e–02[a*]
Henry's law constant ($atm\text{-}m^3/mole$)	1.51e–10[a*]	1.51e–10[a*]	1.51e–10[a*]
Water solubility (mol/L)	5.25e–3[a]	1.21e–3[a*]	1.21e–3[a*]
PKa	0.4[a*]	0.4[a*]	0.4[a*]
LogP	4.15[a]	7.11[a*]	6.84[a*]
Soil adsorption coefficient (L/kg)	398[a*]	398[a*]	398[a*]
Bioconcentration factor (BCF) (L/kg)	49.3[a]	29.5[a*]	29.5[a*]

*Predicted value.

[a]U.S. EPA (2019b) U.S. EPA CompTox Chemicals Dashboard: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=PFDA. When available, average experimental values are provided in the table but average predicted values that may be less reliable are included in the absence of experimental data. All values from the U.S. EPA CompTox Chemicals Dashboard were accessed on January 17, 2024.

1.1.2. Sources, Production, and Use

PFAS are not naturally occurring in the environment (ATSDR, 2021). They are synthetic compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. This class of chemicals has been used in consumer products including stain-resistant fabrics for clothing, carpets, and furniture; nonstick cookware; and personal care products (e.g., dental floss, cosmetics, and sunscreen) (ATSDR, 2024, 2021, 2018a).

PFDA has been used in stain and grease-proof coatings on food packaging, furniture, upholstery, and carpet (Harbison et al., 2015). Kotthoff et al. (2015) analyzed a variety of consumer products for PFAS. PFDA was detected in nano- and impregnation-sprays, outdoor textiles, carpet, gloves, paper-based food contact materials, ski wax, and leather.

EPA has been working with companies in the fluorochemical industry since the early 2000s to phase out the production and use of long-chain PFAS such as PFDA (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS). However, the production and use of PFAS has resulted in their release to the environment through various waste streams. Also, because products containing PFAS are still in use, they may continue to be a source of environmental contamination due to disposal or breakdown in the environment (Kim and Kannan, 2007).

No Chemical Reporting Data (CDR) on production volume are available in EPA's ChemView (U.S. EPA, 2019a) for PFDA or its salts. As part of the National Defense Authorization Act for Fiscal Year 2020 (see Section 7321), 172 per- and polyfluoroalkyl substances, including PFDA, were added to the EPA's Toxic Release Inventory (TRI) list (https://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals). The reporting requirements apply to a de minimus limit of 1% and a manufacture, process, or otherwise use threshold of 100 lbs. The 2022 TRI Report documented 3,400 pounds of PFDA disposed on-site or otherwise released for all industries (https://enviro.epa.gov/triexplorer/release_chem?p_view=USCH&trilib=TRIQ1&sort=_VIEW_&sort_fmt=1&state=All+states&county=All+counties&chemical=0000335762&industry=ALL&year=2022&tab_rpt=1&fld=RELLBY&fld=TSFDSP).

Wang et al. (2014b) estimated global emissions of PFDA from direct and indirect (i.e., formation from degradation of precursors) sources between 1951 and 2030 at 8 metric tons based on a lower estimate and 222 metric tons based on a higher estimate. The lower estimate assumes that producers cease production and use of long-chain PFCAs and their precursors in line with global transition trends. The higher estimate assumes that the emission scenario in 2015 remains constant until 2030.

1.1.3. Environmental Fate and Transport

Long-chain PFAS, including PFDA, are considered extremely stable and persistent in the environment (ATSDR, 2024, 2018a; Harbison et al., 2015), and can be found worldwide in the

environment, wildlife, and humans (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS). Long-chain PFAS, including PFDA, have been found at private and federal facilities and have been associated with various sources, including aqueous film-forming foam (AFFF) for fire suppression and PFAS manufacturers and industries that use PFAS (e.g., textiles) (ATSDR, 2021).

Although specific data on PFDA are lacking, PFAS that are released to air have been found to exist in the vapor phase in the atmosphere and resist photolysis, but particle-bound concentrations have also been measured (Kim and Kannan, 2007). Wet and dry deposition are potential removal processes for particle-bound PFAS in air (ATSDR, 2024, 2018a).

In soil, the mobility of PFAS will vary depending on their soil adsorption coefficients (see Table 1-1), with PFDA being moderately mobile. Uptake of soil PFAS to plants has been shown to occur for similar, long-chain PFAS such as PFOA (ATSDR, 2024, 2018a). Yoo et al. (2011) estimated a grass-soil accumulation factor (grass concentration divided by soil concentration) of 0.10 for PFDA that was based on samples collected from a site with bio-solids-amended soil.

The potential for PFAS to bioaccumulate in aquatic organisms is dependent on their bioconcentration factors (see Table 1-1), with the potential being high for PFDA to bioaccumulate compared with most of the other PFAS for which these data are available.

1.1.4. Potential for Human Exposure, Including Populations and Lifestages with Potentially Greater Exposure

The general population may be exposed to PFAS via inhalation of indoor or outdoor air, ingestion of drinking water and food, and dermal contact with PFAS-containing products (ATSDR, 2024, 2018a; NLM, 2017, 2013). Exposure may also occur via hand-to-mouth transfer of materials containing these compounds (ATSDR, 2024, 2018a). However, the oral route of exposure has been considered the most important route of exposure among the general population. This conclusion is based on several studies that have investigated the various routes of PFAS exposure (Sunderland et al., 2019). Other authoritative sources on exposure assessment (e.g., ATSDR) continue to conduct human biomonitoring studies on PFAS, including PFDA, and those sources should be consulted for the most up-to-date information on PFDA exposure in humans.

Gebbink et al. (2015) modeled exposure to PFDA among the adult general population. Intermediate exposure (i.e., based on median inputs for all exposure parameters) from direct and indirect (i.e., precursor) sources was estimated at 67 pg/kg-day. Of the pathways evaluated (i.e., ingestion of dust, food, water; inhalation of air), direct intake of PFDA in the diet accounted for the largest portion (51.3%) of exposure for the intermediate scenario. From a systematic evidence map published in 2023 (Holder et al., 2023), the most common pathways for PFDA exposure were food, dust, and drinking water, in accordance with the number of studies that detected the chemical in 50% or more of samples.

The presence of PFAS in human blood provides evidence of exposure among the general population. PFAS have been monitored in the human population as part of the National Health and

Nutrition Examination Survey (NHANES). PFDA was measured in serum samples collected in 2013–2014 from more than 2,000 survey participants (CDC, 2022a, b). The results of these analyses are presented in Table 1-2. Olsen et al. (2017) analyzed human plasma samples from 616 American Red Cross (AMC) donors for PFAS in 2015. The results were compared with results of similar analyses conducted in 2000–2001, 2006, and 2010. Geometric mean concentrations of PFDA declined 50% from 2000–2001 to 2015. Examination of the trend over time from the NHANES dataset also shows a decrease in PFDA levels over time, with a median (25th and 75th percentile) serum concentration that fell from 0.3 (0.2, 0.5) ng/mL in 2005–2006 to 0.2 (0.1, 0.3) ng/mL in 2017–2018. PFDA has also been detected in cord blood and human milk (ATSDR, 2024, 2018a). For example, Lankova et al. (2013) detected PFDA in 10% of human milk samples collected from 50 Czech women at concentrations ranging from <6 to 12 pg/mL, indicating that breastmilk is a potential route of exposure for infants. Exposure can also occur through hand-to-mouth transfer of materials containing these compounds (ATSDR, 2021) or in infants through ingestion of formula reconstituted with contaminated drinking water.

Populations that may experience exposures greater than those of the general population may include individuals in occupations that require frequent contact with PFAS-containing products, such as firefighters or individuals who install and treat carpets (ATSDR, 2024, 2018a). Also, because PFDA can be found in ski wax, individuals who engage in professional ski waxing may be more highly exposed because PFAS in dust may become airborne and inhaled during this process (Harbison et al., 2015). Nilsson et al. (2010) observed a significant correlation between the number of years individuals had worked as ski wax technicians and their blood levels of PFDA.

Populations living near fluorochemical facilities where environmental contamination has occurred may also be more highly exposed (ATSDR, 2024, 2018a). Yamada et al. (2014) estimated exposure to PFDA and other PFAS among high seafood consumers and high freshwater fish consumers in France. Depending on how nondetects were handled (set to zero or the limit of detection), mean estimates for PFDA were 0.16 to 0.73 ng/kg-day for high seafood consumers and 0.42 to 0.96 ng/kg-day for high freshwater fish consumers, compared with the adult general population (0.00 to 0.34 ng/kg-day). Thus, populations with a large portion of their diet from fish, including some tribal groups, may experience disproportionally greater PFDA exposure.

Table 1-2. Serum PFDA concentrations based on NHANES 2013–2014 data (µg/L)

Population group[a]	Value
Total population (N = 2,168)	
Geometric mean	0.185
50th percentile	0.200
95th percentile	0.700

Population group[a]	Value
3 to 5 yr (N = 181)	
Geometric mean	–[b]
50th percentile	0.100
95th percentile	0.370
6 to 11 yr (N = 458)	
Geometric mean	–[a]
50th percentile	<LOD[c]
95th percentile	0.350
12 to 19 yr (N = 402)	
Geometric mean	0.136
50th percentile	0.100
95th percentile	0.400
20 yr and older (N = 1,766)	
Geometric mean	0.193
50th percentile	0.200
95th percentile	0.800

LOD = limit of detection.

[a]This table provides only general context on serum PFDA levels from a single study and within a narrow period (environmental PFDA levels are changing over time). Note that PFDA is expected to bioaccumulate over a lifetime (see Sections 1.1.3 and 3.1). Up-to-date information from authoritative bodies should be used in any decisional context.

[b]Not calculated because the proportion of results below the limit of detection was too high to provide a valid result.

[c]Limit of detection was 0.1.

Source: CDC (2022b). Fourth National Report on Human Exposure to Environmental Chemicals.

Air and Dust

PFDA is not currently listed as a hazardous air pollutant under the Clean Air Act and has not been evaluated under the National Air Toxics Assessment (https://www.epa.gov/national-air-toxics-assessment) or the Air Toxics Screening Assessment (https://www.epa.gov/AirToxScreen). However, PFDA was measured at concentrations ranging from 0.13 to 1.56 pg/m^3 in the vapor phase and from 0.13 to 0.49 pg/m^3 in the particle phase of air samples collected from an urban area of Albany, New York, in 2006 (Kim and Kannan, 2007).

PFAS, including PFDA, have also been measured in indoor air and dust and may be associated with the indoor use of consumer products such as PFAS-treated carpets or other textiles (ATSDR, 2024, 2018a). For example, Strynar and Lindstrom (2008) analyzed dust samples from 110 homes and 10 daycare centers in North Carolina and Ohio in 2000–2001 and detected PFDA in 30.4% of the samples. Similar analyses were conducted by Karásková et al. (2016) who collected 56 dust samples from 41 homes in the Czech Republic, Canada, and the United States in 2013. PFDA was detected in more than 80% of the samples with mean concentrations of 5.2, 8.5, and 6.9 ng/g for the Czech Republic, Canada, and the United States, respectively. Knobeloch et al. (2012) collected vacuum cleaner dust from 39 homes in Wisconsin in 2008 and detected PFDA in 72% of

the samples at a median concentration of 5.7 ng/g. Fraser et al. (2013) analyzed dust samples collected from offices (n = 31), homes (n = 30), and vehicles (n = 13) in Boston, Massachusetts, in 2009. PFDA was detected in 97% of the office samples at concentrations ranging from 5.3 to 492 ng/g, 43% of the home samples at concentrations ranging from 7.0 to 26.8 ng/g, and 69% of the vehicle samples at concentrations ranging from 5.4 to 70.1 ng/g. Indoor air samples (n = 4) from a town in Norway collected between 2005 and 2006 had a mean concentration of 3.4 pg/m^3 for PFDA (Barber et al., 2007).

Water

EPA conducted monitoring between 2013 and 2015 for several PFAS (but not PFDA) in drinking water as part of the third Unregulated Contaminant Monitoring Rule (UCMR3). The fifth Unregulated Contaminant Monitoring Rule (UCMR5) requires public water systems to monitor for PFDA and 28 other PFAS (as well as lithium) in drinking water, with sampling collection occurring between 2023 and 2025 (U.S. EPA, 2019c, 2016c). As of April 11, 2024, PFDA has occurred at or above the UCMR 5 minimum reporting level (MRL) (0.003 µg/L) in 13 out of approximately 24,000 samples at 5 out of approximately 4,650 PWSs (UCMR 5 Data Summary (epa.gov). The UCMR 5 dataset is not complete and will be updated on a quarterly basis until completion of data reporting in 2026. Data are added and possibly removed or updated over the course of this reporting cycle following further review by analytical laboratories, PWSs, states, and EPA. Kim and Kannan (2007) analyzed lake water, rainwater, snow, and surface water from Albany, New York, and reported concentrations of PFDA ranging from undetected to 8.39 ng/L. Konwick et al. (2008) observed elevated PFDA concentrations (30–113 ng/L) in a river in Georgia near the site of a wastewater land application system associated with carpet manufacturing. Washington et al. (2010) analyzed soil samples from agricultural fields in Decatur, Alabama, where wastewater treatment sludges had been applied. PFDA was the PFAS with the highest concentration with a maximum of 990 ng/g.

Aqueous Film-Forming Foam Training Sites

PFDA was detected at an Australian training ground where AFFFs had been used (Baduel et al., 2015), and Bräunig et al. (2017) suggested that PFAS were distributed via groundwater to biotic and abiotic matrices in an Australian town impacted by PFAS from a nearby fire-fighting training site. Mean concentrations of PFDA were 0.12 µg/L in water, 0.4 µg/kg dry weight in soil, <0.2 µg/kg wet weight in grass, 0.24 ng/g in egg yolk, 0.21–9.7 µg/L in cow, sheep, and horse serum, and 0.4 µg/L in human serum.

Military Sites

PFDA was detected at 10 U.S. military sites in 67.0% of the surface soil samples and in 48.5% of the sediment samples (ATSDR, 2024, 2018a; Anderson et al., 2016). Table 1-3 provides the concentrations of PFDA in soil, sediment, surface water, and groundwater at these military sites.

Table 1-3. PFDA levels at 10 military installations

Media	Value
Surface soil	
Frequency of detection (%)	67.03
Median (µg/kg)	0.980
Maximum (µg/kg)	15.0
Subsurface soil	
Frequency of detection (%)	12.50
Median (µg/kg)	1.40
Maximum (µg/kg)	9.40
Sediment	
Frequency of detection (%)	48.48
Median (µg/kg)	1.90
Maximum (µg/kg)	59.0
Surface water	
Frequency of detection (%)	52.00
Median (µg/kg)	0.067
Maximum (µg/kg)	3.20
Groundwater	
Frequency of detection (%)	34.78
Median (µg/kg)	0.023
Maximum (µg/kg)	1.80

Source: Anderson et al. (2016); ATSDR (2024, 2018a).

Other Exposures

Schecter et al. (2012) collected 31 food samples from five grocery stores in Texas in 2009 and analyzed them for persistent organic pollutants, including PFDA, which was not detected (limit of detection = 0.2 ng/mL) in any of the foods. Chen et al. (2018b) analyzed PFAS, including PFDA, in foods in Taiwan. PFDA was detected in a wide range of foods at geometric mean concentrations ranging from 0.94 ng/mL in milk to 22.2 ng/g in eggs. Heo et al. (2014) analyzed a variety of foods and beverages in Korea for PFAS. PFDA was detected in 1.3% of the fruit and vegetable samples at a mean concentration of 0.0002 ng/g; 12.8% of the meat samples at a mean concentration of 0.132 ng/g; 13.5% of the dairy samples at a concentration of 0.041 ng/g; 19.0% of the beverage samples at a mean concentration of 0.019 ng/L; and 45.5% of the fish and shellfish samples at a mean concentration of 0.056 ng/g. Heo et al. (2014) also detected PFDA in tap water and bottled water in Korea at mean concentrations of 1.19 and 0.014 ng/L, respectively. Pérez et al. (2014) analyzed PFAS in 283 food items (38 from Brazil, 35 from Saudi Arabia, 36 from Serbia, and 174 from Spain). PFDA was detected in 4.5%, 3.4%, and 2.1% of the samples from Brazil, Serbia, and Spain, respectively. The mean concentrations of PFDA in foods from these countries were 170, 267, and 772 pg/g, respectively. Stahl et al. (2014) characterized PFAS in freshwater fish from 164 U.S. urban river sites and 157 near-shore Great Lakes sites. PFDA was detected in fish from 20% of the

urban river samples (median = <method detection limit; maximum = 28.5 ng/g) and from 92% of the Great Lakes samples (median = 0.68 ng/g; maximum = 13.0 ng/g).

1.2. SUMMARY OF ASSESSMENT METHODS

This section summarizes the methods used for developing this Toxicological Review. A more detailed description of the methods for each step of the assessment development process is provided in the systematic review protocol (see Appendix A). The protocol includes additional problem formulation details, including the specific aims and key science issues identified for this Toxicological Review.

1.2.1. Literature Search and Screening

The detailed search approach, including the query strings and populations, exposures, comparators, and outcomes (PECO) criteria (see Table 1-4), is provided in Appendix B. The results of the current literature search and screening efforts are documented in Section 2.1. Briefly, a literature search was first conducted in 2017 and regular yearly updates are performed. The most recent literature search update that was fully incorporated into the assessment is from April 2022. The literature through March 2023 was screened while the document was undergoing public comment. The results of this literature update and any additional unscreened studies identified during public comment and external peer review were screened against the PECO criteria and presented in Tables I-1, I-2, and I-3 of Appendix I in the assessment. The tables provide the identified studies that met PECO criteria or certain supplemental evidence categories (i.e., in vivo mechanistic or MOA studies, including non-PECO routes of exposure and populations; in vitro and in silico models; and absorption, distribution, metabolism, and excretion [ADME] and pharmacokinetic [PK] studies) and EPA's judgment and supporting rationale on whether the studies have a material impact on the assessment conclusions (i.e., identified hazards or toxicity values) presented in the public comment draft. New studies judged influential in informing assessment conclusions and data gaps were incorporated into the relevant section of the assessment prior to finalization.

The literature search queried the following databases (no date or language restrictions were applied):

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- Toxline (National Library of Medicine)
- TSCATS (Toxic Substances Control Act Test Submissions)

In addition, relevant literature not found through database searching was identified by:

- Review of studies cited in any PECO-relevant studies and published journal reviews; finalized or draft U.S. state, U.S. federal, and international assessments (e.g., the draft Agency for Toxic Substances and Disease Registry [ATSDR] assessment released publicly in 2018). In addition, studies included in ongoing IRIS PFAS assessments (PFHxS and PFNA) were also scanned for any studies that met PFDA PECO criteria.

- Searches of published PFAS systematic evidence maps (SEMs) (Carlson et al., 2022; Pelch et al., 2022) starting in 2021.

- Review of studies submitted to federal regulatory agencies and brought to the attention of EPA; for example, studies submitted to EPA by the manufacturers in support of requirements under the Toxic Substances Control Act (TSCA).

- Identification of studies during screening for other PFAS. For example, epidemiology studies relevant to PFDA were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the IRIS Program.

- Other gray literature (e.g., primary studies not indexed in typical databases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to the attention of EPA.

All literature is tracked in the U.S. EPA Health and Environmental Research Online (HERO) database (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614). The PECO criteria (see Table 1-4) identify the evidence that addresses the specific aims of the assessment and guide the literature screening process.

Table 1-4. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	Human: Any population and lifestage (occupational or general population, including children and other sensitive populations). The following study designs will be included: controlled exposure, cohort, case control, and cross-sectional. (Note: Case reports and case series will be tracked as potential supplemental material.) Animal: Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Other: In vitro, in silico, or nonmammalian models of genotoxicity. (Note: Other in vitro, in silico, or nonmammalian models will be tracked as potential supplemental material.)
Exposures	Human: Studies providing quantitative estimates of PFDA exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational setting measures (e.g., water levels or air concentrations, residential location

PECO element	Evidence
	and/or duration, job title, or work title). (Note: Studies that provide qualitative, but not quantitative, estimates of exposure will be tracked as supplemental material.) Animal: Oral or inhalation studies including quantified exposure to PFDA based on administered dose, dietary level, or concentration. (Note: Nonoral and noninhalation studies will be tracked as potential supplemental material.) PFDA mixture studies are included if they employ an experimental arm that involves exposure to a single PFDA. (Note: Other PFDA mixture studies are tracked as potential supplemental material.) Studies must address exposure to the following: PFDA (CASRN 335-76-2), or PFDA ammonium salt (CASRN 3108-42-7) or PFDA sodium salt (CASRN 3830-45-3).
Comparators	Human: A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. Animal: Includes comparisons to historical controls or a concurrent control group that is unexposed, exposed to vehicle only or air only exposures. (Note: Experiments including exposure to PFDA across different durations or exposure levels without including one of these control groups will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)
Outcomes	All cancer and noncancer health outcomes. (Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing toward toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol]). Functional immune measures (e.g., antibody responses) are considered relevant phenotypic outcomes in accordance with immunotoxicity guidance from the World Health Organization (IPCS, 2012).

In addition to those studies meeting the PECO criteria and studies excluded as not relevant to the assessment, studies containing supplemental material potentially relevant to the specific aims of the assessment were inventoried during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues (see Appendix A.2.4) and other potential scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as "potentially relevant supplemental material" included the following:

- In vivo mechanistic or mode-of-action (MOA) studies, including non-PECO routes of exposure (e.g., intraperitoneal injection) and populations (e.g., nonmammalian models)
- In vitro and in silico models
- ADME and PK studies (excluding models)[8]

[8]Given the known importance of ADME data, this supplemental tagging was used as the starting point for a separate screening and review of pharmacokinetics data (see Appendix A.9.2 for details).

- Exposure assessment or characterization (no health outcome) studies
- Human case reports or case-series studies

The literature was screened by two independent reviewers with a process for conflict resolution, first at the title-and-abstract level and subsequently the full-text level, using structured forms in DistillerSR (Evidence Partners; https://distillercer.com/products/distillersr-systematic-review-software/). Literature inventories for PECO-relevant studies and studies tagged as "potentially relevant supplemental material" during screening were created to facilitate subsequent review of individual studies or sets of studies by topic-specific experts.

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiologic and animal toxicological studies used in the PFDA assessment are provided in the systematic review protocol (see Appendix A). The general approach for evaluating PECO-relevant health effect studies is the same for epidemiology and animal toxicological studies, although the specifics of applying the approach differ; thus, they are described in detail in Appendices A.6.2 and A.6.3, respectively. Approaches for study evaluation for mechanistic studies are described in detail in Appendix A.6.5.

The key concerns for the review of epidemiology and animal toxicological studies are potential bias (systematic errors or deviations from the truth related to internal validity 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 and can lead to a false negative). For example, any types of random measurement error that may lead to attenuation of study results (i.e., bias toward the null). In evaluating individual studies, two or more reviewers independently arrived at judgments regarding the reliability of the study results (reflected as study confidence determinations; see below) regarding each outcome or outcome grouping of interest; thus, different judgments were possible for different outcomes within the same study. The results of these reviews were tracked within EPA's version of the Health Assessment Workplace Collaborative (HAWC). To develop these judgments, each reviewer assigned a rating of *good*, *adequate*, *deficient* (or *not reported*, which generally carried the same functional interpretation as *deficient*), or *critically deficient* (listed from best to worst methodological conduct; see Appendix A.6 for definitions) related to each evaluation domain representing the different characteristics of the study methods that were evaluated based on the criteria outlined in HAWC.

Once all evaluation domains were evaluated, the identified strengths and limitations were collectively considered by the reviewers to reach a final study confidence classification:

- *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.

- *Medium* confidence: Possible deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree or to have a notable impact on the results.

- *Low* confidence: Deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. *Low* confidence results were given less weight than *high* or *medium* confidence results during evidence synthesis and integration (see Sections 1.2.4 and 1.2.5).

- *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

Using the HAWC platform, the two reviewers reached a consensus judgment regarding each evaluation domain and overall (confidence) determination with conflict resolution by an additional reviewer, as needed. The specific limitations identified during study evaluation were carried forward to inform the synthesis (see Section 1.2.4) within each body of evidence for a given health effect.

1.2.3. Additional Epidemiology Considerations

While the detailed methods for epidemiology study evaluation are described in the systematic review protocol (see Appendix A.6.2.1), a few considerations that have been developed further are described in this section.

As noted above, study sensitivity is an important consideration given that it could lead to a false negative (i.e., null) result (Type II error) if a study is underpowered or not designed with adequate sensitivity to detect an association that may exist. A key element for study sensitivity, along with others described in the systematic review protocol, is whether exposure contrasts/gradients are sufficient across populations to detect differences in risk. For example, measurement errors that result in inaccurate exposure estimates can lead to exposure misclassification but can also influence the ability to detect an association or an exposure-response relationship, which may be evidence of a biologic gradient.

Confounding across PFAS is a potential source of uncertainty when interpreting the results of epidemiology studies of individual PFAS (e.g., quantifying the effect of an individual PFAS can potentially be confounded by other PFAS). For confounding to occur, copollutants would have to be associated with PFAS of interest, associated with the endpoint, and not act as an intermediate in the causal pathway. One way to begin to assess whether coexposure is occurring is through examination of correlations. In a preliminary analysis of 22 studies in the inventory reporting correlations, correlations differed across the PFAS (see Appendix A.6, Figure 6-2). While some pairs have correlation coefficients consistently above 0.6 (e.g., PFNA and PFDA), the correlations for most vary from 0.1 to 0.6 depending on the study. For this reason, it was not considered appropriate to assume that coexposure to other PFAS was necessarily an important confounder in all studies. The potential for confounding across PFAS is incorporated in individual study evaluations and assessed

across studies in evidence synthesis. In most studies, it is difficult to determine the likelihood of confounding without considering additional information not typically included in individual study evaluation (e.g., associations of other PFAS with the outcome of interest and correlation profiles of PFAS within and across studies). In addition, even when this information is considered or the study authors perform analyses to adjust for other PFAS, it is often not possible to fully disentangle the associations due to high correlations. This stems from the potential for amplification bias in which bias can occur following adjustment of highly correlated PFAS (Weisskopf et al., 2018). Thus, in most studies, there may be some residual uncertainty about the risk of confounding by other PFAS. A "Good" rating for the confounding domain is reserved for situations in which concern is minimal for substantial confounding across PFAS as well as other sources of confounding. Examples of this situation include results for a PFAS that predominates in a population (such as a contamination event) or studies that demonstrate robust results following multi-PFAS adjustment, which would also indicate minimal concern for amplification bias. Because of the challenge in evaluating individual studies for confounding across PFAS, this issue is also assessed across studies during the evidence synthesis phase (as described in the systematic review protocol; Appendix A, Section 9), primarily when there is support for an association with adverse health effects in the epidemiological evidence (i.e., *moderate,* or *robust* evidence in humans, as described below). Analyses used include comparing results across studies in populations with different PFAS exposure mixture profiles, considering results of multipollutant models when available, and examining strength of associations for other correlated PFAS. In situations in which there is considerable uncertainty regarding the impact of residual confounding across PFAS, it is captured as a factor that decreases the overall strength of evidence (see Appendix A.10).

1.2.4. Data Extraction

The detailed data extraction approach is provided in Appendix A.8. Briefly, data extraction and content management were carried out using HAWC for all health effects for animal studies and some health effects for epidemiological studies (for which data visualizations were necessary to understanding the evidence synthesis judgments). Data extraction elements that were collected from epidemiological, animal toxicological, and in vitro studies is described in HAWC (https://hawcprd.epa.gov/about/). Not all studies that meet the PECO criteria went through data extraction: Studies evaluated as being *uninformative* were not used to inform assessment judgments and therefore did not undergo full data extraction. All findings from informative studies were considered for extraction, regardless of the statistical significance of their findings. The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). For quality control, data extraction was performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction were resolved by discussion or consultation within the evaluation team.

1.2.5. Evidence Synthesis and Integration

For the purposes of this Toxicological Review, evidence synthesis and integration are considered distinct but related processes (see Appendix A, Sections 9 and 10 for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from Hill (1965). Mechanistic evidence is also synthesized as necessary to help inform key decisions regarding the human and animal evidence; processes for synthesizing mechanistic information are covered in detail in Appendix A, Section 9.2.

The syntheses of the human and animal health effects evidence focus on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies. Thus, data permitting, the evidence synthesis emphasizes studies of *high* and *medium* confidence. Correspondingly, during data extraction when a relative abundance of medium and *high* confidence studies was available for a given health outcome the *low* confidence studies did not generally undergo full data extraction. Documentation of when this approach was taken is noted in the specific health effect sections. When possible, results across studies are compared using graphs and charts or other data visualization strategies. The synthesis of mechanistic information informs the integration of health effects evidence for both hazard identification (e.g., biological plausibility or coherence of the available human or animal evidence; inferences regarding human relevance, or the identification of susceptible populations and lifestages across the human and animal evidence) and dose-response evaluation (e.g., selection of benchmark response levels, selection of uncertainty factors). Evaluations of mechanistic information typically differ from evaluations of phenotypic evidence (e.g., from routine toxicological studies) primarily because mechanistic data evaluations consider the support for and involvement of specific events or sets of events within the context of a broader research question (e.g., support for a hypothesized MOA; consistency with known biological processes), rather than evaluations of individual apical endpoints considered in relative isolation.

Following the synthesis of human and animal health effects data and mechanistic data, integrated judgments are drawn across all lines of evidence for each assessed health effect. During evidence integration, a structured and documented two-step process is used, as follows:

- Building from the separate syntheses of the human and animal evidence, the strength of the evidence from the available human and animal health effect studies is summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Sir Bradford Hill (Hill, 1965). This process is conceptually similar to that used by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Morgan et al., 2016; Guyatt et al., 2011; Schünemann et al., 2011), which arrives

at an overall integration conclusion based on consideration of the body of evidence. These summaries incorporate the relevant mechanistic evidence (or MOA understanding) that informs the biological plausibility and coherence within the available human or animal health effect studies. The terms associated with the different strength of evidence judgments within evidence streams are *robust*, *moderate*, *slight*, *indeterminate*, and *compelling evidence of no effect*.

- The animal, human, and mechanistic evidence judgments are then combined to draw an overall judgment that incorporates inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed. The final output is a summary judgment of the evidence base for each potential human health effect across evidence streams. The terms associated with these summary judgments are ***evidence demonstrates***, ***evidence indicates (likely)***, ***evidence suggests***, ***evidence inadequate***, and ***strong evidence of no effect***. The decision points within the structured evidence integration process are summarized in an evidence profile table for each considered health effect.

As discussed in the protocol (see Appendix A), the methods for evaluating the potential carcinogenicity of PFAS follow processes laid out in the EPA cancer guidelines (U.S. EPA, 2005); however, for PFDA, data relevant to cancer were sparse, which limited the extent of possible analysis (see Section 3.3).

1.2.6. Dose-Response Analysis

The details for the dose-response employed in this Toxicological Review can be found in Appendix A.11. Briefly, a dose-response assessment was performed for noncancer health hazards, following exposure to PFDA via the oral route, as supported by existing data. For oral noncancer hazards, oral reference doses (RfDs) are 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). The derivation of a reference value like the RfD depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer outcomes, a dose-response assessment was conducted for evidence integration conclusions of ***evidence demonstrates*** or ***evidence indicates (likely)***. In general, toxicity values are not developed for noncancer hazards with ***evidence suggests*** conclusions (see Appendix A, Section 10.2 for exceptions). Consistent with EPA practice, the PFDA assessment applied a two-step approach for dose-response assessment that distinguishes analysis of the dose-response data in the range of observation from any inferences about responses at lower environmentally relevant exposure levels (U.S. EPA, 2012a, 2005):

- Within the observed dose range, the preferred approach was to use dose-response modeling to incorporate as much of the dataset as possible into the analysis. This modeling to derive a point of departure (POD) ideally includes an exposure level near the lower end of the range of observation, without significant extrapolation to lower exposure levels.

- As derivation of cancer risk estimates and reference values nearly always involves extrapolation to exposures lower than the POD; the approaches applied in these assessments are described in more detail in Section A.11.2.

When sufficient and appropriate human and laboratory animal data are available for the same outcome, human data are generally preferred for the dose-response assessment because use of human data eliminates the need to perform interspecies extrapolations. For reference values, this assessment will derive a candidate value from each suitable dataset. Evaluation of these candidate values will yield a single organ/system-specific value for each organ/system under consideration from which a single overall reference value will be selected to cover all health outcomes across all organs/systems. While this overall reference value represents the focus of these dose-response assessments, the organ/system-specific values can be useful for subsequent cumulative risk assessments that consider the combined effect of multiple PFAS (or other agents) acting at a common organ/system. For noncancer toxicity values, uncertainties in these estimates are characterized and discussed.

For dose-response purposes, EPA has developed a standard set of models (http://www.epa.gov/bmds) that can be applied to typical datasets, including those that are nonlinear. In situations in which there are alternative models with significant biological support (e.g., toxicodynamic models), those models are included as alternatives in the assessment(s) along with a discussion of the models' strengths and uncertainties. EPA has developed guidelines on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (see the EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a)). For each modeled response, a POD from the observed data was estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses. The POD is used as the starting point for subsequent extrapolations and analyses. For noncancer effects, the POD is used in calculating the RfD.

2. LITERATURE SEARCH RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 1,330 unique records, including records identified from additional sources, such as posted National Toxicology Program (NTP) study tables, review of reference lists from other authoritative sources (ATSDR, 2018b), searches of published per- and polyfluoroalkyl substances systematic evidence maps (Pelch et al., 2022) and studies submitted to EPA during public comment and external peer review (see Figure 2-1). Of the 1,330 studies identified, 426 were excluded during title-and-abstract screening that did not meet PECO and did not contain potentially relevant supplemental information, and 627 were reviewed at the full-text level. Of the 627 studies screened at the full-text level, 372 were considered to meet the populations, exposures, comparators, and outcomes (PECO) eligibility criteria (see Table 1-4). The PECO criteria identify the evidence that addresses the specific aims of the assessment and focuses the literature screening, including study inclusion/exclusion. In addition to those studies meeting the PECO criteria, studies containing supplemental material potentially relevant to the specific aims of the assessment were tagged during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues and other major scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as "potentially relevant supplemental material" included the following:

- In vivo mechanistic or mode-of-action studies, including non-PECO routes of exposure (e.g., intraperitoneal injection) and non-PECO populations (e.g., nonmammalian models);
- In vitro and in silico models;
- Absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) studies (excluding models);
- Exposure assessment or characterization (no health outcome) studies; and
- Human case reports or case-series studies

The studies meeting PECO at the full-text level included 342 epidemiologic studies, 14 animal studies, 2 physiologically based pharmacokinetic (PBPK) model and 8 in vitro/in vivo genotoxicity studies, and 9 accessory records or supplementary materials included in epidemiological and animal studies. Of the 1,330 studies screened, 498 were identified as supplemental material during title-and-abstract or full-text screening and tagged by topic area

(e.g., in vivo mechanistic or MOA, ADME). High-throughput screening data on perfluorodecanoic acid (PFDA) are currently available from the EPA's Chemicals Dashboard U.S. EPA (2022a), (data were retrieved in November 2022) and relevant information is presented and analyzed in Appendix E. The last literature search update used for the Toxicological Review was April 2023.

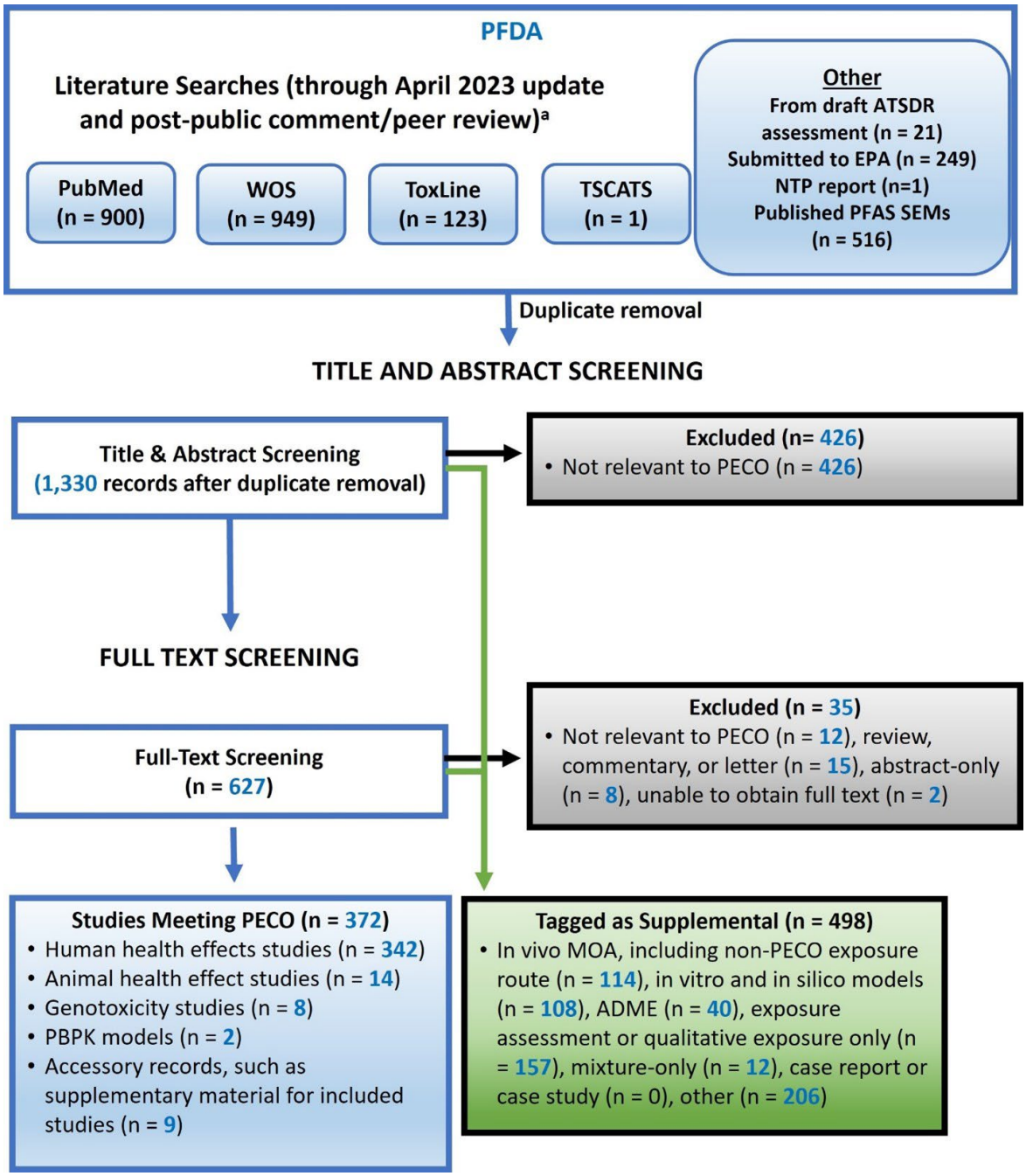


Figure 2-1. Literature search for perfluorodecanoic acid and related salts.[a] The numbers for the different litarature databses and other sources do not account for duplicates. See the HERO page for this assessment for more details.

2.2. SUMMARY OF STUDIES MEETING PECO CRITERIA

Human and animal studies have evaluated potential effects to the liver, immune system, developing fetus, male and female reproductive systems, endocrine, cardiometabolic, neurodevelopmental, urinary, general toxicity, and other organ systems (e.g., hematology) following exposure to PFDA. The evidence base for these outcomes is synthesized in Sections 3.2.1–3.2.11. A limited number of available studies in humans and animals informative of potential carcinogenic effects with PFDA exposure are summarized in Section 3.3. The two identified PBPK models are discussed in Section 3.1.

Three hundred and forty-two epidemiological studies were identified that reported on the potential association between PFDA and noncancer and cancer human health effects (list of studies filterable by health effect available at: https://hawcprd.epa.gov/summary/visual/assessment/100500072/Epi-studies-of-PFDA-health-effects/). The database of animal toxicity studies for PFDA consists of oral exposure studies (see Table 2-1), including five dietary exposure studies in rats exposed for 7–14 days (Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991); two drinking water studies in mice exposed for 12–49 days (Li et al., 2022; Wang et al., 2020), two 28-day gavage studies in rats and/or mice (Frawley et al., 2018; NTP, 2018), one 14-day oral study (presumed to be gavage) in mice (Lee and Kim, 2018), and one gestational exposure study in mice via gavage with two exposure windows (GD 10–13 and 6–15) (Harris and Birnbaum, 1989). In addition, three single exposure studies in animals via the oral route were identified with limited utility for the evaluation of repeat-dose toxicity and the derivation of oral reference dose (RfD) values (Kawabata et al., 2017; Brewster and Birnbaum, 1989; Harris et al., 1989).

Table 2-1. Animal toxicity studies examining health effects after PFDA administration

Author (year) reference	Species, strain (sex)	Exposure route and duration	Dose range[a]
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Oral gavage; daily over 28 d	0, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/kg-d
Frawley et al. (2018)	Rat, Harlan Sprague-Dawley (female)	Oral gavage; daily over 28 d	0, 0.125, 0.25 and 0.5 mg/kg-d
Frawley et al. (2018)	Mouse, B6C3F1/N (female)	Oral gavage; weekly over 28 d	0.04464, 0.0893, 0.179, 0.36 and 0.71 mg/kg-d (reported as 0, 0.3125, 0.625, 1.25, 2.5 and 5 mg/kg-wk)
Takagi et al. (1991)	Rat, F344 (male)	Diet; daily over 14 d	0, 10 mg/kg-d (reported as 0% and 0.01%)

Author (year) reference	Species, strain (sex)	Exposure route and duration	Dose range[a]
Lee and Kim (2018)	Mouse, ICR (male)	Uncharacterized (presumed to be oral gavage); days 9, 11, and 13 over 14 d	0 and 21.4 mg/kg-d (reported as 0 and 100 mg/kg)
Li et al. (2022)	Mouse, C57BL/6J (female)	Drinking water; daily for 14 d	0 and 25 mg/kg-d
Wang et al. (2020)	Mice, CD-1 (male)	Drinking water; daily over 12–49 d	0, 13 mg/kg-d (reported as 0 and 0.1 mM)
Permadi et al. (1993)	Mouse, C57BL/6 (male)	Diet; daily over 10 d	0, 37.8 mg/kg-d (reported as 0% and 0.02%)
Kawashima et al. (1995)	Rat, Wistar (male)	Diet; daily over 7 d	0, 1.15, 2.3, 4.6, and 9.22 mg/kg-d (reported as 0, 00125%, 0.0025%, 0.005%, and 0.01%)
Yamamoto and Kawashima (1997)	Rat, Wistar (male)	Diet; daily over 7 d	0 and 4.6 mg/kg-d (reported as 0% and 0.005%)
Takagi et al. (1992)	Rat, Fisher F344 (male)	Diet; daily over 7 d	0 and 10 mg/kg-d (0% and 0.01%)
Harris and Birnbaum (1989)	Mouse, C57BL/6N (female)	Oral gavage; GD 10–13	0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 mg/kg-d
Harris and Birnbaum (1989)	Mouse, C57BL/6N (female)	Oral gavage; GD 6–15	0, 0.03, 0.1, 0.3, 1, 3, 6.4, and 12.8 mg/kg-d

GD = gestational day.

Doses are presented as adjusted daily doses (ADD). Additional details on the ADD conversions can be found in the HAWC project page for PFDA.

Graphical representations of outcome-specific study evaluations are presented and discussed within the hazard sections outlined above. Detailed rationales for each domain and overall confidence rating are available in Health Assessment Workspace Collaborative (HAWC).

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND INTEGRATION

3.1. PHARMACOKINETICS

Perfluorodecanoic acid (PFDA) and its salts have characteristics of absorption, distribution, metabolism, and excretion (ADME) comparable to other perfluoroalkyl acids (PFAAs) in that they are readily absorbed by gastrointestinal tract following oral exposure irrespective of sexes and species. Both animal and human data suggest that PFDA has a high affinity for protein binding and efficient renal reuptake. Therefore, PFDA tends to accumulate in organs to the extent similar to or greater than that of other PFAAs and has relatively slow clearance (Dzierlenga et al., 2019; Fujii et al., 2015; Zhang et al., 2013b). In general, PFDA accumulates primarily in liver, followed by kidney, blood, and other tissues. PFDA is specifically a perfluorocarboxylic acid (PFCA), which is a subset of PFAAs. Similar to other PFAAs, PFDA is also metabolically inert and therefore most PFDA is eliminated unchanged in urine and feces.

Of note, growing mechanistic evidence (both animal and human) suggests that renal clearance becomes less efficient as the perfluorocarbon chain length increases (Dzierlenga et al., 2019; Kudo, 2015; Lau, 2015). The findings support previous reports indicating that fecal elimination may play an increasingly important role in elimination of long-carbon chain length of PFAAs like PFDA (C10) compared with shorter chain PFAAs (C ≤ 8) (Vanden Heuvel et al., 1991). Collectively, these PFDA pharmacokinetic data support the conclusions of Kudo (2015) and Lau (2015) that PFDA has a much longer half-life than shorter chain PFAAs (e.g., PFHxA). While female rats administered PFDA tended to have a higher dose-normalized area under the plasma concentration time curve (AUC) than males, (Dzierlenga et al., 2019) suggested there was no sex difference in PFDA half-life. From a Bayesian analysis using a classical pharmacokinetics (PK) model structure evaluated across studies, doses, and routes (see Appendix G.1), EPA obtains a population mean volumes of distribution (Vd) of 431 mL/kg in male rats and 300 mL/kg in females (see Table 3-1) in female rats, but mean clearance of 3.6 mL/kg-day in males versus 3.4 mL/kg-dayin females (see Table 3-2), leading to half-life estimates of 83 and 61 days in male and female rats, respectively (see Table 3-3). Thus, there appear to be some sex differences, but of no more than 40%. The elimination half-life of PFDA is much longer in humans (mean values of 4.5–12 years estimated by Zhang et al. (2013b); 4.7 years in this analysis (see Table 3-3)) than in rats (18–110 days, as suggested by multiple studies reviewed below; shorter in female than male rats) or mice (1.4–4 days, beta phase (Fujii et al., 2015)). By comparison, Lau (2015) provided estimated half-lives of 2.3–3.8 years for PFOA in humans (modestly lower than PFDA), 5.4 years for PFOS (comparable to PFDA), but only 32 days for PFHxA. In male rats, PFOA has a half-life of 2–6 days,

PFOS a half-life of 38–71 days, and PFHxA 0.4–1.6 hours, compared with 23–110 days for PFDA. Therefore, the qualitative trend with chain length and structure is similar across species, although there is an order of magnitude difference in elimination of PFOA versus PFOS in rats whereas in humans the difference between PFOA and PFOS is no more than a factor of 2.

3.1.1. Absorption

Bioavailability (or fractional absorption) is typically estimated by comparing the AUC of blood concentrations observed after an oral dose with the AUC for same dose given by intravenous (i.v.) injection. When the oral and i.v. doses are different, AUCs normalized to the respective doses can be compared. If the pharmacokinetics are linear with dose and the oral uptake is less than 100%, the AUC/dose after oral dosing will be less than that after i.v. dosing, and the fraction absorbed (F_{abs}) is estimated as [AUC/dose (oral)]/[AUC/dose (i.v.)].

In the most recent animal study by Dzierlenga et al. (2019), Hsd: Sprague-Dawley (SD) rats were given PFDA or one of two other PFAA (perfluorohexanoic acid [PFHxA] and perfluorooctanoic acid [PFOA]) by i.v. injection at 2 mg/kg or oral gavage (2, 10, and 20 mg/kg). It was found that the time to peak concentration (Tmax) increases with the chain length of PFAAs and slightly with dose levels of oral administration for both sexes. For PFDA, Tmax (mean ± standard error of mean, hour) increases from 8.27 ± 0.63 to 10.0 ± 0.06 hour, and from 9.01 ± 0.80 to 10.8 ± 1.2 hour, with increased gavage doses (2–20 mg/kg) of PFDA for males and females, respectively. Peak concentration, Cmax (normalized with dose levels, mM/mmol/kg), also appeared to be somewhat higher with increasing oral doses in female rats, but not male rats. Oral bioavailability for PFDA was estimated to be 160%–180% for both sexes because the AUC/dose was higher after oral dosing than i.v. dosing. The nominal observation of >100% absorption may be the result of enhanced reabsorption by intestinal transporters (Dzierlenga et al., 2019). Other aspects of the results do not indicate nonlinearity; for example, the AUC/dose did not change significantly among oral doses of 2, 10, and 20 mg/kg. But the peak concentration after the i.v. dose (2 mg/kg), measured just 5 minutes after dosing, was lower than the value estimated from the oral dose data, occurring 8–9 hours after the dose. There is no clear explanation for this behavior or for the observation of the AUC/dose being so much higher after oral versus i.v. dosing. Given the consistency of the AUC/dose for the oral doses, it seems most likely that the unexpected result is due to some other difference in PK mechanisms for the i.v. versus oral doses.

Kim et al. (2019) estimated F_{abs} = 0.87 ± 0.25 and 0.65 ± 0.08 in female and male SD rats, respectively. On the other hand, Dzierlenga et al. (2019) reported F_{abs} of 1.58–1.72 and 1.70–1.79 for male and female rats, respectively, for 2–20 mg/kg doses, based on the serum AUC after oral versus i.v. dosing (i.e., values much greater than 100%). It is unclear how to interpret these data nor how to resolve the discrepancy between the two papers. It is possible that immediately after i.v. dosing as performed by Dzierlenga et al. (2019), binding to plasma proteins was less than the equilibrium fraction and therefore the rates of distribution and clearance were higher, whereas the slower absorption after oral dosing allowed a higher fraction to bind before PFDA circulated to the

kidneys and other tissues, resulting in a lower AUC/dose after i.v. than oral dosing. The standard approach for estimating F_{abs} using the AUC (oral)/AUC (i.v.) implicitly assumes that clearance is identical after dosing, which would not be true if this hypothesis is correct. Data for the rate of binding to serum proteins would be needed to evaluate this hypothesis but are not available.

In the Bayesian PK analysis of the rat data described in Appendix G.1, EPA estimated F_{abs} separately in male and female rats, but as a population-level parameter assumed to be the same across all available studies, since data from both oral i.v. dosing must be analyzed simultaneously to determine the value. The Bayesian prior for F_{abs} was strictly bounded to ≤1 given that a larger value is not physically possible. The resulting mean (90% CI) was 0.84 (0.68–0.99) in male rats and 0.87 (0.73–0.99) in female rats.

Fujii et al. (2015) measured PFDA PK in male and female FVB/NJcl mice dosed with 0.313 μmol/kg by i.v. administration and 3.13 μmol/kg by oral gavage. A shortcoming of the experimental design is that serum concentration data were only collected up to 24 hours, making it harder to estimate the PK parameters. However, based on the reported parameters the Tmax after oral dosing was 12 and 15 hours in male and female rats, respectively. Fujii et al. (2015) reported the ratio of dose-adjusted AUC after oral and i.v. exposures as 1.1 and 1.2 (i.e., 110% and 120%) in male and female mice, respectively, indicating complete absorption. That these values are slightly greater than 1 may not only be due to experimental variability, but also because clearance might have been slightly slower for the oral dose, which was 10 times higher than the i.v. dose. Because values of AUC/dose reported by Fujii et al. (2015) are not significantly different for oral versus i.v. dosing, the difference among them is presumed to be due to experimental variability and the results are interpreted as showing 100% bioavailability (F_{abs} = 1) in mice.

Although there is no direct evidence of oral absorption of PFDA in humans, it can be inferred from observations in epidemiological studies that identified positive associations between PFDA concentrations in human tissues (e.g., blood or placenta) and environmental levels (e.g., drinking water) (Stubleski et al., 2016). Given the results for rats and mice and the lack of controlled PK studies in humans, F_{abs} = 1 will be used for humans.

No data on absorption of PFDA through the respiratory tract or skin has been found. While oral ingestion is considered the primary route of exposure, the contribution from these other routes would need to be better evaluated in the scientific literature to determine their significance.

Evidence Synthesis for Absorption

Data from PK studies in rats and mice indicate a high level of PFDA oral bioavailability in those species but the apparent F_{abs} > 1 reported in some cases indicates that assumptions implicit in the typical calculation of F_{abs}, i.e., that distribution and clearance are identical once a chemical enters the blood, are not valid for PFDA. The mechanism involved is unknown. EPA's analysis of the rat data, which constrained F_{abs} to ≤1, yielded mean (90% CI) F_{abs} = 0.84 (0.68–0.99) in male rats and 0.87 (0.73–0.99) in female rats. Reported Tmax values ranged from 8 to 11 hours in rats, indicating that absorption was essentially complete in less than 12 hours (Dzierlenga et al., 2019).

The limited PK data for mice indicate F_{abs} of ~1 and a Tmax after oral exposure of ~12 hours (Fujii et al., 2015). PK data in humans that could be used to quantify oral absorption are not available, thus F_{abs} is assumed to be 1 for humans; however, given the range estimated for rats, it seems plausible that F_{abs} in humans could be as low as 0.7.

3.1.2. Distribution

General Considerations

Upon absorption, PFDA moves rapidly through the body via the bloodstream to various organs and tissues, mainly liver, lung, and kidney and, to a lesser extent, brain, and bone (Dzierlenga et al., 2019; Vanden Heuvel et al., 1991). In general, PFDA tends to accumulate in organs to an extent greater than or similar to that of other PFAS. It has been suggested that the extent of the covalent binding of PFDA with biological matrices (e.g., serum proteins) in blood and tissues is critical to its distribution and bioaccumulation (Kudo, 2015; Vanden Heuvel et al., 1992). For instance, Kim et al. (2019) measured binding of PFDA to plasma proteins in vitro to incorporate this factor into a physiologically based pharmacokinetic (PBPK) model and reported that more than 99.7% was bound to protein in rat and human plasma. These measured values were in line with animal experimental data reported by Ylinen and Auriola (1990) that 99% of PFDA was bound with the serum proteins in Wistar rats with a single intraperitoneal (IP) dose of 20 mg/kg PFDA. However, if distribution to tissues is assumed to be limited by the product of free fraction and tissue blood flow, the PK distribution phase is predicted to be much longer than observed. Hence, the plasma binding must be labile, not strictly limiting its distribution or clearance.

Of note, the degree of protein binding with PFDA affects not only its distribution but also the elimination. Specifically, the PFAS–serum protein complex mediates glomerular filtration since only the unbound fraction is expected to be filtered (Kudo, 2015). PFAS can then be extensively resorbed as fluid carrying them passes down the renal tubules, with this resorption mediated by other PFAS-protein complexes, specifically by organic anion transporter (Oat) proteins (Kudo, 2015). For instance, Weaver et al. (2010) investigated the roles of rat renal Oat proteins in the deposition of perfluorinated carboxylates with different chain lengths of carbons (C2–C18). The transport of PFDA (C10) was measured from 10 to 300 mM with renal Oat proteins (Chinese hamster ovary cell line and kidney RNA from SD rats). Of five Oat proteins (Oat1, Oat2, Oat3, Urat1, and Oatp1a1), Oatp1a1 appears to be the major Oat protein responsible for the reabsorption of C8 through C10, with highest affinities for C9 and PFDA (C10). These data collectively suggest that chain length is a factor in the extent to which PFAAs are substrates of various basolateral and apical transporters in renal proximal tubule cells, which in turn impacts the rate of elimination. Moreover, since saturation of these transporters will lead to nonlinearity in elimination, one can expect that PFAAs, which are significant substrates, will have greater nonlinearity in their elimination (as a function of exposure level) compared with PFAAs for which the transporters have lesser affinity. While this is a general expectation, the PK data of Dzierlenga et al. (2019) did not exhibit nonlinear elimination

with single doses in the range of 2–20 mg/kg, although it is not known if transporter saturation would occur with higher doses or multiple doses (leading to accumulation of PFDA) in this dose range.

Animals (Rats and Mice)

Distribution in rats and mice was examined in multiple toxicological studies of PFDA. Vanden Heuvel et al. (1991) specifically evaluated [1–14C] PFDA pharmacokinetics in rats and observed distribution into all tissues examined, including liver, kidney, heart, and gonads. Tissue levels outside of the liver were less than 1% of the administered dose in male rats and less than 2% of the dose in female rats. Vanden Heuvel et al. (1992) then examined what they described as the covalent binding of PFDA to protein in male rat at 2 hours and at 1 and 4 days after intraperitoneal dosing with 4.8 mg/kg [1–14C] PFDA and reported that ~0.1% of the administered dose was bound in plasma and liver and ~0.25% was bound in testes (results independent of sample time). This is the only report of covalent binding of PFAS encountered by EPA and the compounds are otherwise understood to be chemically inert. The fact that it was a small fraction of the PFDA and only identified by radioactivity, and not chemical identity, suggests that a 14C-labeled contaminant of the PFDA is actually responsible for the binding and therefore the observation is not mechanistically meaningful for PFDA. However, even though only a small fraction of the 14C was covalently bound, the quantity could be enough to interfere with estimation of long-term clearance or half-life based on measurements of remaining 14C activity.

Other investigators measured distribution into multiple tissues, most commonly kidney, liver, and brain (Dzierlenga et al., 2019; Kim et al., 2019; Fujii et al., 2015). Although PFDA can be found in the brain, the accumulation of PFDA was generally lower in the brain than in other organs or tissues, while the highest levels were found in liver. For instance, Kawabata et al. (2017) observed that the hepatic concentration of PFDA (mg/g tissue) was ~60 times higher than that of the brain in Wistar rats given a single oral dose of 50 mg PFDA/kg. This measurement was made 9 days after the dose was administered, which should be a sufficient time for distribution among the tissues to equilibrate but is short enough compared with overall clearance to represent a significant portion of the administered dose.

Volume of Distribution in Rats and Mice

Ohmori et al. (2003) estimated the volume of distribution (V_d) for PFDA in male and female Wistar rats (three each sex) after i.v. administration (48.64 mmol/kg BW) as 347.7 ± 15.2 and 441.1 ± 55.1 mL/kg, respectively, for male and female Wistar rats (three each sex). The V_d of PFDA obtained by Ohmori et al. (2003) only varied slightly by sex although up to twofold larger than those of other PFAAs tested in the same experiment (PFHA, PFOA, or PFNA). This sex difference is in contrast with two more recent studies showing that V_d was larger in males than in females (Dzierlenga et al., 2019; Kim et al., 2019). For instance, Dzierlenga et al. (2019) investigated the disposition of PFDA in Hsd: SD rats administered 2 mg/kg PFDA by i.v. and found V_d for the central

compartment (V1) was slightly larger in males (274 ± 28 mL/kg) than in females (238 ± 35 L/kg) whereas the peripheral (V2) distribution was almost twice as large in males (355 ± 69 mL/kg) than in females (186 ± 57 mL/kg). Summing V1 and V2 for these results from Dzierlenga et al. (2019), the total V_d in males is estimated to be 50% higher than in females. Dzierlenga et al. (2019) also obtained a larger V_d in males versus females when PFDA was given by oral gavage, similar to their results from i.v. dosing.

Kim et al. (2019) reported total volumes of distribution (i.e., not normalized to BW) for their i.v. exposure: 0.1182 L and 0.0584 L for male and female rats, respectively. However, if one assumes a BW of 0.25 kg, then the V_d obtained is consistent with the reported Cmax values, i.e., V_d = dose/Cmax. Given these absolute volumes of distribution and 0.25 kg BW, V_d values were estimated to be 472.7 and 233.8 mL/kg for male and female rats for Kim et al. (2019), which are quite similar to the values reported by Dzierlenga et al. (2019) (see Table 3-1).

There are limited data on ADME properties of PFDA in mice. Fujii et al. (2015) evaluated the PK of PFDA in FVB/NJc mice aged 8–10 weeks using single i.v. dose (0.31 μmol/kg) and oral gavage (3.13 μmol/kg). Unlike rats, while the V_d (mean ± standard deviation, mL/kg) was slightly larger in males (250 ± 60) than in females (200 ± 50) after i.v. administration, the difference in PFDA distribution was not significant. Of note, once entering the body via i.v. administration, most of PFDA were retained in the liver of mice (64%–80% for males, 46%–55% for females). The overall distribution profiles of gavage route were similar to those of i.v. route (Fujii et al., 2015).

The V_d values from the mouse and rat studies are summarized in Table 3-1 along with results for rats from a hierarchical Bayesian analysis from partial pooling of the data, described in Appendix G.1.

Table 3-1. Volume of distribution values reported for animal studies

Study	Strain	Route	Dose (mg/kg)	Volume of distribution (mL/kg)[a]
Male rats				
Dzierlenga et al. (2019)	Hsd: SD	i.v.	2	629 ± 97 *842.7 (473.8–1,235)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	2	586 ± 57 *435.7 (259.7–591.1)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	10	411 ± 46 *324.4 (221.1–421.8)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	20	456 ± 35 *350.2 (236.4–459.4)*
Kim et al. (2019)	SD	i.v.	1	472.7 ± 37.2[b] *441.5 (391.4–454.2)*
Kim et al. (2019)	SD	Oral	1	*464.2 (298.5–576.7)*[c]
Ohmori et al. (2003)	Wistar	i.v.	25	*350.7 (335.1–363.6)*
Population mean (90% CI)				***430.8 (303.3–551.7)***
Female rats				
Dzierlenga et al. (2019)	Hsd: SD	i.v.	2	424 ± 92 *350.5 (296–394.1)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	2	277 ± 35 *240.9 (151.5–327.7)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	10	264 ± 36 *230.4 (144.6–311.8)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	20	270 ± 40 *230.3 (143.9–310.6)*
Kim et al. (2019)	SD	i.v.	1	233.7 ± 17.8[b] *279.9 (234.9–333.5)*
Kim et al. (2019)	SD	Oral	1	383.4 (259.8–496.8)[c]
Ohmori et al. (2003)	Wistar	i.v.	25	441.1 ± 55.1 *439.9 (347.4–506.5)*
Population mean (90% CI)				**–299.6 *(216.9–381.9)***
Male mice				
Fujii et al. (2015)	FVB/NJc1	i.v.	0.16	250 ± 60
Female Mice				
Fujii et al. (2015)	FVB/NJc1	i.v.	0.16	200 ± 50

[a]Values in plain text are as reported for each study unless otherwise noted. Values in italics are the mean (90% credible interval) for the total of the central and tissue compartments for EPA's PK model, values from the Bayesian analysis described in Appendix G.

[b]Kim et al. (2019) reported V_d as 118.18 ± 9.31 and 58.42 ± 4.46 mL for male and female rats, respectively, after i.v. exposures. These were normalized to an assumed 0.25 kg BW, which is consistent with V_d calculated as dose/Cmax, given that Cmax is the initial concentration for i.v. dosing.

[c]Kim et al. (2019) did not report V_d for oral doses.

Distribution of PFDA in mice and rats during pregnancy/gestation has not been evaluated.

Distribution in Humans

While PFDA is distributed throughout the body, tissue concentrations are expected to be less than 50% of the concentration in blood plasma based on the animal data presented above. Despite the relatively low fraction in any given tissue, this concentration ratio suggests that most of the PFDA mass may be in various tissues since they constitute over 90% of the body. Pérez et al. (2013) measured PFAS levels in multiple tissues from cadavers in a specific region of Spain. While PFDA was detectable in 70% of the brain tissue samples, it was below the limit of detection in all liver and bone samples, 68% of lung samples, and 90% of kidney samples (Pérez et al., 2013), severely limiting any interpretation of the results for PFDA. From the reported mean and median values, it appears that PFDA preferentially accumulates in brain tissue followed by lung and kidney tissue, with much lower accumulation in liver and bone tissue (Pérez et al., 2013). In contrast, Wang et al. (2018a) evaluated the ratio of PFAS in cerebrospinal fluid (CSF) and serum of 113 patients and showed only limited distribution to the CSF with a median CSF/serum ratio of 0.013. Mamsen et al. (2019) evaluated tissue levels in human fetuses and did not report quantifiable levels in the fetal brain, although they did in fetal liver and lung (further results on gestational distribution are discussed below.) These other results put into question the reported value for brain by Pérez et al. (2013), although the results of Mamsen et al. (2019) support a high distribution to lung.

Pan et al. (2019) observed a significant correlation between PFDA in human semen and serum concentrations, with a mean ratio of 0.02.

Because the mass apportionment of PFDA to specific tissues in humans is uncertain, it is appropriate that blood PFDA concentration has been applied to assess PFDA exposure for humans and was used in this review to estimate the relationship between exposure and internal dose. Dosimetry in blood has been more thoroughly evaluated in both humans and experimental animals, so can best be correlated with dose.

A recent study evaluated levels of several PFAS, including PFDA, in human serum as a function of various measures of body composition as well as localized measurements of adipose content throughout the body generated by dual-energy X-ray absorptiometry (DXA) and whole-body magnetic resonance imaging (WB-MRI) (Lind et al., 2022). In women, the study showed a negative correlation between serum PFDA concentration and many measures of body fat, as well as with the volume of areas of the body with high fat fractions, although much less so with the volume of these regions. For example, there is a negative correlation between serum PFDA and the volume of hips and inner thighs in women, but no correlation with the fat content of these regions (Lind et al., 2022). In men, the study showed no association between serum PFDA concentration and measures of body composition. Given the minimal distribution of PFDA to adipose tissues seen in rats (Kim et al., 2019), one might expect essentially no effect of the volume of these tissues on serum levels, as was seen in men. However, one would predict a negative correlation between V_d and body fat, and in fact the results in women appear to be consistent with that prediction if glomerular filtration increases with body mass or surface area, as discussed in the excretion

section. It is also possible that the correlation is due to variation in exposure related to body fat, wherein the male population exposure (per kg BW) was constant with body fat but for some reason exposure decreased with body fat among women. Matched estimates of exposure from dietary surveys or samples, or matched measures of urinary clearance (PFAS concentrations in urine) are ultimately needed to determine if the correlations actually reflect PK variation.

Human Distribution during Pregnancy

PFDA can also be found in human breast milk, placenta, embryo/fetal tissues, and cord blood {Monroy, 2008, 2349575; Kärrman, 2010, 3121276;Liu, 2011, 2919240;Zhang, 2013, 3859792;Mamsen, 2019, 5080595;Mamsen, 2017, 3858487}. Mamsen et al. (2019) and Mamsen et al. (2017) examined fetal tissues after voluntary abortions (first trimester) or intrauterine fetal death (second and third trimester; (Mamsen et al., 2019)). More specifically, Mamsen et al. (2017) reported time-matched maternal serum and fetal tissue levels from fetuses between 36 and 65 days of age (i.e., between 5 and 10 weeks); the data appeared to show an increasing trend in tissue concentration with fetal age, but the trend was not statistically significant. When tissues were analyzed separately, PFDA concentration in placenta, liver and lung were likewise found to increase with trimester, but were not detected in heart, CNS, or adipose tissue (Mamsen et al., 2019). Also, the first trimester data were from women with a mean age of 26.5 years, while the second and third trimester were from women with a mean age of 32.5 years. Interestingly, first trimester maternal serum concentrations (mean 0.34 ng/mL) were somewhat higher than the second and third trimester concentrations (mean 0.26 and 0.27 ng/mL, respectively), although the difference was not statistically significant. The ratio of placenta concentration to first trimester maternal serum indicates a strong time-trend in distribution to the placenta, but this trend was also not statistically significant and the ratio of fetal liver and lung to placenta did not show a consistent pattern with trimester (Mamsen et al., 2019). In summary, while some of the data are indicative of a time-dependence in the ratio of placental and fetal tissue to maternal serum levels, none of those results are statistically significant and other aspects of the data indicate that the ratio is constant.

Pan et al. (2017) performed a longitudinal study in 100 pregnant women and observed a significant decline between the first and third trimesters of all PFAS evaluated, including PFDA. This result is consistent with the expectation that distribution to the fetus and overall increase in body mass of the mother, fetus, placenta, etc. results in a larger distribution volume, for which the rate of increase in this volume is more rapid than intake from ongoing exposure. However, while the decline was statistically significant, there was only a 16% decline in the median and 13% decline in the geometric mean between the first and third trimester for PFDA. Pan et al. (2017) also found a negative correlation between the ratio of cord serum at birth and third trimester maternal serum (C/T3) and pregnancy body mass index (BMI).

To compare the distribution between tissues and maternal blood matrices among different studies, adjustment should be made to correct for the distribution among blood components. Specifically, Poothong et al. (2017) measured a mean ratio of 1.7 for serum: whole blood and 1.3 for

plasma: whole-blood concentrations of PFDA. These factors will be used to adjust the subsequent tissue: blood matrix ratios to tissue: plasma, when reported for whole blood or serum. If the ratio of serum: whole-blood concentration is 1.7 and hematocrit (hct) is 45%, then the mass fraction of PFDA in plasma, given this ratio, would be Fp = 1.7 × (1 − hct) = 93.5%. Using the reported plasma: whole-blood ratio and the same calculation, one obtains Fp = 1.3 × (1 − hct) = 71.5%. Partitioning of PFDA and other PFAAs between human plasma and blood cells were also investigated by Jin et al. (2016). The estimated mass fraction in plasma (human samples) increased among perfluoroalkyl carboxylates as the carbon chain length increased from C6 (mean 0.24) to C11 (0.87) with the mean of 0.82 for PFDA (C10), which corresponds to a plasma: whole-blood concentration ratio of 0.82/(1 − hct) = 1.5. Because this value is intermediate between the serum:whole blood and plasma:whole-blood values reported by Poothong et al. (2017), it will be used to convert tissue partitioning data relative to whole-blood concentrations to serum-based concentrations below.

While the placenta shares circulation from the mother and fetus, it is the only tissue for which PFDA concentrations in adult humans can be compared with matched plasma samples to evaluate overall distribution. Mamsen et al. (2017) reported time-matched maternal serum and placenta tissue levels from fetuses between 37 and 68 days of age (i.e., between 5 and 10 weeks), and obtained mean placenta: maternal plasma ratio of 43%. The results for this ratio from Mamsen et al. (2019), shown graphically, indicate mean values of about 40% and 55% for the second and third trimester, respectively.

The ratio of placenta to maternal serum (estimated from blood) at birth measured by Zhang et al. (2013a) was 34% (both mean and median ratio; n = 32). The observed ratio ranges from 21%–53%, with most data between 30% and 40%.

Mean concentrations in lung and intestines were slightly greater than placenta (shown graphically in Figure 2 of Mamsen et al. (2017)), while other tissues were below, and the reported mean ratio of fetal tissue to maternal plasma was 27%. This indicates that distribution into the fetus as a whole is 50%–80% of the range in placenta (34%–55%). Similarly, Bao et al. (2022) measured PFDA in matched samples of 50 samples collected at delivery and reported a median placenta/maternal serum ratio of 0.48. These results for the placenta are generally consistent with the volume of distribution (L/kg) measured in female rats, described above. Given that the population mean V_d in female rats obtained above (0.3 L/kg) is based on a larger set of studies, which show a fair amount of variability between them (indicating that results from a single study may not be reliable), and accounts for distribution to all tissues, the V_d in humans will be assumed to be the same as in rats; with the results for male rats being used for men and results for female rats for women.

Studies of the volume of distribution in newborns are not available, but one can reasonably assume that it is similar to fetal tissues. Mamsen et al. (2017) specifically reported PFDA concentrations in first trimester fetal liver, heart, intestine, lung, connective tissue, spinal cord, ribs, and extremities. Results for individual tissues were only shown graphically, but most fetal tissues

had a mean concentration lower than the placenta, with mean maternal plasma concentration of 0.28 ng/g, placenta of 0.09 mg/g (43% of plasma), and fetal tissue of 0.05 ng/g (27% of plasma). While Mamsen et al. (2019) had fewer data for PFDA in older fetuses, from their supplemental data mean levels in first trimester fetal livers were less than those in the placenta (Mamsen et al., 2017). As discussed in the section on PK modeling below, the impact on distribution of the pregnant mother with her fetus will not be large, but these results are informative of distribution within the fetus, which will be imputed to newborns.

Specific mechanisms known to impact the distribution of substances during pregnancy are the changes in blood volume (hemodynamics) and concentration of serum proteins. A review by Feghali et al. (2015) states that serum concentration of albumin decreases 13% by gestation week (GW) 32, but that the overall serum volume increases by 42% by GW 38. The net impact of both these changes is then a 0.87 × 1.42 = 1.24-fold increase in the total amount of albumin in the serum, which would suggest 24% lower distribution to various tissues (since there is greater total binding in the serum), including the fetus, compared with what one would otherwise predict. However, the evaluation of fetal distribution just described is based on empirical fetal concentration data, which already depend on and therefore implicitly accounts for variation in maternal serum binding. Further, since we lack precise measurements of the V_d in human adults (specifically, the pregnant mother) versus the fetus and amniotic fluid, and there are no data on excretion (clearance) during pregnancy, the specific contribution of these changes in maternal blood volume and albumin concentrations to the overall empirically observed PFDA concentration changes cannot be quantified.

Several studies evaluated the cord serum: maternal serum ratio in humans at childbirth, with the following median (mean) values reported or calculated from the reported median (mean) concentrations in each matrix:

Liu et al. (2011): 0.42 (0.39);
Needham et al. (2011): 0.29 (mean not reported);
Zhang et al. (2013a): 0.28 (0.25);
Han et al. (2018): 0.38 (0.38 geometric mean [GM] ratio);
Yang et al. (2016a): 0.25 (0.35);
Yang et al. (2016b): 0.39 (0.43);
Li et al. (2020a) (preterm): 0.23;
Li et al. (2020a) (full-term): 0.35; and
Bao et al. (2022): 0.38 (mean ratio not reported).

The average of the median values from these studies is 0.33, indicating that the placenta creates a significant barrier for PFDA between maternal and fetal blood. But beyond this overall average, (Li et al., 2020a) observed a significant increase in the cord/maternal serum ratio between preterm and full-term pregnancies, from a median ratio of 0.23 to 0.35. The authors evaluated the correlation of the cord/maternal serum ratio with multiple placental transporters and identified a

significant, positive correlation with p-glycoprotein (MDR1) and multidrug resistance-associated protein 2 (MRP2). These positive correlations, significant for full-term but not preterm pregnancies, indicate that the placenta acts as a passive barrier to PFDA in early pregnancy and this function is partly defeated by the expression of MDR1 and MRP2 transporters late in pregnancy. Pan et al. (2017) observed a negative correlation of C/T3 serum with maternal serum albumin concentration and a positive correlation of this transfer ratio with fetal (cord) serum albumin concentration, indicating the influence of serum binding on the transfer.

However, if the ratio of fetal serum to maternal serum is 0.33 (33%) and the ratio of fetal tissue to maternal serum is 27%, then the ratio of fetal tissue to fetal serum would be 27%/33% = 82%, a much higher level of distribution than observed in adult rats (43% in males, 30% in females) and human placenta/maternal serum ratios estimated above.

Because the total body burden of PFDA in the human PK studies is unknown, it is not possible to directly estimate V_d in humans. For male and female rats, the estimated (geometric mean) V_d values are 448 and 287 mL/kg, respectively (see Table 3-1). As described above, the fetal tissue: maternal plasma ratio varied between 0.25 and 0.55, with Mamsen et al. (2017) reporting a mean fetal tissue: maternal plasma ratio of 0.27, which is 90% of the average V_d in female rats (assuming 1 L/kg body density). These data indicate that fetal tissue levels are close to maternal levels: if the maternal V_d was that of female rats (0.300 L/kg) and fetal: maternal serum was 0.33, that implies similar average concentration in the fetus as the mother, which is not indicated by the comparison of fetal tissue and placenta concentration.

One can then ask if the somewhat different distribution into the fetus would impact the overall distribution in the mother and fetus together (e.g., for PK modeling during pregnancy). If one presumes that distribution into the fetus is fast compared with the rate of fetal development, such that the concentration in maternal and fetal tissues remains at equilibrium, and one recognizes that the fetus is less than 5% of the combined maternal and fetal mass, then the impact of slightly lower distribution into the fetus on distribution in the mother and fetus as a whole will be minimal. Hence, human maternal V_d is likely to be unchanged during pregnancy. The available data do not indicate a difference greater than 10% or 20%.

Given that PFDA binds strongly to serum proteins, one possible explanation for the apparently higher distribution between fetal serum and tissues is that the fetus has a much lower level of these proteins than an adult, allowing for a greater proportion of PFDA in fetal tissue versus fetal serum. However, data to support this hypothesis, i.e., measurements of PFDA binding in cord blood, are not available. Pharmacokinetic modeling of PFOA dosimetry in humans by Goeden et al. (2019) suggests another hypothesis: that the greater amount of extracellular water in the tissues of fetuses and children (Friis-Hansen, 1961) leads to a greater distribution of PFAS into these tissues. The amount of extracellular water in newborns was estimated to be 2.4 times higher than adults (Friis-Hansen, 1961). Multiplying the volume of distribution from female rats (30%) by 2.4, one obtains 72%, which is much closer to the estimate of 82% obtained here. Hence, while the

mechanism by which distribution in a fetus, which we assume also applies to newborns, might not be the difference in extracellular tissue water, the available quantitative data for extracellular water can provide a reasonable prediction for the difference between newborns and adults, as well as the transition between them (see Figure 3-1).

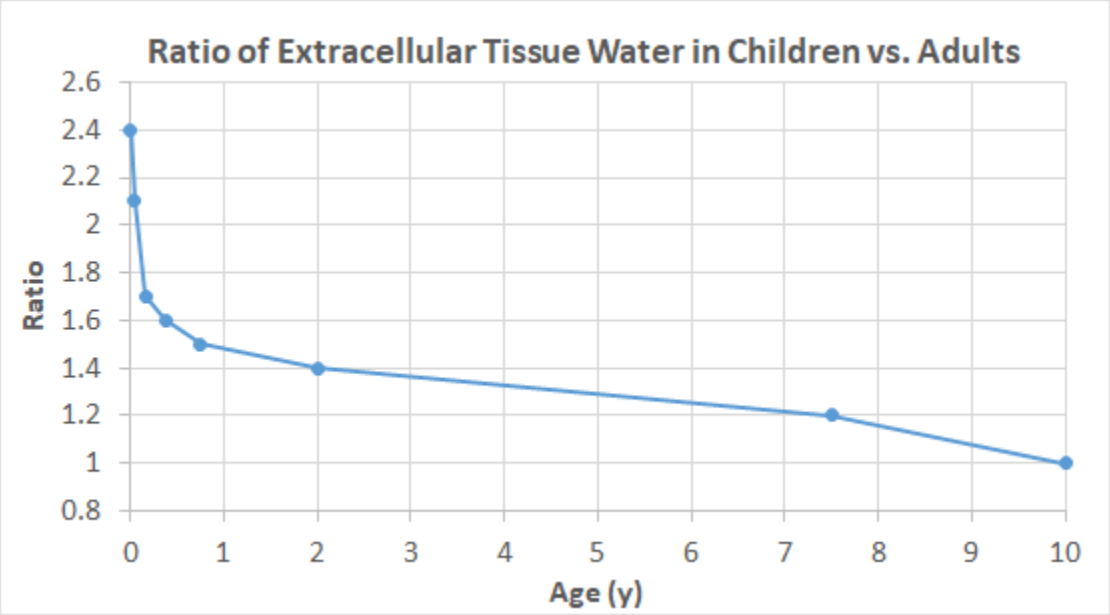


Figure 3-1. Ratio of extracellular water (% of body weight) in children versus adults. Values (points) are calculated from results in **Friis-Hansen (1961)** and plotted at the midpoint for the corresponding age ranges evaluated.

The interpolation function shown in Figure 3-1 can be multiplied by the adult V_d (L/kg) to obtain the corresponding value for children under 10 years of age, as was done by Goeden et al. (2019). However, an opposing factor is the ~20% larger blood volume as a fraction of BW in young children compared with older children and adults (Darrow et al., 1928), given that a high fraction of PFDA is bound to blood proteins. Hence, the extent and even the direction of any change in V_d with age are uncertain and will require further PK studies to address.

Human Lactational Distribution

A recent evaluation of women with children 2–5 years of age by Kim et al. (2020b) found that PFDA is decreased in women who have breastfed by a factor of 1.3% (95% CI: 0.5, 2.1%) per month of breastfeeding, indicating that this is a significant route of distribution from the mothers, and correspondingly a source of exposure for their children.

Liu et al. (2011) also investigated correlations between PFDA concentrations in matched maternal serum and breast milk samples collected from their subjects. The median value for the concentration ratio between milk and maternal serum was 0.03:1. It should be noted that this empirically measured ratio implicitly accounts for the level of serum binding in the breastfeeding

mother, that is, the extent to which that may differ from women outside of the gestational and lactational lifestages. While this low ratio indicates a rather limited level of lactational transfer to infants, the total intake by a breastfed infant will also depend on the milk ingestion rate. For an exclusively breastfed infant, the total exposure by this route could be quite large. Considering that breast milk is a key source of nutrition for infants and the lack of studies demonstrating a specific hazard from this route of exposure versus in utero exposure and other possible routes for infants, the best option for limiting developmental exposure to PFDA is to limit maternal exposure, which will reduce both in utero and lactational exposure of the offspring. Additional information on PFAS and breastfeeding are available on EPA's website https://www.epa.gov/pfas/meaningful-and-achievable-steps-you-can-take-reduce-your-risk#:~:text=Mothers%20and%20Breastfeeding.

Effect of Liver Disease on Human Distribution

In a cross-sectional study by Yeung et al. (2013), the authors investigated the role of liver disease in the deposition of PFDA by analyzing the distribution of PFDA in serum and liver using samples from patients with hepatocellular carcinoma (HCC) and cirrhosis due to chronic hepatitis C viral infection, (HCV); while the mean and median liver:serum ratios were higher in HCC (0.65 and 0.66) patients than in HCV (0.41 and 0.33, respectively), the difference was not significantly different. While the ratio of liver-to-serum PFDA concentration were not evaluated in control subjects of this study, the comparison of absolute liver concentrations of other PFAS in healthy versus diseased samples indicated that pathological changes in diseased livers can alter the liver:serum PFAS distribution.

Evidence Synthesis for Distribution

While PFDA was found to distribute into all tissues evaluated in rat PK studies, the largest concentrations were found in the liver and then the kidney, with extremely low concentrations in other tissues (Dzierlenga et al., 2019; Kim et al., 2019). Distribution to the body as a whole is quantified by the V_d, for which EPA estimated mean values of 0.43 and 0.30 L/kg in male and female rats, respectively, reflecting the tissue-specific data. Fujii et al. (2015) reported V_d in male and female mice of 0.25 and 0.20 L/kg, respectively, indicating low distribution to most specific tissues similar to that seen in rats.

Controlled human PK studies or other analyses that could be used to directly evaluate tissue distribution are not available. Pérez et al. (2013) reported tissue levels measured in human cadavers and found the highest concentration in brain tissue, followed by lung and kidney, with liver concentrations being below the method limit of detection, which is in significant contrast to the data from rats. The results may be explained if most PFDA exposure is by inhalation (e.g., via contaminated dust), which allows uptake to the olfactory bulb from the nose, but when seeking to incorporate these data into a PBPK model Fàbrega et al. (2015) did not consider inhalation to be a significant route of exposure. Mamsen et al. (2019) measured PFDA tissue levels in human fetuses and found significant levels in both the liver and lung, but not in fetal spinal cord (first trimester) or

brain (second or third trimester). Mamsen et al. (2019) also found that fetal liver concentrations exceeded the lung in the second and third trimester. These results support the observation of high lung tissue concentrations in adults but indicate possible errors in the measurements for liver and brain by Pérez et al. (2013).

Chiu et al. (2022) estimated the V_d for four PFAS in humans and obtained population median values ranging from 0.19 L/kg for PFNA to 0.43 L/kg for PFOA, which are similar to corresponding values estimated in rats. The median ratio of third trimester fetal liver and lung tissue to maternal serum concentrations observed by Mamsen et al. (2019) for PFDA was ~0.6 and 0.4, respectively, and PFDA concentrations in fetal heart, CNS and adipose were much lower than liver and lung, indicating an overall fetal volume of distribution less than 0.5, i.e., also in a range similar to that observed in rats. Therefore, while not required for the dosimetric calculations used in this review, EPA concludes that the V_d for PFDA in adult humans (and in the human fetus vs. maternal blood) can be assumed to match that estimated for rats (0.43 L/kg for males and 0.30 L/kg for females) as described above.

Given the observations for distribution between maternal serum and the fetus, as well as the placenta (Mamsen et al., 2019), EPA also concludes that V_d in a pregnant woman (including her fetus) will not be significantly different from that in a nonpregnant woman.

EPA estimated an average ratio of cord/maternal blood of 0.33 or 33%. Assuming that cord blood concentration equals that circulating in the fetus, given the observations for fetal tissue versus maternal serum, it appears that distribution between fetal blood and fetal tissues is higher than in adults. This observation could be explained by a higher fraction of extracellular water in fetal tissues (Friis-Hansen, 1961) but may also result from lower serum binding in the fetal/cord blood.

Finally, the median ratio of PFDA in human breast milk to maternal serum was reported to be 0.03 (Liu et al., 2011). While this is a relatively small fraction, the resulting exposure to a breastfed infant could be significant, given the rate of milk ingestion per kg BW of the child, and it was found that breastfeeding reduced the serum concentrations in mothers 2–5 years after giving birth by 0.5%–2% per month of breastfeeding (Kim et al., 2020b).

3.1.3. Metabolism

Vanden Heuvel et al. (1991) examined the metabolism of PFDA in male and female Wistar rats administrated with a single IP dose of [1-14C] PFDA (9.4 μmol/kg, 5 mg/kg). Only parent PFDA was found in urine or feces, suggesting no appreciable metabolism of PFDA. The findings are expected since PFDA is a long-chain (C10) PFAA with chemical stability similar to that of other shorter length PFAA chemicals (e.g., perfluorohexane sulfonic acid, PFHxS). Although there have been no studies of PFDA biotransformation following inhalation or dermal exposure, metabolism by these administration routes is similarly not expected.

3.1.4. Excretion

In general, excretion is one component of overall elimination of substances in the body, the other being metabolism. Total elimination is often evaluated by observing the decline in concentration of a compound in the blood or other tissues. Because PFDA does not undergo appreciable metabolism, as discussed just above, the elimination data discussed below are interpreted as measures of total excretion.

Excretion in Animals (Rats and Mice)

As observed for other PFAS, sex-specific elimination of PFDA was observed in rats. For example, after i.v. administration (2 mg/kg PFDA), the AUC/dose was significantly higher in female rats (3,065 mM-h/mmol/kg) than in male rats (1,875 mM-h/mmol/kg) (Dzierlenga et al., 2019). Similar results were obtained for oral exposures of 2–20 mg/kg, with AUC/dose in female rats being 5,200–5,500 mM-h/mmol/kg versus 2,960–3,320 mM-h/mmol/kg in male rats (Dzierlenga et al., 2019). These observations collectively suggest that elimination is slower in female rats than in males, perhaps because renal reuptake of PFDA is more efficient in female than male rats. These observations are in directional contrast, however, with results for other PFAS such as PFHxA and PFOA for which clearance is significantly more rapid in female than male rats (Dzierlenga et al., 2019).

As noted earlier, the fecal excretion becomes increasingly important in elimination of long-carbon chain length of PFAAs like PFDA (C10) compared with shorter chain PFAS. For instance, Kudo et al. (2001) attempted to evaluate the elimination of PFDA in Wistar rats (both sexes) with intraperitoneal administration of PFDA using a single dose of 20 mg/kg. It was found that PFDA was slowly excreted in urine, with only 0.2% of the dose being eliminated within 120 hours. More of the administered PFDA (~4%) was found in feces, indicating fecal excretion was a major route of the elimination of PFDA for both sexes. Fecal excretion remained as the major route when rats were intravenously injected with a dose of 25 mg/kg. Similarly, Vanden Heuvel et al. (1991) evaluated the elimination of PFDA after 5 mg/kg intraperitoneal doses to male and female rats. Fecal elimination accounted for 51% and 24% of the administered dose to the males and females, respectively, over 28 days, while urinary excretion was less than 5% of the dose. These results are partly contradicted by the data of Kim et al. (2019), who observed slightly over 3% total excretion in urine and feces after 120 hours, but that urine accounted for 25% of this excretion in male rats (and 38% at 150 days) while urine was over or close to 50% of excretion in females. The difference in fecal versus urinary excretion between the previous studies and Kim et al. (2019) may be a result of the much higher doses used (5–25 mg/kg vs. 1 mg/kg) but a more systematic evaluation of excretion versus dose in a single study would be needed to clearly determine if that was the case. Dzierlenga et al. (2019) also found that the total clearance (CL_{tot}) of PFDA was extremely low compared with short-chain PFAA compounds (e.g., PFHxA) in both male and female Hsd:Sprague-Dawley rats. These results were in line with previous findings of Vanden Heuvel et al. (1991),

Ohmori et al. (2003), and Kim et al. (2019), that PFAAs with shorter carbon chain length tended to show higher CL_{tot}.

Reported values of CL_{tot} for rats and mice are listed in Table 3-2. While the respective ranges of study-specific reported CL_{tot} values for male and female rats indicate a degree of interlaboratory variability in the method of determination, the studies are all considered of adequate quality and therefore there is no reason to preclude any one of them from an overall analysis. Therefore, a hierarchical Bayesian analysis from partial pooling of all these data, described in Appendix G.1, was performed to obtain overall population mean values and intervals for male and female rats, listed in Table 3-2. These values (intervals) for CL_{tot} are considered robust estimates of average clearance in rats (and uncertainty therein).

Table 3-2. PFDA total clearance in rats and mice

Citation	Dose (mg/kg)	Route	CL_{tot}* (mL/d/kg)	n
Male rats				
Ohmori et al. (2003)	25	i.v.	5.2 ± 1.3 *6.37 (5.92–6.89)*	3
Kim et al. (2019)	1	i.v.	3.04 ± 0.40[a] *0.8 (0.53–1.09)*	5
Kim et al. (2019)	1	Oral	*1.66 (1.08–2.27)*	5
Dzierlenga et al. (2019)	2	i.v.	12.82 ± 0.74 *7.06 (5.44–8.62)*	3[b]
	2	Oral	7.44 ± 0.31 *3.71 (2.29–5.19)*	3[b]
	10	Oral	7.94 ± 0.31 *4.8 (3.41–6.12)*	3[b]
	20	Oral	8.11 ± 0.22 *4.62 (3.22–5.99)*	3[b]
Population mean (90% credible interval)	–		***3.61 (1.84–5.54)***	
Female rats				
Ohmori et al. (2003)	25	i.v.	5.3 ± 0.2 *5.39 (3.66–7.14)*	3
Kim et al. (2019)	1	i.v.	3.24 ± 0.24[a] *1.97 (1.67–2.24)*	5
Kim et al. (2019)	1	Oral	*1.95 (1.31–2.61)*	5
Dzierlenga et al. (2019)	2	i.v.	7.85 ± 0.58 *7.25 (6.61–7.89)*	3[b]
	2	Oral	4.61 ± 0.22	3[b]

Citation	Dose (mg/kg)	Route	CL_{tot}* (mL/d/kg)	n
			3.01 (2.18–3.81)	
	10	Oral	4.37 ± 0.24 *2.89 (2.14–3.64)*	3[b]
	20	Oral	4.61 ± 0.24 *3.04 (2.25–3.83)*	3[b]
Population mean (90% credible interval)	–		***3.39 (2.09–4.56)***	
Male mice				
Fujii et al. (2015)	0.16	i.v.	3.9[c]	9
Female mice				
Fujii et al. (2015)	0.16	i.v.	2.2[c]	9

CL_{tot} = total clearance; i.v. = intravenous.
*Values in plain text are as reported for each study unless otherwise noted. Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix G.
[a]Reported absolute CL (mL/d) was divided by 0.25 kg; value is consistent with dose/AUC_{inf} reported.
[b]Dzierlenga et al. (2019) indicates three rats/timepoint used.
[c]Total of urinary and fecal clearance; see text below for details.

While Vanden Heuvel et al. (1991) also evaluated the elimination of PFDA in rats, they did not report clearance values nor AUC values that could be used to calculate clearance. The half-lives estimated from the decline in total body burden (based on 14C activity) were 23 and 43 days in males and females, respectively, while the half-lives based on blood concentrations were 22 and 29 days, respectively (Vanden Heuvel et al., 1991). These female half-lives are comparable to the beta-phase half-lives reported for female rats by Dzierlenga et al. (2019) (18–44 days), although somewhat lower than reported for female rats by Kim et al. (2019) (50–75 days). The half-life estimates of Vanden Heuvel et al. (1991) for male rats are between the alpha-phase (1.7–2.1 days) and beta-phase values (80–110 days) reported by Kim et al. (2019), and somewhat less than those reported by Dzierlenga et al. (2019) (36–68 days beta- or single-phase half-life). This range of half-life values reflects the fact that half-life estimates are sensitive to noise in the experimental data and study design, with Vanden Heuvel et al. (1991) having only measured elimination for 28 days, while Dzierlenga et al. (2019) measured plasma concentrations to 105 days and Kim et al. (2019) to 150 days. Hence the results of Vanden Heuvel et al. (1991) appear to be generally consistent with the other studies described here but will not be used in quantitative evaluation of clearance.

Only Fujii et al. (2015) evaluated the urinary and fecal clearance of PFDA in FVB/NJcl mice using single i.v. dose (0.16 mg/kg) and oral gavage (1.6 μmol/kg). PFDA appeared to have smaller total (feces and urine) clearance than short-chained PFAAs (C ≤ 8) (Fujii et al., 2015). Mouse urinary and fecal clearance were determined by dividing the total amounts excreted in the urine and feces during a 24-hour period by the AUC of the serum concentration of each PFCA between 0

to 24 hours. Fecal elimination appeared to the primary elimination route regardless of exposure routes (i.v. and oral gavage). For i.v. administration, there were no marked differences in total clearance between sexes: 2.2 (1.4 and 0.8 mL/kg-day fecal and urinary clearance, respectively) and 2.8 mL/day/kg (1.8 and 1.0 mL/ kg-day fecal and urinary clearance, respectively) for male and female mice, respectively. In comparison, the total clearances for gavage-administered of PFDA were 3.9 mL/kg-day for male (3.6 and 0.3 mL/kg-day fecal and urinary clearance, respectively) and 2.2 mL/kg-day for females (1.9 and 0.3 mL/kg-day fecal and urinary clearance, respectively) (Fujii et al., 2015). Because the toxicological studies being evaluated used oral exposure, the oral PK results are considered most relevant and sex-specific PK parameters are therefore suggested for calculating HEDs from corresponding points of departure in male and female mice. The beta-phase half-lives obtained for male and female mice after oral gavage are 1.4 day and 4.1 day, respectively (calculates as ln(2)/λ2 from Table 1 of Fujii et al. (2015)). However, since clearance was only observed for 24 hours in the Fujii study, these half-life estimates are considered uncertain and are not used for HED calculation. Instead, the CL_{tot} values in Table 3-2, which are determined from the amount of PFDA excreted in urine and feces, were used. While it would be preferable to have PK data from at least one other study in mice, the results of Fujii et al. (2015) are considered adequate for evaluating the relative clearance in mice versus humans.

Excretion in Humans

Fujii et al. (2015) also estimated the elimination of PFDA in humans using 24-hour urine samples collected from 10 healthy volunteers (five male, five female) and bile samples from five patients (three female, two males) who underwent biliary drainage, and matched blood samples from both the healthy volunteers and patients. The five bile-sample patients were between 68 and 90 years of age, with one being treated for carcinoma of the head and pancreas and the other four being treated for gallstones. The clearance rate to urine and bile from these data involves a straightforward calculation of the ratio of the daily amount excreted by the route to the matched blood sample in a subject. However, the fecal clearance rate is based on an estimate of 98% resorption from the intestine (i.e., enterohepatic recirculation), which they obtained by comparing their results for PFOA with direct observation of PFOA half-lives in humans by Olsen et al. (2007): 98% intestinal resorption is required to match the total (urinary and biliary) excretion otherwise estimated for PFOA with the previously measured PFOA human half-life and a V_d of 200 mL/kg estimated previously for PFOA in mice. Their estimate indicates that fecal excretion accounts for 76% of total excretion in humans. It may be reasonable to assume that PFDA and PFOA are resorbed in the intestines to a similar extent, but this assumption is made in combination with use of biliary excretion data from five elderly, diseased patients and an estimate of V_d from mice. It is possible that anchoring the estimated fecal clearance to the data from Olsen et al. (2007) in healthy subjects corrects for possible effects of biliary disease, but these results should be considered with some caution. Data on PFDA concentrations in human feces versus serum that would otherwise be needed to directly evaluate its fecal excretion are not available.

The PFDA urinary, biliary, fecal, and total clearances (sum of urinary and fecal clearance) estimated by Fujii et al. (2015) for humans were: 0.015 ± 0.01, 2.51 ± 2.1, 0.050 ± 0.04, and 0.066 ± 0.05 (mean ± standard deviation, mL/kg-d). The difference between biliary clearance (not included in the total) and fecal clearance is presumed to be the result of resorption after biliary elimination. There was considerable variability in the results for biliary excretion, as reflected by large standard deviation (e.g., 2.51 ± 2.1 mL/kg-d), despite the fact that samples were collected for 24 hours. On the other hand, collecting 24-hour urine data provides a much better estimate of clearance by that route than extrapolating from a single spot sample.

In general, the total clearance profiles (urinary and fecal) of Fujii et al. (2015) were comparable between humans and mice: total clearance in humans decreased as a function of chain length for C7 to C9, then increased only slightly as the length increased further to C13, while mice showed a clear decrease from C7 to C10 followed by a clear increase with chain length from C10 to C13. In humans, the pattern in total clearance for C7 and higher was due to a shifting balance as fecal clearance increased with chain length, but urinary clearance decreased.

Zhang et al. (2013b) estimated the urinary clearance of PFDA from matched urine and blood or serum samples from 86 healthy volunteers. The resulting median clearance rate in young females (age ≤50 years, n = 20), was 0.047 mL/kg/day and the median for the male and older (age >50 years) group (n = 60) was 0.035 mL/kg-day. The result for younger women is three times higher and the result for men and older women is more than double the urinary clearance estimated by Fujii et al. (2015). Chen et al. (2022) likewise evaluated human urinary clearance in 14 men and 6 women, aged 20–25, based on matched serum and urine samples, and observed a mean (and median) CL of 0.17 mL/kg-day, 3.6 times higher than the value for younger women from Zhang et al. (2013b) and more than 10 times higher than Fujii et al. (2015). The reason for the discrepancies between the results of Chen et al. (2022), Zhang et al. (2013b), and Fujii et al. (2015) is unclear, but a possible factor is that Zhang et al. (2013b) and Chen et al. (2022) used single urinary voids ("spot samples") to estimate clearance while Fujii et al. (2015) collected 24-hour urine samples, which avoids assumptions required to extrapolate from a spot sample to total daily excretion, including the potential issue of intraday variability in urine concentration. However, given the slow clearance of PFDA, there should be minimal intraday variation in its excretion and the larger sample sizes of Zhang et al. (2013b) and Chen et al. (2022) should have balanced the impact of variability to some extent. Therefore, in the absence of a clear reason to select any of these studies as superior, a population sample-size weighted mean urinary clearance from the three studies (including both subpopulations from Zhang et al. (2013b)) was computed. To summarize, the mean (group) urinary PFDA clearances and sample sizes from each study, along with the overall weighted mean urinary CL, are:

Fujii et al. (2015): 0.015 mL/kg-day (n = 10);

Zhang et al. (2013b): 0.066 mL/kg-day (young females, n = 19);

Zhang et al. (2013b): 0.096 mL/kg-day (males and older females, n = 60);

Chen et al. (2022): 0.168 mL/kg-day (n = 20); and

Population-weighted average: 0.097 mL/kg-day.

To further evaluate the role of fecal and other possible routes of elimination in humans, EPA compared urinary CL results from Zhang et al. (2013b) for the male and older female group for PFNA and PFOA, two others slowly eliminated PFAS, to the total CL estimated for these compounds by Chiu et al. (2022). Despite the low urinary clearance, these two PFAS were selected for comparison because Zhang et al. (2013b), Fujii et al. (2015), and Chiu et al. (2022) all evaluated their clearance. The population of males and older females from Zhang et al. (2013b) was specifically selected for comparison because it is more comparable to that of Chiu et al. (2022) and there is stronger evidence for more rapid clearance in the younger female population for some PFAS.

For PFOA (sum of all isomers), Zhang et al. (2013b) estimated a GM CL of 0.027 mL/kg-day in the male and older female group, while Chiu et al. (2022) estimated a population GM total CL of 0.095 mL/kg-day. The difference between these two, which could be attributed to fecal and other routes of CL, is 0.068 mL/kg-day, while the mean fecal CL estimated by Fujii et al. (2015) for PFOA was 0.052 mL/kg-day. However, for PFNA Zhang et al. (2013b) estimated a GM urinary CL of 0.10 mL/kg-day in the male and older female group, while Chiu et al. (2022) estimated a population GM total CL of only 0.056 mL/kg-day. So, one cannot draw a similar conclusion for the results of PFNA, unless it has zero fecal CL, which seems unlikely. However, the mean fecal CL estimated by Fujii et al. (2015) for PFNA was 0.024 mL/kg-day, less than half as much as they estimated for PFOA. This analysis illustrates the variability and uncertainty in any of these analyses, but that fecal CL estimates of Fujii et al. (2015) appear to be in the correct range. The only other fecal elimination data for PFDA are from animal studies and extrapolation of those to humans also has uncertainty. Therefore, the best estimate of fecal CL for PFDA is judged to be the estimate of Fujii et al. (2015): 0.050 mL/kg-day. Together with the weighted mean urinary CL obtained above, a total CL by the two routes is estimated to be 0.147 mL/kg-day.

Maternal excretion via breastfeeding

As described briefly in the "Distribution in Humans" section (in Section 3.1.2), a recent evaluation of women with children 2–5 years of age by Kim et al. (2020b) found that PFDA is decreased in women who have breastfed by a factor of 1.3% (95% CI: 0.5, 2.1%) per month of breastfeeding, indicating that this is a significant route of elimination for such mothers. Assuming the V_d of female rats (300 mL/kg) this rate of elimination is comparable to a clearance of 0.13 mL/kg-day, indicating that elimination roughly doubles during breastfeeding. The specific bioassays being extrapolated from animals to humans only involved exposure to young adult animals or during an initial pregnancy, when lactational excretion would not be a factor. However, it could be significant for the estimation of dosimetry in human children, useful for the interpretation of epidemiological data. The elimination that occurs during breastfeeding would reduce the body burden in a mother who then becomes pregnant again, hence the risk to her

subsequent children. While reduction due to breastfeeding would not be predicted for women who formula-feed their children, some reduction in maternal PFDA would also be expected due to distribution to the fetus, along with the placenta, umbilical cord and amniotic fluid that are lost at childbirth, independent of how the child is subsequently fed.

Analysis of menstrual blood loss as a route of excretion

Another factor to be considered is clearance through menstrual blood and serum loss. As there is no known mechanism for resorption of PFDA from menstrual blood and serum (unlike urinary and biliary/fecal pathways), one might assume that any fluid lost by this process would carry with it the PFDA it contained.

Zhang et al. (2013b) calculated a rate for menstrual clearance assumed to apply for all PFAS based on a study of PFOA and PFOS that estimated menstrual blood loss using measurements of the blood quantity excreted (Harada et al., 2005). This estimate was not specific to PFOA or PFOS and might also be applied to PFDA. However, Harada et al. (2005) cite Hallberg et al. (1966) as the source for a menstrual blood loss of 70 mL per cycle, but according to Hallberg, "the upper normal limit of the menstrual blood loss is situated between 60–80 mL." Thus, 70 mL/cycle appears to be closer to an upper bound for healthy women. On the other hand, Verner and Longnecker (2015) reviewed Hallberg et al. (1966) who evaluated both blood loss and total fluid loss from menstruation and concluded that the fluid lost in addition to blood was likely to be serum, with the corresponding serum binding proteins and associated PFAS. Including this serum loss and assuming 12.5 menstrual cycles per year, Verner and Longnecker (2015) estimated an average yearly total serum loss of 868 mL. Assuming a standard human body weight of 80 kg, the corresponding average rate of clearance is 868 mL/(365 days)/(80 kg) = 0.030 mL/kg-day.

Lorber et al. (2015) examined the effects of ongoing blood loss through menstruation or through frequent blood withdrawal as a medical treatment. Male patients with frequent blood withdrawal had serum concentrations 40%–50% less than males from the general population for the chemicals observed in the study (PFOA, PFNA, PFDA, PFHxS, and PFOS). Female patients also had a lower serum concentration than females from the general public. Although the trend of lower PFAS serum concentration in patients compared with the general public was consistent, there was not a clear trend in relation to the number of recent blood draws or in the recency of the last blood draw. This study's analysis of the impact of menstrual blood loss was purely a modeling exercise, which was performed for PFOA and PFOS. The authors estimated a monthly blood loss of 35 mL (which is similar to the median loss reported by Hallberg et al. (1966)), 50% of which was serum, resulting in a clearance of 17.5 mL/month, or 0.0073 mL/kg-day in an 80 kg woman. This value is also chemical-independent.

Glynn et al. (2020) evaluated PFAS levels in fifth grade children (age 11–12 years) and reported a moderate but significant difference between serum PFDA concentrations in girls (median 0.22 ng/mL, n = 92) and boys (media 0.25 ng/mL, n = 108). However, only 5 (6%) of the girls had begun menstruation and this was not a significant factor in relation to PFDA serum

concentration among the girls. Because of the small sample and limited duration of menstruation, a significant impact of menstruation is unlikely.

Given the low level of urinary clearance reported by the several studies described above (Chen et al., 2022; Fujii et al., 2015; Zhang et al., 2013b), if younger women had additional menstrual clearance of 0.03 mL/kg-day (Verner and Longnecker, 2015; Harada et al., 2005), a significant difference in serum levels of PFDA would be observed. EPA evaluated data from the National Health and Nutrition Examination Survey (NHANES) for PFDA. Specifically, EPA analyzed the collection of NHANES waves from 2003–2004 through 2017–2018. Participants were included if they were age 12 and above and if they had measured PFAS levels but were excluded if they were pregnant or if they were currently breastfeeding. For all waves except 2003–2004, this information on reproductive status was available only for women aged 20–44. This resulted in a total of 16,162 measurements. In the case for which a serum concentration was below the limit of detection (LOD), the value was imputed with the LOD/$\sqrt{2}$. Overall, 26.4% of the PFDA measurements were below the LOD. This analysis was carried out in R (R Core Team, 2022) and the R package "survey" was used to incorporate the NHANES survey strategy into the analysis and generate results applicable to the U.S. population (Lumley, 2023, 2004). A consistent, meaningful difference in serum levels in men versus women was not reported for PFDA (see Figure 3-2) while larger differences were found for PFNA (see Figure 3-2) and PFHxS (results not shown).

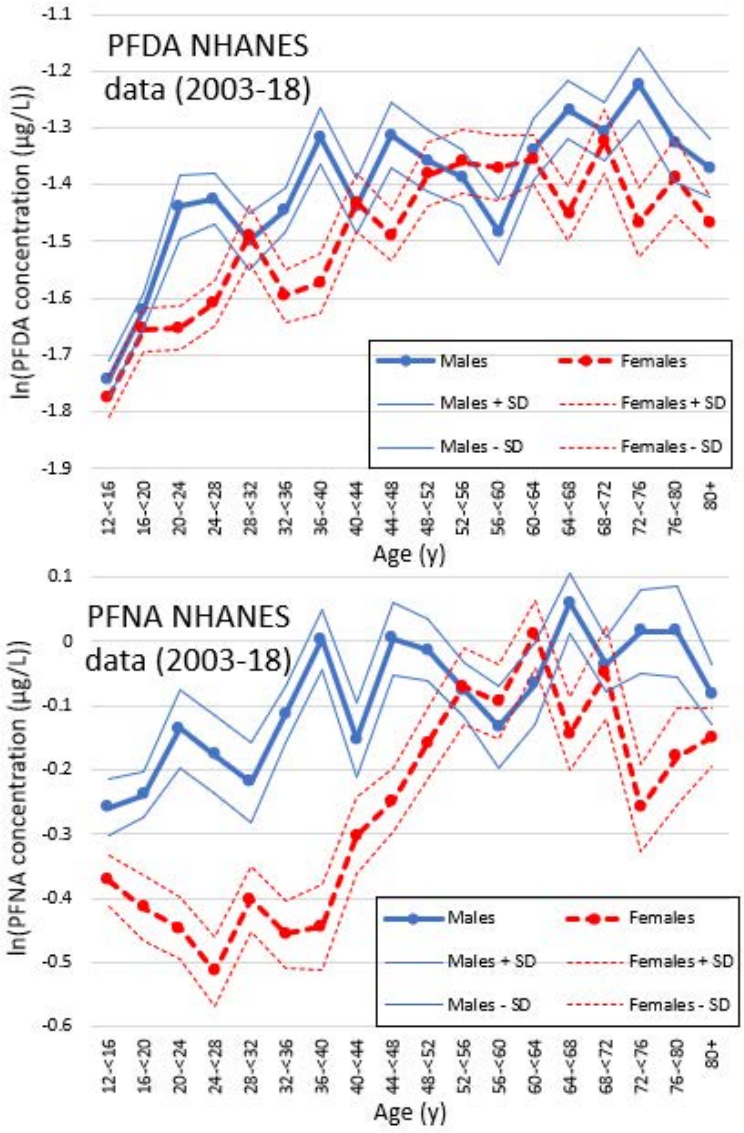


Figure 3-2. Serum concentrations of PFDA and PFNA in U.S. males versus females as a function of age. Data are from NHANES cycle years 2003–2018. Mean and standard deviation (SD) were calculated for each age range and sex after log-transforming the data.

The observed concentration differences between men and women for PFNA and PFHxS, but not PFDA, could be the result of a differences in exposure to men versus women of reproductive age. However, the difference in PFNA urinary clearance observed by Zhang et al. (2013b) (median CL in younger women more than twice the median for men and older women) suggests another mechanism that could differ in effect across PFAS. Specifically, renal transporters involved in the resorption of PFAS are known to be under hormonal control and the affinity of PFAS of varying chain lengths differ for given transporters (Weaver et al., 2010). So, hormonal regulation of urinary

resorption could explain differences in total clearance and observed serum levels between men and women for specific PFAS.

While the observations in Figure 3-2 indicate significant male-female differences in CL of PFDA for some age categories, these are not consistent over the entire reproductive age range. Further, not all women menstruate regularly for various reasons. Given these observations, menstrual clearance as a specific mechanism will not be evaluated further as a clearance pathway for PFDA. Further, the comparable CL in male versus female rats (see Table 3-2) indicates the lack of a large sex-dependent mechanism in that species. While median urinary CL of PFDA among young women was found to be 34% higher than the corresponding median for men and older women (Zhang et al., 2013b), there was a large overlap in urinary CL between the two populations, with almost identical GMs and a GM in the young female group. In contrast, the mean, GM, and median CL for PFNA were all higher for PFNA CL in the young females than in males and older females (Zhang et al., 2013b). Therefore, sex- and age-dependent differences in CL of PFDA among humans, either as a result of menstrual fluid loss or hormonally regulated urinary excretion, is not considered by EPA to be adequately supported by the available data and the subsequent analysis will only estimate a single average value for human CL.

Excretion in infants

Yao et al. (2023) estimated the urinary CL of PFDA and other PFAS in infants by comparing concentrations in cord blood with the amount collected in diaper gel pads during the first week of life. While there are uncertainties due to the fact that infant serum levels may change over the week during which the urine was collected, the results indicate that the infant urinary CL (median 0.047, mean 0.082 mL/kg-day) are in the same range as adult values. Hence, total clearance in children is assumed to be the same as in adults.

Summary for total clearance in humans

In summary, the total estimated urinary plus fecal clearance based on Chen et al. (2022), Fujii et al. (2015), and Zhang et al. (2013b) is 0.147 mL/kg-day for males and females of all ages. Specifically, a population-size weighted mean urinary CL of 0.097 mL/kg-day was calculated from the results for all three studies and the estimated fecal CL of 0.050 mL/kg-day from Fujii et al. (2015) was added to the urinary CL estimate. This value is considered appropriate for calculation of human equivalent doses (HEDs), either from animal-human dose extrapolation or from human serum PFDA levels identified from epidemiological studies (see Section 3.1.7).

Evidence Synthesis for Excretion

PFDA is excreted in both feces and urine of rats and mice with much of the data indicating a higher fraction of the excretion being in feces. That Kim et al. (2019) observed higher urinary excretion may be due to dose dependence, with urinary excretion being higher at higher doses. Unlike other PFAS, total clearance of PFDA was somewhat lower in female rats than in male rats but

the difference was small, with a mean value of 3.6 mL/kg-day in the males versus 3.4 mL/kg-day in the females from EPA's Bayesian analysis of multiple studies. In mice, the estimated CL_{tot} was 3.9 mL/kg-day in males versus 2.2 mL/kg-day in females (Fujii et al., 2015).

In humans, estimates of urinary clearance varied widely from a study mean of 0.015 mL/kg-day reported by Fujii et al. (2015) to the mean of 0.168 mL/kg-day reported by Chen et al. (2022). In the absence of a clear rationale for selecting among the available data, an overall population-size-weighted mean of 0.097 mL/kg-day was calculated for urinary clearance in humans.

Only one study estimated fecal clearance in humans that was based on biliary clearance measured in five adult patients (Fujii et al., 2015). Besides the limited, health-compromised patient population, the estimate involved use of a V_d estimated for mice (0.2 L/kg) and read-across from results for PFOA. However, comparisons of the estimated fecal clearance for other PFAS by Fujii et al. (2015) to estimates of total clearance of those PFAS reported in the literature indicated that the estimates of Fujii et al. are in a range consistent with other data. Therefore, the fecal clearance of Fujii et al. (2015), 0.050 mL/kg-day, is assumed applicable to humans.

The total clearance of PFDA in humans is then estimated as the sum of urinary (0.097 mL/kg-day) and fecal clearance (0.050 mL/kg-day), i.e., 0.147 mL/kg-day.

Menstrual fluid loss has been considered as a route of elimination of persistent chemicals, including PFAS (Verner and Longnecker, 2015; Zhang et al., 2013b). However, EPA's evaluation of population PFDA serum concentration data from NHANES (see Figure 3-2) did not reveal a difference between men and never-pregnant women that is consistent in magnitude with a clearance of 0.03 mL/kg-day (menstrual fluid loss estimated from Verner and Longnecker (2015)) over the course of female reproductive age, although such a difference was observed for PFNA (see Figure 3-2) and PFHxS (not shown). If menstrual fluid loss was a common mechanism of PFAS clearance, a consistent impact would be observed. Therefore, EPA concluded that menstrual fluid loss is not a specific mechanism of PFAS clearance, although the total clearance rates for some other PFAS do appear to be significantly larger in women of reproductive age than in the rest of the population.

3.1.5. Summary of Pharmacokinetic Parameters

Summary rat, mouse, and human pharmacokinetic parameters (clearance, volume of distribution, and F_{abs}) from the preceding analyses are provided in Table 3-3, along with overall half-lives calculated from the clearance and volume of distribution.

Table 3-3. Rat, mouse, and human pharmacokinetic parameters

Sex and species	Clearance (mL/kg-d)	Volume of distribution (mL/kg)	$T_{1/2}$[a] (d)	References
Male rats	3.61	430.8	83	Kim et al. (2019) Dzierlenga et al. (2019)
Female rats	3.39	299.6	61	

Sex and species	Clearance (mL/kg-d)	Volume of distribution (mL/kg)	$T_{1/2}$[a] (d)	References
Rats (M + F)[b]	3.5	365	72	Ohmori et al. (2003)
Male mice	3.9	250	44	Fujii et al. (2015)
Female mice	2.2	200	63	
Mice (M + F)[b]	3.1	225	50	
Humans	0.147	365[c]	1.721 (4.7 yr)	Chen et al. (2022) Fujii et al. (2015) Zhang et al. (2013b)

[a]$T_{1/2}$ = (volume of distribution [mL/kg]) × ln (2)/(clearance [mL/kg-d]).
[b]Average of separate male and female values.
[c]V_d in humans assumed equal to the (average) value for male and female rats.

Some mechanistic insight can be gained by comparing the clearance values shown in Table 3-3 with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding. Davies and Morris (1993) summarized GFR for multiple species. Using 0.25 kg as the species average BW for the rat, the GFR/BW for rats is 7.55 L/kg-day, which is 2,100 and 2,200 times higher than the population mean clearance in male and female rats, respectively. Considering that (Davies and Morris, 1993) reported on human physiological data collected before 1990, when the average BW was less than today, it seems appropriate to use their value for average human BW, 70 kg, which results in an estimated GFR/BW of 2.57 L/kg-day in humans, which is more than 17,000 times greater than the estimated total clearance and more than 26,000 times the estimated urinary clearance of PFDA for humans. Thus, GFR itself is not a limiting factor for PFDA clearance in rats or humans.

Binding to serum proteins likely plays a role in these sizable differences. As discussed above in the context of distribution, PFDA binds to albumin with high affinity, which mediates glomerular filtration since only the unbound fraction is filtered (Kudo, 2015), in addition to any role played by renal transporters. Kim et al. (2019) measured reported PFDA free fractions (f_{free}) of 0.00118 and 0.000112 in male and female rat plasma. Using these values, GFR × f_{free} = 8.9 and 0.85 mL/kg-day in male and female rats. This alternative estimate of clearance for male rats is only 2.5 times higher than the empirical population mean in Table 3-3 (6.8 mL/kg-d), which could be interpreted as implying that there is moderate renal resorption. However, for female rats GFR × f_{free} is fourfold lower than the empirical clearance of 3.4 mL/kg-day. Section 3.1.6 notes that the PBPK model of Kim et al. (2019), which assumes that tissue distribution is similarly limited by the free fraction, underpredicts the short-term distribution of PFDA in rats. Hence, while it is expected that serum protein binding limits renal excretion (and tissue distribution) to some extent, the reduction appears to be less than predicted under the assumption that clearance is strictly limited to the

equilibrium free fraction. Alternatively, there could simply be an error in the measured free fraction.

Kim et al. (2019) also measured and reported average PFDA f_{free} values of 0.00157 and 0.00123 in human males and females, respectively, which leads to GFR × f_{free} = 4 and 3 mL/kg-day for men and women, which are still 40 and 30 times greater than the estimated urinary clearance value (0.097 mL/kg-day for both men and women). Thus, it appears likely that there is significant renal resorption of PFDA in humans, which acts beyond the limitation predicted based on measured serum protein binding.

According to EPA's $BW^{3/4}$ guidelines (U.S. EPA, 2011) use of chemical-specific data for dosimetric extrapolation, such as described above, is preferable to the default method of $BW^{3/4}$ scaling. However, for the purpose of comparison, using the standard species BWs of 0.25 kg in rats and 80 kg in humans, the clearance in humans is predicted to be 4.2 times lower than that of rats. Given clearance rates of 3.6 and 3.4 mL/kg-day in male and female rats, one would then predict clearance rates of 0.86 mL/kg-day in men and 0.81 mL/kg-day in women, 5.9 and 5.5 times higher than the total clearance estimated from human PK data. Thus, given the PFDA-specific PK data, use of $BW^{3/4}$ could lead to an overprediction of human elimination, hence an overprediction of HEDs of approximately sixfold.

3.1.6. Evaluation of Physiologically Based Pharmacokinetic (PBPK) and Pharmacokinetic PK Modeling

The PFAS protocol (see Appendix A) recommends the use of PBPK models as the preferred approach for dosimetry extrapolation from animals to humans, while allowing for the use of data-informed extrapolations (such as the ratio of serum clearance values) for PFAS that lack a scientifically sound and/or sufficiently validated PBPK model. Only if chemical-specific information is not available does the protocol recommend that doses be scaled allometrically using body weight $(BW)^{3/4}$ methods. Selection from among this hierarchy of decisions considers both the inherent and chemical-specific uncertainty (e.g., data availability) for each approach option. This hierarchy of recommended approaches for cross-species dosimetry extrapolation is based on EPA's guidelines on using allometric scaling for the derivation of oral reference doses (U.S. EPA, 2011). This hierarchy preferentially prioritizes adjustments that result in reduced uncertainty in the dosimetric adjustments (i.e., preferring chemical-specific values to underpin adjustments versus use of default approaches).

A PBPK model is available for PFDA in rats and humans Kim et al. (2019). The computational code for this model was obtained from the model authors and evaluated for consistency with the written description in the published paper, the PK data for PFDA, known physiology, and the accepted practices of PBPK modeling. The code was converted from the Berkeley Madonna language in which it was written to the same R/MCSim platform as used for other modeling for IRIS, and by Bernstein et al. (2021), but as a completely independent model (a "stand-alone" version, i.e., without implementation in EPA's PBPK Template model). The model was

also implemented in the PBPK Template by setting parameters to match that of the model. Using both the stand-alone version and EPA's PBPK Template with matching parameters, EPA was able to exactly reproduce the model simulations shown in Kim et al. (2019) (Bernstein et al., 2021). Unfortunately, this process revealed several flaws in the model. One flaw, an error in the balance of blood flow through the liver, had only a moderate impact on model predictions. A much larger issue is that the model had only been calibrated to fit the oral PK data for rats and the set of model parameters selected by the model authors to match those data included an oral bioavailability (BA) lower than is otherwise supported by the empirical PK data. For example, the fraction absorbed by the male rat was effectively set to 25% in the Kim et al. model when the empirical PK analysis showed 65 ± 8% bioavailability and EPA's analysis yielded a mean estimate of 84%. Further, when the model was used to simulate the intravenous PK data, which are data to which a PK model should be calibrated, the parameters were found to be completely inconsistent with these data. Figure 3-3 compares results obtained with a replication of the PBPK model, which exactly matches the published PBPK model results for oral dosimetry, with the data and empirical PK fit for a 1 mg/kg i.v. dose to male rats. Additional details of EPA's analysis are provided by Bernstein et al. (2021).

The overprediction (approximately three to four times higher than these key pharmacokinetic data for male rats) of the i.v. data by the Kim et al. (2019) model indicates that distribution into the body is significantly underpredicted by the model, which was offset in the simulations of oral dosimetry data by using an unrealistically low oral bioavailability. Initial efforts to re-fit the model to the data did not produce acceptable fits to both the i.v. and oral dose PK data and involved changing model assumptions in a way that would require separate experimental validation before use. It was therefore determined that the published model structure and underlying assumptions did not allow a sufficiently sound calibration of the model to the PK data, given the currently available data.

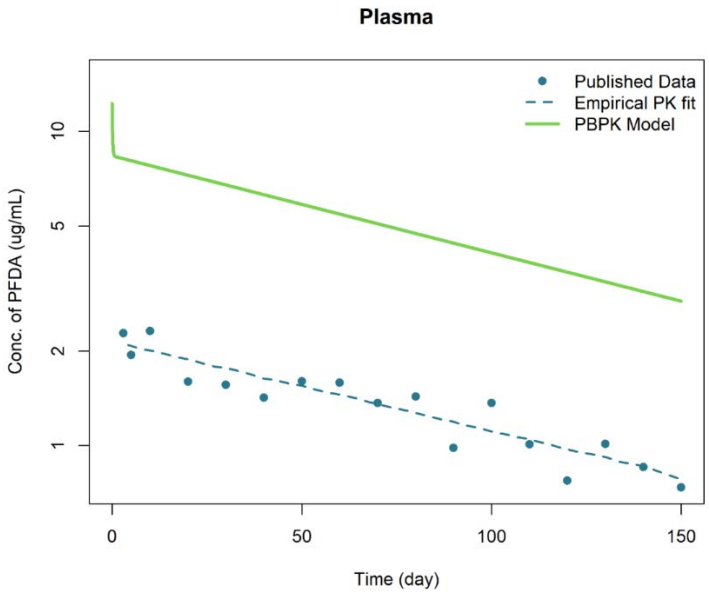


Figure 3-3. Comparison of PFDA PBPK model predictions to i.v. dosimetry data (circles) of Kim et al. (2019) for a 1 mg/kg dose.

"Empirical PK fit" is the result of an empirical PK analysis shown by Kim et al. (2019) (digitized). EPA's replication of the PBPK model exactly reproduces the PBPK model results of Kim et al. (2019) for oral dosimetry hence is considered an accurate reproduction of the model. The discrepancy between the PBPK model prediction for a 1 mg/kg dose and the data demonstrates that the published model structure and parameters are highly inconsistent with the empirical data, leading to the assertion that there is a significant flaw in the model.

Fàbrega et al. (2015) also described a PBPK model parameterized for multiple PFAS in humans, including PFDA. However, this model makes use of the same key assumption regarding PFAS distribution as Kim et al. (2019), which EPA considers to be critically flawed due to the resulting error in prediction of dosimetry after i.v. exposure as shown in Figure 3-3. Further, Fàbrega et al. (2015) estimated the equilibrium blood:tissue partition coefficients by comparing tissue concentrations measured in cadavers (autopsy subjects, (Pérez et al., 2013)) with blood concentrations from living donors reported 6 years later. In general, EPA considers this comparison of blood and tissue levels in nonmatched subjects (albeit from the same geographic region), reported 6 years apart, to be a highly uncertain method for the estimation of tissue distribution. It is also unknown whether the time between death and autopsy sampling can result in changes in PFDA tissue concentrations, although the delay was no more than 24 hours (Pérez et al., 2013). Further, Pérez et al. (2013) reported that PFDA tissue levels were below the LOD in liver and bone, for which Fàbrega et al. (2015) used the LOD as the tissue concentration, and only above the LOD in 32% of lung samples and 10% of kidney samples, making estimation of the corresponding PCs even more uncertain. Hence, the PBPK model of Fàbrega et al. (2015) was not considered further for use in this review.

EPA also evaluated the use of a classical PK model to explicitly describe the time-dependent dosimetry of PFDA in rats. Specifically, the PK parameters from the Bayesian PK analysis described in Appendix G.1 were incorporated into a two-compartment PK model and evaluated against independent PK data, specifically end-of-study serum levels from the NTP (2018) bioassay. Further details of the model and evaluation are provided in Appendix G.2. While EPA considers the model predictions to be of generally acceptable quality (mean predictions were within a factor of 2 of the mean measured levels), there was a bias toward underprediction of the observed data.

Another factor in considering use of a PK model is the potential to extrapolate across lifestages. However, as described in "Distribution in Humans" above in Section 3.1.2, there are only limited data on PFDA distribution during gestation, the interpretation of which is not entirely clear. There are only limited PK data in young children, none in young animals to evaluate differences in that lifestage, and none on clearance during pregnancy versus nonpregnant adults. Hence, even if the model were judged to be adequate for low-dose extrapolation of dosimetry in adult animals, extrapolation of a PK model such as that described in Appendix G to other lifestages would involve additional uncertainty. Given the alternative approach described below for estimating internal doses in adult rats under bioassay conditions, the two-compartment PK model will not be used for dosimetric extrapolation despite its potential promise.

3.1.7. Approach for Pharmacokinetic Extrapolation of PFDA among Rats, Mice, and Humans

Empirical PK data from all published studies, including Kim et al. (2019), were evaluated and summarized above to obtain values for the volume of distribution (V_d, mL/kg) and total clearance (CL_{tot}, mL/kg-day) in male and female rats and mice, women of childbearing age (<50 years of age) and men and older women (see Table 3-3). However, evaluation of a published PBPK model (Kim et al., 2019) and a two-compartment PK model showed significant errors in the PBPK model, and that the simpler PK approach produced biased predictions of PFDA serum concentrations measured at the end of the NTP bioassay (also see Appendix G). Two alternatives to use of PK (or PBPK) models for dosimetric extrapolation are algebraic interpolation of the serum concentrations measured in the NTP bioassay and use of data-derived extrapolation factors (DDEFs). Given the measured serum PFDA concentrations in rats, it is preferable to use those PK data rather than a less informed estimate of internal dose. Because the PK model predicted the measured rat serum levels from the NTP bioassay reasonably well, although biased to underprediction (see Figure G-9), the qualitative features of those PK model predictions were used to guide an interpolation approach described in Appendix G.2.2. In summary, the end-of-study serum concentrations for various exposure PODs were estimated by linear interpolation between the observed concentrations, and then the average serum concentration over the 28-study was estimated to be 50% of this final concentration since the expected time-course of PFDA in rats as shown in Figure G-8 is an approximately linear increase over the 28 days. Given a linear increase over time, the average concentration will just be 50% of the final concentration. These rat internal dose estimates were also assumed to be valid for Frawley et al. (2018), who used the same rat

strain, dosing schedule, and duration as NTP but did not measure the end-of-study concentrations. The resulting estimates of average serum concentration are then used as internal dose POD (POD_{int}) values for calculation of human equivalent dose POD (POD_{HED}) values as described below.

However, in-study PK data were not available for the mouse developmental study of Harris and Birnbaum (1989), making both evaluation of PK model predictions and interpolation of observed serum concentrations impossible. As stated in EPA's guidance for DDEFs (U.S. EPA, 2014), use of these factors "maximize the use of available data and improve the scientific support for a risk assessment." As discussed above in the section on excretion (3.1.4), the estimated population average values of CL for mice and humans are considered sufficiently sound for use in such extrapolation and use of the alternative (default) approach, $BW^{3/4}$ scaling, would lead to significant errors in HED calculations. Therefore, a DDEF calculated from the CL values for female mice and humans listed in Table 3-3 is considered the next preferred option for extrapolation of developmental endpoints observed in mice to humans (the CL for female mice is used specifically since exposure to the mouse fetus occurs through dosing to the dam and it is the dam's CL that determines her internal dose). As described in Appendix G.2.3, the POD_{HED} can be calculated from the F_{abs} and CL in the animal and humans as:

$$POD_{HED} = POD_A \times (F_{abs,A}/F_{abs,H}) \times CL_H/CL_A, \quad (3\text{-}1)$$

where $F_{abs,H}$ and CL_H are the fraction absorbed and clearance in humans, while $F_{abs,A}$ and CL_A are the fraction absorbed and clearance in the animal. The DDEF is then $(F_{abs,A}/F_{abs,H}) \times CL_H/CL_A$. As discussed in Section 3.1.1, F_{abs} was estimated to be 100% in mice while $F_{abs,H}$ is assumed to be 100%. With CL = 2.2 mL/kg-day in female mice and 0.147 mL/kg-day in humans (see Table 3-3), the DDEF for female mouse to human extrapolation is 0.067.

When a POD_{int}, specifically a serum concentration, is obtained from rat toxicity studies as described above or from human epidemiological studies (Budtz-Jørgensen and Grandjean, 2018a; Grandjean et al., 2012), the POD_{HED} will likewise be calculated as:

$$POD_{HED} = POD_{int} \times CL_{H,} \quad (3\text{-}2)$$

using CL_H = 0.147 mL/kg-day = 1.47×10^{-4} L/kg-day (see Table 3-3). This is the exposure rate for which the steady-state human serum concentration will equal POD_{int}.

Uncertainty Analysis for HED Calculations for PFDA

Given that rat internal doses were based on observed serum concentrations from the NTP bioassay, and that these concentrations increase almost linearly with applied dose (see Figure G-9), interpolations of PODs other than the applied doses are presumed to introduce minimal uncertainty in the end-of-study value. However, the subsequent estimate that the average concentration during a 28-day study is 50% of the final concentration uses the PK model in a qualitative manner (i.e., its prediction that serum concentrations increased linearly with exposure

day over the course of the study) as shown in Figure G-8. These predictions in turn follow from the estimated half-lives of PFDA in male and female rats, 83 and 61 days, respectively (see Table 3-3). If the actual half-lives are longer than these estimates, linear accumulation over 28-days of exposure would still be predicted, so the average serum concentration should not be underpredicted. If the half-lives are shorter than EPA's estimates, the serum concentration time-course would be more concave downward, that is, higher than linear accumulation if consistent with the observed end-of-study values. EPA's upper confidence limit on CL in female rats is 4.56 mL/kg-day (see Table 3-2) and the lower confidence limit in V_d is 216.9 mL/kg, leading to a lower bound on half-life of 33 days. Given that half-life, the animals would still only be at about 60% of steady state after 28 days of dosing and calculating an average value for an exponential curve versus a straight line to the same final value at 29 days results in only a 10% difference. Even if the error is twice this, the interpolation used would only underpredict the average concentration by 20%. Hence, the overall error in estimates of the average blood concentrations for rats in the bioassays is judged to be less than 20%.

The urinary clearance value used for humans was based on the results of Fujii et al. (2015), Zhang et al. (2013b)and Chen et al. (2022), resulting in a population-weighted average of 0.097 mL/kg-day, which is over sixfold higher than estimate of urinary CL from Fujii et al. (2015) (0.015 mL/kg-d). The contribution of fecal clearance was taken from Fujii et al. (2015) as the best option available, resulting in an estimated total CL for humans of 0.147 mL/kg-day. A more modest correction for fecal absorption (using the ratio of fecal/urinary elimination observed in rats after i.v. dosing) could be applied versus the rate estimated by Fujii et al. (2015), which was roughly threefold higher. Specifically, the ratio of fecal/urinary CL reported for female rats by Kim et al. (2019) is 0.742. Using this ratio to obtain a lower bound estimate of fecal clearance, and then applying it to the urinary CL of Fujii et al. (2015), one obtains a total CL estimate of 0.026 mL/kg-day, 5.6-fold lower than that used. On the other hand, if the urinary CL of Chen et al. (2022) and the fecal CL of Fujii et al. (2015) are combined, the estimated CL_H is 0.218 mL/kg-day, 50% higher than the estimate used. As discussed above, the uncertainty in the rat internal doses is judged to be less than 20%, i.e., a factor of 1.2, but in the direction of higher internal dose, which would result in higher POD_{HED} values, while this uncertainty in CL_H would result in lower POD_{HED} values. The overall uncertainty in CL_H is therefore judged to be less than a factor of 6—POD_{HED} values should be over-estimated by no more than sixfold—while the POD_{HED} is unlikely to be underestimated by more than a factor of 1.5 × 1.2 = 1.8- or 2-fold.

Uncertainties in the extrapolation to developmental exposure and dosimetry in children remain, given that developmental PK studies have not been conducted in rats and mice and developmental PK data are limited for humans. (As described in "Distribution in Humans" in Section 3.1.2, the available data for distribution in human fetuses indicate it is similar to distribution in adult female rats, so there is no indication of a marked lifestage difference in the volume of distribution.) There are likewise no data on clearance or excretion in early lifestages in

comparison with adult animals or humans, so there is uncertainty in the extent to which such differences may exist.

Studies evaluating the impact of breastfeeding on other PFAS have shown that it is a significant route of exposure for the infant. For example, Koponen et al. (2018) showed a significant increase in the serum concentration of PFOS, PFOA, PFNA, and PFHxS in children at 1 year of age with months of breastfeeding. A linear regression of the data estimated an approximately threefold increase in PFNA and eightfold increase in PFHxS concentration from 12 months of breastfeeding versus children who were not breastfed. A significant decline was then observed in serum concentrations in children at 6 and 10.5 years of age compared with 1 year (Koponen et al., 2018). Although the median ratio of PFDA concentration in breast milk to maternal serum was only 0.03 (3%) (Liu et al., 2011), breastfeeding was found to reduce PFDA serum concentrations of women who had breastfed by an average of 1.3% per month of breastfeeding (Kim et al., 2020b). For comparison, the estimated average human half-life of 4.7 years corresponds to a decline of about 1.2% per month. This rate of lactational transfer is going from an adult woman who has accumulated PFDA over her lifetime to a child who is 5%–10% of her body mass and thus represents a significant exposure to the child. However, the exact extent of this transfer and the resulting time-course of PFDA in the child is unknown. The range of PFDA concentrations in breast milk found by Liu et al. (2011) was <0.001–0.070 ng/mL, over 70-fold, while the range in maternal serum was 0.052–1.271 ng/mL, about 24-fold. Two other sources of variability in the lactational transfer of PFDA to children is the source and exclusivity of breastfeeding in the child's nutrition. Not only do these results indicate wide variability in the amount of PFDA in breast milk, but in the transfer rate and efficiency from the mother by that route.

Because only one endpoint is extrapolated from developmental exposures of PFDA in animals (specifically, mice) to humans and that was for decreased fetal body weight (Harris and Birnbaum, 1989), lactational transfer is not an issue for animal-human extrapolation. However, the data of Koponen et al. (2018) indicate that elevated levels of long-half-life PFAS persist in children until age 6, when median levels of PFHxS and PFNA were still higher than those in 1-year-old children who had not been breastfeed. Hence, the bolus of exposure from breastfeeding could result in serum concentrations above the steady-state level in a 5-year-old child, which means that calculation of a POD_{HED} using CL_H and the observed serum concentration for the analysis of immune effects by Grandjean et al. (2012) could overestimate the exposure that led to those observations. Given the data of Koponen et al. (2018), such an overestimate would be largest for a child who was breastfed for a full 12 months and serum concentrations at age 6 are only two to three times higher than observed at age 10.5, when they are likely to be at or below steady state. Thus, the extent of this overestimate for serum concentration data in 5-year-olds should be no more than a factor of 3.

The PFDA serum concentrations reported by NHANES (see Figure 3-2) show concentrations increasing significantly between age 12 and early to late 20s, which does not indicate that clearance is significantly lower in children than in adults. If clearance was lower in children, the opposite

trend would be expected. Likewise, the results of Yao et al. (2023) indicate that urinary CL of PFDA in infants is similar to adults. The decline in serum concentrations from age 1 to 10.5 for multiple PFAS observed by (Koponen et al., 2018) is consistent with the ending of lactational exposure and growth of the children, which is expected to dilute the body burden present at age 1, with clearance being constant. Hence, the available data do not indicate significant lifestage variation in clearance and the uncertainty from extrapolation of the CL_H estimated for adults across lifestages is judged unlikely to be greater than is accounted for by application of the standard human interindividual uncertainty factor (UF_H), of which a factor of 3 is typically attributed to pharmacokinetic uncertainty.

While the PK parameter estimates seek to make the best use of the available chemical- and species-specific data, there are also many uncertainties noted above, in particular for humans. Therefore, we also evaluated the use of default $BW^{3/4}$ scaling of total clearance (CL × BW), i.e., if $CL_H = CL_{rat} \times (BW_{rat}/BW_H)^{0.25}$. The resulting clearance values for men and women (scaled from male and female rats, respectively) are approximately six times higher than the value estimated from human data (see Section 3.1.5). Hence, estimates of human equivalent doses using $BW^{3/4}$ scaling of clearance would be significantly less health protective than using the estimated CL_H. Hence, although the available human PK data are limited, and other uncertainties discussed above must be acknowledged, the clearance values obtained from chemical-specific data are preferred and used in the derivations below because they are based on direct observation of human excretion. On the other hand, estimated internal doses for 28-day rat bioassays are considered to have a minimal uncertainty of less than 20%. The use of the PK model for mice has greater uncertainty but is only applied to a single endpoint, which is not meaningful for the final RfD. Hence, that approach for the mouse endpoint is considered acceptable and still preferable to $BW^{3/4}$ scaling.

Evidence Synthesis for Pharmacokinetic Extrapolation

For extrapolation of short-term animal bioassays for which noncancer toxic effects have been observed, EPA considers the best measure of internal dose to be the average serum concentration of PFDA over the study duration (28 days) for effects in adult rats or over the entirety of gestation for effects on birth weight observed in mice. The use of average concentration presumes that the induced effect is the result of exposure throughout the study period for birth weight throughout all of gestation and essentially assumes that if the animal had been exposed in such a way that they had a constant serum level equal to that average over the period evaluated, the effect would be the same.

For birth weight effects, if a constant internal concentration was maintained for half of gestation, with no exposure for the other half, the effect on pup birth weight is presumed to be less than if the same constant internal concentration was maintained for all of gestation. Although the animals were only dosed for a portion of the time, calculating the average gestational dose over all of gestation accounts for this expectation by reducing the average on the basis of the number of days with no exposure. The internal dose calculated for the animal is appropriately lower

because the human equivalent dose is calculated with the assumption that human exposure occurs for all of gestation, not a fraction of it.

EPA's hierarchy of approaches for animal-human extrapolation is to first choose a PBPK model if a sufficiently sound and reliable one is available. The second-best option is to make use of chemical-specific PK data, perhaps as incorporated into a classical PK model, to estimate the human exposure that would yield the same internal dose as occurs in the animal model when exposed at a toxicological point-of-departure (POD) dose.

EPA's evaluation of existing PBPK models for PFDA and other PFAS, as well as for PFDA PK data for rats for which the blood AUC/dose is lower after i.v. dosing than after oral dosing, indicates that fundamental aspects or mechanisms of PFDA distribution and excretion are not completely understood. Absent a sound understanding of the mechanisms and corresponding quantitative data to inform a PBPK model that incorporates those mechanisms, EPA concludes that existing PBPK models for PFDA and other PFAS are not adequate for application in dosimetric extrapolation and interpretation of the corresponding toxicological data. EPA also notes that the published PFDA PBPK models have not been parameterized for gestation, lactation, or the development of young offspring in rats, mice, or humans.

EPA conducted a hierarchical Bayesian analysis of the available rat PK data and the results, described in Appendix G.1., appeared successful in matching the observed kinetics and the range of variability in the data. However, when the posterior parameter distribution was then sampled and used to predict the PFDA concentrations in rats at the end of a 28-day study, the median results systematically underpredicted the mean observed concentrations (see Appendix G.2.1). While the extent of the discrepancy between the model predictions and the observed concentration for each dose was in a range that would generally be considered acceptable, the consistent bias to underprediction indicates that the discrepancy is not just due to random variation. That the actual accumulation in rat serum was greater than predicted suggests that either clearance or distribution to various tissues decreased with multiple dosing versus the single doses used for PK data. Such a change is not consistent with the generally recognized PK mechanisms for PFAS. For example, saturation of renal resorption would lead to lower accumulation after multiple doses than predicted based on single-dose PK, not the opposite. Hence, EPA does not have a specific hypothesis for the source of the discrepancy.

While EPA's classical PK model systematically underpredicted the observed concentrations in rats at the end of the NTP 28-day bioassay, EPA considers the qualitative model behavior (i.e., the shape of the predicted time-course over the 28 days of exposure) to be a reasonably accurate prediction. More specifically, model predictions in Figure G-8 show an essentially linear increase in serum PFDA over the course of the study, which is consistent with the mean estimated half-lives of 83 and 61 days in male and female rats, respectively (see Table 3-3). If the actual time-course is similar to the predicted time-course, then the average serum concentration over the course of a 28-day bioassay, which EPA considers to be a reasonable measure of internal dose for use in animal-

human extrapolation, will simply be one-half the final concentration. Hence, EPA concluded that assuming this relationship while using the measured end-of-study PFDA concentrations would provide a more accurate prediction of the average concentration than calculating the average using the PK model. For rat PODs that are between the doses used in the NTP bioassay (i.e., obtained from BMD modeling), the presumed end-of-study serum concentration was then obtained by linear interpolation between the mean observed concentrations. Given the fairly linear relationship between exposure and the end-of-study concentrations (data in Figure G-9), such interpolation is expected to be quite accurate.

For the mouse developmental endpoint (body weight at birth), end-of-study PFDA concentrations were not measured. Therefore, application of EPA's PK model using the reported PK parameters for female mice to estimate the average serum concentration in the mouse dams is considered the best option. Given the estimated half-life of 63 days in female mice and that dosing was only started after mating, PFDA is expected to accumulate in the pregnant dams over the entire course of gestational dosing. The PK model accounts for this accumulation and median predictions for rats in the NTP 28-day bioassay were within a factor of 2 of the observed mean concentrations, so a similar degree of accuracy is expected from application of the PK model for mice.

3.2. NONCANCER HEALTH EFFECTS

For each potential health effect discussed below, the synthesis describes the evidence base of available studies meeting the PECO criteria, as well as the supplemental studies that most directly inform questions relating to coherence, MOA, biological plausibility, or human relevance during evidence integration.

For this section, evidence to inform organ/system-specific effects of PFDA in animals following developmental exposure are discussed in the individual organ/system-specific sections (e.g., liver effects in adult animals after gestational exposure are discussed in the liver effects section). Given that spontaneous abortion and preterm birth are informative of both female reproductive and developmental toxicity, these endpoints are also discussed in the sections for developmental (see Section 3.2.3) and female reproductive effects (see Section 3.2.5). General toxicity effects, including body weights and survival, were summarized to aid in interpretation of other potential health effects considering the association between PFDA exposure and induction of wasting syndrome (rapid and marked reductions in body weight and food consumption) in animals (refer to Section 3.2.10 for more details).

3.2.1. Hepatic Effects

Methodological Considerations

Serum enzymes and other clinical markers of hepatocellular and biliary function were evaluated across human and animal studies. For the animal studies, the results were interpreted together with histopathology and liver weight measures to aid in the assessment of potential

adverse liver effects in response to PFDA exposure. Elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indicators of hepatocellular damage that result in the release of these enzymes into the blood, with ALT considered more specific and sensitive (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Alkaline phosphatase (ALP) is localized to the bile canalicular membrane and is, therefore more indicative of hepatobiliary damage. Increases in circulating ALP, γ-glutamyltransferase ([GGT]; another canalicular enzyme) and bile components (bilirubin and bile salts/acids) are associated with obstruction of hepatic bile flow and damage to biliary epithelial cells (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Blood proteins such as albumin, globulin, and total protein (amount of albumin and globulin) are routinely evaluated in clinical chemistry. Albumin is synthesized in the liver and then excreted into the bloodstream, where it is bound by fatty acids, cations, bilirubin, thyroxine (T4), and other compounds. Globulins, a collection of blood proteins larger than albumin, is made by both the liver and immune system. Decreased levels of these blood proteins can be good indicators of protein loss due to kidney disease or impaired synthesis as a result of liver damage (Whalan, 2015).

Human Studies

Serum biomarkers

Eleven epidemiological studies (12 publications) reported on the relationship between PFDA exposure and liver serum biomarkers. As discussed above, ALT and AST are considered reliable markers of hepatocellular function/injury, whereas levels of ALP (Boone et al., 2005), bilirubin, and γ-GGT are routinely used to evaluate hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). In addition, one study examined clinical disease, specifically nonalcoholic fatty liver disease.

Of the studies of liver enzymes, nine were *medium* confidence, including five studies in general population adults (five cross-sectional and one cohort), one pregnancy cohort, and one cohort (analyzed cross-sectionally) of children, and one cross-sectional study examined all ages (3–79 years) (see Figure 3-4). One study, a cross-sectional study of a population in proximity to a fluoropolymer plant was *low* confidence because of limited adjustment for confounding (Yao et al., 2020) while another study, a cross-sectional study of pregnant women, also was considered uninformative because of lack of consideration of potential confounding (Jiang et al., 2014). All studies measured liver enzymes using standard laboratory approaches.

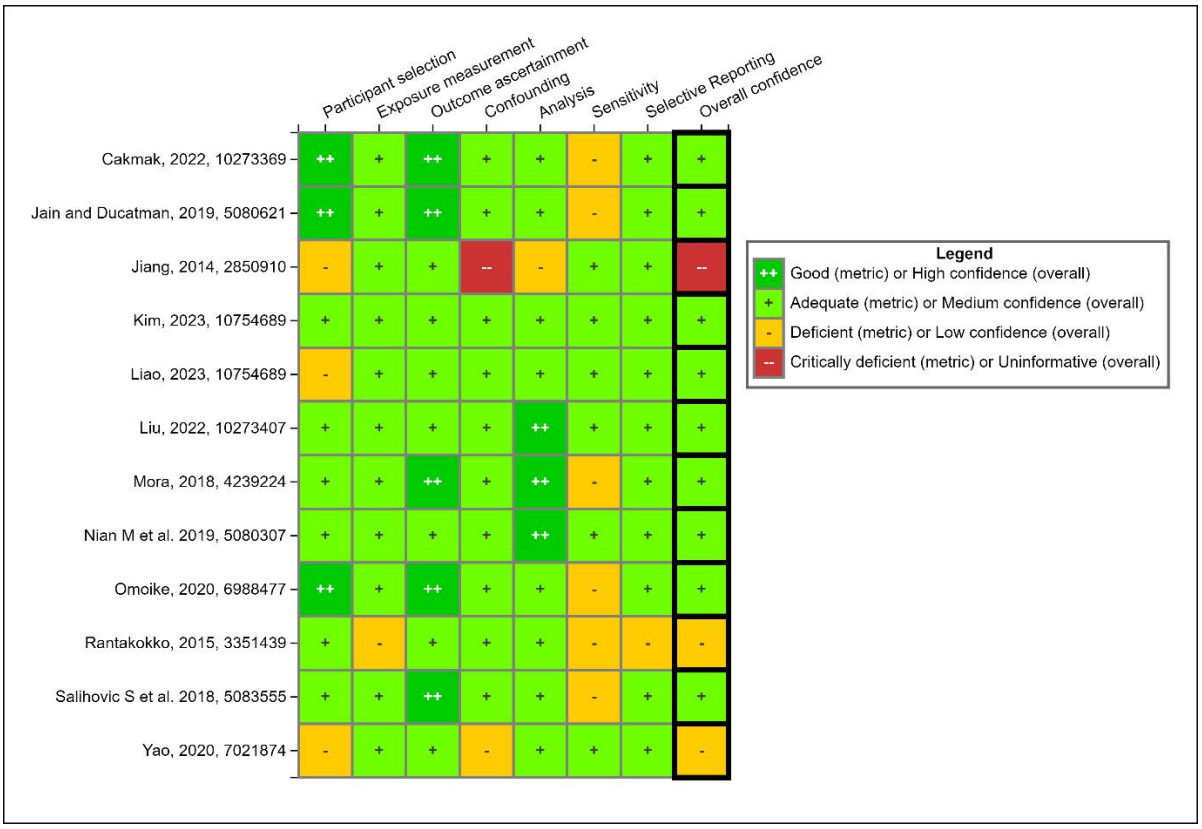


Figure 3-4. Evaluation results for epidemiological studies assessing effects of PFDA exposure on liver serum biomarkers. Refer to HAWC Human Hepatic Effects for details on the study evaluation review.

The results for the nine *medium* confidence studies (10 publications) are summarized in Table 3-4. There are generally consistent positive associations with ALT in adults, with six of seven studies reporting higher ALT with higher exposure (four were statistically significant). The associations for other liver markers such as AST, ALP, GGT, and total bilirubin were also mostly in the positive direction, but there was some inconsistency, with most of the studies reporting an inverse association with one of these markers (specific marker differed by study). While most of the studies were cross-sectional, Salihovic et al. (2018) examined changes in liver function with changes in PFDA exposure over 10 years in elderly adults. They observed positive associations with ALT, ALP, and GGT, but an inverse association with total bilirubin. In the only available study in children, the only marker examined (ALT) was found to be lower with higher exposure in girls, and no change was observed in boys (Mora et al., 2018).

It is possible that the observed associations (primarily in adults) could be due to confounding by co-occurring PFAS. In the studies that reported correlations across PFAS, the correlations between PFDA and PFNA, PFOA, and PFOS were moderate to high. Correlations with PFNA were highest, ranging from 0.44 to 0.89, while PFOA and PFOS were generally less than 0.6. Most of the studies did not perform multipollutant modeling, but five studies did present mixture

results using various methods. In each study, the analyses were not designed to identify the association for PFDA with and without adjustment for other PFAS, but rather to examine the effect of a mixture of PFAS. However, weights for each PFAS in the mixture provide an indication of which PFAS(s) were most influential on the association with liver enzymes. In one study, PFNA and PFOA had the greatest contribution/weight in the mixture (Borghese et al., 2022), while PFNA was the strongest driver for ALT and GGT in Kim et al. (2023). In Liu et al. (2022), PFOS was the dominant component to the combined effect, while in (Liao et al., 2023), PFBS and PFHxS were the top contributors, depending on the specific liver enzyme. These results indicate potential for the PFDA results to be confounded by other PFAS, particularly given the high correlation between PFNA and PFDA exposure. However, these analyses are not evidence that PFDA does not have an effect on liver enzymes, as the weights indicate only that in these models PFDA does not contribute much beyond what is contributed by other chemicals in the model.

The one available study of nonalcoholic fatty liver disease (NAFLD) was evaluated as *low* confidence because of concerns that exposure measured concurrent with this chronic outcome does not represent an etiologically relevant period. Rantakokko et al. (2015) used histological findings from biopsies obtained during elective gastric bypass operation and reported an inverse association with PFNA exposure (OR 0.05, 95% CI <0.01, 0.83 for 2–4 foci vs. none per 200× field).

Overall, there is reasonably consistent evidence of a positive association between exposure to PFDA and ALT in adults, including positive associations in six of seven available studies (four statistically significant) and an exposure-response gradient observed in one of the two studies that examined quartiles of exposure. Evidence for other biomarkers of hepatic function, particularly ALP and total bilirubin, is less consistent but still generally coherent with the results for ALT. Nevertheless, the potential for confounding of the association by other PFAS reduces certainty in the evidence. Further, there is some uncertainty as to the biological significance of the observed changes because of the small magnitude of effect in most studies, particularly given that the two available studies of more functional hepatic endpoints (e.g., histopathology) are *low* confidence and the results are inconsistent across studies. However, abnormally elevated serum ALT indicates impaired liver functioning and even small increases can be predictive of liver disease (U.S. EPA, 2022c; Valenti, 2021; Park et al., 2019).

Table 3-4. Associations between PFDA and serum biomarkers of hepatic function in *medium* confidence epidemiological studies

Reference	Population	Median exposure (IQR) or as specified in ng/mL	Effect estimate	Markers of hepatocellular injury		Markers of hepatobiliary injury		
				ALT	AST	ALP	GGT	Total bilirubin
Adults								
Cakmak et al. (2022) Borghese et al. (2022)	Cross-sectional (2007–2017); Canada; 6,109 adults and children	Plasma 0.2	% change (95% CI) per 1 GM increase	3.0 (−0.1, 6.2)	2.2 (−0.6, 5.0)	1.0 (−3.3, 5.6)	**15.5 (2.2, 30.4)***	1.6 (−7.8, 11.9)
	1,404 adults		% change (95% CI) for doubling	NR	1.7 (−1.1, 4.4)	−0.8 (−3.1, 1.5)	3.3 (−4.8, 11.9)	NR
Jain and Ducatman (2019b)	Cross-sectional (NHANES 2011–2014); U.S.; 2,883 adults	Serum 0.2	β (*p*-value) per log-unit change	Nonobese 0.003 (0.9) Obese 0.01 (0.5)	Nonobese −0.009 (0.6) Obese −0.01 (0.5)	Nonobese **−0.03 (0.01)*** Obese −0.006 (0.7)	Nonobese −0.003 (0.9) Obese 0.003 (0.9)	Nonobese **0.05 (0.02)*** Obese 0.02 (0.3)
Omoike et al. (2020)	Cross-sectional (NHANES 2005–2012); U.S. 6,652 adults	Serum 0.3 (20th–80th: 0.1–0.5)	% change (95% CI) per 1% increase	NR	NR	NR	NR	0.01 (−0.0, 0.03)

Reference	Population	Median exposure (IQR) or as specified in ng/mL	Effect estimate	Markers of hepatocellular injury		Markers of hepatobiliary injury		
				ALT	AST	ALP	GGT	Total bilirubin
Salihovic et al. (2018)	Cohort (2001–2014); Sweden; 1,002 elderly adults	Plasma at baseline (70 yr) 0.3 (0.2–0.4)	β (95% CI) for changes in liver function with change in ln-PFDA over 10 yr (mixed random effects)	**0.02 (0.01, 0.04)***	NR	**0.2 (0.1, 0.2) ***	0.06 (0.0,0.1)	**−2.3 (−2.7, −1.9)***
Liao et al. (2023)	Cross-sectional analysis within cohort (2015–2019); Canada, 420 pregnant women	0.5 (0.3–0.8)	β (*p*-value) for tertiles vs. T1	1.89 (−1.32, 5.09)	0.83 (−1.51, 3.18)	NR	**2.08 (0.35, 3.82)***	0.12 (−1.27, 1.52)
Liu et al. (2022)	Cross-sectional (2018–2019); China; 1,303 adults	Serum 0.9 (0.5–1.4)	% difference (95% CI) for quartiles vs. Q1	**Q2: 3.46 (1.84, 5.09)* Q3: 8.03 (4.33, 11.86)* Q4: 15.51 (6.44, 25.35)***	**Q2: 1.16 (0.13, 2.21)* Q3: 4.63 (2.27, 7.05)* Q4: 11.75 (5.91, 17.91)***	Q2: −0.75 (−1.63, 0.13) **Q3: −2.09 (−4.00, −0.14)*** Q4: −4.40 (−8.72, 0.13)	**Q2: 3.31 (1.41, 5.24)* Q3: 8.97 (4.57, 13.55)* Q4: 19.37 (8.37, 31.48)***	**Q2: 2.67 (1.50, 3.86)* Q3: 6.23 (3.56, 8.98)* Q4: 12.03 (5.53, 18.94)***
Nian et al. (2019)	Cross-sectional (2015–2016); China; 1,605 adults	Serum 0.9 (0.5–1.5)	% change (95% CI) per ln-unit change	**3.1 (0.1, 6.1)***	1.0 (−0.9, 3.0)	**−3.8 (−5.4, −2.2)***	2.2 (−0.9, 5.3)	**4.3 (2.1, 6.6) ***

Reference	Population	Median exposure (IQR) or as specified in ng/mL	Effect estimate	Markers of hepatocellular injury		Markers of hepatobiliary injury		
				ALT	AST	ALP	GGT	Total bilirubin
Kim et al. (2023)	Cross-sectional (2015–2017), Korea; 1,404 adults	Serum 1.0 (0.7–1.5)	% change (95% CI) for doubling	**3.4 (0.1, 6.8)***	**2.6 (0.4, 4.8)***	NR	4.6 (−0.1, 9.6)	NR
Children								
Mora et al. (2018)	Cross-sectional analysis of cohort (1999–2002), U.S.; 682 children (7–8 yr)	Plasma 0.3 (0.2–0.5)	β (95% CI) with IQR increase	−0.3 (−1.2, 0.5) Boys: 0.1 (−1.3, 1.4) Girls: −0.9 (−1.8, −0.1)*	NR	NR	NR	NR

*$p < 0.05$.

IQR: interquartile range (i.e., 25th–75th percentile); NR = not reported.

Animal Studies

The toxicity database for PFDA liver effects in experimental animals consists of two 28-day gavage studies (Frawley et al., 2018; NTP, 2018), five short-term studies (≤14 days) via the diet (Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991), one short-term study via drinking water (Wang et al., 2020) and one developmental study via gavage (Harris and Birnbaum, 1989). The studies included several strains of rats (SD, Wistar and Fischer 344) and mice (C57BL/6N, B6C3F1/N and CD-1[ICR]) and measured endpoints considered informative for evaluation of liver toxicity, such as histopathology, serum biomarkers of effects, and organ weights.

Histopathology

Liver histopathology was examined across five short-term oral exposure studies: two *high* confidence studies in rats exposed via gavage (Frawley et al., 2018; NTP, 2018), a *low* confidence study in mice exposed via the diet (Kawashima et al., 1995), and two *low* confidence studies in mice exposed via drinking water (Li et al., 2022; Wang et al., 2020). The primary issue contributing to the *low* confidence rating for the Kawashima et al. (1995), Li et al. (2022), Wang et al. (2020) studies was the incomplete reporting of histopathology data (no information on incidence or severity) (see Figure 3-5). Additional deficiencies were identified in the study evaluation domains for allocation (nonreporting of randomization), nonreporting of blinding practices, and chemical administration and characterization in Kawashima et al. (1995).

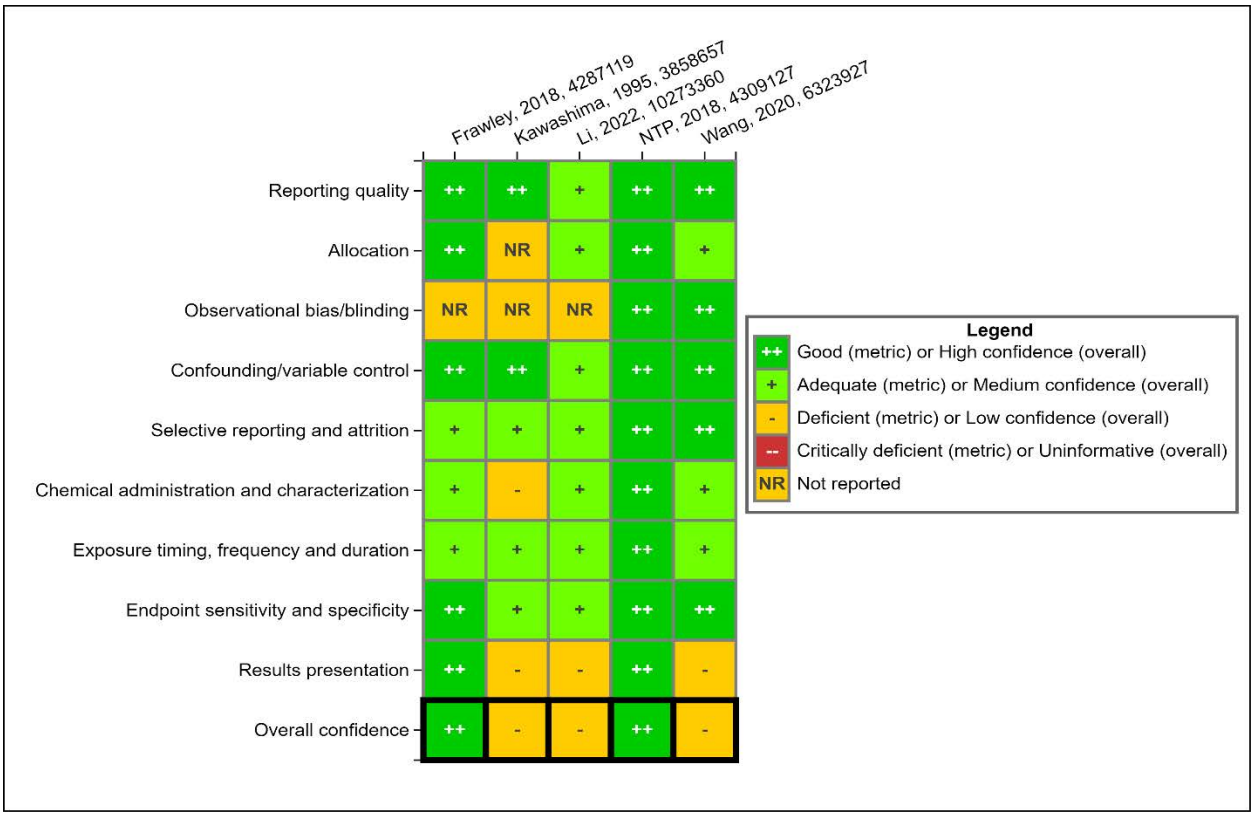


Figure 3-5. Evaluation results for animal studies assessing effects of PFDA exposure on liver histopathology. Refer to HAWC for details on the study evaluation review.

Hepatocyte lesions were identified in male and female SD rats at exposure doses of 0.5–2.5 mg/kg-day across the two *high* confidence studies with 28-day exposure and these lesions were not present in control animals (Frawley et al., 2018; NTP, 2018) (see Table 3-5 and Figure 3-6). Cytoplasmic alterations that consisted of accumulation of eosinophilic granules within the cytoplasm of centrilobular hepatocytes were observed in nearly all rats at doses of 0.625–2.5 mg/kg-day in the study by NTP (2018). Cytoplasmic vacuolation that was largely centrilobular in distribution and characterized by accumulation of microvacuoles within the cytoplasm was also reported in males and females at 1.25 and 2.5 mg/kg-day (10%–100% incidence rate) (NTP, 2018). Hepatocyte hypertrophy (i.e., increase in the size of primarily centrilobular hepatocytes) was significantly elevated in these animals at similar doses (80%–100% incidence) (NTP, 2018). The severity of these lesions increased with dose, ranging from minimal to marked in males and minimal to moderate in females. Minimal hepatocyte necrosis was increased in rats across studies and sexes (Frawley et al., 2018; NTP, 2018) with incidence rates ranging from 10% to 40%; a statistically significant trend was reported in females at doses ≥1.25 mg/kg-day in one study (NTP, 2018). Frawley et al. (2018) characterized the changes as centrilobular, single cell hepatocyte necrosis occurring in female rats (males were not tested in the study). Hepatocyte necrosis in male and female rats was described in the (NTP, 2018) report as "a few widely scattered, variably sized,

randomly distributed foci of necrotic hepatocytes within the hepatic parenchyma mixed with variable numbers of mononuclear inflammatory cells."

PFDA treatment had no appreciable effect on cellular infiltration in the liver in either male or female rats up to a dose of 2.5 mg/kg-day after 28-day exposure (NTP, 2018) (see Figure 3-6). The *low* confidence studies also observed hepatocyte changes in animals at higher PFDA doses (4.6–25 mg/kg-day); however, data were only summarized qualitatively and, therefore, are not displayed in Figure 3-6 or Table 3-5 (Li et al., 2022; Wang et al., 2020; Kawashima et al., 1995). Kawashima et al. (1995) described increases in lipid droplets, cell size (hypertrophy), peroxisome proliferation and vacuolated nuclei in male Wistar rats in the two high-dose groups after 7-day exposure via the diet (4.6 and 9.22 mg/kg-day). Similarly, increased hypertrophy and lipid accumulation was reported in the liver of female C57BL/6J mice after exposure to 25 mg/kg-day for 14 days via drinking water. Additionally, liver necrosis, steatosis, edema, and degeneration were found in male CD-1 mice exposed to a PFDA dose of 13 mg/kg-day via drinking water for 12 days (Wang et al., 2020). Although there is no information on incidence or severity, the findings from the Kawashima et al. (1995), Li et al. (2022) and Wang et al. (2020) studies are coherent with observations from the *high* confidence 28-day studies (e.g., vacuolation, hypertrophy and necrosis).

Altogether, PFDA induced a spectrum of morphological changes in rodent hepatocytes that included cytoplasmic alterations and vacuolization, hypertrophy, and some evidence of structural degenerative lesions (minimal necrosis accompanied in some cases by evidence of possible inflammation) after short-term exposure. Furthermore, a general pattern of increased severity (within and across lesions) was apparent with increasing dose.

Table 3-5. Incidence and severity of hepatocyte lesions in Sprague-Dawley rats exposed to PFDA in 28-day gavage studies

Animal group	Dose (mg/kg-d)								
	0	**0.125**	**0.156**	**0.25**	**0.312**	**0.5**	**0.625**	**1.25**	**2.5**
Cytoplasmic alterations									
NTP (2018) – Female (n = 10 in all groups)	0		0		0		**8 (minimal)**	**10 (minimal)**	**10 (mild)**
NTP (2018) – Male (n = 10 in all groups)	0		0		0		**10 (minimal)**	**10 (marked)**	**10 (marked)**
Cytoplasmic vacuolization									
NTP (2018) – Female (n = 10 in all groups)	0		0		0		0	1 (minimal)	**10 (moderate)**
NTP (2018) – Male (n = 10 in all groups)	0		0		0		0	**9 (mild)**	**10 (moderate)**
Hypertrophy									
NTP (2018) – Female (n = 10 in all groups)	0		0		0		0	**8 (minimal)**	**10 (moderate)**
NTP (2018) – Male (n = 10 in all groups)	0		0		0		2 (mild)	**10 (moderate)**	**10 (moderate)**
Necrosis									
Frawley et al. (2018) – Female (n = 8 in all groups)	0	0		0		3 (minimal)			
NTP (2018) – Female (n = 10 in all groups)	0		0		0		0	1 (minimal)	**4 (minimal)**
NTP (2018) – Male (n = 10 in all groups)	0		1 (minimal)		0 (minimal)		1 (minimal)	3 (minimal)	1 (minimal)

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies. For example, the dose of 0.125 mg/kg-day was not used in the (NTP, 2018) study. Severity was normalized to a four-point scale by the study authors as follows: minimal, mild, moderate, and marked.

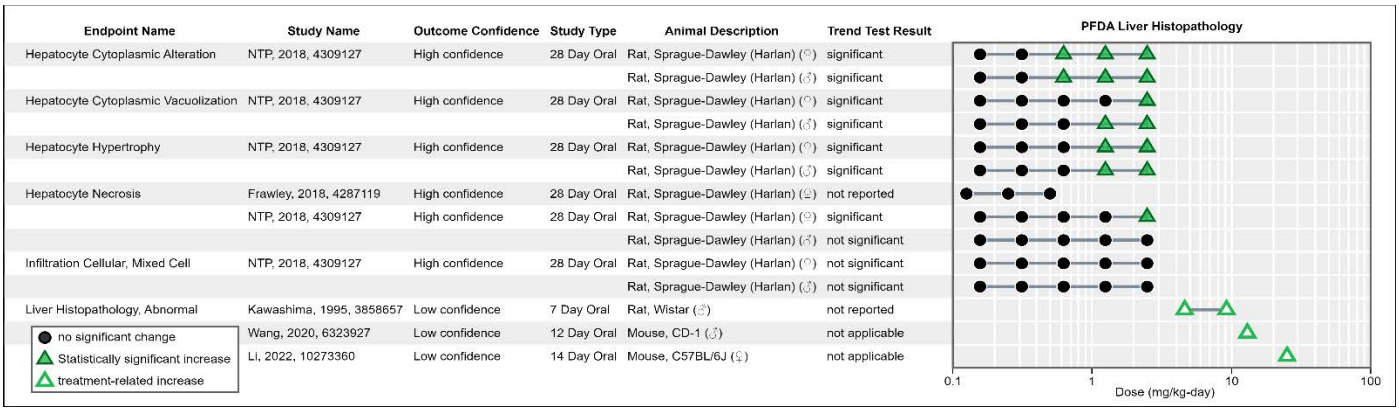


Figure 3-6. Effects on liver histopathology following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Serum biomarkers

Effects on serum biomarkers of liver function, including serum enzymes (ALT, AST, ASP), biliary components (bile salts and bilirubin) and blood proteins (albumin, globulin, and total protein) were evaluated in rodents across two short-term oral exposure studies (Wang et al., 2020; NTP, 2018). The studies were considered *high* confidence with no notable concerns with respect to risk of bias or sensitivity. Outcome-specific study evaluations are displayed in Figure 3-7.

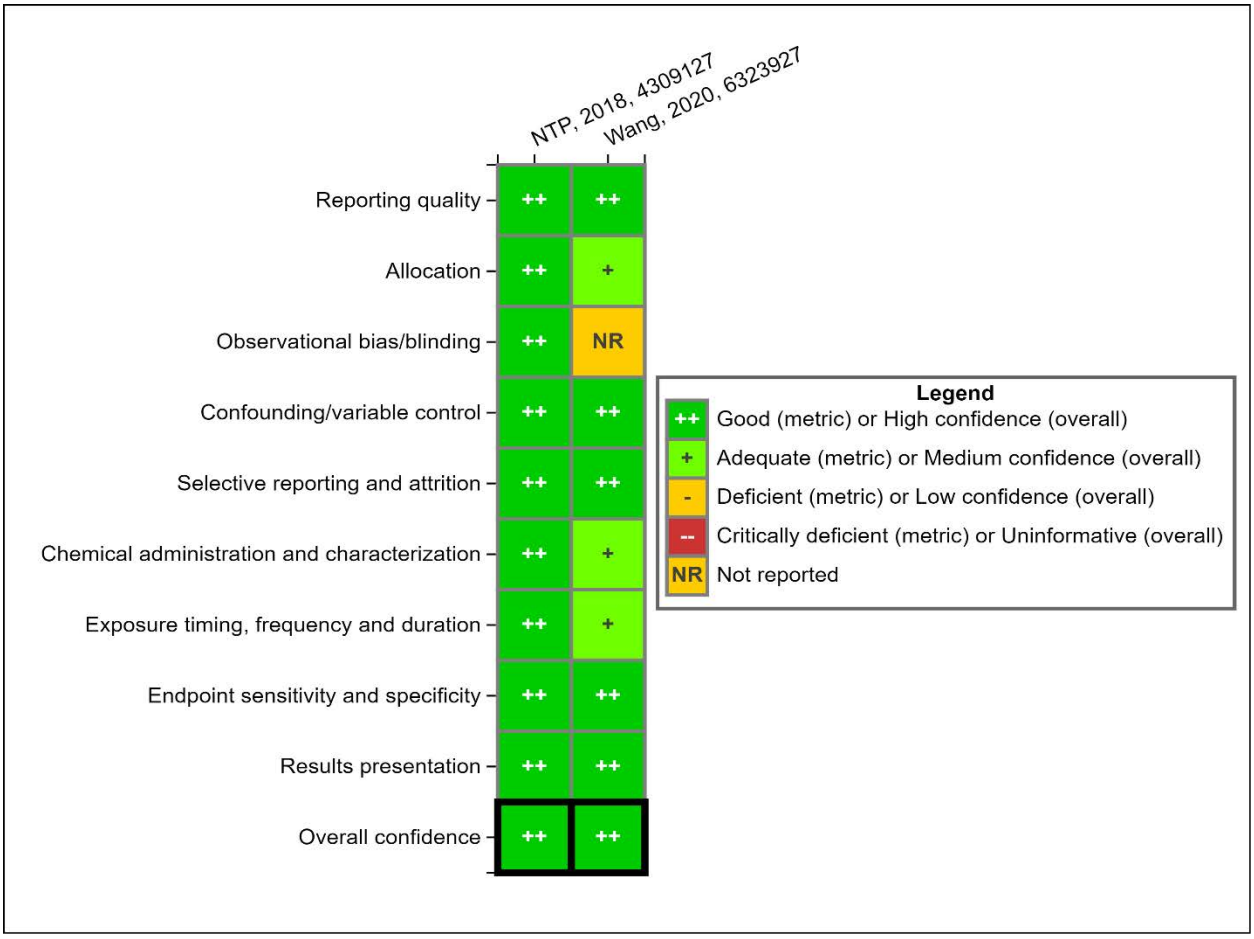


Figure 3-7. Evaluation results for animal studies assessing effects of PFDA exposure on liver serum biomarkers. Refer to HAWC for details on the study evaluation review.

Increases in ALT and AST, two markers of hepatocellular damage, were consistently reported in SD rats with 28-day gavage exposures and CD-1 mice exposed for 12 days via drinking water (Wang et al., 2020; NTP, 2018) (see Table 3-6 and Figure 3-8). Increased ALT was reported in male and female rats, although only effects in females showed a significant trend with 44% and 20% changes from controls occurring at 1.25 and 2.5 mg/kg-day, respectively. AST levels increased in a dose-dependent manner in both sexes, reaching statistical significance at all exposure doses in males (13%–42% compared with controls across 0.156–2.5 mg/kg-day) and at 1.25 and 2.5 mg/kg-day in females (31% and 80% compared with controls, respectively). In mice exposed to a higher PFDA dose (13 mg/kg-day), these enzymes were similarly elevated, increasing by 338% and 649% relative to controls for ALT and AST, respectively (Wang et al., 2020).

Table 3-6. Percent change relative to controls in hepatocellular serum markers in short-term animal studies after PFDA exposure

Animal group	Dose (mg/kg-d)					
	0.156	0.312	0.625	1.25	2.5	13
Alanine aminotransferase (ALT)						
28-d gavage; female SD rats (NTP, 2018)	−3	3	13	**44**	**20**	
28-d gavage; male SD rats (NTP, 2018)	21	**45**	**46**	28	7	
12-d drinking water; male CD-1 mice (Wang et al., 2020)						**338**
Aspartate aminotransferase (AST)						
28-d gavage; female SD rats (NTP, 2018)	−3	−8	1	**31**	**80**	
28-d gavage; male SD rats (NTP, 2018)	**13**	**18**	**25**	**34**	**42**	
12-d drinking water; male CD-1 mice (Wang et al., 2020)						**649**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors. Shaded cells represent doses not included in the individual studies.

Markers of hepatobiliary function including ALP, bile salts/acids and bilirubin (total, direct and indirect) were also altered in SD rats after a 28-day exposure (NTP, 2018) (see Table 3-7 and Figure 3-8). ALP levels increased significantly at doses ≥0.312 mg/kg-day in both males and females (22%–106% compared with controls). Levels of bile salts/acids and bilirubin (total, direct and indirect) were elevated in male and female rats at doses ≥1.25 mg/kg-day; the effects showed a significant trend and were large in magnitude (205%–1,207% and 28%–733% compared with controls for bile salts/acids and bilirubin, respectively).

Table 3-7. Percent change relative to controls in hepatobiliary serum markers in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Alkaline phosphatase (ALP)					
Female SD rats	14	**34**	**35**	**106**	**92**
Male SD rats	9	**22**	**41**	**90**	**41**
Bile salts/acids					
Female SD rats	−6	55	34	**205**	**658**

Animal group	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Male SD rats	−53	−39	37	**440**	**1207**
Total bilirubin					
Female SD rats	−6	−9	−10	**28**	**356**
Male SD rats	4	5	13	**46**	**350**
Direct bilirubin					
Female SD rats	0	4	4	**104**	**700**
Male SD rats	−22	−4	−7	**78**	**733**
Indirect bilirubin					
Female SD rats	−7	−11	−14	**10**	**275**
Male SD rats	11	7	19	**37**	**255**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

Albumin, globulin, and total protein were examined in SD rats after 28-day exposure (NTP, 2018) (see Table 3-8 and Figure 3-8). Dose-related decreases in albumin were reported in males, decreasing by 8% and 20% at 1.25 and 2.5 mg/kg-day, respectively. In females, statistically significant increases in albumin levels were reported at 0.312 (11%) and 0.625 (13%) mg/kg-day but there was no dose-response gradient. A significant trend for globulin levels was found in both males and females, with decreases of 9%–42% at ≥0.156 mg/kg-day. The albumin and globulin findings corresponded well with a decrease in total protein and increase in albumin/globulin (A/G) ratio in animals. Statistically significant increases in the A/G ratio (13%–47%) occurred in males and females at all exposure doses (0.156–2.5 mg/kg-day) and total protein decreased significantly (4%–28%) in males at similar doses. In females, total protein decreased by 2% and 12% at the highest doses (1.25 and 2.5 mg/kg-day, respectively), but a significant trend was not established.

Table 3-8. Percent change relative to controls in serum proteins in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Albumin					
Female SD rats	7	**11**	**13**	7	−10
Male SD rats	1	3	0	**−8**	**−21**
Globulin					
Female SD rats	−9	**−18**	**−18**	**−21**	**−14**
Male SD rats	**−13**	**−19**	**−27**	**−36**	**−42**
Albumin/globulin ratio					
Female SD rats	**17**	**36**	**36**	**36**	**13**
Male SD rats	**15**	**27**	**40**	**47**	**36**
Total protein					
Female SD rats	3	3	3	−2	−12
Male SD rats	**−4**	**−5**	**−10**	**−17**	**−28**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

In summary, coherent effects across serum enzymes, biliary system components, and blood proteins that are consistent with altered liver function were reported in rats and mice after short-term PFDA exposure. In mice, the serum enzyme changes were accompanied by a 40% reduction in body weights at the high-PFDA dose tested (13 mg/kg-day) (Wang et al., 2020). Although the 28-day rat study reported significant body weight reductions at ≥1.25 mg/kg-day, dose-related changes in some serum biomarkers of hepatic injury occurred at doses lower (0.156–0.625 mg/kg-day) than those associated with marked systemic toxicity (NTP, 2018).

Endpoint Name	Study Name	Outcome Confidence	Experiment Name	Animal Description	Trend Test Result
Alanine Aminotransferase (ALT)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Wang, 2020, 6323927	High confidence	12 Day Oral	Mouse, CD-1 (♂)	not applicable
Aspartate Aminotransferase (AST)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
	Wang, 2020, 6323927	High confidence	12 Day Oral	Mouse, CD-1 (♂)	not applicable
Alkaline Phosphatase (ALP)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Bile Salt/Acids	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Direct Bilirubin	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Indirect Bilirubin	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Total Bilirubin (TBILI)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Albumin (A)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Albumin/Globulin (A/G) Ratio	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Globulin (G)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant
Total Protein (TP)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
				Rat, Sprague-Dawley (Harlan) (♂)	significant

Figure 3-8. Effects on serum liver biomarkers following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Organ weight

The studies evaluating liver weight changes in animals consisted of four *high* confidence studies (see Figure 3-9): two 28-day gavage studies using female B6C3F1/N mice or male and female SD rats (Frawley et al., 2018; NTP, 2018), one 14-day study in female C57BL/6J mice exposure via drinking water (Li et al., 2022), and one developmental study measuring effects in female P_0 C57BL/6N mice exposed via gavage during gestational days (GD) 6–15 or 10–13 (Harris and Birnbaum, 1989). The 28-day rat study by Frawley et al. (2018) included three cohorts exposed to similar experimental conditions. There are also four *medium* confidence studies that administrated PFDA via the diet for 7–14 days in male Wistar rats (Kawashima et al., 1995), male Fischer F344 rats (Takagi et al., 1992, 1991), or male C57BL/6N mice (Permadi et al., 1993).[9] Overall confidence in these shorter duration studies was reduced to medium based primarily on uncertainties surrounding the characterization of the test compound (no analytical verification methods) and administered doses (lack of information on food consumption for estimating dietary exposure doses) (see Figure 3-9). A *low* confidence study with incomplete reporting on liver weight data (no information on sample size) is also available in Wistar rats exposed via the diet for 7 days (Yamamoto and Kawashima, 1997) (see Figure 3-9).

[9]An additional study from the same laboratory Permadi et al. (1992) was identified that reported identical liver weight data; therefore, it was considered an accessory record to the Permadi et al. (1993) study summarized in this assessment.

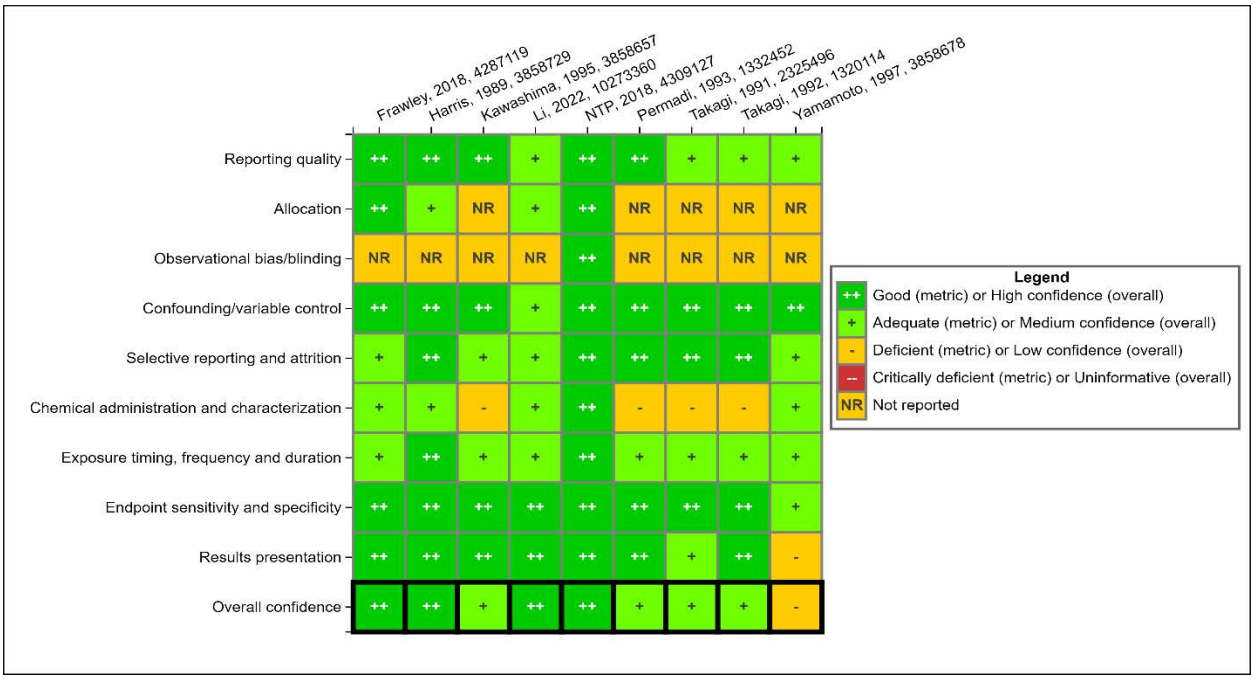


Figure 3-9. Evaluation results for animal studies assessing effects of PFDA exposure on liver weight. Refer to HAWC for details on the study evaluation review.

Increased liver weight was consistently reported across all studies, species, strains, and sexes (see Table 3-9 and Figure 3-10). Relative liver weight is often preferred over absolute liver weight as it accounts for variations in body weight that may mask organ weight changes (Bailey et al., 2004). Statistically significant increases in relative liver weights were reported in rats and mice at ≥0.089 mg/kg-day across the short-term studies, while reductions in terminal body weight occurred in these animals at higher doses (≥1.25 mg/kg-day) (see Section 3.2.10 for more details). In general, the changes in relative liver weights demonstrated a dose and time dependency. For example, dose-related increases in relative liver weights of 17%–56% compared with controls were reported in male Wistar/Fisher rats at doses 1.15–10 mg/kg-day after 7–14 days across three studies with dietary exposure (females were not examined) (Kawashima et al., 1995; Takagi et al., 1992, 1991). In female P_0 C57BL/6N mice exposed during gestion (GD 10–13 and 6–15), relative liver weights increased by 12%–127% compared with controls at doses of 1–16 mg/kg-day (Harris and Birnbaum, 1989). At a longer exposure duration (28 days), similar magnitudes of relative liver weight increases were observed in female B6C3F1/N mice and male/female SD rats but at lower PFDA doses (16%–81% at 0.089–0.71 mg/kg-day and 10%–102% at 0.125–2.5 mg/kg-day, respectively). Further, in the studies that evaluated liver weight and other relevant liver toxicity endpoints, the increases in liver weight corresponded with the reported observations of hepatocellular histopathology (Frawley et al., 2018; NTP, 2018) and alterations in serum biomarkers of hepatocellular/biliary function (NTP, 2018).

Table 3-9. Percent change relative to controls in liver weight (relative to body weight) due to PFDA exposure in short-term oral toxicity studies

Animal group	Dose (mg/kg-d)											
	0.03–0.045	0.089	0.1–0.179	0.25–0.36	0.5–0.71	1.0–1.25	2.0–3	4–4.6	6.4–8	9.22–12.8	16–25	32–37.8
7 d; male Wistar rats (Kawashima et al., 1995)						**17**	**28**	**42**		**27**		
7 d; male Fisher F344 rats (Takagi et al., 1992)										**56**		
14 d; male Fisher F344 rats (Takagi et al., 1991)										**56**		
28 d; male SD rats (NTP, 2018)			**11**	**20**	**28**	**54**	**91**					
28 d; female SD rats (NTP, 2018)			**12**	**20**	**32**	**52**	**102**					
28 d; female SD rats – Histopathology cohort (Frawley et al., 2018)			1	**8**	**16**							
28 d; female SD rats – MPS cohort (Frawley et al., 2018)			**10**	**13**	**23**							
28 d; female SD rats – TDAR to SRBC cohort (Frawley et al., 2018)			2	**19**	**35**							
GD 10–13; pregnant P_0 female C57BL/6N mice (Harris and Birnbaum, 1989)				−4	**3**	**12**	**15**	**45**	**72**		**93**	**106**
GD 6–15; pregnant P_0 female C57BL/6N mice (Harris and Birnbaum, 1989)	0		3	1		**18**	**54**		**106**	**127**		
10 d; male C57BL/6N mice (Permadi et al., 1993)												**100**
14 d; Female C57BL/6 J mice (Li et al., 2022)											**219**	
28 d; female B6C3F1/N mice (Frawley et al., 2018)	4	**16**	**27**	**51**	**81**							

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Endpoint Name	Study Name	Outcome Confidence	Experiment Name	Animal Description	Trend Test Result
Liver Weight, Relative	Kawashima, 1995, 3858657	Medium confidence	7 Day Oral	Rat, Wistar (♂)	not reported
	Takagi, 1992, 1320114	Medium confidence	7 Day Oral	Rat, Fischer F344 (♂)	not applicable
	Takagi, 1991, 2325496	Medium confidence	14 Day Oral	Rat, Fischer F344 (♂)	not applicable
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative (Histopathology Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative (MPS Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative (TDAR to SRBC Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative	Harris, 1989, 3858729	High confidence	Gestational Oral (GD 10-13)	P0 Mouse, C57BL/6n (♀)	significant
			Gestational Oral (GD 6-15)	P0 Mouse, C57BL/6n (♀)	significant
	Permadi, 1993, 1332452	Medium confidence	10 Day Oral PFDA	Mouse, C57Bl/6 (♂)	not applicable
	Li, 2022, 10273360	High confidence	14 Day Oral	Mouse, C57BL/6J (♂)	not applicable
Liver Weight, Relative (Hematology Study)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	significant

Figure 3-10. Effects on relative liver weight following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

The liver effects in response to oral exposure to PFDA in short-term animal studies consisted of increased serum biomarkers of liver function, increased liver weight and increased incidence of hepatocellular lesions (e.g., cytoplastic alterations, vacuolation, and to a lesser extent necrosis). Increased liver weight and hepatocellular hypertrophy can be associated with changes that are adaptive in nature (Hall et al., 2012), and not necessarily indicative of adverse effects unless observed in concordance with other clinical, pathological markers of overt liver toxicity (see PFAS Protocol; Appendix A). As discussed in the systematic review protocol, Hall et al. (2012) was focused on framing liver effects in the context of progression to liver tumors so additional information was considered when evaluating noncancer liver effects for PFDA exposure. The additional information consists of multiple in vitro and in vivo mechanistic studies in rodents (including peroxisome proliferator activated receptor alpha (PPARα)-null mice) and limited studies in human-relevant models (mostly in vitro systems but also studies in animal models with reduced PPARα sensitivity) as well as evidence from other PFAS that help elucidate possible modes of action of PFDA liver toxicity.

Summary of Mechanistic Studies for PFDA

Mechanistic evidence relevant to potential PFDA-induced liver effects was collected from the peer-reviewed literature and from in vitro high-throughput screening (HTS) assays accessed through the EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard)((U.S. EPA, 2022a); data were retrieved on November 03, 2022). Given the relatively abundant evidence base compared with most other PFAS, the available in vitro and in vivo mechanistic studies on PFDA were considered in the context of what is known about the mode of action (MOA) for hepatic effects elicited by related PFAS, including PFOA and PFOS, the most well-studied PFAS. MOA information for PFOS and PFOA was based primarily on published reviews. As discussed in the systematic review protocol (see Appendix A), an Adverse Outcome Pathway (AOP)-type approach was employed to organize and discuss the evidence according to the following levels of biological

organization: molecular interactions, cellular effects, organ effects, and organism effects. A summary of the mechanistic and supplemental evidence related to the potential mechanisms of hepatotoxicity for PFDA is provided below. A detailed description of the methodology and results of the analysis undertaken herein can be found in Appendices D and E.

Mechanistic evidence from in vivo and in vitro rodent cell models indicates that PFDA can activate (potentially directly) several xenobiotic-sensing nuclear receptors and other cell signaling pathways, namely PPARα, constitutive androstane receptor (CAR)/pregnane × receptor (PXR), nuclear factor erythroid 2 related factor 2 (Nrf2), tumor necrosis factor alpha (TNFα), nuclear factor kappa B pathway (NFκB) and c-Jun-N-terminal kinase (JNK)/activating transcription factor 2 (ATF-2) (see Appendix D.3.1 on molecular initiating events for more details). PFDA exposure was also associated with alterations in the hepatic expression and activity of xenobiotic metabolizing enzymes (XMEs), reactive oxygen species (ROS) production and markers of oxidative damage (DNA oxidation and lipid peroxidation), disruption of mitochondrial functions, induction of inflammatory responses, cellular damage/stress and abnormal liver metabolic functions related to bile acid, glucose, and lipid metabolism in animals (see Appendix D.3.2 on cellular effects for more details). These molecular and cellular mechanisms are associated with chemical-induced liver disorders such as steatohepatitis and fibrosis (Angrish et al., 2016; Cao et al., 2016; Joshi-Barve et al., 2015; Wahlang et al., 2013) and provide support for the biological plausibility of the observed liver effects in rats and mice after short-term PFDA exposure (see synthesis of animal studies in this section for more details).

The available mechanistic information in human models is limited to a few in vitro studies in the peer-reviewed literature and ToxCast and Tox21 HTS assay results (U.S. EPA, 2022a). The available evidence suggests some concordance with responses evaluated in animal models. PFDA could modulate the activity of many human nuclear receptor pathways potentially relevant to its mechanism(s) of hepatotoxicity. For example, PFDA activated PPARα in primary and immortalized human liver cell lines ((Rosenmai et al., 2018; Buhrke et al., 2013; Rosen et al., 2013) and Table E-2 for in vitro HTS assay results) and exhibited direct binding toward the human PPARα in vitro (Ishibashi et al., 2019). However, reduced or no sensitivity toward the human PPARα versus other mammalian species (i.e., mouse, Baikal seal and polar bear PPARα isoforms) in terms of binding and transcriptional activity have been well documented in some studies (Ishibashi et al., 2019; Routti et al., 2019; Wolf et al., 2012; Wolf et al., 2008). Reduced PPARα sensitivity in human versus rodent models (i.e., rats and mice) has been previously demonstrated in studies with other perfluorinated compounds (Corton et al., 2018; Wolf et al., 2012; Wolf et al., 2008).

PFDA activated nuclear receptors other than PPARα in human liver cell lines (i.e., PPARγ, PXR and FXR) and displayed high potency toward the human FXR in a receptor-ligand binding assays (Buhrke et al. (2013), Rosen et al. (2013), Zhang et al. (2017) and Table E-2 for in vitro HTS assay results). At the cellular level, PFDA elevated ROS production and induced markers of cellular

stress and cytotoxicity in human hepatoma HepG2 cells (see Appendix D.3.2 on cellular effects for more details).

PPARα activation is described as one of the mechanisms through which perfluorinated compounds induce liver toxicity in animals (U.S. EPA, 2024b; ATSDR, 2021; U.S. EPA, 2016a, b). PPARα appears to be important for disruption of bile acid homeostasis and downstream effects related to bile acid synthesis and transport mechanisms, as well as signaling pathways associated with cellular stress and anti-inflammatory responses in PFDA-exposed mice (Luo et al., 2017). However, other responses appear to occur, at least in part, independently of PPARα. Rosen et al. (2013) reported transcriptional induction of PPARα-dependent and -independent genes in primary human hepatocytes exposed to PFDA. Lim et al. (2021) showed that PFDA-mediated transcriptional regulation of transporters involved in metabolism and xenobiotic biotransformation in HepaRG cells was more consistent with activation of the ROS-sensitive transcription factor Nrf2 as opposed to PPARα or CAR. Increased liver weight and activation of Nrf2 were reported after PFDA treatment in both WT and PPARα-null mice (Luo et al., 2017; Maher et al., 2008). PFDA-mediated induction of hepatic Mrp transporters involved in cholestasis was attenuated in mice devoid of Nrf2 or Kupffer cell function (Maher et al., 2008). A study that evaluated PFDA animal models known to be generally resistant to PPARα activation (i.e., guinea pigs and/or Syrian hamsters) displayed histological responses indicative of hepatocellular stress, mitochondrial damage, hepatic lipid accumulation and liver enlargement with PFDA exposure (Van Rafelghem et al., 1987b). Noteworthy, hepatic lipid accumulation was characterized as more pronounced in guinea pigs and Syrian hamsters compared with rats and mice and the opposite was found for peroxisome proliferation (Van Rafelghem et al., 1987b). Finally, the NTP (2018) study that reported PFDA-induced liver effects in rats exposed for 28 days, also evaluated the effects of the potent PPARα inducer, Wyeth-14,643, on the liver. Similar to PFDA, Wyeth-14,643 caused increases in liver weights, hepatocyte hypertrophy and changes in serum liver biomarkers (e.g., increased ALT, ALP, and AST) in rats; however, unlike PFDA, Wyeth-14,643 exposure was not associated with any structural degenerative changes (i.e., hepatocyte necrosis).

Overall, the mechanistic evidence supports the biological plausibility of liver effects observed in animal bioassays. Further, the available data indicate a likely role for both PPARα-dependent and -independent mechanisms in the hepatotoxicity of PFDA in animals. Existing evidence from in vitro studies and animal models considered more relevant to humans with respect to PPARα sensitivity suggest that some responses may be conserved across species (including activation of relevant nuclear receptor pathways [PPARα/γ, PXR, and FXR] and outcomes related to hepatocellular stress, mitochondrial damage, lipid accumulation, and liver enlargement). Taken together, these data provide some support for the potential human relevance of the observed hepatic effects in animals. Some uncertainties remain based on differences in experimental design and/or confounding effects with cytotoxicity in in vitro test systems, as well as limited information available from in vivo models to characterize the putative involvement of PPARα and other cell

signaling pathways in the mechanisms of hepatotoxicity of PFDA in animals and humans (see Appendix D.3 and E.1 for more details).

Evidence from Related PFAS

Given the limitations in the mechanistic evidence for PFDA described above, studies investigating the effects of structurally related long-chain PFAS (perfluoroalkyl sulfonic acids containing ≥6 carbons or PFCAs with ≥7 carbons) are summarized herein, focusing on studies conducted in null and humanized animal models identified from literature searches conducted for other ongoing EPA PFAS IRIS assessments (i.e., PFHxS and PFNA) or in final EPA human health assessments (i.e., PFOA and PFOS). Data in these models available for short-chain PFAS (e.g., PFBA) are not summarized herein, as they were considered less relevant to PFDA exposure than those data available for long-chain PFAS, although extrapolations from other PFAS are all inherently uncertain.

Gene expression profiling in response to exposure to several long-chain PFAS has been evaluated in wild-type and PPARα-null mice and the results indicate a role for both PPARα-dependent and independent pathways in the liver effects of these compounds. Gene expression changes induced by PFOA, PFHxS, and PFNA in wild-type mouse livers were largely attributable to PPARα; however, a subset of transcriptional changes related to lipid metabolism, inflammation, and xenobiotic metabolism occurred in PPAR-α null mice that reflect potential activation of additional nuclear receptors such as CAR and PPARγ (Rosen et al., 2017; Rosen et al., 2010; Rosen et al., 2008).

Consistent with transcriptional regulation, the data support that tissue-level responses induced by these long-chain PFAS are likely to be mediated by PPARα–dependent and independent mechanisms. Increases in liver weight and hepatocyte hypertrophy and/or proliferation were reported in PPARα wild-type and null mice exposed to PFOA (Das et al., 2017; Wolf et al., 2008). Similarly, hepatomegaly (characterized by increased liver weight and cell size and decreased DNA content) and hepatic lipid accumulation (indicating or leading to steatosis) were observed with PFHxS or PFNA exposure in wild-type mice and mice devoid of PPARα function (Das et al., 2017). In contrast, these liver effects were only induced in wild-type animals treated with the prototype PPARα agonist, Wyeth 14,643. Nakagawa et al. (2012) showed elevated levels of hepatic triglycerides in wild-type, PPARα-null and humanized PPARα (hPPARα) mouse strains exposed to ammonium perfluorooctanoate, but macrovesicular and/or microvesicular steatosis in PPARα-null and hPPARα mice only. Additionally, PFOS and PFHxS decreased triglyceride and cholesterol levels in plasma and increased triglycerides in the liver of APOE*3-Leiden CETP mice, which exhibit attenuated clearance of apoB-containing lipoproteins and human-like lipoprotein metabolism on a Western diet (Bijland et al., 2011). Likewise, PFDA exposure was associated with marked increases in hepatic lipid content (including triglyceride levels) and accumulation in rats and mice (Kudo and Kawashima, 2003; Adinehzadeh and Reo, 1998; Kawashima et al., 1995; Sterchele et al., 1994; Brewster and Birnbaum, 1989; Harrison et al., 1988; Van Rafelghem et al., 1988b; Van Rafelghem et

al., 1987a), as well as, guinea pigs and Syrian hamsters (Van Rafelghem et al., 1987b), which like humans, appear to be less responsive to PPARα activation.

The precise mechanism(s) of how these long-chain PFAS induced hepatic lipid accumulation and the potential association of this accumulation with progression to steatosis remain unclear. Das et al. (2017) showed that PFOS, PFHxS, and PFNA, which are known to induce significant hepatic lipid accumulation in animals, alter the expression of genes involved in fatty acid synthesis and oxidation in mouse livers, and that these transcriptional changes are partly independent of PPARα (Das et al., 2017). The authors hypothesized that perfluorinated compounds disrupt the balance of fatty acid synthesis and oxidation in favor of accumulation, which leads to steatosis. In contrast, exposure to potent PPARα activators such as Wyeth 14,643, is not associated with steatosis-like changes because, these compounds likely favor fatty acid oxidation over synthesis/accumulation (Das et al., 2017).

Collectively, studies in PPARα null and humanized animal models for structurally related long-chain PFAS are consistent with the plausible PPARα-dependent and independent MOA for PFDA liver toxicity and add further support to the potential human relevance of the observed liver effects in animals. Further, the evidence suggests that these perfluorinated compounds have the potential to induce steatosis, a well-known chemical-induced response that can progress to steatohepatitis, fibrosis, and impaired liver function (Al-Eryani et al., 2015).

Considerations for Potentially Adaptive Versus Adverse Responses

Increases in liver weight and hepatocyte hypertrophy were observed in rodents with PFDA administration in short-term oral studies (see Figures 3-6 and 3-10 above). Enlargement of the liver and/or individual hepatocytes is a common chemical-induced response that can involve lipid accumulation (e.g., micro- or macrovesicular steatosis), organellar growth and proliferation (e.g., peroxisomes, endoplasmic reticulum), increased intracellular protein levels (e.g., Phase I and II enzymes), and altered regulation of gene expression (e.g., stress response, nuclear receptors) (reviewed by Batt and Ferrari (1995)). Hepatocyte hypertrophy related to organelle growth and proliferation in response to activation of xenobiotic-sensing receptors (primarily PPARα) is often considered an adaptive response (Hall et al., 2012). However, hepatocyte swelling is also associated with cell death processes, oncosis, or oncotic necrosis (Kleiner et al., 2012), which occurs in several liver diseases or conditions, such as ischemia-reperfusion injury, drug-induced liver toxicity, and partial hepatectomy (Kass, 2006; Jaeschke and Lemasters, 2003). Furthermore, mechanistic evidence for PFDA and other long-chain PFAS suggests that in addition to PPARα induction, these compounds activate non-PPARα-related mechanism of liver toxicity (see Appendix D.3 and E.1 for more details on the synthesis of PFDA-induced mechanisms of hepatotoxicity).

Hall et al. (2012) indicated that concordant histopathological evidence of degenerative or necrotic changes (e.g., hepatocyte necrosis, fibrosis, inflammation, steatosis, biliary degeneration, necrosis of resident cells within the liver) can be used to support the argument that liver weight/hepatocyte enlargement are adverse (Hall et al., 2012). In addition to increases in liver

weight and/or hepatocyte hypertrophy, PFDA caused cytoplasmic alterations and vacuolization as well as necrosis in rat hepatocytes across two *high* confidence 28-day gavage studies (Frawley et al., 2018; NTP, 2018). Cytoplasmic alterations of minimal to marked severity were observed in nearly all male and female rats at ≥0.625 mg/kg-day (NTP, 2018). Cytoplasmic vacuolization of minimal/mild to moderate severity occurred in males and females at ≥1.25 mg/kg-day (NTP, 2018). Minimal necrosis was reported in females in the two 28-day studies with statistically significant increases at the highest dose, 2.5 mg/kg-day (Frawley et al., 2018; NTP, 2018). Male rats were only tested in one study, showing increased incidence of hepatocyte necrosis, but the effect was not dose-dependent (NTP, 2018). The lesions show a clear pattern of increased hepatocyte damage/injury with dose, ranging from cytoplasmic changes to hypertrophy to necrosis (NTP, 2018). The necrotic lesions were accompanied in some cases by evidence of an initial inflammatory response (NTP, 2018) and, although these changes were characterized as minimal, the findings indicate some degree of structural degeneration considered adverse and that may progress to more severe liver pathologies with increasing dose or exposure duration. Consistent with these observations, steatosis, necrosis, edema, and degeneration were reported in mice at 13 mg/kg-day and extensive lipid accumulation was reported in rats at 9.22 mg/kg-day in *low* confidence short-term studies with PFDA administered orally (Wang et al., 2020; Kawashima et al., 1995). Acute i.p. studies provide additional support for the accumulation of lipids in the liver with PFDA exposure (see Synthesis of Metabolic Effects in Appendix D.3.2), which is a key event leading to hepatic steatosis (Angrish et al., 2016). As discussed above, steatosis is a common liver response in animals associated with exposure to perfluorinated compounds such as PFOA, PFHxS, or PFNA. Sustained steatosis can progress to steatohepatitis and other adverse liver diseases such as fibrosis and cirrhosis (Angrish et al., 2016).

Alterations in serum liver biomarkers were also present in rats that exhibited increases in liver weight, hepatocyte hypertrophy, and other histological lesions (i.e., necrosis) after 28-day gavage exposure to PFDA (NTP, 2018). According to Hall et al. (2012), clinical markers of liver damage and function can provide evidence in support of the adversity of concomitant increases in liver weight/hepatocyte hypertrophy. These authors suggested that a weight-of-evidence approach should be applied when evaluating clinical marker data, considering dose-dependent and biologically significant changes in at least two of the following parameters: two- to threefold increase in ALT; a biologically significant change in biomarkers of hepatobiliary damage (e.g., AST, ALP, and γ-glutamyltranspeptidase [γGT]); a biologically significant change in biomarkers of liver dysfunction (e.g., albumin, bilirubin, bile acids/salts, and coagulation factors). PFDA increased ALT levels in female rats at ≥1.25 mg/kg-day (NTP, 2018); similar changes were observed in male rats, but the effects did not show a significant trend. Although the increases in circulating ALT levels in females were relatively small (20%–44% or 1.2- to 1.4-fold), concordant changes in other clinical biomarkers occurred in these animals that are consistent with the Hall et al., (2012) criteria for adversity. Dose-dependent increases in AST and ALP were found in male and female rats at

≥0.156 mg/kg-day (13%–80% or 1.1-to-1.8 fold for AST and 22%–106% or 1.1-to-2.1 fold for ALP). Similarly, levels of bile salts/acids and bilirubin were elevated in rats of both sexes at ≥1.25 mg/kg-day, exhibiting marked changes (205%–1,207% or 3.1- to 13.1-fold for bile acids/salts and 28%–733% or 1.3- to 8.3-fold for bilirubin). Further, ALT (338% or 4.4-fold) and AST (649% or 7.5-fold) were elevated in mice that exhibited liver lesions after exposure to a high dose of PFDA (13 mg/kg-day) (Wang et al., 2020).

Overall, the available evidence for PFDA meets all the Hall et al. (2012) criteria for adversity and supports the conclusion that PFDA exposure has multiple and coherent effects on liver histopathology, serum biomarkers, and liver weights in exposed animals (primarily rats) that support the findings of adverse liver effects in animals.

Evidence Integration

There is *slight* evidence of an association between PFDA exposure and hepatic effects in humans based on associations with liver biomarkers in the blood. Positive associations between exposure to PFDA and ALT were observed in most studies of adults. However, there is uncertainty because of the potential for confounding of the association by other PFAS.

The evidence for PFDA-induced liver effects from short-term animal studies via the oral route is considered *moderate* based on coherent effects across multiple endpoints relevant to the assessment of liver toxicity (serum biomarkers, histopathology, and organ weight) (see Figures 3-6, 3-8, and 3-10 above and HAWC summary visual of coherent PFDA liver effects). Increases in serum biomarkers of hepatocellular/hepatobiliary injury (ALT, AST, ALP, bile salts/acids, and bilirubin) (NTP, 2018) and liver weights were reported in male and female SD rats at ≥0.156 mg/kg-day after 28-day gavage exposure (Frawley et al., 2018; NTP, 2018). In general, the responses were consistent in directionality across sexes and dose groups, exhibiting a clear dose-response gradient. Furthermore, the evidence for increased liver weights was consistent across several species (rats and mice), strains (SD, Wistar, Fischer F344, C57BL/6N, C57BL/6J, and B6C3F1/N) and exposure designs (gavage and dietary) (see synthesis of organ weight in this section for more details). At higher doses (≥0.5 mg/kg-day), a consistent pattern of hepatocellular lesions was observed in SD rats that included cytoplastic alterations and vacuolization, hypertrophy, and necrosis (Frawley et al., 2018; NTP, 2018). The pattern of hepatocellular changes showed a progression in severity within and across lesions with an increase in exposure dose, which adds certainty to the interpretation of the evidence. In combination with the histopathological findings, alterations in serum biomarkers and liver weights support the development of adverse liver effects in rats after continuous PFDA exposure (see section on considerations for potentially adaptive versus adverse responses, above). The evidence base for liver effects in animals consists primarily of two *high/medium* confidence 28-day studies in SD rats conducted by NTP that showed concordant effects. Other available short-term studies provided support for PFDA-induced liver effects across laboratories and species but had issues with incomplete reporting that resulted in a *low* confidence rating, evaluated limited endpoints, and/or tested higher doses associated with general systemic

toxicity, which add some uncertainty. Additional studies via relevant exposure routes and experimental designs (most prominently subchronic and chronic exposure studies) examining potential liver effects of PFDA exposure are needed to increase confidence in the evidence base.

Analysis of mechanistic and supplementary data from in vivo and in vitro rodent models provide experimental (e.g., liver weight changes after i.p. exposure) and biological support for the phenotypic effects reported in the short-term oral studies summarized above. Exposure to PFDA was associated with the activation of several molecular signaling pathways and altered cellular functions hypothesized to be involved in the MOA for liver toxicity of related perfluorinated compounds (see summary on mechanistic and supplementary studies for PFDA in this section and Appendices D and E for more details). Additionally, the evidence for PFDA-mediated liver effects implicates both PPARα-dependent and -independent mechanisms.

The activation of PPARα in the MOA for noncancer liver effects in rodents has implications to human health assessment based on perceived differences in PPARα response between rats/mice versus humans. PFDA can activate the human PPARα in vitro but it exhibits less/no sensitivity toward the human isoform in comparison with other mammalian species in some studies. PFDA also interacts with other nuclear receptors and cell signaling pathways relevant to its potential mechanism of hepatotoxicity in both human and animal models. Furthermore, some hepatic responses in animals occurred at least in part independent of PPARα or were found to be activated in human in vitro assays or animal models that are more relevant to humans with respect to PPARα sensitivity (see summary on mechanistic studies for PFDA in this section and Appendices D and E.1 for more details). These observations are consistent with studies in PPARα null and humanized animals for other long-chain PFAS such as PFOA, PFHxS, and PFNA that suggest non-PPARα mechanisms of liver toxicity (see evidence for other PFAS in this section for more details). Given that the precise role of PPARα in the noncancer liver effects of PFDA remains largely unknown and the possible involvement of PPARα-dependent and independent pathways, the effects observed in animals are considered potentially relevant to humans. This assumption is consistent with EPA's review of RfD/RfC methodology from 2002 (U.S. EPA, 2002).

Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause hepatotoxicity in humans given sufficient exposure conditions[10] (see Table 3-10). This conclusion is based primarily on coherent liver effects in rats (and, to a lesser extent, mice) exposed to doses ≥0.156 mg/kg-day for 28 days. Alterations in serum liver biomarkers were reported in animals and in epidemiological studies, although the latter results are more uncertain. The available mechanistic information overall provides support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.

[10]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-10. Evidence profile table for PFDA exposure and liver effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.1: Human Studies)					⊕⊕⊙ ***Evidence indicates (likely)*** *Primary basis:* Two *high* confidence studies in rats at ≥0.156 mg/kg-d after short-term exposure *Human relevance:* Effects in rats are considered relevant to humans (**see "Mechanistic Studies and Supplemental Evidence" in Section 3.2.2**) *Cross-stream coherence:* Alterations in serum liver biomarkers were reported in animals and in epidemiological studies. *Susceptible populations and lifestages:* None identified, although individuals with preexisting liver disease could potentially be at greater risk. *Other inferences:* the MOA for PFDA-induced liver effects is unknown, although the available evidence indicates the involvement of PPARα-dependent and -independent mechanisms
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Serum biomarkers</u> Nine *medium* confidence studies (7 in adults, 1 in adults and children, 1 in children)	• Six of seven studies in adults reported positive associations between ALT and PFDA exposure in adults.	• Consistency across studies in adults	• Potential for confounding by other PFAS • Unclear biological significance of small changes in ALT	⊕⊙⊙ *Slight* Evidence of a positive associations between PFDA exposure and ALT in adults, but there is uncertainty due to potential confounding	

Evidence stream summary and interpretation					Evidence integration summary judgment
Liver disease One *low* confidence study	• Strong inverse association with nonalcoholic fatty liver disease	• No factors noted	• Lack of coherence with serum biomarkers		
Evidence from in vivo animal studies via the oral route (see Section 3.2.1: Animal Studies)					
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Histopathology Two *high* and 3 *low* confidence studies in adult rats or mice • 7-d dietary • 12- and 14-d drinking water • 28-d gavage (2×)	• Hepatocellular lesions (ranging from cytoplasmic alterations to necrosis) at ≥0.5 mg/kg-d across *high* confidence studies. • Other liver lesions (i.e., lipid accumulation and edema) were found in *low* confidence studies at higher doses (≥4.6 mg/kg-d)	• Consistency across two *high* confidence studies • Coherent pattern of hepatocellular lesions across all studies • Increased severity (within and across lesions) with increasing exposure	• No factors noted	⊕⊕⊙ *Moderate* Consistent and coherent changes in serum biomarkers, histopathology, and liver weights, with the strongest evidence in rats at ≥0.156 mg/kg-d although data are limited to short-term studies (see HAWC summary visual of coherent PFDA liver	

Evidence stream summary and interpretation					Evidence integration summary judgment
Serum biomarkers Two *high* confidence studies in adult rats or mice • 12-d drinking water • 28-d gavage	• Increased serum markers of liver and hepatobiliary toxicity at ≥0.156 mg/kg-d	• *Consistency* across *high* confidence studies • *Coherence* across serum markers • *Dose-response* gradient for most effects	• No factors noted	effects). Taken together, the coherent changes across markers of hepatic injury were judged as adverse (see "Considerations for potentially adaptive versus adverse responses")	
Organ weight Four *high*, 4 *medium* and 1 *low* confidence studies in adult rats and mice. • 7–14-d dietary (5×) • 14-d drinking water (1×) • 28-d gavage (2×) • Gestational gavage (1×)	• Increased relative liver weights at ≥0.089 mg/kg-d	• *Consistency* across all studies, including multiple species and both sexes. • Dose-response gradient • *Coherence* with serum markers and histopathology	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated** **Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Molecular initiating events— PPARα and other cell signaling pathways	*Key findings and interpretation:* • Evidence of activation of PPARα, CAR/PXR, Nrf2, TNFα, NFκB and JUNK/ATF-2 in rodent hepatic in vivo and/or in vitro models. • Some evidence of activation of PPARα/γ, PXR and FXR in human liver cells and/or cell-free binding assays • The human FXR was a sensitive target for PFDA in vitro. • Reduced sensitivity toward the human PPARα compared with Baikal seal, polar bear, and mouse PPARα isoforms in vitro.			Evidence of PPARα-dependent and -independent pathways in studies in rodents and human in vitro models that support the biological plausibility of PFDA-induced liver effects.	

Evidence stream summary and interpretation			Evidence integration summary judgment
	Limitations: Lack of humanized in vivo models. Some inconsistencies in the vitro results may be due to differences in experimental model and/or design or confounding issues with cytotoxicity.		
Cellular effects	*Key findings and interpretation:* • Alterations in hepatic XMEs, oxidative stress, cell and mitochondrial damage, inflammation, and liver metabolic functions in rodents. • PPARα appears to be important for disrupting bile acid homeostasis in mice and associated downstream effects. • Activation of Nrf2 in wild-type and KO PPARα mice and observations of hepatocellular stress, mitochondrial damage and lipid accumulation in animal models known to be less responsive to PPARα activation (i.e., guinea pigs and/or Syrian hamsters) support involvement of PPARα-independent mechanisms. • PFDA increased ROS production and markers of cellular stress/cytotoxicity in human hepatoma HepG2 cells. *Limitations:* Few studies examining the role of PPARα and other cell signaling pathways and no evidence in humanized vivo models. Inconsistencies in the in vivo results are likely attributable to differences in experimental model and/or design features.		
Organ-level effects	*Key findings and interpretation:* • Increased liver weights in rats, mice (both WT and PPARα-KO animals) and in a rodent species known to be resistant to PPARα activation (i.e., Syrian hamsters). *Limitations:* Lack of evidence examining other organ-level effects, including histological evidence.		

3.2.2. Immune Effects

Methodological Considerations

Immune-related health effects evaluated from human and animal studies are grouped according to immunotoxicity guidance from the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) and considered for evidence of major categories of immunotoxicity: (1) immunosuppression, (2) immunostimulation, (3) sensitization and allergic response, or (4) autoimmunity and autoimmune disease (IPCS, 2012). Evidence for potential immune effects is considered within these four categories because of common and related mechanisms. Within each category, health effects data are organized and discussed from most to least relevant for drawing hazard conclusions about immunotoxicity (IPCS, 2012). For human data, clinical studies on disease or immune function assays (e.g., antibody responses) are considered most relevant, then general/observational immune assays (lymphocyte phenotyping or cytokines), and finally endpoints such as hematology (i.e., blood leukocyte counts) are considered least relevant. Similarly, animal data are presented from most to least relevant for immunotoxicity assessment as described by WHO/IPCS as follows: host resistance, immune function assays, general/observational immune assays, blood leukocyte counts, and immune organ histopathology and weights (IPCS, 2012). The available human and animal evidence provide relevant information for the assessment of immunosuppression and sensitization or allergic response. However, the available evidence is lacking or inappropriate to specifically address the potential for immunostimulation and autoimmunity following PFDA exposure; therefore, these categories of potential immunotoxicity are not discussed further.

Human Studies

Epidemiology studies examining immune effects of PFDA exposure include studies on antibody response, infectious diseases, and hypersensitivity-related outcomes, which includes asthma, allergies, and atopic dermatitis. Outcomes related to immunosuppression were considered within two subcategories: antibody response and infectious disease incidence. Several different outcomes were included in the sensitization and allergic response category. The health effects evidence from human studies is summarized below for each category.

Antibody response outcomes

The production of antigen-specific antibodies in response to an immune challenge (e.g., vaccination in humans or injection with an antigen [e.g., sheep red blood cells] in rodents) is a well-accepted measure of immune function included in risk assessment guidelines and animal testing requirements for immunotoxicity (ICH Expert Working Group, 2005; U.S. EPA, 1998; IPCS, 1996). Antibodies are proteins circulating in blood and other body fluids that bind to antigens and thereby identify them for destruction or removal. The production, release, and increase in

circulating levels of antigen-specific antibodies are important for protection against infectious agents and preventing or reducing severity of influenza, respiratory infection, colds, and other diseases as part of the humoral immune response. Vaccine immune titers are thus functional measures of adaptive immune response and important indicators of immunotoxicity; reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied). A blood concentration for tetanus or diphtheria antibodies of 0.1 IU/mL is sometimes cited in the literature as a "protective level" but Galazka et al. (1993) argues that there is "no absolute protective level of antitoxin and protection results when there is sufficient toxin-neutralizing antibody in relation to the toxin load" as (see Appendix C.1 for additional details).

Five studies (six publications) examined PFDA exposure and antibody responses following vaccination for diphtheria or tetanus in children and adults; study evaluations are summarized in Figure 3-11 and Table 3-11. These included three independent prospective birth cohorts in the Faroe Islands, all *medium* confidence, one with enrollment in 1997–2000 and subsequent follow-up to age 7 (Grandjean et al., 2012) and age 13 (Grandjean et al., 2017a), one with enrollment in 2007–2009 and follow-up to age 5 (Grandjean et al., 2017b), and one with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021). Shih et al. (2021) also examined antibody response to Hepatitis types A and B vaccination. These three cohorts are all separate study populations born in the Faroe Islands and enrolled at different times and thus considered independent of each other. The analyses in Grandjean et al. (2017b) combined new data from the cohort born in 2007–2009 with new follow-up data from the cohort born in 1997–2000 (Grandjean et al., 2012), which are labeled in the results table. There was also a cross-sectional study of children in Greenland (Timmermann et al., 2021). These studies were generally well conducted, but exposure contrast was a concern in most of them, with median exposure levels ~0.3 ng/mL and interquartile ranges ~0.2 ng/mL (exposure contrast was slightly better in (Timmermann et al., 2021)). Potential for confounding across PFAS was considered in individual study evaluations as well as across studies in evidence synthesis (see below). In addition to these developmental exposure studies, one study in healthy adult volunteers in Denmark was considered *low* confidence because of limited information provided on recruitment of study subjects, lack of consideration of confounders, and a small study population (12 individuals) leading to concerns with potential selection bias, confounding, and low sensitivity (Kielsen et al., 2016).

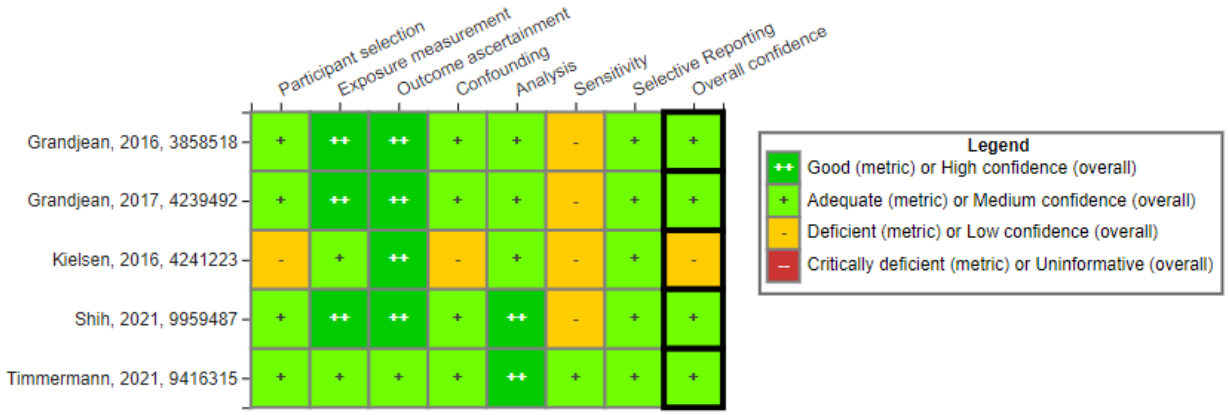


Figure 3-11. Evaluation results for epidemiological studies assessing effects of PFDA exposure on antibody response to vaccination. Refer to HAWC Human Vaccine Response Effects for details on the study evaluation review.

Domain and overall confidence ratings may vary by outcome; outcome-specific ratings and rationales are available in HAWC and described in the relevant sections below. Multiple publications of the same study: Grandjean et al. (2017a) also represents Grandjean et al. (2012).

The two prospective birth cohorts in the Faroe Islands with antibody levels measured during childhood reported inverse associations between higher concentrations of serum PFDA and lower antivaccine antibody levels for diphtheria and tetanus (see Table 3-11). Although results were not always statistically significant, the general trend toward lower antibody levels was apparent. Antibody levels were measured in individuals of several age groups (and therefore different lengths of time since their initial vaccination or booster vaccination) and compared with serum PFDA concentrations also measured at different ages. Although results were not always statistically significant, inverse associations were observed in most (but not all) of these comparisons. No biological rationale is understood as to whether one period is more predictive of an overall immune response and, given the long half-life of PFDA, there are reasonably high correlations across periods (Grandjean et al., 2017a). Antibodies to diphtheria decreased with increasing PFDA concentrations in 11 of the 13 exposure and outcome measurement timing combinations assessed. One of the two results that did not support the trend was a statistically significant increase in diphtheria antibodies in children at 5 years of age (before receiving the 5-year booster) associated with increases in PFDA concentrations at 18 months of age. This increase appears to be a response in this specific exposure and outcome timing combination in the 2007–2009 cohort as there was an increase with all PFAS measured at 18 months and outcome measured at 5 years of age in the 2007–2009 cohort. However, the 1997–2000 cohort from the same population and all other exposure and outcome timing combinations, including in the 2007–2009 cohort when exposure was measured at birth, resulted in a decrease of diphtheria antibodies (Grandjean et al., 2017b). There is no clear explanation for the discrepant findings for this specific exposure and outcome timing combination in the 2007–2009 cohort. The only other result that did

not show a decrease in diphtheria antibodies was among 7-year-olds based on maternal PFDA concentration Grandjean et al. (2012). However, because a decrease in diphtheria antibodies was observed within 7-year-olds when PFDA concentrations were measured at age 5, the lack of effect may be explained by differences in the long-term influence of the maternal exposure measurement.

Similar to the diphtheria results, tetanus antibodies had a decreasing trend with increasing PFDA concentrations with few exceptions (10 of the 13 combinations indicated decreased antibody levels). One of the exceptions is a statistically significant increase in tetanus antibodies in 7-year-olds with increasing maternal PFDA concentrations (similar to the discrepancy observed for diphtheria for a similar exposure-outcome combination). Tetanus antibody levels at 13 years of age were also increased with increasing PFDA concentrations measured in the children at ages 7 and 13 years of age (Grandjean et al., 2017a). This observation may indicate that by 13 years of age, the effect of maternal and childhood exposure is less relevant to tetanus antibody levels.

The other two studies of developmental exposure and antibody response to vaccination reported less consistent findings. The cross-sectional results in Timmermann et al. (2021) differed in direction of association based on the covariate set selected (with or without adjustment for area of residence). The exposure measurement in these analyses may not have represented an etiologically relevant window; cross-sectional analyses in the Faroe Islands studies at similar ages also found weaker associations than analyses for some other exposure windows. However, a subset of the study population did have maternal samples available, and those results were also inconsistent by vaccine. On the other hand, this study was the only one to examine the odds ratio (OR) for "not being protected against diphtheria (antibody concentrations <0.1 IU/mL)", and they reported an OR of 5.08 (95% 1.32, 19.51) among children with known vaccination records (adjusted for area of residence, consistent with continuous antibody results). Shih et al. (2021), which examined antibody levels at age 28 with exposure measures at multiple time points, reported inconsistent associations across exposure windows and vaccines. Results also differed by sex, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes men). Results were similarly inconsistent for antibodies to Hepatitis A and B (not shown). Similar to the results in 13-year-olds in the other Faroe Islands cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Lastly, one *low* confidence study examined exposure to PFDA in adulthood and found inverse associations with antibodies to both diphtheria and tetanus (statistically significant for diphtheria) (Kielsen et al., 2016).

It is plausible that the observed associations with PFDA exposure could be partially explained by confounding across the PFAS or cumulative effects, although several analyses and observations indicate that this is unlikely. Exposure levels to other PFAS in the Faroe Islands populations were considerably higher (PFOS 17 ng/mL, PFOA 4 ng/mL, PFNA 1 ng/mL, PFDA 0.3 ng/mL at age 5 years in Grandjean et al. (2012), and there was a high correlation between PFDA and PFNA (r = 0.78) and moderate correlations with PFOS and PFOA (r = 0.39 and 0.35, respectively). The authors assessed the possibility of confounding in a follow-up paper (Budtz-

Jørgensen and Grandjean, 2018a) that reanalyzed data from both Grandjean et al. (2012) and (Grandjean et al., 2017b) for benchmark analysis. In this reanalysis, effect estimates for PFDA were adjusted for PFOS and PFOA. Details of the analytic results were provided to EPA by the authors (Budtz-Jørgensen and Grandjean, 2018b). There were variable attenuation of the observed effect estimates across the different analyses, and PFNA (the PFAS with the strongest correlation with PFDA) was not adjusted for in these models. However, details of the regression modeling result (Budtz-Jørgensen and Grandjean, 2018b) show that PFNA was a nonsignificant predictor of either tetanus or diphtheria antibody concentrations with associations just 15% the strength of the PFDA association and thus PFNA could not have been a meaningful confounder. Further, adjustment of the PFDA association by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations. The details of the effects of PFDA with, and without, control of PFOS and PFOA are shown in Appendix C.1.1 with discussion of the impact and implications of multiple confounder control. Overall, while it is not possible to rule out confounding across PFAS, the available evidence suggests that it is unlikely to explain the observed effects. Other sources of potential confounding, including possible coexposures such as PCBs, were controlled appropriately. However, Grandjean et al. (2012) showed the correlation of PCBs with PFDA in their Table 2 at age 5 years as r = 0.14; the low correlation with PFDA means that PCBs could not have been a meaningful confounder of the PFDA effect estimate

Overall, in the two birth cohorts examining effects in children in the Faroe Islands, of the 26 paired antibody-to-PFDA exposure evaluations of diphtheria and tetanus antibody responses, 21 of them support a decrease in antibodies with increasing PFDA concentration (see Table 3-11).[11] Although the results were not always statistically significant, the decreases were generally large, with decreases in antibody concentration ranging from 2% to 25% per doubling of PFDA concentration. The variability in some of the results could be related to differences in etiological relevance of exposure measurement timing, differences in timing of the boosters since this was uncontrolled by the study (children were vaccinated according to the official Danish/Faroese vaccination program), as well as differences in timing of antibody measurements in relation to the last booster and PFDA exposure measurement. This evidence is considered relevant to the U.S. population absent a clear explanation for why the study population is not applicable (e.g., a genetic polymorphism unique to that population) to ensure protection for vulnerable and susceptible groups. In addition, a cross-sectional study of children in Greenland reported a large OR for lack of protection against diphtheria following vaccination (Timmermann et al., 2021), and similar decreases in both diphtheria and tetanus antibodies were also observed in a small study in adults (n = 12) from Denmark based on a reduced change in antibodies after a booster shot (Kielsen et al., 2016). These associations were observed despite poor sensitivity resulting from narrow exposure

[11]Additional results based on the (Grandjean et al., 2012) and (Grandjean et al., 2017a) cohorts were reported in {Budtz-Jørgensen, 2018, 11146378} and those results were used to derive candidate toxicity values using a different regression model. Complete details are provided in Appendix C.1.

contrasts in all three studies, which increases confidence in the association. There is some remaining uncertainty resulting from variability in the response by age of exposure and outcome measures as well as vaccination (initial and boosters) in the Faroe Islands childhood cohorts, and due to potential for confounding across PFAS. There is also uncertainty due to inconsistent results in Timmermann et al. (2021) as well as a birth cohort with follow-up to young adulthood in the Faroe Islands (Shih et al., 2021). However, the findings in children in the Faroe Islands are based on both outcome measurement in childhood and prospective exposure measurement, and the inconsistency may conceivably be attributed to these differences.

Table 3-11. Summary of PFDA exposure and selected data on antibody response in humans

Reference, N, confidence	PFDA exposure timing and concentration in ng/mL[a]	Outcome measure timing	Diphtheria vaccine (% change in antibodies with increase in PFDA[b])	Tetanus vaccine (% change in antibodies with increase in PFDA[b])
Grandjean et al. (2012)[c], Faroe Islands, N = 380–537, medium Grandjean et al. (2017a)[c] Faroe Islands, 1997–2000 cohort[c]	Maternal; mean (IQR): 0.3 (0.2–0.4)	Children (age 5 yr), prebooster	**−21.7 (−35.7, −4.8)**	−2.5 (−18.5, 16.8)
		Children (age 5 yr), postbooster	**−18.8 (−30.5, −5.0)**	−6.1 (−23.5, 15.3)
		Children (age 7 yr)	0.7 (−18.2, 24.0)	16.4 (−6.7, 45.2)
	Children (age 5 yr); mean (IQR): 0.3 (0.2–0.4)	Children (age 5 yr), prebooster	−16.0 (−29.6, 0.3)	−13.6 (−26.3, 1.4)
		Children (age 5 yr), postbooster	−8.7 (−20.6, 5.0)	**−19.9 (−33.1, −3.9)**
		Children (age 7 yr)	−14.4 (−28.4, 2.4)	**−22.3 (−35.8, −5.8)**
	Children (age 13 yr); mean (IQR): 0.3 (0.2–0.4)	Children (age 13 yr)	−3.7 (−22.0, 18.9)	18.7 (−11.8, 59.8)
Grandjean et al. (2017b)[c], Faroe Islands, N = 349, medium 2007–2009 cohort (unless specified)	At birth, not reported	Children (age 5 yr), prebooster	−3.54 (−23.19, 21.15)	−8.40 (−26.27, 13.79)
	Infant (18 mo); median (25th–75th percentile): 0.3 (0.2–0.4)	Children (age 5 yr), prebooster	2007–2009 cohort **25.52 (2.00, 54.48)** 1997–2000 cohort −22.87 (−60.92, 52.24)	2007–2009 cohort −5.78 (−23.56, 16.13) 1997–2000 cohort −14.47 (−56.88, 69.66)

Reference, N, confidence	PFDA exposure timing and concentration in ng/mL[a]	Outcome measure timing	Diphtheria vaccine (% change in antibodies with increase in PFDA[b])	Tetanus vaccine (% change in antibodies with increase in PFDA[b])
	Children (age 5 yr); median (25th–75th percentile): 0.3 (0.2–0.5) ng/mL	Children (age 5 yr), prebooster	−8.99 (−23.63, 8.46)	−1.76 (−16.73, 15.91)
Shih et al. (2021), Faroe Islands, N = 281, medium	Cord blood; median (IQR) 0.07 (0.06)	Adults (age 28 yr)	Total: 7.29 (−11.2, 29.6) Women: −1.39 (−24.8, 29.2) Men: 16.16 (−10.6, 51.0)	Total: −12.9 (−25.0, 1.2) Women: −17.0 (−33.0, 3.0) Men: −8.8 (−26.0, 12.4)
	Children (age 7 yr); 0.22 (0.16)		**Total: 37.89 (1.8, 86.8)** Women: 30.99 (−16.5, 105.4) Men: 43.8 (−4.4, 116.3)	Total: 3.2 (−18.5, 30.7) Women: −2.6 (−31.3, 38.0) Men: 8.3 (−21.1, 48.7)
	Children (age 14 yr); 0.28 (0.17)		Total: −7.2 (−35.1, 32.7) Women: 39.4 (−28.7, 172.8) Men: −20.5 (−47.4, 20.3)	Total: −22.6 (−42.9, 4.9) Women: −41.0 (−66.6, 4.1) Men: −14.3 (−39.7, 21.9)
	Adults (age 22 yr); 0.39 (0.26)		**Total: 34.9 (4.9, 73.6)*** **Women: 39.0 (2.2, 89.0)*** Men: 27.2 (−17.6, 96.3)	Total: −5.2 (−22.9, 16.4) Women: −4.0 (−25.4, 23.5) Men: −7.6 (−35.1, 31.6)
	Adults (age 28 yr); 0.34 (0.25)		Total: 19.6 (−1.2, 44.9) Women: 24.7 (−2.9, 60.0) Men: 12.8 (−16.2, 51.8)	Total: −5.3 (−18.7, 10.3) Women: −1.9 (−19.6, 19.8) Men: −9.9 (−28.8, 14.2)
Timmermann et al. (2021), Greenland, N = 314, medium	Children (age 7–12 yr)	Children (age 7–12 yr)	Adjusted for time since vaccine booster, breastfeeding duration 126 (32, 289) Additionally adjusted for area of residence −39 (−70, 27)	Adjusted for time since vaccine booster, breastfeeding duration 74 (12, 169) Additionally adjusted for area of residence −29 (−61, 28)
	Maternal (N = 57)	Children (age 7–12 yr)	−39 (−84, 133)	95 (−45, 591)

Reference, N, confidence	PFDA exposure timing and concentration in ng/mL[a]	Outcome measure timing	Diphtheria vaccine (% change in antibodies with increase in PFDA[b])	Tetanus vaccine (% change in antibodies with increase in PFDA[b])
Kielsen et al. (2016), Denmark N = 12, low	Adult (10 d post vaccination); median (IQR): 0.3 (0.2–0.3) ng/mL	Adult – change from 4 d to 10 d post vaccination	**−18.18 (−29.52, −5.00)**	−8.31 (−18.10, 2.66)

Bold font indicates $p < 0.05$.

[a]Exposure timing is organized into groups based on maternal exposure, childhood exposure (including from birth through age 13), and adult exposure.

[b]Linear regression (β or % change in antibody per twofold increase of PFDA). Numbers in parentheses are 95% confidence intervals.

[c]{Budtz-Jørgensen, 2018, 11146378}provided additional modeling of these data. Complete details are provided in Appendix C.1.

Infectious disease

Direct measures of infectious disease incidence or severity such as respiratory tract infections, pneumonia, or otitis media are useful for evaluating potential immunotoxicity in humans. Increases in incidence or severity of infectious disease can be a direct consequence of impaired immune function whether the specific functional deficit has been identified or not. Given the clear adversity of most infectious diseases, they are generally considered good measures for how immunosuppression can affect individuals and communities but can be difficult to measure. Physician diagnosis is the most specific way to assess infectious diseases, but these are usually only available for severe diseases and are less likely for diseases for which treatment is not sought. Self-reported incidence or severity of disease may be less reliable but may be the only way to assess diseases such as the common cold or gastroenteritis, which while less adverse, are more common and can thus provide information about immunosuppression and susceptibility to more severe infections. In general, symptoms of infection alone are not considered reliable measures of disease because of their lack of specificity. Antibody levels in response to infection are also included in this section (differentiated from antibody levels in response to vaccination, described above); the utility of these measures depends on the study design and population due to various factors such as potential confounding and prevalence of infection.

Six studies examined PFDA exposure and infectious disease outcomes in children and one study examined disease severity in adults (see Figure 3-12). Three of these focused on the number of episodes of infectious disease. One was a *medium* confidence prospective birth cohort study in Japan that looked at the association of PFDA exposure with total infectious disease (including otitis media, pneumonia, RS virus, and varicella) from birth to age 4 (Goudarzi et al., 2017), with outcomes ascertained using a questionnaire identifying physician diagnosed disease incidents. A second *medium* confidence birth cohort in China identified cases of common cold or bronchitis/pneumonia reported by parents with verification with medical records (Wang et al.,

2022). A *low* confidence cohort with PFDA exposure measured in childhood examined number of episodes of parent-reported lower respiratory tract infections and common colds based on parent reports using an unvalidated questionnaire (Kvalem et al., 2020). Another prospective birth cohort examined days of infectious disease symptoms (fever, diarrhea, coughing, nasal discharge, vomiting) with follow-up at 1–4 years (Dalsager et al., 2016). This study was considered *low* confidence due to the nonspecific nature of the symptoms reported, which may not represent infectious disease. In the same birth cohort in Denmark, but with a larger sample size, hospitalizations due to infectious disease were identified from a national registry (Dalsager et al., 2021a). These two studies were evaluated separately due to their different samples and outcomes measurement methods but should not be considered fully independent samples. Also in children, one study examined antibody response to hand, foot, and mouth disease (HFMD) infection. This birth cohort in China (Zeng et al., 2019b) measured antibody levels in infants at birth and 3 months of age, a period expected to reflect passive immunity from maternal antibodies. This study is *low* confidence because the outcome is broad and difficult to interpret and there are concerns for confounding by timing of HFMD infection as well as other limitations. Lastly, one study examined severity of COVID-19 illness in Denmark using biobank samples and national registry data (Grandjean et al., 2020). There was concern for selection bias in this study due to the expectation that biobank samples were more likely to be available for individuals with chronic health concerns, some of which may be associated with PFAS exposure either causally or via changes in PFAS elimination. In addition, severity of COVID-19 is not a direct measure of immune suppression as other factors may contribute to illness severity.

The results for this set of studies are summarized in Table 3-12. Results were overall inconsistent. Positive associations (although mostly not statistically significant) between PFDA exposure and specific infectious diseases were observed in some studies (diarrhea, common cold, and lower respiratory infection in Wang et al. (2022), lower respiratory infections in Kvalem et al. (2020), upper respiratory tract infections in Dalsager et al. (2021a), fever in Dalsager et al. (2016)), but inverse associations were observed in other studies. When two studies were available for a given infectious disease, the results were generally not in the same direction. The single study of HFMD antibodies reported lower levels of protective antibody concentrations with higher PFDA exposure and higher odds of having antibody levels below a clinically protective level (Zeng et al., 2019b). Exposure contrast was limited across studies, which makes it difficult to interpret the null findings. Associations were slightly stronger in Wang et al. (2022), the only *medium* confidence study with adequate sensitivity (due to slightly higher exposure levels and contrast), but this likely does not fully explain the inconsistency in direction of association across studies. While these results do not provide coherence with the observed antibody response effects, they do not decrease certainty as they are expected to be biased toward the null. Infectious diseases are difficult to measure given the reliance on recall, the subjectivity of symptoms, and variability in physician seeking behavior, and so nondifferential outcome misclassification is expected. Further, there were

concerns about sensitivity in most of the studies due to limited exposure contrast; while the PFDA concentrations may have been adequate to cause decreases in antibody response, it is possible that they were too limited to result in the more downstream infectious disease effects.

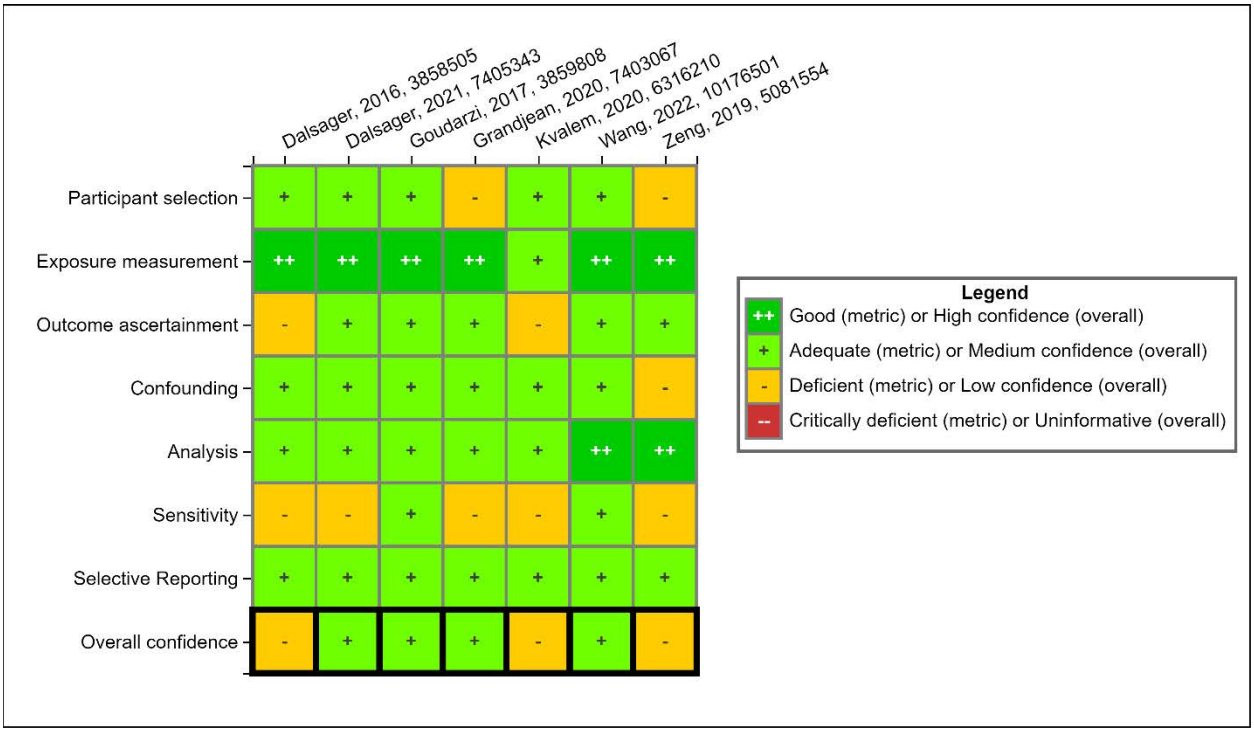


Figure 3-12. Evaluation results for epidemiological studies assessing of PFDA exposure on infectious disease. Refer to HAWC Human Infectious Disease Effects for details on the study evaluation review.

Table 3-12. Studies on PFDA and infectious disease in humans

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA results
Total infectious disease[a]	Goudarzi et al. (2017) *medium*	Maternal; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 4 yr	OR (95% CI): Q1: Ref Q2 1.00 (0.73, 1.35) Q3 0.89 (0.66, 1.21) Q4 0.80 (0.59, 1.08)
	Dalsager et al. (2021a), *medium*	Maternal; median: 0.3	From birth to age 4 yr	HR (95% CI) 1.06 (0.93, 1.22)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA results
Lower respiratory tract infection[b]	Wang et al. (2022), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1 yr	OR (95% CI) for event during first year of life per log10 increase: 1.84 (0.36, 9.49) IRR (95% CI) for count of events per log10 increase: 0.85 (0.26, 2.79)
	Dalsager et al. (2021a), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	From birth to age 4 yr	HR (95% CI) 1.06 (0.85, 1.32)
	Kvalem et al. (2020) *medium*	Child age 10; median (IQR): 1.3 (0.9)	Age 10–16 yr	RR (95% CI) per IQR increase 1.09 (0.86, 1.39)
			Age 16 yr (last 12 mo)	1.34 (0.84, 2.14)
Diarrhea	Dalsager et al. (2016) *low*	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3 yr	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 0.91 (0.53, 1.56) High: 0.91 (0.52, 1.57)
	Wang et al. (2022), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1 yr	OR (95% CI) for event during first year of life per log10 increase: 3.36 (0.90, 12.63) IRR (95% CI) for count of events per log10 increase: **2.16 (1.23, 3.79)***
	Dalsager et al. (2021a), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	From birth to age 4 yr	HR (95% CI) for GI 0.81 (0.46, 1.43)
Common cold (No. episodes/ frequency)	Wang et al. (2022), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1 yr	OR (95% CI) for event during first year of life per log10 increase: 1.66 (0.48, 5.75) IRR (95% CI) for count of events per log10 increase: 1.05 (0.65, 1.68)
	Dalsager et al. (2021a), *medium*	Maternal; median (IQR): 0.6 (0.4–0.8)	From birth to age 4 yr	HR (95%) for upper respiratory tract infection 1.16 (0.95, 1.42)
	Kvalem et al. (2020), *medium*	Child age 10; median (IQR): 1.3 (0.9)	Age 10–16 yr	OR (95% CI) per IQR increase: Reference 1–2 colds

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA results
				3–5 colds: 1.69 (0.46, 6.18) >5: 1.36 (0.39, 4.80)
			Age 16 yr (last 12 mo)	Reference 0 colds 1–2 colds: 0.78 (0.55, 1.09) **≥3: 0.56 (0.37, 0.84)***
Cough	Dalsager et al. (2016) *low*	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3 yr	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 0.63 (0.37, 1.07) High: 0.85 (0.50, 1.46)
Fever	Dalsager et al. (2016) *low*	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1–3 yr	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 1.07 (0.63, 1.81) High: 1.45 (0.85, 2.49)
Hand Foot and Mouth Disease Virus Antibodies	Zeng et al. (2019b), *low*	Cord; median (IQR): 0.1 (0.01–0.2)	Birth and age 3 mo	OR (95% CI) for HFMD antibody concentration below clinically protective level Cord blood: 1.19 (0.82, 1.71) **3 mo: 2.22 (1.42, 3.47)***
COVID-19 severity	Grandjean et al. (2020), *medium*	Biobank prior to illness; median (IQR): 0.1 (0.1–0.2)	Adulthood	OR (95% CI) for 1 unit increase Increased severity based on hospitalization, admission to intensive care and/or death 0.53 (0.10, 2.84)

Bolded values are statistically significant. *$p < 0.05$.
[a]Includes Otitis media, pneumonia, RS virus, Varicella.
[b]Lower respiratory tract infections include bronchitis, bronchiolitis, and pneumonia.

Sensitization and allergic response

Another major category of immune response is the evaluation of sensitization-related or allergic responses that are a result of aggravated immune reactions (e.g., allergies or allergic asthma) to foreign agents (IPCS, 2012). A chemical may be either a direct sensitizer (i.e., promote a specific IgE-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is

prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. While these responses are mediated by circulating factors such as T-cells, immunoglobulin (Ig)E, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of measurements of health effects such as allergies or asthma, and skin prick test responses. Observational tests such as measures of total IgE levels measure indicators of sensitivity and allergic responses but are not a direct measurement of the response. The section is organized by the different types of measurements, starting with functional measures as the most informative.

Seven cohorts (10 publications) examined hypersensitivity outcomes in children. Study evaluations are summarized in Figure 3-13 and Table 3-13. Study sensitivity was a concern across most of the studies, due to narrow exposure contrasts that make interpretation of the null findings difficult.

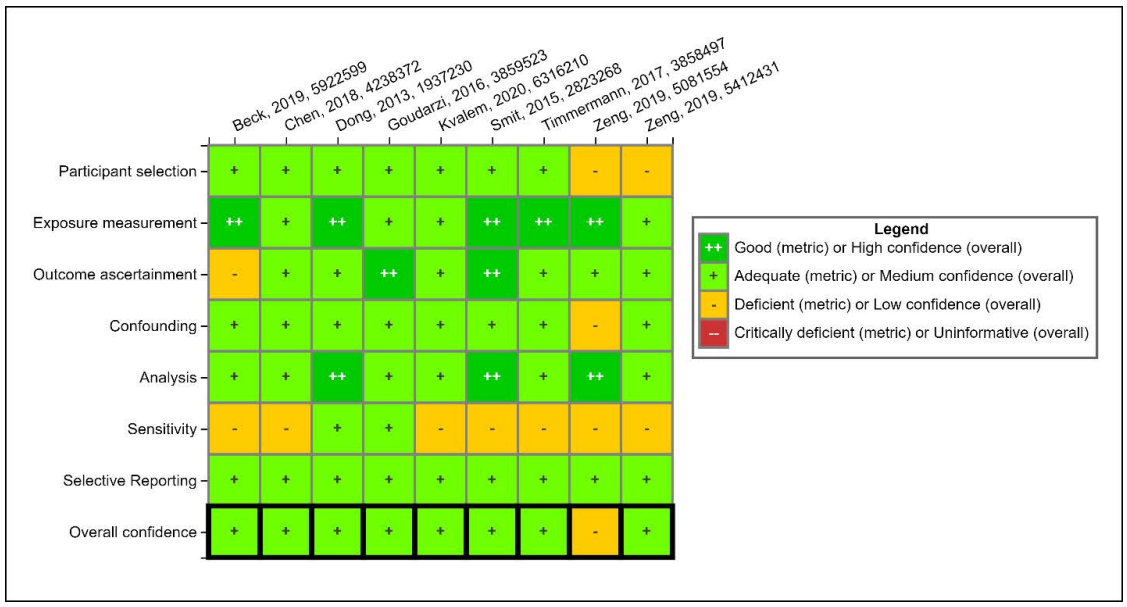


Figure 3-13. Evaluation results of epidemiological studies assessing effects of PFDA exposure on sensitization or allergic response. Refer to HAWC Human Hypersensitivity Effects for details on the study evaluation review.

Functional immune measures of sensitization or allergic response

Asthma

Six studies (eight publications) evaluated any asthma-related outcome in relation to PFDA exposure. One case-control study in Taiwan examined asthma incidence (i.e., physician diagnosis within the past year, identified from two hospitals), which is the most specific measure but may result in under-ascertainment; this study was considered *medium* confidence (Zhou et al., 2017b;

Zhu et al., 2016; Dong et al., 2013). Most available studies examined asthma prevalence (ever diagnosed asthma) and were also considered *medium* confidence including four birth cohorts with prenatal or cord PFDA blood measurements (Beck et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017a; Smit et al., 2015) and one study with PFDA exposure measured in childhood (Kvalem et al., 2020).

Positive associations with asthma were observed in Dong et al. (2013) and Timmermann et al. (2017a) (see Table 3-14), including an exposure-response gradient observed in Dong et al. (2013). However, in Timmermann et al. (2017a), the association was observed only in a small number of subjects (4%, n = 22) that did not receive an MMR vaccine; the effects were statistically significant when both the outcome and PFDA exposure were evaluated when the children were 5 years of age. There remained an increased risk for asthma diagnosis when these same children were 13 years old. No association with childhood exposure was observed in the rest of the study population (that received MMR vaccine), but a positive association was suggested ($p > 0.05$) when using maternal PFDA concentrations as an indication of prenatal exposure (Timmermann et al., 2017a). The Taiwan case-control study used the child's current PFDA concentrations and observed increased ORs in the highest quartile compared with the lowest quartile (concentrations not reported for the quartiles) and in boys and girls with low or high testosterone or high estradiol as well as in boys with low estradiol, indicating there was a modifying effect of sex hormones (Zhou et al., 2017b; Zhu et al., 2016; Dong et al., 2013). Associations were stronger in boys than in girls. Dong et al. (2013) also observed a significant increase in asthma severity scores based on a 13-item questionnaire assessing frequency, use of medicine, and hospitalizations in the highest quartile with a significant increasing trend, but there was no difference in the asthma control test (five-item questionnaire assessing control of asthma symptoms). The other four studies (Kvalem et al., 2020; Beck et al., 2019; Zeng et al., 2019a; Smit et al., 2015) reported no increase in asthma with PFDA exposure. The inconsistency may be accounted for at least in part by study sensitivity, as the Taiwan study with a clear association (Dong et al., 2013) had the highest PFDA exposure levels and was based on asthma incidence within the past year. Asthma incidence is a more specific definition, less likely to suffer from outcome misclassification, than whether the child ever had asthma ("ever asthma"). Nonetheless, overall, there is considerable uncertainty due to the lack of association with asthma in most studies.

Dermal allergic measures – eczema

Four *medium* confidence birth cohorts from different geographic locations in five publications (Chen et al., 2018a; Timmermann et al., 2017a; Goudarzi et al., 2016; Smit et al., 2015; Okada et al., 2014) and one study with exposure measured in childhood (Kvalem et al., 2020) evaluated dermal allergic measures. While the studies used different terminology including eczema, atopic eczema, and atopic dermatitis, all assessed presence of an itchy rash that was coming and going for at least 6 months using the International Study of Asthma and Allergies in Childhood

questionnaire with the exception of Kvalem et al. (2020), which used a different questionnaire. The dermal response conditions can represent hypersensitivity to antigen exposure by way of any exposure route. None of the studies found a significant association between PFDA exposure (either prior exposure, based on maternal or the child's earlier PFDA measurement, or current exposure) and dermal allergic effects (see Table 3-14). However, a nonstatistically significant positive association for eczema was observed in Chen et al. (2018a) and for the children without MMR vaccine in Timmermann et al. (2017a). An inverse association ($p > 0.05$) was observed in multiple studies (Kvalem et al., 2020; Timmermann et al., 2017a; Smit et al., 2015; Okada et al., 2014) (in children with MMR vaccine for Mamsen et al. (2017)). This inconsistency is not clearly explained by study confidence or other factors.

Allergic sensitization/skin prick test

Two *medium* confidence studies conducted skin prick tests. In Timmermann et al. (2017a), they examined five common allergens (birch/grass pollen, dog/cat dander, and house dust mites) in 13-year-old children from the Faroe Islands. A positive result was noted if the subjects developed a wheal ≥3 mm in diameter. In Kvalem et al. (2020), a positive result was noted if there was at least one positive test ≥3 mm at 10 and 16 years but the allergens tested were not described. The relative risk of a positive test was slightly higher ($p > 0.05$) with PFDA exposure in Kvalem et al. (2020) but there was no increase in the odds of having a positive test related to PFDA exposure regardless of when the PFDA was evaluated (i.e., maternal, child at 5 years of age, or current measurement at 13 years of age) in Timmermann et al. (2017a). Both studies had similar exposure contrast.

Observational immune measures of sensitization or allergic response

Two studies also analyzed observational measures including total IgE, eosinophil counts, or eosinophil cationic protein (Timmermann et al., 2017a; Zhu et al., 2016; Dong et al., 2013); of these, IgE measures are considered the most informative. Dong et al. (2013) observed a statistically significant increase in total IgE, eosinophilic cationic protein concentration, and absolute eosinophilic count with increasing current child PFDA concentrations in asthmatics, as well as increased eosinophilic cationic protein concentrations in nonasthmatic people in a population in Taiwan. In the same *medium* confidence study, Zhu et al. (2016) evaluated this further and found that the positive association with IgE was observed in boys and girls with asthma, but only statistically significant in boys. Zhu et al. (2016) expanded the evaluation to additional cytokines (interferon gamma [IFN-γ], interleukin [IL]-2, IL-4, and IL-5) in subjects with and without asthma. While there were occasional statistically significant decreases in the lower quartiles compared with quartile 1 (i.e., IL-2 in males and IFN-γ in females) there was no consistency or trend. In the second *medium* confidence study, Timmermann et al. (2017a) did not find any significant association between IgE levels in cord blood or blood samples from children at age 7 and PFDA concentrations (either maternal concentrations or child's concentration at age 5) in children from the Faroe Islands.

Table 3-13. Studies on PFDA and hypersensitivity-related outcomes in humans

Reference	Study design (location/study)	n	Exposure measure timing	Disease assessment timing	Hypersensitivity outcomes assessed	Study confidence
Maternal exposure						
Beck et al. (2019)	Prospective (Denmark birth cohort)	981	Maternal	Age 5 yr	Asthma (ever)	*Medium*
Chen et al. (2018a) Zeng et al. (2019a)	Prospective (Shanghai Birth Cohort)	687	Cord blood (log-transformed)	Age 2 yr	Eczema	*Medium*
		358		Age 5 yr	Asthma (ever)	
Goudarzi et al. (2016) Okada et al. (2014)	Prospective (Japan/Hokkaido Study of Environment and Children's Health cohort 2003–2013)	1,558	Maternal (quartiles)	Age 4 yr	Total allergic disease, wheeze, eczema, rhinoconjunctivitis symptoms	*Medium*[a]
		2,062	Maternal (quartiles)	From birth to age 2 yr	Wheeze, allergic rhinoconjunctivitis symptoms, eczema, total allergic diseases	
Smit et al. (2015)	Prospective (Greenland, Ukraine/ INUENDO birth cohort)	1,024	Maternal (log-transformed)	Children age 5–9 yr	Asthma (ever), eczema, wheeze	*Medium*
Timmermann et al. (2017a)	Prospective (Faroe Island cohort; 1997–2000)	559	Maternal; child (age 5–13; log-transformed)	Age 5, 7, 13 yr	Total IgE, asthma (ever), allergies, allergic rhinoconjunctivitis, eczema, skin prick test	*Medium* (*low* for asthma)
Child exposure						
Zhou et al. (2017b); Zhu et al. (2016); Dong et al. (2013)	Case-control (Taiwan/ Genetic and Biomarker study for Childhood Asthma)	asthma (231) non (225)	Child: current (quartiles)	Children age 10–15 yr	Asthma incidence and control, total IgE, eosinophil count, eosinophil cationic protein	*Medium*
Kvalem et al. (2020)	Prospective (Norway Environment and Child Asthma)	378	Child: 10 yr	Age 10 and 16 yr	Asthma (ever/current), Eczema, skin prick test	*Medium*

[a]*Medium* vs. *high* confidence based primarily on sensitivity.

Table 3-14. Summary of PFDA and selected data on hypersensitivity in humans

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Asthma			
Smit et al. (2015)	Maternal, mean gest wk 24 or 25; geometric mean (5th–95th percentile): Ukraine 0.16 (0.07–0.35) ng/mL, Greenland 0.42 (0.16–1.16) ng/mL	Child (age 5–9 yr)	Ever asthma Ukraine: 0.80 (0.37, 1.75) per 1 SD change Greenland: 0.93 (0.73, 1.19) per 1 SD change Combined: 0.92 (0.73, 1.16) per 1 SD change
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10 yr)	Ever asthma RR: 0.95 (0.78, 1.15)
		Child (age 10–16 yr)	Asthma between 10 and 16 yr RR: 0.89 (0.67–1.16)
		Child (age 16 yr)	Current asthma (last 12 mo) RR: 0.93 (0.71, 1.22)
Timmermann et al. (2017a)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 5 yr)	Ever asthma 1.09 (0.72, 1.65)
		Child (age 13 yr)	1.26 (0.83, 1.92)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 5 yr)	Ever asthma **No MMR: 4.04 (1.05, 15.50)[c]** Yes MMR: 0.71 (0.48, 1.06), **Interaction *p* = 0.02**
		Child (age 13 yr)	No MMR: 2.87 (0.84, 9.79) Yes MMR: 0.71 (0.48, 1.06), **Interaction *p* = 0.03**
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	Ever asthma 0.84 (0.55, 1.29)
Beck et al. (2019)	Maternal, gest week 8–16; median (IQR): 0.3 (0.2–0.4) ng/mL	Child (age 5 yr)	Ever doctor-diagnosed asthma 0.9 (0.60, 1.44) Ever self-reported asthma (≥episodes of wheezing lasting more than a day in past 12 mo) 1.44 (0.87, 2.41)
Zeng et al. (2019a)	Cord blood median (IQR): 0.4 (0.2–0.5)	Child (age 5 yr)	Ever asthma 0.63 (0.23, 1.72) Girls: 0.21 (0.03, 1.47) Boys: 1.09 (0.26, 4.50)
Dong et al. (2013)	Children, current; range: <0.1–5.0 ng/mL	Child (age 10–15 yr)	Asthma incidence Q2: 1.02 (0.58, 1.80) Q3: 1.30 (0.72, 2.33) Q4: 3.22 (1.75, 5.94), *p*-trend < 0.001

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Zhou et al. (2017b)	Children, current; median (IQR): 1.1 (0.9–1.5) ng/mL with asthma, 1.0 (0.8–1.2) ng/nL without asthma	Child (age 10–15 yr)	Asthma incidence Low Testosterone: M: 1.71 (0.75, 3.90); F: 1.24 (0.60, 2.56) High Testosterone: **M: 3.16 (1.21, 8.25)**; F: 1.37 (0.63, 3.02) Low Estradiol: M: 1.21 (0.60, 2.46); F: 0.76 (0.27, 2.20) High Estradiol: **M: 4.01 (1.46, 11.06);** F: 1.78 (0.94, 3.35) No significant interaction with sex hormone category
Zhu et al. (2016)	Children, current	Child (age 10–15 yr)	Asthma incidence **Q4 vs. Q1** **M: 3.45 (1.51, 7.88); *p*-trend = 0.003** **F: 2.86 (1.16, 7.01); *p*-trend = 0.02**
Allergic sensitization (positive skin prick test)			
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10 yr)	RR: 1.15 (0.99, 1.35)
		Child (age 16 yr)	RR: 1.12 (0.87, 1.45)
Timmermann et al. (2017a)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	1.02 (0.74, 1.41)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	0.79 (0.59, 1.05)
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	0.81 (0.59, 1.13)
Eczema			
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10 yr)	Ever doctor diagnosed: RR: 0.93 (0.79, 1.09)
		Child (age 10–16 yr)	Ever between 10 and 16 yr RR: 0.86 (0.66, 1.12)
		Child (age 16 yr)	Current (last 12 mo) RR: 0.92 (0.68, 1.25)
Chen et al. (2018a)	Cord blood; median (range): 0.36 (<LOD–5.73) ng/mL	Child (age 2 yr)	Ever: 1.22 (0.94, 1.58) per log-unit increase Q2 0.94 (0.55, 1.60) Q3 1.15 (0.68, 1.95) Q4 1.58 (0.94, 2.65), *p*-trend = 0.06
Okada et al. (2014) Goudarzi et al. (2016)	Maternal, gest wk 28–32; median (range): 0.522 (<0.1–2.434) ng/mL	Child (age 1 or 2 yr) Child (age 4 yr)	Ever: Q2 0.80 (0.58, 1.10) Q3 0.78 (0.57, 1.08) Q4 0.85 (0.62, 1.17), *p*-trend = 0.3 Q2: 0.85 (0.59, 1.2) Q3: 0.82 (0.56, 1.18) Q4: 0.93 (0.64, 1.28), *p*-trend = 0.6

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Smit et al. (2015)	Maternal, mean gest wk 24 or 25; geometric mean (5th–95th percentile): Ukraine 0.16 (0.07–0.35) ng/mL, Greenland 0.42 (0.16–1.16) ng/mL	Child (age 5–9 yr)	Current: 0.95 (0.75, 1.20) per 1 SD change Ever: 0.88 (0.73, 1.06) per 1 SD change
Timmermann et al. (2017a)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	Ever: 0.92 (0.64, 1.32)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	Ever: 0.92 (0.64, 1.31)
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13 yr)	No MMR: 401.88 (0.09, 1.84×10^6)[c] Yes MMR: 0.88 (0.58, 1.34), p-interaction = 0.2

Bold font indicates $p < 0.05$.

[a]Exposure timing is organized into groups based on maternal exposure (including cord blood), childhood exposure (including from birth through age 13), and adult exposure.

[b]All estimates are presented as OR (95% CI) for the odds of the outcome per twofold increase in PFDA concentration unless otherwise stated.

[c]Results provided broken down by MMR vaccination status; yes (n = 537) or no (n = 22) when provided; some results were not split by MMR vaccination status

Animal Studies

Animal toxicity studies examining effects on the immune system after PFDA exposure include two 28-day gavage studies using SD rats and/or B6C3F1/N mice (Frawley et al., 2018; NTP, 2018) and a 14-day study in Balb/c mice (inferred as a gavage study based on information provided, although the method of chemical administration was not specified) (Lee and Kim, 2018). Immune effects reported in these studies are discussed according to the immunotoxicity categories and endpoint groupings outlined previously (see Methodological Considerations section above for more details). Most of the available evidence, including host resistance, immune function, and observational assays, were conducted in female mice and rats, since female animals are preferred in immunotoxicity testing due to increased sensitivity (Kadel and Kovats, 2018; Klein and Flanagan, 2016). Further, no chemical-specific information on potential sex-specific differences was identified.

Immunosuppression

Host resistance

Host resistance assays measure the effects of toxicants on the overall immune function in response to a challenge, usually from an infectious agent, and these assays are considered highly relevant to the evaluation of immunotoxicity in the context of human health assessment (IPCS, 2012). Host resistance was evaluated in a 28-day gavage study in female B6C3F1/N mice

considered *medium* confidence primarily due to the lack of reporting on the blinding of investigators during assessment, which raises some concerns for potential observational bias (Frawley et al., 2018) (see Figure 3-14). PFDA did not affect survival of mice challenged with three dilution levels of *Influenza* virus (groups A–C) during the observational period after exposures ended (days 29–50 of the study); exposures ranged from 0.179 to 0.71 mg/kg-day (refer to the interactive HAWC link for additional details). The only effect noted was a slight decrease (7.8%) in body weight at the highest exposure dose (0.71 mg/kg-day) on day 29 in group C, the group challenged with the highest level of influenza. In summary, host resistance appeared to be unaffected by PFDA, although the evidence is limited to a single short-term study in mice.

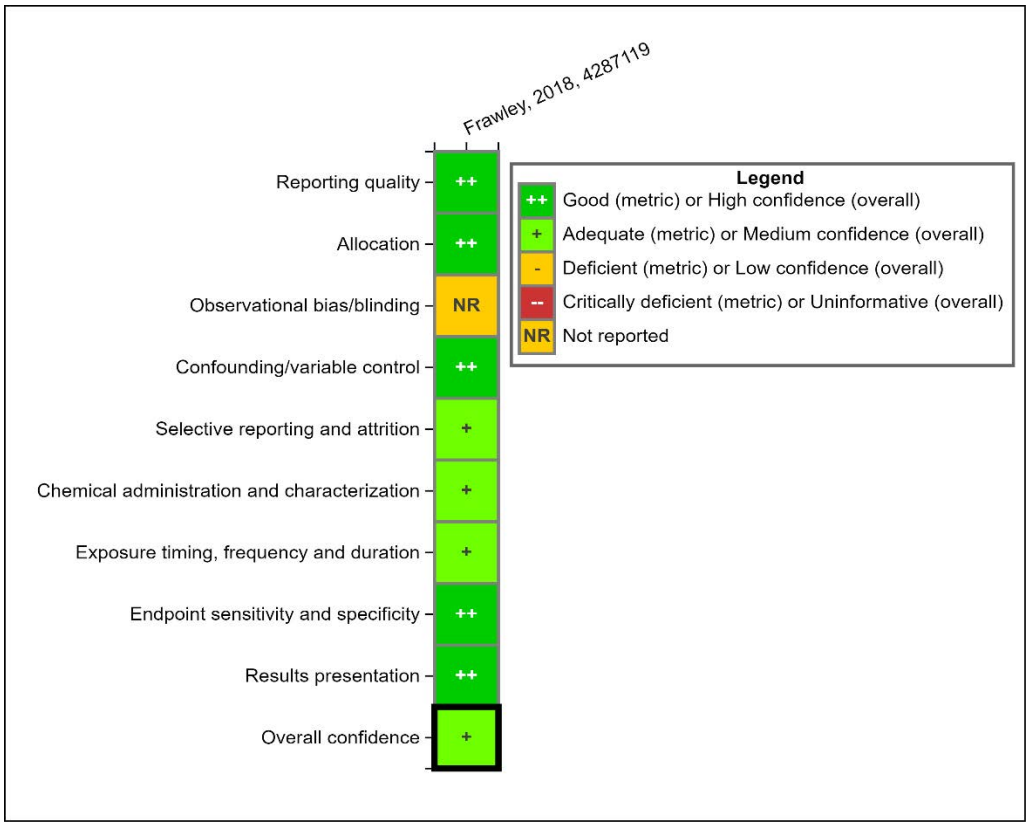


Figure 3-14. Evaluation results for an animal study assessing effects of PFDA exposure on host resistance. Refer to HAWC for details on the study evaluation review.

Immune function assays

Markers of altered immune cell function or damage were evaluated in female B6C3F1/N mice and female SD rats exposed to doses of 0.045–0.71 and 0.125–0.5 mg/kg-day, respectively, for 28 days via gavage (Frawley et al., 2018). Immune function assays included measures of: (1) innate immunity such as mononuclear phagocyte system (MPS) activity in rats and natural killer (NK) cell activity in rats and mice; (2) humoral-mediated immunity such as T-dependent antibody responses

in rats and mice; and (3) cell-mediated immunity such as mixed leukocyte response in mice and delayed-type hypersensitivity in rats and mice. These assays measure specific immune system responses to a stimulus both at the cellular and organism level and can provide clear and direct evidence of immunotoxicity (IPCS, 2012). Overall, study confidence in experiments conducted in both species was high for most endpoints, except delayed-type hypersensitivity (DTH). The absence of information on the blinding or any other strategy used to mitigate potential for observational bias resulted in a *medium* confidence rating for this endpoint (see Figure 3-15).

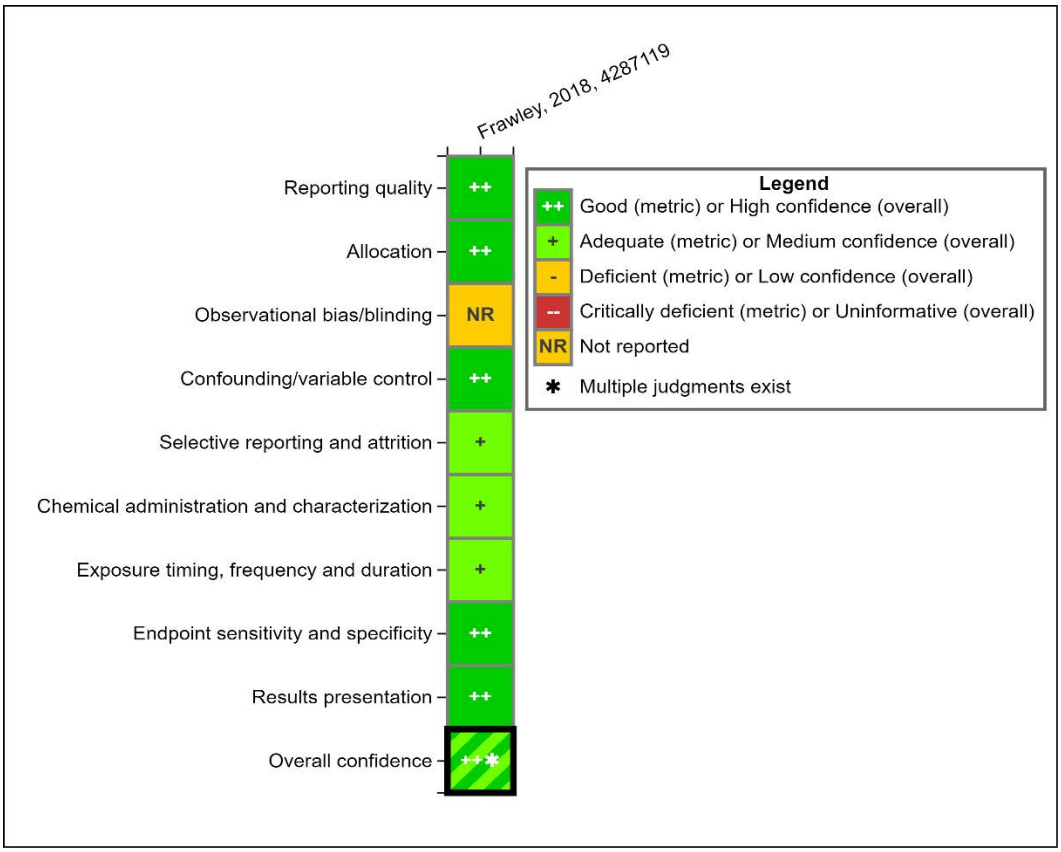


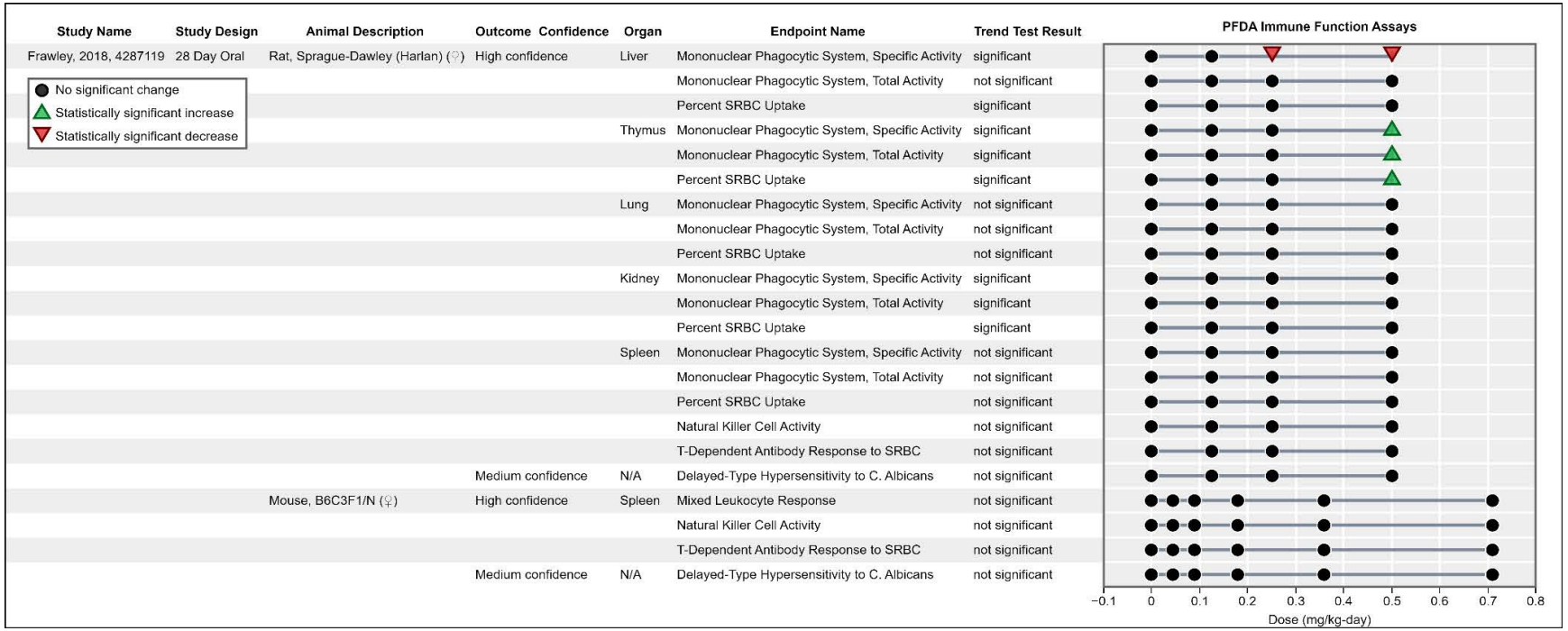
Figure 3-15. Evaluation results for animal studies assessing effects of PFDA exposure on immune function assays. Refer to HAWC for details on the study evaluation review.

Dose-related decreases in specific activity of the MPS (cpm/mg of tissue) were reported in rat liver (MPS was not examined in mice) at 0.125–0.5 mg/kg-day (15%–45% compared with controls), reaching statistical significance at the two highest doses (see Figure 3-16). Alterations in phagocytic activity coincide with the liver histopathology (i.e., hepatocyte necrosis) and increased liver weight (see Section 3.2.1 on liver effects for more details) observed in the exposed animals. Because of the increases in liver weight, it is possible that the effects on specific activity could represent changes in hepatocyte numbers/size rather than alterations in the functional activity of tissue macrophages (Frawley et al., 2018). However, a decreasing trend was also observed for total

MPS activity (p = 0.051) and percent (%) uptake of sheep red blood cell (SRBC) by macrophages in the liver (p = 0.029).

MPS activity was evaluated in other rat tissues such as the thymus, lung, kidney, and spleen. In the thymus, MPS activity (total, specific, and % SRBC uptake) was significantly increased at the highest exposure dose (139%–200% at 0.5 mg/kg-day) (Frawley et al., 2018) (see Figure 3-16). However, the values for total activity and % uptake were two orders of magnitude lower than the negative control tissue (kidney), which raises concerns about the biological significance of these results. No treatment-related effects were found in MPS activity in the lung and spleen of rats (see Figure 3-16).

Apart from the reduced MPS activity in rat liver after PFDA exposure, no treatment-related effects were observed in other immune function assays evaluated in rats and mice (i.e., NK cell activity and T-dependent antibody responses to SRBC in the spleen of rats and mice, mixed leukocyte response in mouse spleen, and DTH response to *Candida albicans* in rats and mice). Despite a general lack of findings from most immune function assays, the mild reductions in phagocytic activity in rat liver suggest potential suppression of innate immunity after short-term PFDA exposure, although uncertainties remain surrounding whether this finding might be attributable to the observed liver toxicity.



Study Name	Study Design	Animal Description	Outcome Confidence	Organ	Endpoint Name	Trend Test Result
Frawley, 2018, 4287119	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	High confidence	Liver	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	significant
				Thymus	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	significant
					Percent SRBC Uptake	significant
				Lung	Mononuclear Phagocytic System, Specific Activity	not significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	not significant
				Kidney	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	significant
					Percent SRBC Uptake	significant
				Spleen	Mononuclear Phagocytic System, Specific Activity	not significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	not significant
					Natural Killer Cell Activity	not significant
					T-Dependent Antibody Response to SRBC	not significant
			Medium confidence	N/A	Delayed-Type Hypersensitivity to C. Albicans	not significant
		Mouse, B6C3F1/N (♀)	High confidence	Spleen	Mixed Leukocyte Response	not significant
					Natural Killer Cell Activity	not significant
					T-Dependent Antibody Response to SRBC	not significant
			Medium confidence	N/A	Delayed-Type Hypersensitivity to C. Albicans	not significant

Figure 3-16. Effects on immune function assays following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

General/Observational Immune Assays

General or observational immune parameters were evaluated in two experiments (reported in one study) using female B6C3F1/N mice and female SD rats after 28-day gavage exposure (Frawley et al., 2018). The 28-day experiments were *high* confidence for most endpoints, except bone marrow colony formation (see Figure 3-17). Key issues regarding observational bias/blinding and results presentation (i.e., ambiguity surrounding sample size) reduced confidence to *medium* for this endpoint. The assays included in the study are spleen cell immunophenotyping (rats and mice), anti-CD3+-mediated T-cell proliferation (rats and mice), bone marrow DNA synthesis (rats and mice), and bone marrow colony formation and differentials (rats only) (Frawley et al., 2018). These assays can indicate changes in immune cell populations and mediators and are often used in support of more predictive measures of immunotoxicity (i.e., host resistance and functional assays) (IPCS, 2012).

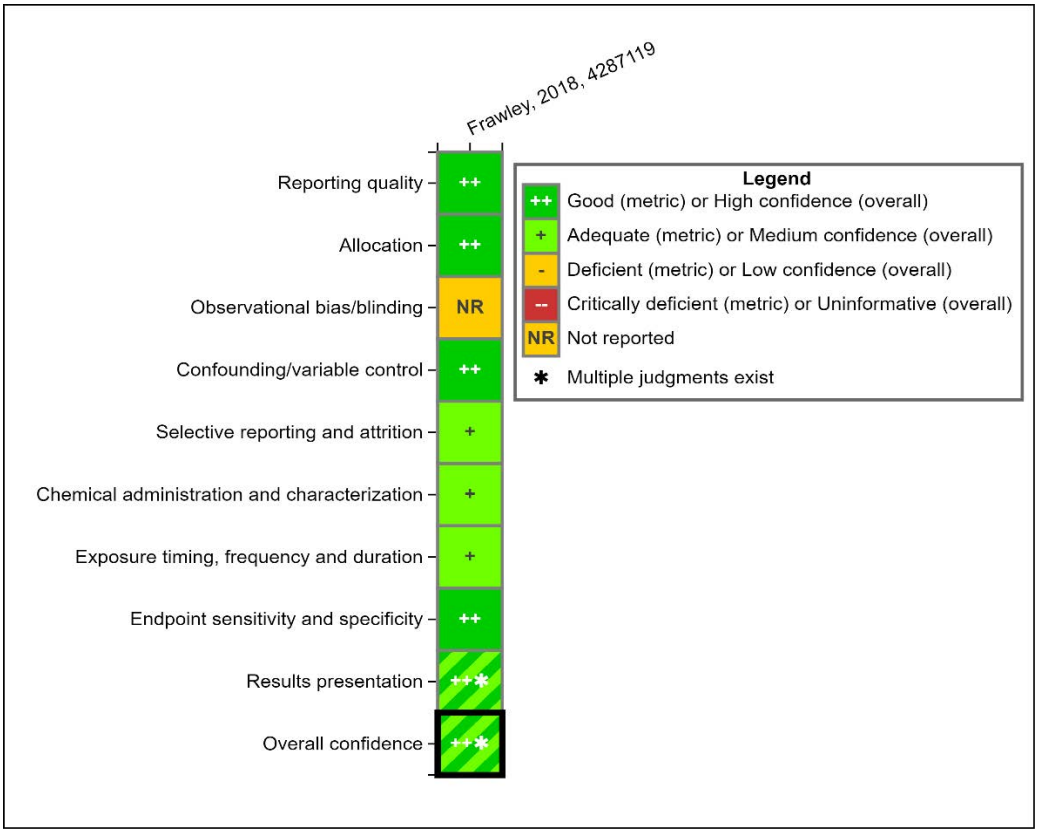


Figure 3-17. Evaluation results for animal studies assessing effects of PFDA exposure on general/observational immune assays. Refer to HAWC for details on the study evaluation review.

PFDA treatment caused dose-related reductions in absolute spleen cell numbers in mice reaching up to 24% decrease compared with controls at the highest dose (0.71 mg/kg-day) (see Table 3-15 and Figure 3-18). Likewise, absolute counts of splenic B-cells, T-cells, T-helper cells,

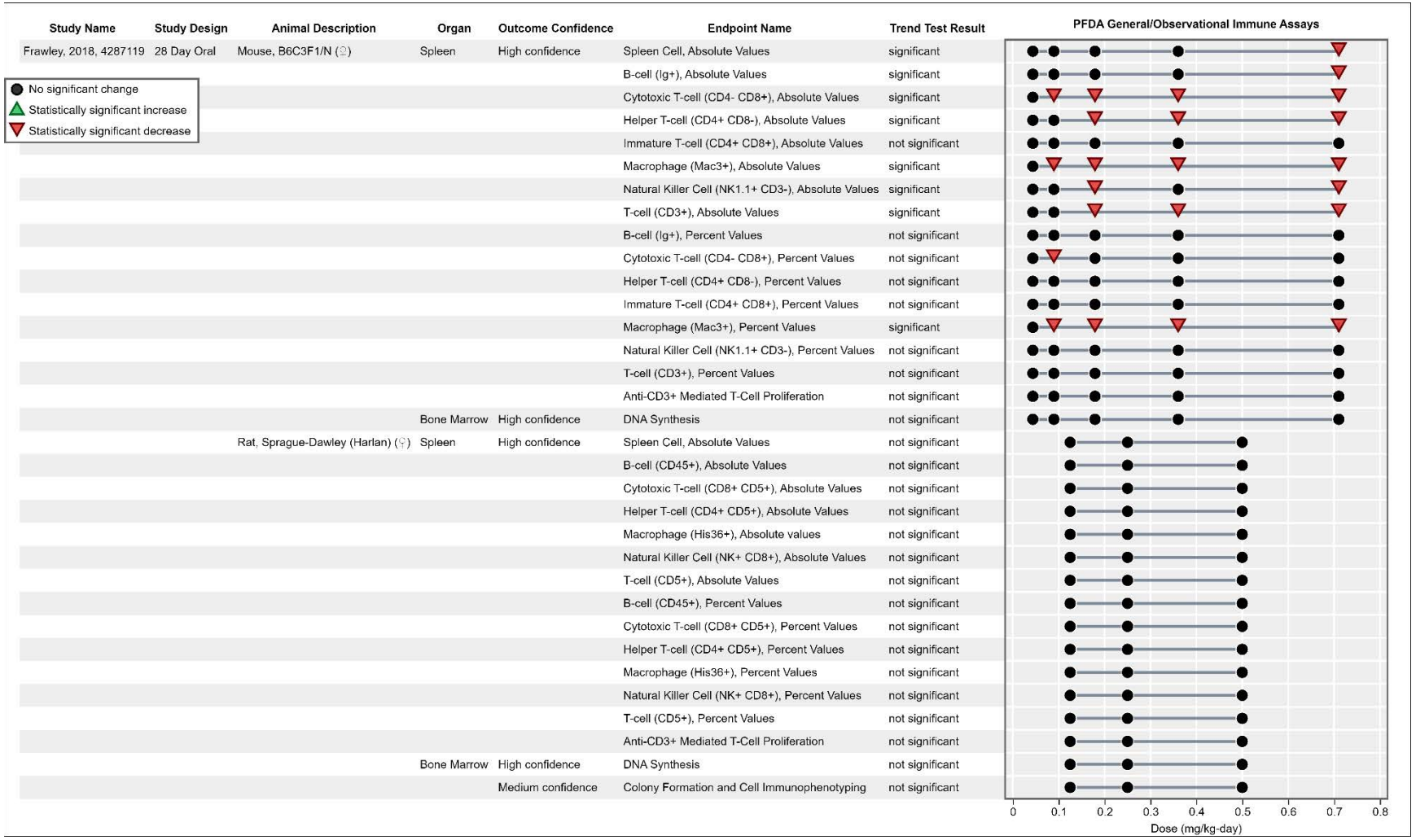
cytotoxic T-lymphocytes, NK cells, and macrophages displayed a decreasing trend and achieved statistical significance at doses ≥0.089 mg/kg-day; absolute counts of immature T-cells were not affected by PFDA exposure (percent changes from controls are summarized in Table 3-15). The relative percentages of spleen immune cell populations in mice were largely unchanged, except for macrophages, which showed dose-related reductions at similar doses (13%–19% relative to controls over 0.089–0.71 mg/kg-day). The mostly null findings in the relative percentage values of spleen cell immunophenotypes likely reflect the observed spleen atrophy in animals (i.e., decreases in spleen cell numbers and spleen weights [see synthesis on Histopathology and organ weights below for more details]) (Frawley et al., 2018). Furthermore, a lack of treatment-related effects was reported for other observational immune assays evaluated in mice (i.e., anti-CD3+-mediated T-cell proliferation and bone marrow DNA synthesis) (see Figure 3-18). In rats, results were null with PFDA exposure (0.125–0.5 mg/kg-day) in assays of spleen cell immunophenotyping (including spleen cell numbers and immune cell populations), anti-CD3+-mediated T-cell proliferation and bone marrow DNA synthesis, colony formation and progenitor cell populations (see Figure 3-18).

The reductions in absolute immune cell populations in mouse spleen provide evidence consistent with potential immunosuppression following short-term PFDA exposure, although uncertainties related to the overt organ toxicity (i.e., spleen atrophy) remain.

Table 3-15. Percent change relative to controls in absolute spleen cell population counts in female B6C3F1/N mice exposed to PFDA exposure for 28-days (Frawley et al., 2018)

Endpoint	Dose (mg/kg-d)				
	0.045	0.089	0.179	0.36	0.71
Spleen cell	−2	−8	−13	−8	**−24**
B-cell (Ig+)	3	0.3	−10	−4	**−27**
Cytotoxic T-cell ($CD4^-$ $CD8^+$)	−10	**−19**	**−22**	**−10**	**−28**
Helper T-cell ($CD4^+$ $CD8^-$)	−11	−13	**−19**	**−12**	**−29**
Immature T-cell ($CD4^+$ $CD8^+$)	−21	−53	−21	−16	−53
Macrophage ($Mac3^+$)	−13	**−21**	**−31**	**−25**	**−39**
Natural killer cell ($NK1.1^+$ $CD3^-$)	−15	−15	**−18**	−16	**−18**
T-cell ($CD3^+$)	−9	−15	**−22**	**−14**	**−28**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies.



Study Name	Study Design	Animal Description	Organ	Outcome Confidence	Endpoint Name	Trend Test Result
Frawley, 2018, 4287119	28 Day Oral	Mouse, B6C3F1/N (♀)	Spleen	High confidence	Spleen Cell, Absolute Values	significant
					B-cell (Ig+), Absolute Values	significant
					Cytotoxic T-cell (CD4- CD8+), Absolute Values	significant
					Helper T-cell (CD4+ CD8-), Absolute Values	significant
					Immature T-cell (CD4+ CD8+), Absolute Values	not significant
					Macrophage (Mac3+), Absolute Values	significant
					Natural Killer Cell (NK1.1+ CD3-), Absolute Values	significant
					T-cell (CD3+), Absolute Values	significant
					B-cell (Ig+), Percent Values	not significant
					Cytotoxic T-cell (CD4- CD8+), Percent Values	not significant
					Helper T-cell (CD4+ CD8-), Percent Values	not significant
					Immature T-cell (CD4+ CD8+), Percent Values	not significant
					Macrophage (Mac3+), Percent Values	significant
					Natural Killer Cell (NK1.1+ CD3-), Percent Values	not significant
					T-cell (CD3+), Percent Values	not significant
					Anti-CD3+ Mediated T-Cell Proliferation	not significant
			Bone Marrow	High confidence	DNA Synthesis	not significant
		Rat, Sprague-Dawley (Harlan) (♀)	Spleen	High confidence	Spleen Cell, Absolute Values	not significant
					B-cell (CD45+), Absolute Values	not significant
					Cytotoxic T-cell (CD8+ CD5+), Absolute Values	not significant
					Helper T-cell (CD4+ CD5+), Absolute Values	not significant
					Macrophage (His36+), Absolute values	not significant
					Natural Killer Cell (NK+ CD8+), Absolute Values	not significant
					T-cell (CD5+), Absolute Values	not significant
					B-cell (CD45+), Percent Values	not significant
					Cytotoxic T-cell (CD8+ CD5+), Percent Values	not significant
					Helper T-cell (CD4+ CD5+), Percent Values	not significant
					Macrophage (His36+), Percent Values	not significant
					Natural Killer Cell (NK+ CD8+), Percent Values	not significant
					T-cell (CD5+), Percent Values	not significant
					Anti-CD3+ Mediated T-Cell Proliferation	not significant
			Bone Marrow	High confidence	DNA Synthesis	not significant
				Medium confidence	Colony Formation and Cell Immunophenotyping	not significant

Figure 3-18. Effects on general/observational immune assays following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Blood leukocyte counts

Hematological evaluations of potential alterations in blood leukocyte (white blood cell) counts with PFDA treatment comes from three *high* confidence experiments (reported in two studies) with gavage exposure for 28 days: one in female B6C3F1/N mice (Frawley et al., 2018) and two in male and female SD rats (Frawley et al., 2018; NTP, 2018) (see Figure 3-19). The parameters evaluated included leukocyte counts and differentials (basophils, eosinophils, lymphocytes, monocytes, and neutrophils). For lymphocytes, both absolute counts and total counts (absolute plus large lymphocytes such as lymphoblasts or reactive lymphocytes) were provided.

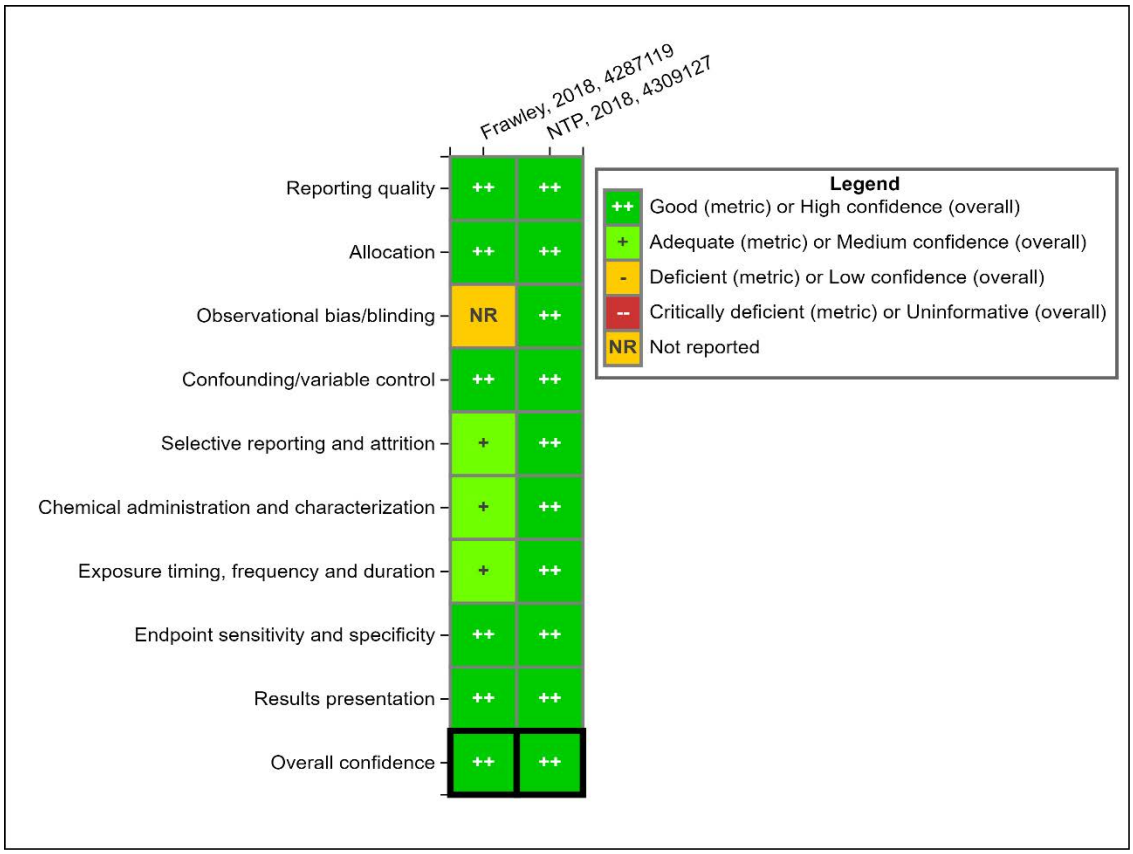


Figure 3-19. Evaluation results for animal studies assessing effects of PFDA exposure on blood leukocyte counts. Refer to HAWC for details on the study evaluation review.

The effects of PFDA exposure on blood leukocyte counts in animals are unclear (see Table 3-16 and Figure 3-20). Frawley et al. (2018) found no treatment-related effects on blood leukocyte numbers and differentials in female mice and female rats with exposures up to 0.71 and 0.5 mg/kg-day, respectively (males were not examined). In a separate study by NTP (2018), statistically significant changes were noted in circulating leukocytes in female rats (but not males) at higher doses (≥1.25 mg/kg-day). Specifically, the number of basophils increased by 157% and

71% compared with controls at doses of 1.25 and 2.5 mg/kg-day, respectively, while the number of monocytes increased by 41% at the high-dose group (2.5 mg/kg-day). Leukocyte and lymphocyte (total and absolute) numbers were elevated at 1.25 mg/kg-day (37%–41% compared with controls) but not at 2.5 mg/kg-day (0% compared with controls). Conversely, eosinophil counts decreased up to 64% at a dose of 2.5 mg/kg-day. In general, the hematological data suggests increases in blood leukocyte counts and populations in female rats. The biological significance of these findings is uncertain given the inconsistencies in the directionality of changes across dose groups in the (NTP, 2018) study and, more importantly, the lack of coherent evidence in other endpoints supportive of a potential immunostimulatory response following PFDA exposure. Additionally, the observed hematological changes occurred mostly at high PFDA doses (≥1.25 mg/kg-day) associated with adverse systemic effects (see Section 3.2.10 for more details).

Table 3-16. Percent change relative to controls in blood leukocyte counts in female Sprague-Dawley rats exposed to PFDA exposure for 28-days (NTP, 2018)

Endpoint	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Basophils	61	14	43	**157**	**71**
Eosinophils	−27	−27	−18	−9	**−64**
Leukocytes	15	11	2	**37**	0
Lymphocyte (absolute)	18	14	3	**41**	0
Lymphocyte (total)	19	15	3	**41**	0
Monocytes	0	6	−12	24	**41**
Neutrophils	−9	−17	−6	9	0

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies.

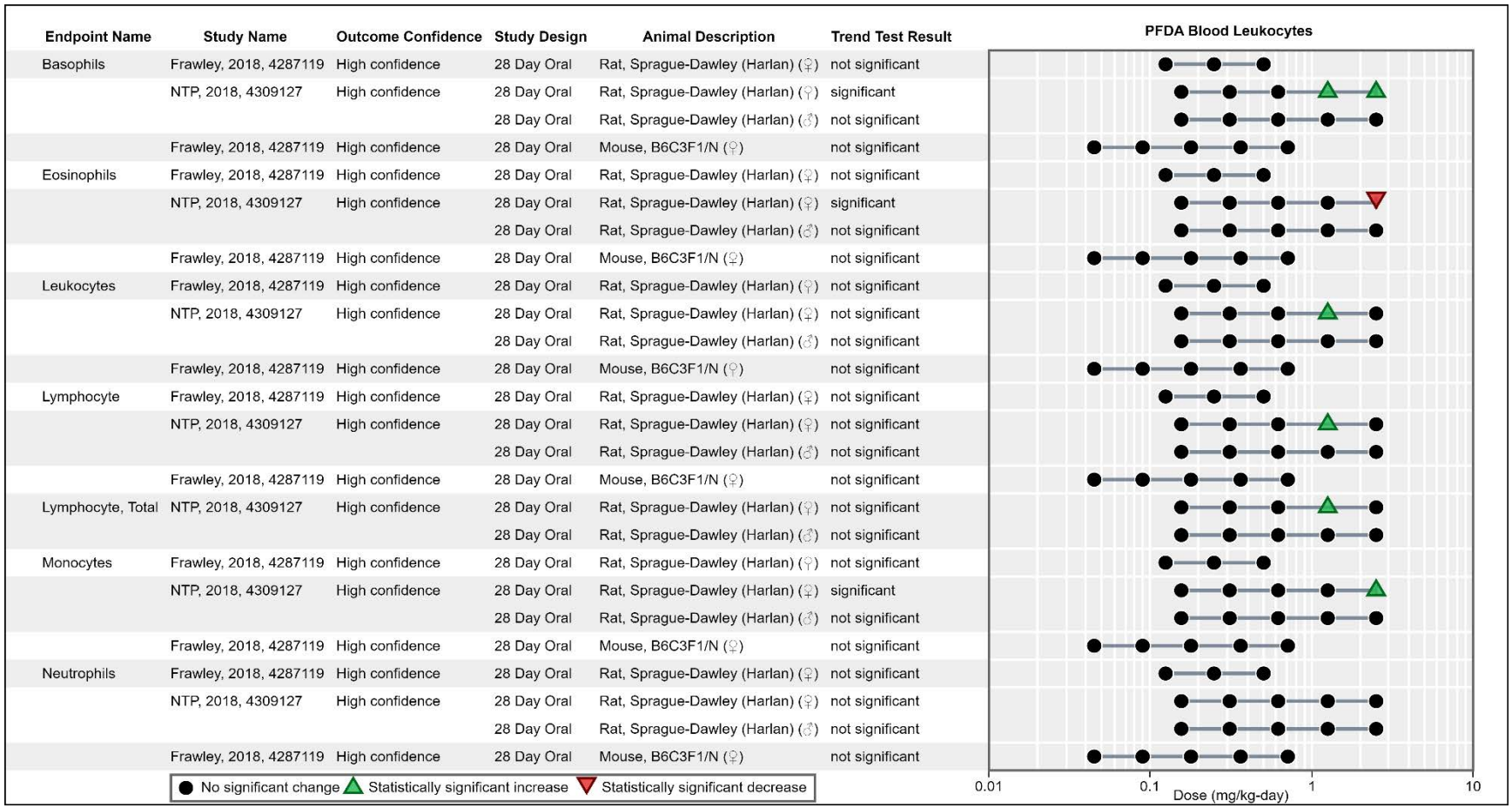


Figure 3-20. Effects on blood leukocyte counts following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Histopathology and organ weights

The data on immune histopathology and organ weights is described in one study using female B6C3F1/N mice and female SD rats (Frawley et al., 2018) and one study using male and female SD rats (NTP, 2018), both with exposure to PFDA for 28 days via gavage. The NTP (2018) study was *high* confidence for histopathology and organ weight measures. Frawley et al. (2018) was considered *high* confidence for the evaluation of organ weight but exhibited deficiencies in the presentation and discussion of histopathological findings (lack of quantitative data), which resulted in a *medium* confidence rating for this endpoint (see Figure 3-21).

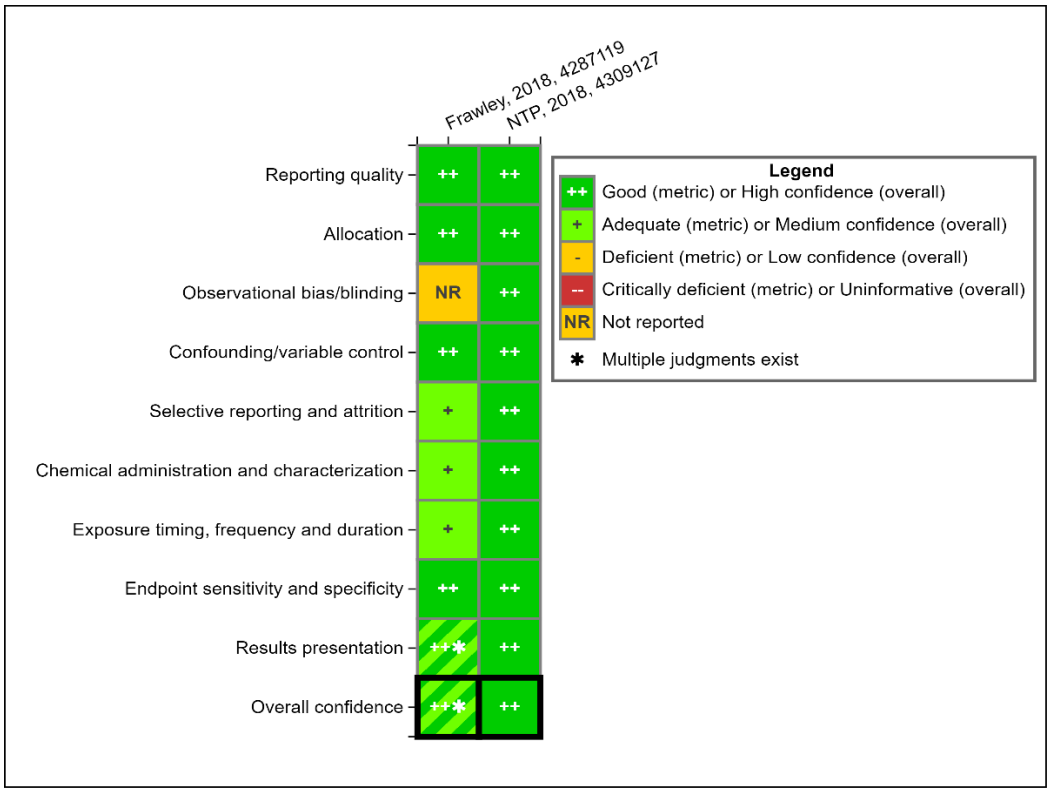


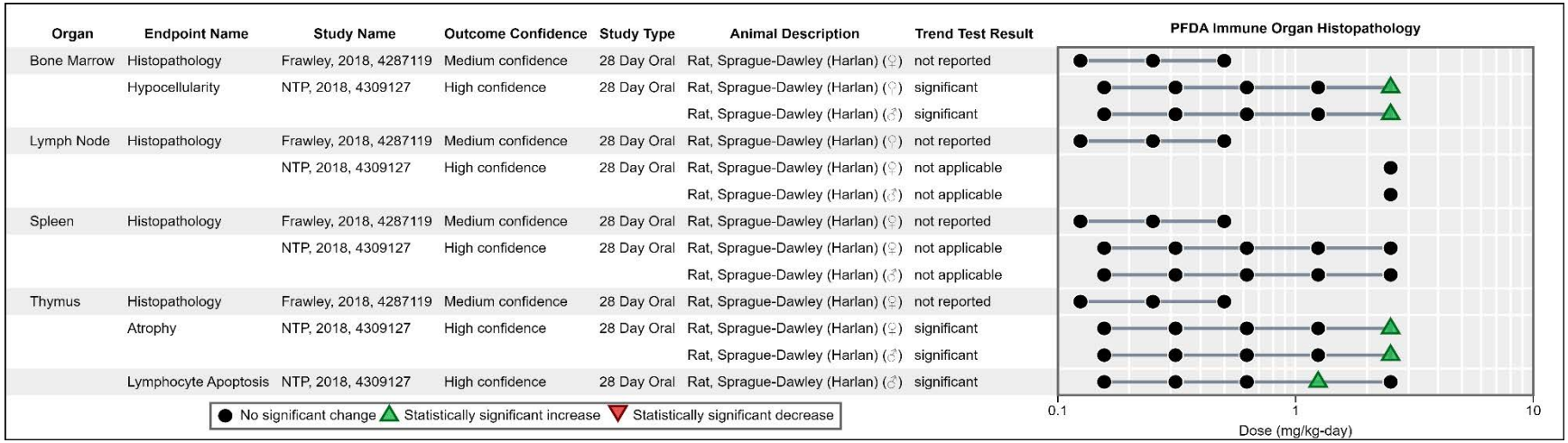
Figure 3-21. Evaluation results for animal studies assessing effects of PFDA exposure on immune histopathology and organ weights. Refer to HAWC for details on the study evaluation review.

Animal toxicity studies provide some evidence of immune organ histopathology (see Figure 3-22). The bone marrow, lymph nodes, spleen, and thymus were examined histologically in male and female rats exposed to doses ranging from 0.125 to 2.5 mg/kg-day (Frawley et al., 2018; NTP, 2018). No treatment-related effects were found in any of these organs at doses ≤0.625 mg/kg-day across the two rat studies, but morphological changes were observed in bone marrow and thymus in the study that tested higher doses (≥1.25 mg/kg-day) (NTP, 2018). Increased incidences of bone marrow hypocellularity (10/10 in males and females) and thymic atrophy (9/10 in males and 8/10 in females) were observed in rats at the highest dose

(2.5 mg/kg-day), while incidence of lymphocyte apoptosis in the thymus was increased in males only at a dose of 1.25 mg/kg-day (8/10 rats). The aforementioned lesions ranged from mild to moderate in severity and did not occur in the controls or in other exposure groups.

Changes in immune organ weights were reported in female mice and male/female rats across two 28-day gavage studies (Frawley et al., 2018; NTP, 2018) (see Table 3-17 and Figure 3-23). The rat study by Frawley et al. (2018) included three cohorts exposed to similar experimental conditions. Statistically significant decreases in spleen weights (absolute and relative) were observed across species and sexes at ≥0.179 mg/kg-day, reaching 55% in rats and 22% in mice relative to controls at the highest doses tested (2.5 and 0.71 mg/kg-day, respectively) (Frawley et al., 2018; NTP, 2018). Although there were no notable histopathological findings in the spleen, the organ weight reductions in mice are concordant with alterations in spleen cell numbers and populations previously described (see synthesis on general/observational immune assays above for additional details). Absolute and relative thymus weights decreased in a dose-dependent manner (8–75% compared with controls) at ≥1.25 mg/kg-day in rats that exhibited thymic lesions (atrophy and apoptosis) and marked body weight reductions in one study (NTP, 2018). In contrast, another study reported increases in absolute and relative thymus weights in rats at lower PFDA doses (0.125–0.5 mg/kg-day) but the results were not consistent across study cohorts and in most cases did not show a dose-response dependency (Frawley et al., 2018) (see Table 3-21). As such, the significance of the increases in thymus weights in rats is uncertain. Thymus weights in mice were not affected by PFDA treatment (up to 0.71 mg/kg-day) in one study (Frawley et al., 2018).

In summary, histopathological lesions were found in the bone marrow and thymus of rats and decreased spleen and thymus weights were reported in mice and/or rats after short-term PFDA exposure. The effects on spleen weights are coherent with reductions in spleen cell counts and populations in mice at ≥0.089 mg/kg-day. The bone marrow and thymus lesions in rats were only observed in the presence of marked reductions in body weight (12%–38% relative to controls) at PFDA doses ≥1.225 mg/kg-day, which provides a significant source of uncertainty. Indeed, bone marrow hypocellularity and thymic atrophy have been linked to diet restriction in short-term rat studies (Levin et al., 1993) and PFDA-induced wasting syndrome characterized by decreased food consumption and rapid weight loss has been well documented in animals (see Section 3.2.10 for more details). As such, the toxicological significance of the histopathological findings is uncertain.



Organ	Endpoint Name	Study Name	Outcome Confidence	Study Type	Animal Description	Trend Test Result
Bone Marrow	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Hypocellularity	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
					Rat, Sprague-Dawley (Harlan) (♂)	significant
Lymph Node	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
		NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
					Rat, Sprague-Dawley (Harlan) (♂)	not applicable
Spleen	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
		NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
					Rat, Sprague-Dawley (Harlan) (♂)	not applicable
Thymus	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Atrophy	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
					Rat, Sprague-Dawley (Harlan) (♂)	significant
	Lymphocyte Apoptosis	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant

Figure 3-22. Effects on immune organ histopathology following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Table 3-17. Percent change relative to controls in immune organ weights in short-term animal studies after exposure to PFDA

Animal group	Dose (mg/kg-d)						
	0.045	0.089	0.125–0.179	0.25–0.36	0.5–0.71	1.25	2.5
Spleen weight (absolute) Male SD rats NTP (2018)			11	0	−4	**−26**	**−49**
Spleen weight (absolute) Female SD rats NTP (2018)			1	−2	−9	**−36**	**−55**
Spleen weight (absolute) Female C57BL/6N mice Frawley et al. (2018)	−3	2.8	−18	−6	**−20**		
Spleen weight (relative) Male SD rats NTP (2018)			7	1	−1	**−6**	**−19**
Spleen weight (relative) Female SD rats NTP (2018)			−3	−5	**−9**	**−27**	**−30**
Spleen weight (relative) Female C57BL/6N mice Frawley et al. (2018)	−3	−6	**−16**	**−9**	**−22**		
Thymus weight (absolute) Male SD rats NTP (2018)			1	0	−1	**−44**	**−75**
Thymus weight (absolute) Female SD rats NTP (2018)			5	18	9	−20	**−65**
Thymus weight (absolute) Female SD rats; MPS cohort Frawley et al. (2018)			13	23	13		
Thymus weight (absolute) Female SD rats; Histopathology cohort Frawley et al. (2018)			−5	3	−1		
Thymus weight (absolute) Female SD rats; TDAR to SRBC cohort Frawley et al. (2018)			**34**	**30**	21		
Thymus weight (relative) Male SD rats NTP (2018)			−2	0	3	**−29**	**−61**
Thymus weight (relative) Female SD rats NTP (2018)			1	12	9	−8	**−46**
Thymus weight (relative) Female SD rats; MPS cohort Frawley et al. (2018)			18	27	18		
Thymus weight (relative) Female SD rats; Histopathology cohort Frawley et al. (2018)			−7	0	0		
Thymus weight (relative)			**36**	**21**	14		

Animal group	Dose (mg/kg-d)						
	0.045	**0.089**	**0.125–0.179**	**0.25–0.36**	**0.5–0.71**	**1.25**	**2.5**
Female SD rats; TDAR to SRBC cohort Frawley et al. (2018)							

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies.

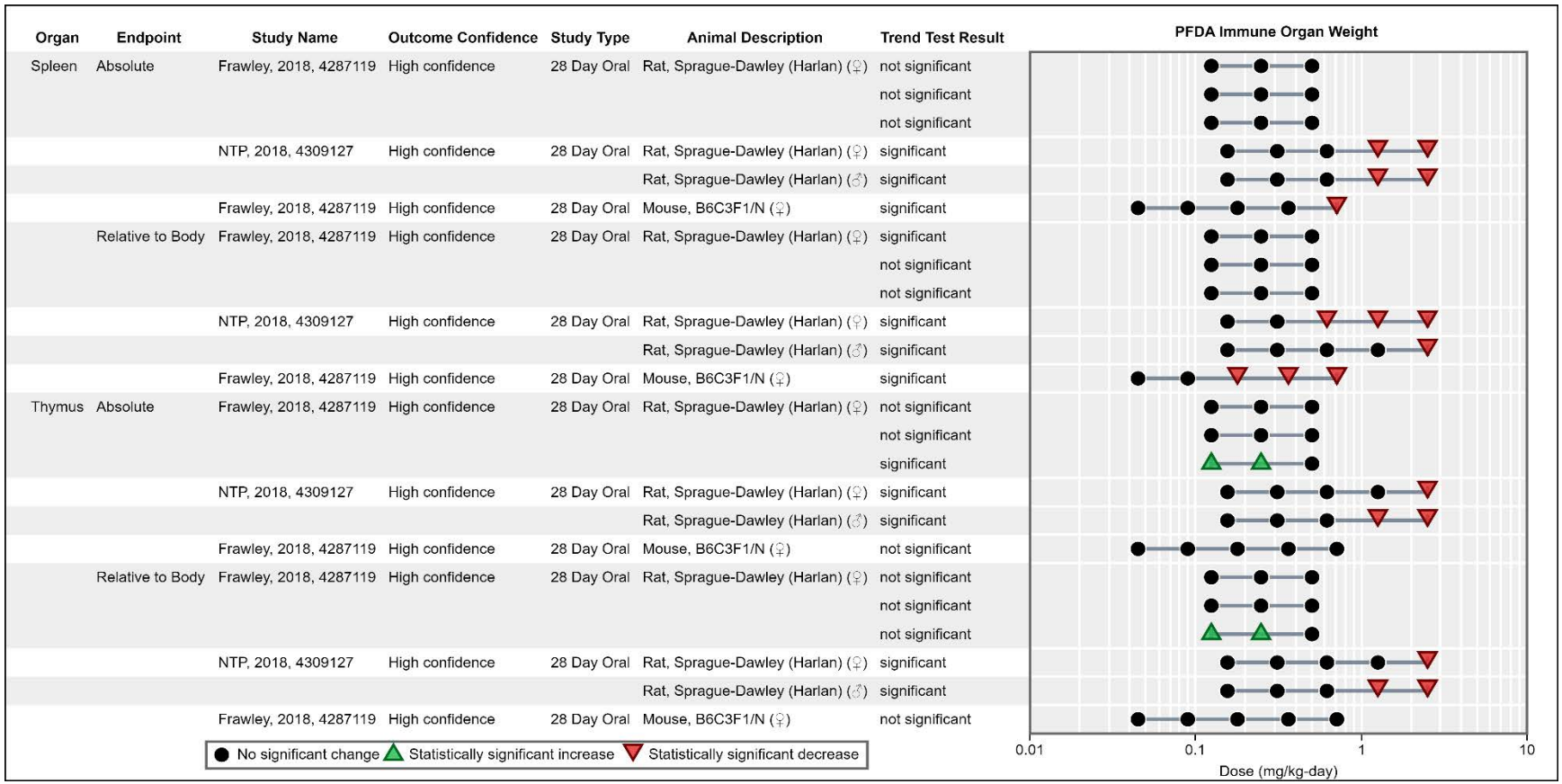


Figure 3-23. Effects on immune organ weights following exposure to PFDA in short-term oral studies in animals. The rat study by **Frawley et al. (2018)** included three cohorts exposed to similar experimental conditions. (Results can be viewed by clicking the HAWC link.)

Sensitization and Allergic Response

Immune function assays

A 14-day study in male ICR mice exposed to a dose of 21.4 mg/kg-day examined the effect of PFDA treatment on ovalbumin (OVA)-induced active systemic anaphylaxis (Lee and Kim, 2018), a well-accepted model for evaluating mast cell function and allergic reactions (Je et al., 2015; Evans et al., 2014; Ribeiro-Filho et al., 2014). The study was rated as *low* confidence due to issues with reporting on potential confounding effects (no information on general systemic toxicity measures; this excessive dose would be expected to cause significant, overt toxicity given observations, including "wasting syndrome," from other short-term studies with similar dosing paradigms; see Section 3.2.10 for more details), experimental groups (no indication of randomization), and the characterization of the test compound (no information on analytical verification or specific method of administration) (see Figure 3-24).

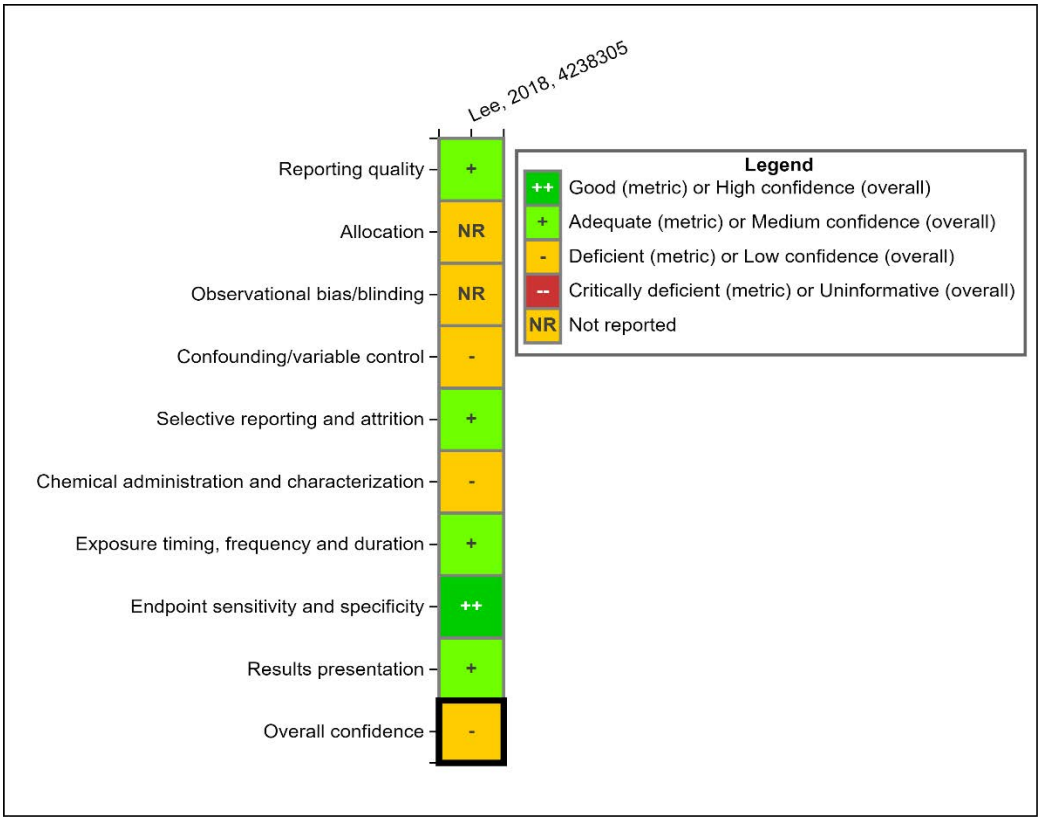


Figure 3-24. Evaluation results for an animal study assessing effects of PFDA exposure on immune function assays for sensitization and allergic response. Refer to HAWC for details on the individual study evaluation review.

PFDA (21.4 mg/kg-day) exacerbated the response to OVA-induced active systemic anaphylaxis in mice as indicated by a significant decrease in rectal temperature (i.e., hypothermia) and significant elevation in serum levels of inflammatory mediators such histamine, TNFα and

immunoglobulins (IgE and IgG1) compared with OVA treatment alone (Lee and Kim, 2018). Histamine is released in response to mast cell degranulation and plays a key role in immediate-type hypersensitivity (Amin, 2012). The findings from the Lee and Kim (2018) study suggest possible induction of immediate-type hypersensitivity, although the exposure dose was high compared with doses associated with immunosuppressive responses in animals (0.089–2.5 mg/kg-day) and raises concerns over potential confounding with general toxicity effects. Although the study provided no information on general toxicity measures, PFDA exposure was associated with significant body weight reductions at doses ≥1.25 mg/kg-day in oral exposure studies and the induction of wasting syndrome in acute, i.p. injection studies at doses ≥20 mg/kg (see Section 3.2.10 for more details).

Mechanistic Studies and Supplemental Evidence

The available supplemental evidence most relevant to interpretation consists of an acute i.p. injection study evaluating immunotoxicity endpoints in exposed rats and a few in vitro studies in human and animal models examining possible mechanisms of immunotoxicity following PFDA exposure.

An acute i.p. injection study investigating potential immune effects of PFDA exposure (20 and 50 mg/kg) in Fischer 344 rats showed reductions in the antibody (i.e., serum Keyhole limpet hemocyanin [KLH]-specific IgG2a levels) and DTH responses to KLH in exposed animals (Nelson et al., 1992); the effects on the DTH response were not statistically significant but showed a decreasing trend with increase in dose at each time point (40%–46% and 38%–47% compared with ad libitum-fed controls after 8 and 30 days respectively). In addition, NK cell activity was increased in rats after PFDA treatment (Nelson et al., 1992). Exposure to PFDA altered immune responses in comparison with both ad libitum- and pair-fed controls with the exception of NK activity, which was similarly elevated in PFDA-exposed rats and pair-fed (but not ad libitum) controls (Nelson et al., 1992). The acute toxicity of PFDA is characterized by a wasting syndrome, which induces rapid and severe reductions in food consumption and body weight in rats at doses similar to those associated with the immunomodulatory effects described above (20–100 mg/kg) (see Section 3.2.10 for more details). The findings suggest that the antibody and DTH responses are directly related to PFDA exposure, while the NK activity is likely a secondary effect of chemical-induced wasting syndrome. Functional alterations in antibody and DTH responses after acute i.p. exposure is supportive of the immunomodulatory effects observed after short-term PFDA administration (see synthesis of animal studies in this section for more details).

Using an in vitro model to study mast cell functions and allergic inflammation, Lee and Kim (2018) showed that PFDA exposure can elevate markers of mast cell degranulation (histamine, β-hexosaminidase and intracellular calcium levels), increase gene expression and secretion of proinflammatory cytokines involved in immune cell recruitment and activation (TNF-α, IL-1β, IL-6, and IL-8) and induce nuclear factor kappa B [NF-kB] transactivation in IgE-stimulated rat basophilic leukemia (RBL-2H3) cells (Lee and Kim, 2018). The data are consistent with the

exacerbation of hypothermia and allergic inflammatory mediators (histamine, TNFα, IgE, and IgG1 levels) in OVA-stimulated mice following continuous high-dose oral exposure to PFDA (see synthesis of animal studies in this section for more details) and suggest a plausible mechanism for PFDA-induced immediate-type hypersensitivity.

Other potential mechanisms of PFDA-induced immune effects were evaluated in two studies conducted in human and animal in vitro cell models. No effects on IgM secretion and surface membrane expression were observed in human (F4 and Hurtwitz) or murine (HPCM2) B-cell lines at noncytotoxic PFDA concentrations, but detergent-like activity (i.e., solubilization of cell membranes) was reported in these lymphoblastoid cell lines at doses that caused significant cytotoxicity (Levitt and Liss, 1986). Another study evaluated the effects of PFDA on cytokine release in human primary and cultured leukocytes (Corsini et al., 2012). Decreases in proinflammatory (TNF-α and IL-6) and anti-inflammatory (IL-10 and IFN-γ) cytokine levels were reported in human peripheral blood leukocytes stimulated with lipolysaccharide (LPS) and phytohemagglutinin, respectively, following PFDA exposure (Corsini et al., 2012). Leukocytes from female donors were generally more susceptible to alterations in cytokine production (primarily TNFα) compared with male counterparts, although differential responses across cytokine measures were apparent and may be explained in part by variability in cell donors (Corsini et al., 2012). Similarly, PFDA decreased TNFα levels and NF-kB activation (measured as I-kB degradation, p65 phosphorylation, and NF-kB gene reporter activity) in human promyelocytic THP-1 cells stimulated with LPS but had no effects on PPARα-mediated transactivation (Corsini et al., 2012). Cell viability measured via the lactase dehydrogenase assay was unaffected in this cell line by PFDA treatment (Corsini et al., 2012). The data suggest that PFDA suppresses cytokine release (i.e., TNFα) by interfering with the NF-kB pathway in stimulated immune cells and that such effects may occur independently of PPARα activation.

Collectively, the mechanistic data indicate that PFDA can modulate NF-kB activation to induce both pro- and anti-inflammatory responses in cultured immune cells, which may have implications for the mechanisms of immunotoxicity of this compound.

Evidence Integration

Studies in humans and animals exposed to PFDA are available for the evaluation of potential immunosuppression and sensitization or allergic responses.

The evidence of an association between PFDA exposure and immunosuppressive effects in human studies is *moderate*. This evidence is based on largely consistent decreases in antibody response following vaccination (against two different infectious agents) in two *medium* confidence studies describing results from two independent birth cohorts in the Faroe Islands with outcome measurement in childhood. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease (IPCS, 2012). The antibody results present a consistent pattern of findings that higher prenatal, childhood, and adult serum

concentrations of PFDA were associated with suppression of at least one measure of the antivaccine antibody response to common vaccines in two well-conducted birth cohorts in the Faroe Islands and supported by a *low* confidence study in adults. An inverse association was observed in 21 of 26 evaluations, with a minimum of a 2% decrease in antibody concentration per doubling of PFDA concentration at levels consistent with the general population in NHANES; six of these evaluations were statistically significant and exhibited a large magnitude of effect (i.e., >18% decrease in response). These associations were observed despite poor study sensitivity, which increases confidence in the findings. There is some remaining uncertainty resulting from variability in the response, including positive associations in a few exposure-outcome combinations, differences in the responses by age of exposure and outcome measures as well as timing of vaccination (initial and boosters), from potential confounding across PFAS, and from inconsistency in two other *medium* confidence studies with outcome measurement in adults and cross-sectional exposure measurement in children. Overall, the evidence supports an association with immunosuppressive-type effects. These results are consistent with hazard identification conclusions from the NTP (2016) monograph on immunotoxicity associated with exposure to PFOS and PFOA, which concluded that PFOA and PFOS are *presumed to be an immune hazard to humans* based largely on evidence of suppression of antibody responses in both human and animal studies (NTP, 2016). Additionally, the Science Advisory Board (SAB) report, *Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS*, agreed with EPA that the human evidence for PFOS and PFOA showed consistent associations between exposure and reduced antibody responses in children indicative of potential immunosuppression (U.S. EPA, 2022b). The SAB panel stated that "Decreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease. The conclusion that suppression of vaccine responses is an adverse finding is widely accepted in the field of immunotoxicology…Moreover, the immunosuppression indicated by the observed antibody decreases are not limited to those specific antigens (e.g., tetanus and diphtheria only), but rather are indicative of modulation of the general immune response." Additionally, the SAB panel concluded that "decreased antibody responses to vaccinations are adverse effects, and that this effect is an appropriate critical effect for deriving RfDs for PFOA and PFOS."

The lack of clear association with infectious disease outcomes does not reduce certainty in this effect. While observing an association with infectious disease would increase certainty based on coherence across outcomes, the lack of coherence is explained because these studies are expected to be biased toward the null due to nondifferential outcome misclassification as described above. Although no effects were observed in T-dependent antibody responses with PFDA in one rat and one mouse study (both *high* confidence), other immunomodulatory responses were observed in animals that indicate potential for immunosuppression (see summary of animal evidence below for more details).

The database of animal studies examining PFDA-induced immunosuppressive responses consists of two *high* or *medium* confidence studies in B6C3F1/N mice (Frawley et al., 2018) and/or SD rats (Frawley et al., 2018; NTP, 2018) exposed via gavage for 28 days. PFDA did not elicit a strong immunotoxic response in animals as evidenced by the absence of treatment-related effects in a host resistance assay and most immune function assays (NK cell activity and T-dependent antibody responses to SRBC, mixed leukocyte response and DTH response to *C. albicans*). Nevertheless, coherent responses that suggest potential immunosuppression by PFDA exposure were observed, which is consistent with the human evidence (see Figures 3-16, 3-18, 3-22, 3-23 and HAWC summary visual for coherent PFDA immune effects). The immunomodulatory responses included dose-related decreases in phagocytic activity of rat liver macrophages (MPS activity) at ≥0.25 mg/kg-day and in immune cell population counts in mouse spleen at ≥0.089 mg/kg-day (Frawley et al., 2018), but issues regarding overt organ toxicity (increased liver weight and hepatocyte necrosis and spleen atrophy, respectively) introduce significant uncertainty (Frawley et al., 2018). Additionally, morphological changes occurred in the bone marrow (hypocellularity) and thymus (atrophy and lymphocyte apoptosis) of rats at PFDA doses associated with systemic toxicity (i.e., decreased body weights at ≥1.25 mg/kg-day) (NTP, 2018); the changes are consistent with the wasting syndrome that PFDA elicits and could represent secondary effects of the accompanying systemic toxicity. In light of the uncertainties in the available database, the evidence for potential immunosuppression from short-term animal studies is considered *slight*.

Mechanistic evidence from a high-dose, i.p. injection study is supportive of potential PFDA-induced immunosuppression (i.e., decreased antibody and DTH responses) in rats at ≥20 mg/kg (Nelson et al., 1992). Furthermore, an in vitro study using stimulated human primary and cultured leukocytes suggests that PFDA is capable of inhibiting NF-κB transcription and suppressing cytokine production (Corsini et al., 2012), which may be relevant to its mechanisms of immunotoxicity. Limitations in the mechanistic information include issues interpreting the exposure context (i.e., acute, high-dose exposure) of the i.p. injection study and general lack of studies in animal and human models that can provide support for the biological plausibility of putative immunosuppression observed in human and animal studies.

There is *slight* evidence for sensitization and allergic responses from studies in humans, but notable limitations and uncertainties in the evidence base remain. In human studies, the available evidence for infectious disease and hypersensitivity was less consistent than the evidence on immunosuppression and had more uncertainties resulting from a limited number of studies, unexplained heterogeneity in outcome or results, variable exposure assessment approaches that considered exposure at different times in relation to outcomes, and in some cases self-reported outcomes. For asthma, two of the three available studies reported no association with PFDA exposure. However, significant associations with asthma diagnosis and asthma-related outcomes, including an exposure-response gradient, were observed in one well-conducted (*medium* confidence) study with adequate sensitivity (Dong et al., 2013). This study also had the most

specific outcome definition, based on asthma incidence in the past year. These differences could account for the inconsistency with other asthma studies, including the other *medium* confidence study that examined "ever asthma." In addition, increases in biomarkers related to asthma were reported in this study, providing biological plausibility to the apical association. Still, the number of available studies is small, and poor sensitivity makes the null results difficult to interpret.

In animals, the single, short-term, *low* confidence study that examined endpoints relevant to sensitization and allergic responses reporting findings coherent with immediate-type hypersensitivity (i.e., exacerbation of hypothermia and markers of mast cell-mediated allergic inflammation in OVA-induced mice) (Lee and Kim, 2018); however, the high-exposure dose used (21.4 mg/kg-day) raises significant concerns about potential confounding effects by indirect systemic toxicity and thus these coherent results were not interpreted to provide biological plausibility for the findings in humans and the animal evidence was considered *indeterminate* (Lee and Kim, 2018).

Altogether, considering the available evidence from human, animal and mechanistic studies, the ***evidence indicates*** that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans, given sufficient exposure conditions[12] (see Table 3-18). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from human epidemiological studies (mostly from two birth cohort studies) at levels of 0.3 ng/mL (median exposure in studies observing an adverse effect). The evidence in animals showed coherent immunomodulatory responses at ≥0.089 mg/kg-day that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. A small number of studies conducted via i.p. injection and in vitro exposure in human and rodent cell culture models add some support for the biological plausibility of the observed phenotypic effects. While there is some evidence that PFDA exposure might also have the potential to affect sensitization and allergic responses, the human evidence underlying this possibility is uncertain and without support from animal or mechanistic studies. Consistent with the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFDA. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental lifestages represents a major source of uncertainty in the immunotoxicity database of PFDA.

[12]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-18. Evidence profile table for PFDA exposure and immune effects

Evidence stream summary and interpretation					Inferences and summary judgment
Evidence from studies of exposed humans (see Section 3.2.2: Human Studies)					⊕⊕⊙ ***Evidence indicates (likely)*** *Primary basis*: Evidence of immunosuppression from human studies indicating reduced antibody response in children at levels of approximately 0.3 ng/mL (*moderate* evidence) and some coherent findings in animals (*slight* evidence) at ≥0.089 mg/kg-d. Overall, other forms of potential PFDA-induced immunotoxicity, including *slight* human evidence for hypersensitivity-related outcomes, were interpreted with less certainty. *Human relevance*: Coherent effects in human and animal studies *Cross-stream coherence*: Evidence of immunosuppression in both animals and humans. *Susceptible populations and lifestages*: Consistent with the antibody response data in humans, children and fetuses may be at higher risk of adverse effects.
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Immunosuppression</u> (antibody response) Four *medium* confidence studies (3 in children) and 1 *low* confidence study	• Three studies in children and one in adults reported *decreased* antibody response following vaccination with higher PFDA exposure	• Consistency overall across vaccine type, timing of vaccination, and age at antibody response measurement including in two *medium* confidence studies with prospective exposure measurement and outcomes in children. • Associations observed despite limited sensitivity	• Potential for confounding across PFAS	⊕⊕⊙ *Moderate* Generally consistent evidence for decreased antibody responses. The inconsistent and *low* confidence evidence on infectious disease did not influence this judgment.	
<u>Immunosuppression</u> (infectious diseases) Three *medium* and 2 *low* confidence cohort studies	• Positive association with infectious diseases in one *medium* and two *low* confidence studies, but inconsistency across studies of the same infections/symptoms	• No factors noted	• Unexplained inconsistency, although limited sensitivity may contribute. • Imprecision		
<u>Sensitization and allergic response</u> Seven *medium* confidence studies in children	• Significantly higher odds of asthma (OR = 3.2) in one *medium* confidence study. One additional study reported *increased* odds of asthma with higher PFDA exposure, but only in a small subgroup that did not receive	• Large effect size for asthma incidence in the only study with adequate sensitivity (based on exposure contrast and outcome definition) • Exposure-response gradient across quartiles in same study	• Potential for confounding across PFAS • Unexplained inconsistency across studies	⊕⊙⊙ *Slight* Sparse evidence for hypersensitivity with some concerns for unexplained inconsistency and potential confounding	

Evidence stream summary and interpretation					Inferences and summary judgment
	MMR vaccine before age 5 • Other studies reported no association with hypersensitivity outcomes				*Other inferences*: MOA is unknown, but some uncertain evidence from human and animal in vitro studies suggests a possible role for NFκB in both pro- and anti-inflammatory responses that may be relevant to the mechanism(s) of immunotoxicity of PFDA.
Evidence from in vivo animal studies (see Section 3.2.2: Animal Studies)					
Studies and confidence	**Summary and Key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Immunosuppression</u> Two *high/medium* confidence studies in mice and/or rats • 28-d gavage (2×)	• Decreased hepatic MPS activity in rats at ≥0.5 mg/kg-d in the presence of liver toxicity (increased liver weight and hepatocyte necrosis) • Decreased absolute spleen cell population counts in mice at ≥0.89 mg/kg-d in the presence of spleen atrophy (decreased spleen weights and total cell counts) • Bone marrow and thymic lesions and decreased thymus weights in rats at ≥1.25 mg/kg-d in the presence of marked body weight reductions • No effects in a host resistance assay in mice or other immune function assays conducted in rats and	• Coherence across immune responses (i.e., MPS activity in rats and spleen cell population counts and spleen weights in mice) • Dose-response gradient for MPS activity, absolute spleen cell counts and spleen weights. • *High/medium* confidence studies	• Lack of effects on host resistance and most immune function assays • Potential confounding with overt organ or systemic toxicity.	⊕⊙⊙ *Slight* Coherent evidence of potential immunosuppression in rats and mice at doses ≥0.089 mg/kg-d across two *high/medium* confidence studies (see HAWC summary visual for coherent PFDA immune effects); however, there is uncertainty due to potential confounding effects with overt organ/systemic toxicity.	

Evidence stream summary and interpretation					Inferences and summary judgment
	mice at doses up 0.71 mg/kg-d				
Sensitization and allergic response One *low* confidence study in mice • 14-d	• Exacerbation of hypothermia and release of serum inflammatory markers (i.e., histamine, TNFα, IgE and IgG1) in OVA-stimulated mice at 21.4 mg/kg-d	• Coherence across markers of allergic inflammation and hypersensitivity	• Potential for confounding by systemic toxicity	⊙⊙⊙ *Indeterminate* *Low* confidence evidence with considerable uncertainty due to potential confounding effects due to high-dose systemic toxicity.	
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Mast cell function and allergic response	*Interpretation*: PFDA may induce mast cell-mediated allergic inflammation via NFκB activation. *Key findings*: • Increases in markers of mast cell degranulation (histamine, β-hexosaminidase and intracellular calcium levels), immune cell recruitment and activation (TNF-α, IL-1β, IL-6, and IL-8 levels) and NFκB transactivation in IgE-stimulated rat RBL-2H3 cells. *Limitations*: Single study available.			A small number of mechanistic studies in human and rodent in vitro models suggest a possible involvement of NFκB in pro- and anti-inflammatory responses that may be relevant to the mechanisms of immunotoxicity of PFDA. Supportive evidence of immunosuppression in rats was reported in an acute, i.p. injection study. Although the available evidence is limited introducing significant uncertainty, the findings provide some support for the biological plausibility of the immune-related responses in humans and animals.	
Other mechanisms	*Interpretation*: PFDA may suppress cytokine production by inhibiting NFκB activation. *Key findings*: • Attenuation of cytokine release (including TNFα) in stimulated human peripheral blood leukocytes (leukocytes from female donors appeared to be more susceptible to these effects); decreases in TNFα release and NFκB activation but no effects on PPARα transactivation in stimulated human promyelocytic THP-1 cells (Corsini et al., 2012). • No effects on IgM secretion and surface expression in human and murine B-cell lines exposed to noncytotoxic PFDA concentrations (Levitt and Liss, 1986). *Limitations*: Few studies available; cell donor variability introduces some uncertainty in interpreting sex-specific differences in cytokine release from exposed human primary leukocytes.				

Evidence stream summary and interpretation			Inferences and summary judgment
Other evidence	*Interpretation*: Results are consistent with immunosuppressive responses observed in oral exposure studies. *Key findings*: • Decreases in antibody response and DTH in KLH-stimulated rats compared with libitum and pair-fed controls; Increase in NK cell activity may be attributable to PFDA-induced anorexia. *Limitations*: Single study with high-dose, one-time i.p. exposure.		

3.2.3. Developmental Effects

Human Studies

Studies of developmental endpoints related to PFDA are available for fetal and postnatal growth restriction, spontaneous abortion, anogenital distance, birth defects, and gestational duration outcomes (i.e., preterm birth and gestational age). Given that spontaneous abortion and preterm birth could be driven by either female reproductive or developmental toxicity, these endpoints are also discussed in the context of coherence in Section 3.2.5 on female reproductive effects.

Forty-eight epidemiological publications (across 46 different studies) examining PFDA exposures in relation to developmental endpoints were identified in the literature search and additional efforts. This included the following: 8 studies on postnatal growth, 12 studies on gestational duration, 6 on fetal loss, 3 on anogenital distance, 2 studies on birth defects, and 31 publications (from 29 different studies) examined fetal growth restriction. Publications based on overlapping populations in the same cohort were included in the synthesis only if they provided some unique data for different endpoints. For example, the Bjerregaard-Olesen et al. (2019) study from the Aarhus Birth Cohort also provided birth length and head circumference measures in the overall population and across sex that were not included in the main study by Bach et al. (2016). Therefore, it is included in the fetal growth restriction count above and considered one study (population) from two publications with separate analyses. This synthesis, and especially the evaluation of consistency across studies, focuses on a primary study to avoid duplicative analyses or overweighting of one study population. Although the results for the smaller sample size in this study are not plotted, in this instance divergent primary birthweight (BWT) results are presented for comparison in the text. Another study by Gyllenhammar et al. (2018) was supplemented by a second publication (Swedish Environmental Protection Agency, 2017) that provided mean BWT data on a larger population from the same cohort. Supplemental data and communication from study authors were used if they provided additional data or information, and EPA calculated confidence intervals when not reported and rescaled study results to provide comparisons based on a ln-unit change to increase comparability.

Additional methodological considerations

As detailed in the PFAS Systematic Review Protocol (see Appendix A) and Section 1.2.2, multiple outcome-specific considerations for study evaluation influenced the domain ratings and the overall study confidence. For the confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Pregnancy hemodynamics represent a source of uncertainty as PFAS biomarkers sampled late in pregnancy may be prone to bias potentially from either confounding or reverse causality. Although not considered as a factor influencing the exposure domain rating, the potential effects of hemodynamic factors later in pregnancy affecting serum PFDA levels and the result from individual

studies is separately discussed in the evidence synthesis section and Appendix F. Among the few fetal growth studies [e.g., (Gyllenhammar et al., 2018) for PFDA] examining the potential for confounding by measures of pregnancy hemodynamics (e.g., plasma albumin or GFR measures), there is little direct evidence that these measures were important confounders (Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Whitworth et al., 2012) across different PFAS. However, given this source of uncertainty, sample timing patterns across studies were also considered here to see if results among studies with early sampling (i.e., studies with any first trimester sampling or preconception) differed from those with later sampling (i.e., maternal samples exclusively from the second trimester through the third trimester, umbilical cord, placental or postpartum maternal samples). There is additional uncertainty across all health endpoints due to potential confounding by co-occurring PFAS (see Appendix A and F for methods and analyses, respectively). For fetal growth restriction and other developmental endpoints, there may be more concern over potential PFAS coexposure confounding because of PFNA given higher correlations with PFDA and associations that are fairly comparable in consistency and magnitude, as detailed in Appendix F. Although there is some uncertainty as to whether other PFAS are plausible confounders, studies were downgraded if the authors did not rule out or account for these or other covariates that may be confounders.

For the exposure domain, all the available studies analyzed PFAS in maternal serum/plasma, umbilical cord, or placenta using standard methods. Given the long half-life of PFDA, samples collected during all three trimesters (and shortly after birth) were considered representative of the most critical in utero exposure windows for fetal growth and gestational duration measures. Various measures of postnatal growth were included based on an assumed fetal programming mechanism (i.e., Barker hypothesis) wherein in utero perturbations or exposures, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders (see more on this topic in De Boo and Harding (2006) and Perng et al. (2016) syntheses for metabolic disorders for other PFAS). There is some evidence that birth weight deficits from in utero exposures can be followed by increased weight gain during rapid growth catch-up periods in early childhood (Perng et al., 2016). Therefore, the most critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero. Thus, studies were downgraded if exposure data were collected later during childhood concurrent with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts (i.e., limited exposure ranges or distributions) or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (especially when sample size is reduced by estimating stratum-specific results such as by sex). In the outcome domain, specific considerations included accuracy of fetal and early childhood anthropometric measures and adequacy of case ascertainment for dichotomized (i.e., binary) outcomes. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect

estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias toward the null if ascertainment of fetal loss is not associated with PFDA exposures (i.e., nondifferential). In some situations, differential loss is possible and bias away from the null and can manifest as an apparent protective effect. Anogenital distance (AGD) is an externally visible marker that has been shown in animal studies to be a sensitive indicator of prenatal androgen exposure (lower androgen levels associated with decreased AGD). It is associated with other reproductive tract abnormalities, including hypospadias and cryptorchidism in human and animal males (Liu et al., 2014; Sathyanarayana et al., 2010; Salazar-Martinez et al., 2004). The primary outcome-specific criteria for this outcome are the use of clearly defined protocols for measurement, ideally multiple measures of each distance (averaged) and minimal variability in the age of participants at measurement. In boys, measures can be taken from the center of the anus to the posterior base of the scrotum (ASD) or from the center of the anus to the cephalad insertion of the penile (APD).

Fetal and childhood growth restriction was examined through several endpoints including low birth weight (LBW), small for gestational age (SGA), abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher-quality endpoints (i.e., considered good in the outcome domain) that were measured in multiple studies to allow for a detailed evaluation of consistency and heterogeneity across studies that may be present. Some of the adverse endpoints of interest examined here included fetal growth restriction endpoints based on birth weight such as mean birth weight reductions (or variations of this endpoint such as standardized BWT z-scores), as well as categorical measures such as SGA births (e.g., lowest decile of BWT stratified by gestational age and other covariates) and LBW (i.e., typically defined as <2,500 g). Overall, BWT measures are considered accurate and, in these studies, were derived predominately from medical records; therefore, the outcome domain judgments reflect the high reliability of these endpoints. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary for these endpoints to be considered good. LBW is a less preferred measure of fetal growth restriction than SGA, especially if analyses include both term and preterm neonates because birth weight is dependent on both the rate of fetal growth and gestational duration, and perturbation in each may arise from different etiologies. Other measures of fetal growth may be subject to measurement error (e.g., head circumference and body length measures) if the measures are less reproducible (i.e., are subject to more interobserver differences). Thus, unless multiple measurements were taken, these endpoints were given a rating of adequate (Shinwell and Shlomo, 2003).

Gestational duration measures were examined in epidemiological studies as either continuous (i.e., per each gestational week) or dichotomized categorical endpoints such as preterm birth (typically defined as gestational age <37 weeks). Although gestational age dating methods such as ultrasounds early in pregnancy are preferred, this approach and others (e.g., last menstrual period recall) are expected to result in some decreased sensitivity as measurement error could impact classification of SGA as well as preterm birth (PTB). Gestational duration measures were, therefore, downgraded if based solely on last menstrual period estimates or if the method(s) were not reported, and less uncertainty is anticipated in studies that compare and adjust for differences between last menstrual period and ultrasound measurements. Any sources of error noted in the classification of these endpoints are anticipated to be nondifferential with respect to PFDA exposure and, therefore, would not be considered a major concern for risk of bias, but could affect precision and study sensitivity. Additional details for domain-specific evaluation of epidemiological studies can be found in the PFAS Systematic Review Protocol, Appendix A.

Growth restriction – neonatal anthropometric measures

Birth weight

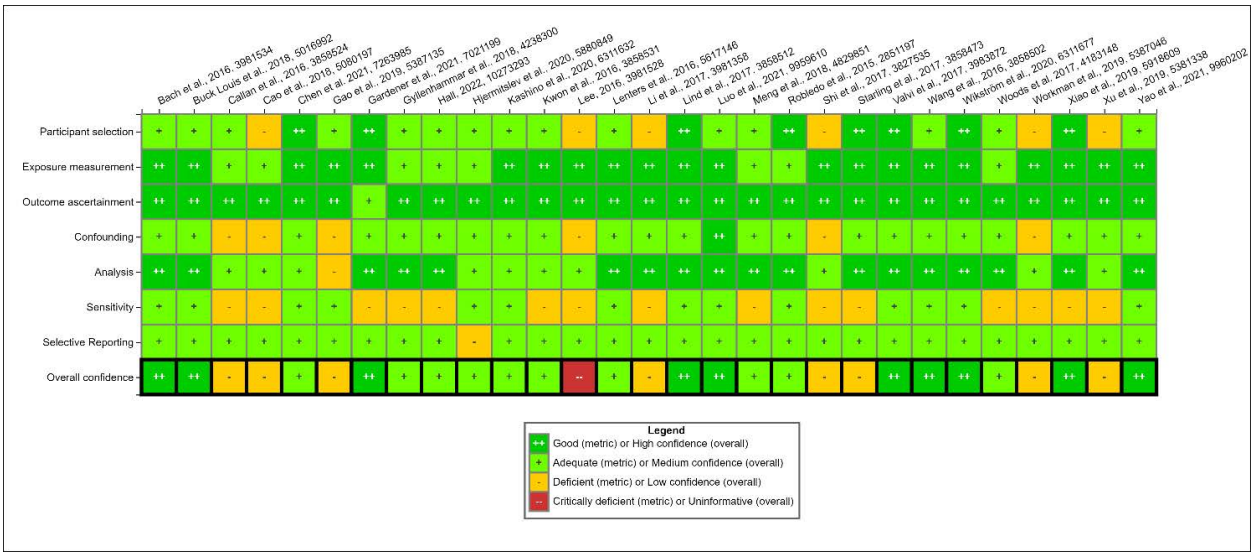


Figure 3-25. Evaluation results for epidemiological studies assessing effects of PFDA exposure on birth weight.[a] Refer to HAWC Human Birth Weight for details on the study evaluation review.

[a]Confidence descriptors based on the mean birth weight or birth weight z-score endpoints.

As shown in Figure 3-25, 29 different studies examined BWT measures (either mean BWT differences or standardized BWT scores) in relation to PFDA exposures. One study that was uninformative (Lee et al., 2016) because of several critical study deficiencies in confounding, selection participation, and study sensitivity is not considered further below. Among the 28 studies

that were included based on maternal, umbilical cord, or placental measures, 8 reported standardized BWT measures such as BWT z-scores (Gardener et al., 2021; Wikström et al., 2020; Workman et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Bach et al., 2016; Wang et al., 2016) with all but 8 (Gardener et al., 2021; Xiao et al., 2019) of these reporting both standardized and mean BWT measures (see Figure 3-26). Twenty-six studies examined mean BWT either in the overall population (i.e., both girls and boys) or both sexes including four (Hall et al., 2022; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015) that reported sex-specific analyses only. Fourteen studies in total reported sex-specific results in both sexes.

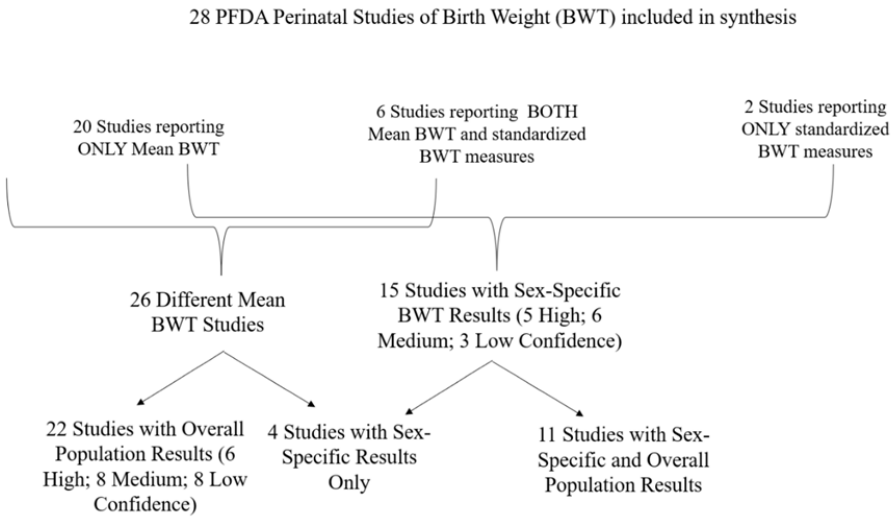


Figure 3-26. Twenty-eight perinatal studies of birth weight measures and subsets considered for different analyses.

Twenty-two of the 28 studies examining either standardized or mean BWT were prospective birth cohort studies, while the remaining 6 (Xu et al., 2019b; Gyllenhammar et al., 2018; Li et al., 2017; Shi et al., 2017; Callan et al., 2016; Kwon et al., 2016) were cross-sectional studies (see Figure 3-26). For evaluation of patterns, studies that collected biomarker samples concurrently or after birth were considered cross-sectional analyses [e.g., (Hall et al., 2022)]. Five of the 28 PFDA studies relied on umbilical cord samples (Xu et al., 2019b; Cao et al., 2018; Li et al., 2017; Shi et al., 2017; Kwon et al., 2016), and exposure characterization was based on PFDA placental measures sampled at birth in the *medium* confidence study by (Hall et al., 2022) . Twenty-two studies had maternal blood measures that were sampled preconception (Robledo et al., 2015) or during the first trimester (Buck Louis et al., 2018; Lind et al., 2017a; Bach et al., 2016), the third trimester (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Kashino et al., 2020; Gao et al., 2019; Xiao et al., 2019; Valvi et al., 2017; Callan et al., 2016; Wang et al., 2016), across multiple trimesters (Chen et al., 2021; Hjermitslev et al., 2020; Wikström et al., 2020; Workman et al., 2019; Meng et al., 2018; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016), or after delivery (Gyllenhammar et al.,

2018). The study by Meng et al. (2018) pooled samples from umbilical cord and multiple maternal samples during trimesters 1 and 2.

Ten of the 28 included studies examining different BWT indices were rated *high* confidence (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Wikström et al., 2020; Xiao et al., 2019; Buck Louis et al., 2018; Lind et al., 2017a; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016), 10 were *medium* confidence (Hall et al., 2022; Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018; Woods et al., 2017; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015), and 8 were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019b; Cao et al., 2018; Li et al., 2017; Shi et al., 2017; Starling et al., 2017; Callan et al., 2016). Among the 28 studies with mean BWT measures, 14 each had adequate and deficient study sensitivity (see Figure 3-26). The evidence syntheses for mean BWT differences detailed below primarily emphasize the results from the 20 *high* or *medium* confidence studies.

Standardized BWT measures

Three of the eight studies reported smaller standardized BWT scores in relation to PFDA exposures including one *medium* (Gyllenhammar et al., 2018) and two *high* (Wikström et al., 2020; Xiao et al., 2019) confidence studies (see Figure 3-27). The study by Gardener et al. (2021) not plotted in Figure 3-27 reported positive associations with increasing PFDA exposures, while four studies reported null associations (Workman et al., 2019; Meng et al., 2018; Bach et al., 2016; Wang et al., 2016). One of the studies showing a null association in quartile 4 (relative to quartile 1) and per each ln-unit increase did show elevated but nonsignificant BWT scores of −0.10 and −0.13 for quartiles 2 and 3 (Bach et al., 2016). Two of the studies (Wikström et al., 2020; Gyllenhammar et al., 2018) with inverse associations in the overall population reported statistically significant BWT z-scores similar in magnitude (β range: −0.14 to −0.15 per each ln-unit increase). The *high* confidence (Xiao et al., 2019) study reported associations about twice as large as these other studies (β = −0.39; 95% CI: −0.94, 0.16), and their results were largely driven by associations in girls (β = −0.62; 95% CI: −1.28, 0.03) (see Figure 3-28). One (Wikström et al., 2020) of two standardized BWT studies with categorical data showed evidence of an inverse exposure-response relationship.

Study sensitivity did not seem to explain the four null studies, as two were adequate (Bach et al., 2016; Wang et al., 2016) and two were deficient (Workman et al., 2019; Meng et al., 2018). No pattern in study results by exposure contrasts was evident either. There was some evidence of potential impact of pregnancy hemodynamics, as two of these three studies were based on later biomarker samples. However, the dearth of available studies precludes a more definitive conclusion being drawn here.

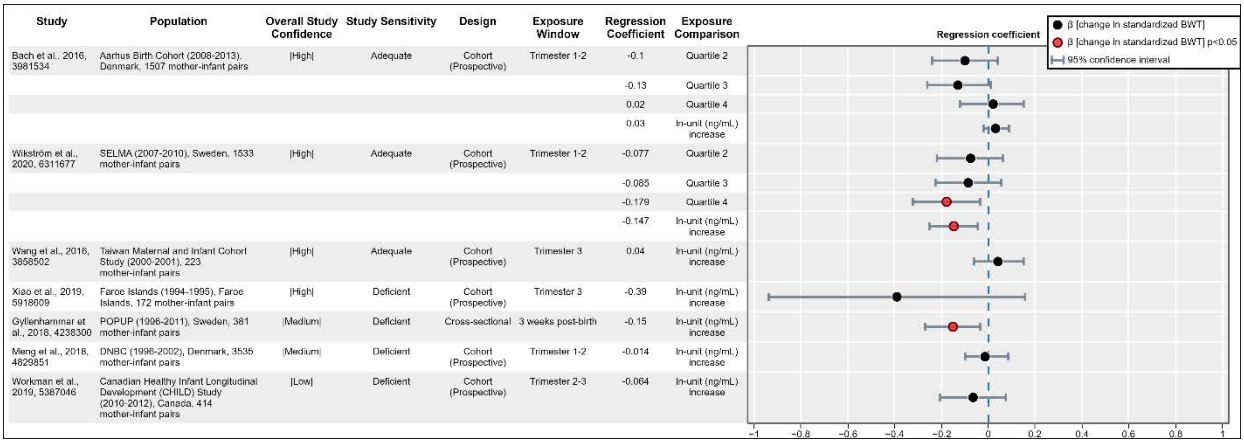


Figure 3-27. PFDA and birth weight z-scores (overall population).[a] Refer to Birth Weight-Z for details on the individual study evaluation review.

BWT = birth weight.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.

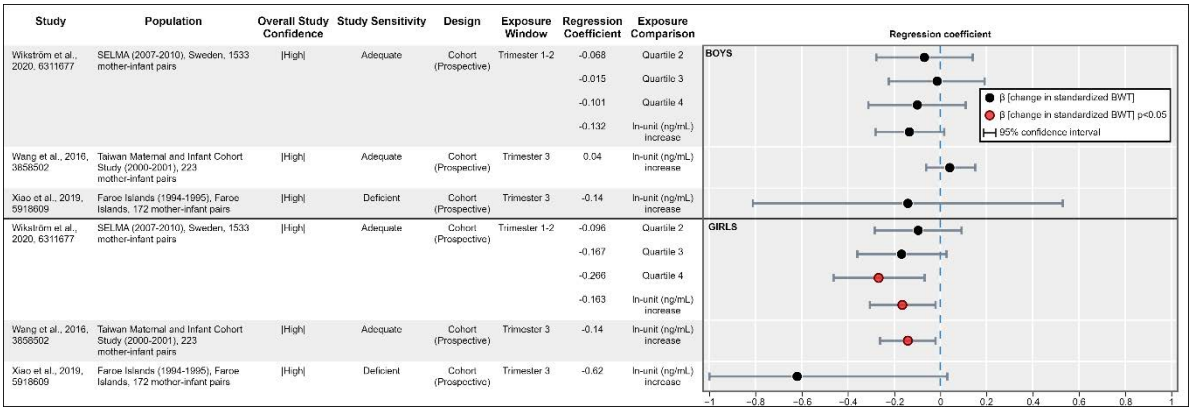


Figure 3-28. PFDA and birth weight z-score (sex-stratified).[a] Refer to Birth Weight-Z Score Sex-Stratified for details on the individual study evaluation review.

BWT = birth weight.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.

Overall population results

As shown in Figure 3-29, 22 studies (6 *high* and 8 each *medium* and *low* confidence) examined mean BWT differences in the overall population (i.e., both sexes combined). Although some of these were not statistically significant, 11 of the 22 studies reported some deficits including 4 *high*, 5 *medium*, and 2 *low* confidence studies. Eight studies in the overall population were null (Chen et al., 2021; Buck Louis et al., 2018; Meng et al., 2018; Shi et al., 2017; Starling et al., 2017; Woods et al., 2017; Bach et al., 2016; Callan et al., 2016) and three others reported increased mean BWT with increasing PFDA exposures (Gao et al., 2019; Xu et al., 2019b; Cao et al., 2018). Five of the six studies with categorical data did not show definitive BWT deficits; however, the one study that

reported deficits did demonstrate an exposure-response relationship in the overall population (Wikström et al., 2020).

There was considerable variability in BWT deficits (β range: −29 to −101 g) per ln-unit increases, with eight studies ranging from 29 to 72 g. The *high* confidence study by Luo et al. (2021) showed a statistically significant larger BWT deficit (β = −96.8 g; 95% CI: −178.0, −15.5 per each ln-unit PFDA increase). For each ln-unit PFDA increase, statistically significant reductions similar in magnitude were reported by the *medium* confidence studies by Swedish Environmental Protection Agency (2017) (β = −94 g; 95% CI: −163, −25) and Kwon et al. (2016) (β = −101 g; 95% CI: −184.8, −17.7). For each ln-unit PFDA increase, smaller nonstatistically significant BWT deficits in two *high* confidence studies by Yao et al. (2021) (β = −46.3 g; 95% CI: −131.1, 38.5) and Valvi et al. (2017) (β = −59 g; 95% CI: −147, 26). The *medium* confidence study by Kashino et al. (2020) reported a deficit between PFDA exposure in the overall population (β = −31.4 g; 95% CI: −60.0, −2.7 per each ln-unit increase). The *medium* confidence study by Lenters et al. (2016) detected a BWT deficit similar in magnitude (β = −31 g; 95%: −75, 12 for each ln-unit PFDA increase) in single-pollutant multivariate models, although PFDA was not selected as an important independent predictor in their multipollutant elastic net model adjusting for other PFAS exposures and phthalate metabolites (see more details in Appendix F).

The associations noted in many studies were evident despite some limitations, such as low exposure levels and/or narrow contrasts, which can decrease study sensitivity and statistical power. In contrast to the *medium* and *high* confidence studies that exhibited associations in the overall population, there was more heterogeneity in the *low* confidence studies often noted by imprecision. Overall, 10 of the 22 studies of the overall population with mean BWT data were deficient in study sensitivity given very low PFDA ranges and median values (from 0.08 to 0.24 ng/mL) (see Table 3-19), which included 5 of the 8 null studies (Meng et al., 2018; Shi et al., 2017; Starling et al., 2017; Woods et al., 2017; Callan et al., 2016). Two (Buck Louis et al., 2018; Bach et al., 2016) of the remaining three null studies also reported low median and IQR values (0.20–0.30); thus, study sensitivity may partially explain some of these null associations given the limited exposure contrasts.

Sex-specific results

Although they were not always consistent across sexes within each study, most studies showed some mean BWT deficits in either or both sexes (see Figure 3-30; 3-31). For example, 9 studies each in girls and boys showed some BWT reductions in relation to PFDA, including 6 of 11 *medium* and *high* confidence studies in boys and 7 of 11 *medium* and *high* confidence studies in girls. Null associations were reported in two studies each for boys (Meng et al., 2018; Robledo et al., 2015) and girls (Hjermitslev et al., 2020; Swedish Environmental Protection Agency, 2017), while increased BWT was reported in three studies in girls (Cao et al., 2018; Lind et al., 2017a; Shi et al., 2017) and boys (Cao et al., 2018; Bach et al., 2016; Wang et al., 2016).

Males

Among the five (two *high*, two *medium*, and one *low* confidence) studies showing BWT deficits in both sexes, three studies reported larger mean BWT deficits in boys (Hall et al., 2022; Kashino et al., 2020; Valvi et al., 2017) while two did in girls (Wikström et al., 2020; Li et al., 2017). The deficits across sexes were quite variable per each unit change in PFDA exposures; with mean BWT deficits ranging from −20 g (Hjermitslev et al., 2020) to −156 g (Swedish Environmental Protection Agency, 2017) in boys. Smaller per ln-unit PFDA changes of −24 g was noted in two studies (Kashino et al., 2020; Meng et al., 2018) for girls compared with very large changes of −140 g (Wang et al., 2016) and −254 g observed in Robledo et al. (2015). The *medium* confidence study by Hall et al. (2022) reported nonsignificant deficits only in tertile 3 for boys (β = −73.2 g; 95% CI: −307.2, 160.8) and girls (β = −50.3 g; 95% CI: −185.3, 84.7) relative to tertile 1.

Females

The *high* confidence study by Wang et al. (2016) reported a mean birth weight decrease among girls only (β = −140 g; 95% CI: −260, −20) per each ln increase. Among these girls, they also reported large mean BWT deficits in PFDA quartile 3 (β = −120 g; 95% CI: −330, 100) and 4 (β = −230 g; 95% CI: −440, −10) compared with the quartile 1 referent. The *high* confidence study by (Wikström et al., 2020) reported an exposure-response relationship among girls with BWT deficits ranging from −42 to −116 g but only in quartile 4 (β = −27 g; 95% CI: −118, 64) for boys. Although deficits were not seen in the *high* confidence (Bach et al., 2016) study among 743 girls based on continuous exposure expressions, large nonmonotonic deficits were noted across all three upper PFDA quartiles. In contrast, their sister publication (not shown on Figure 3-31) by Bjerregaard-Olesen et al. (2019) did report BWT deficits of 43 g (95% CI: −102, 16) per each ln-unit increase in a subset of 334 girls.

Overall, patterns were limited in results across sexes or across study characteristics. Among the studies showing mean BWT associations, six of nine studies in girls and five of nine studies in boys were based on biomarker samples later in pregnancy or postpartum. This might be indicative of potential bias related to pregnancy hemodynamics. Study sensitivity was limited in half the studies but did not appear to explain the four null studies (two each were adequate and deficient).

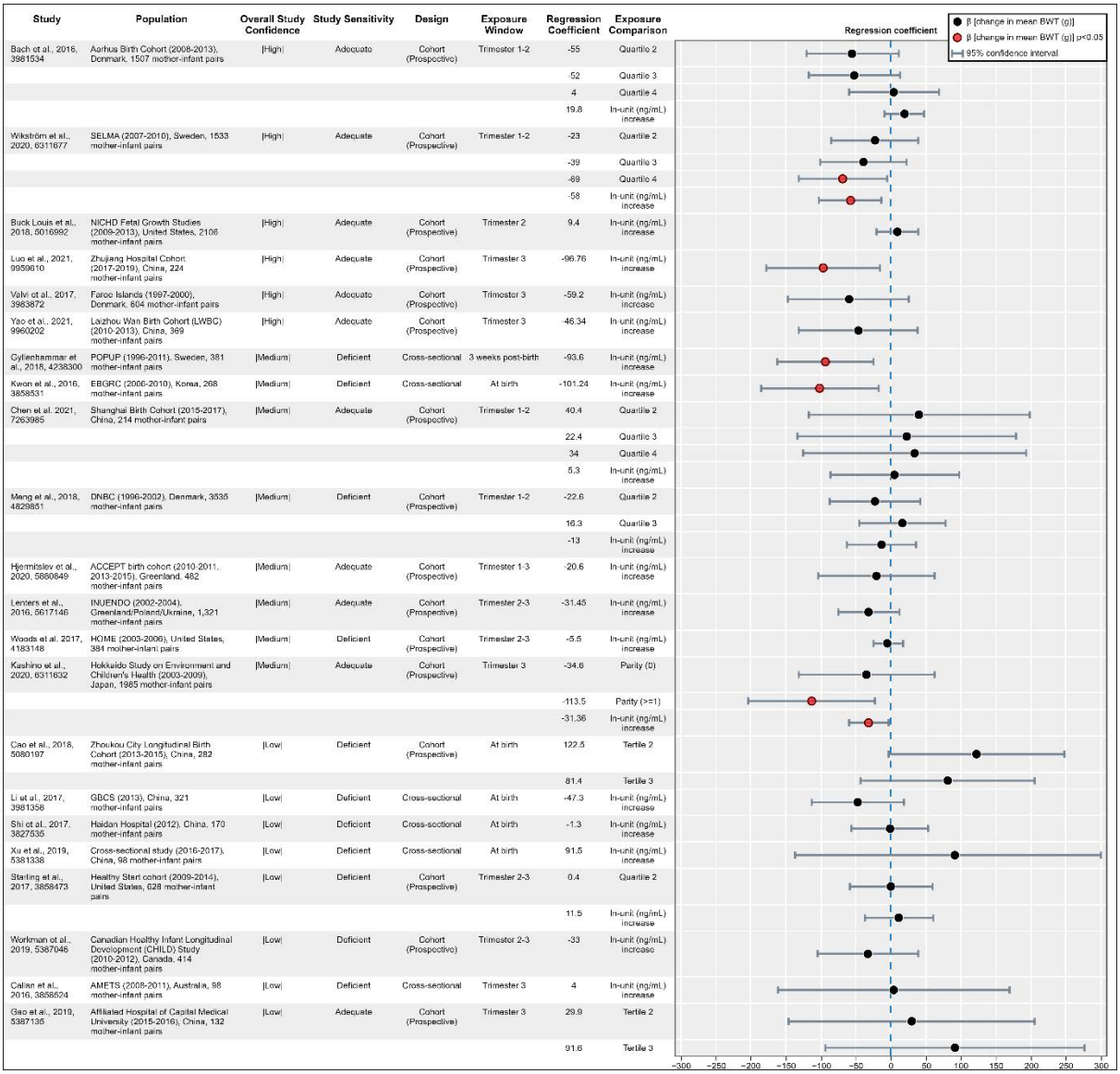
Birth weight summary

Eighteen of 28 studies examining mean or standardized BWT measures in the overall population or in each sex reported inverse associations with PFDA. Seventeen of the 26 studies examining mean BWT measures also reported inverse associations, including 11 of 22 studies (and 9 of 14 *medium* and *high* confidence) examining mean BWT in the overall population. Although there was not a clear sex-specific effect of PFDA, eight studies each in girls and boys showed some mean BWT reductions; four studies showed deficits in both sexes. Few studies examined nonlinear relationships between PFDA and mean BWT. The lone study that reported deficits across categories

demonstrated an exposure-response relationship for mean BWT, while one of two studies showed this for standardized BWT measures.

Eleven of the 22 studies of the overall population were deficient in study sensitivity with particularly low PFDA contrasts, which may partially explain some of these null associations. For example, among the eight null studies examining mean BWT measures in the overall population, there was a slight preponderance of deficient study sensitivity (five compared with three with adequate study sensitivity). There was a definitive pattern by sampling timing as only 2 of the 11 studies (including 2 of 9 *medium*/*high* studies) reporting BWT deficits in the overall population had early sampling biomarkers measures during pregnancy. The majority of sex-specific studies reporting BWT deficits were also based on later biomarker sampling (defined here as from the second trimester exclusive onward).

Despite reasonably consistent evidence of an association between PFDA and different BWT-related measures, and more mixed findings for other endpoints, there is considerable uncertainty given that sample timing differences may explain at least some of the reported fetal growth restriction deficits.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Bach et al., 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	-55	Quartile 2
						-52	Quartile 3
						4	Quartile 4
						19.8	ln-unit (ng/mL) increase
Wikström et al., 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	-23	Quartile 2
						-39	Quartile 3
						-69	Quartile 4
						-58	ln-unit (ng/mL) increase
Buck Louis et al., 2018, 5016992	NICHD Fetal Growth Studies (2009-2013), United States, 2106 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 2	9.4	ln-unit (ng/mL) increase
Luo et al., 2021, 9959610	Zhujiang Hospital Cohort (2017-2019), China, 224 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-96.76	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-59.2	ln-unit (ng/mL) increase
Yao et al., 2021, 9960202	Laizhou Wan Birth Cohort (LWBC) (2010-2013), China, 369 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	46.34	ln-unit (ng/mL) increase
Gyllenhammar et al., 2018, 4238300	POPUP (1996-2011), Sweden, 381 mother-infant pairs	Medium	Deficient	Cross-sectional	3 weeks post-birth	-93.6	ln-unit (ng/mL) increase
Kwon et al., 2016, 3858531	EBGRC (2006-2010), Korea, 268 mother-infant pairs	Medium	Deficient	Cross-sectional	At birth	-101.24	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-2	40.4	Quartile 2
						22.4	Quartile 3
						34	Quartile 4
						5.3	ln-unit (ng/mL) increase
Meng et al., 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	Medium	Deficient	Cohort (Prospective)	Trimester 1-2	-22.6	Quartile 2
						16.3	Quartile 3
						-13	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-3	-20.6	ln-unit (ng/mL) increase
Lenters et al., 2016, 5617146	INUENDO (2002-2004), Greenland/Poland/Ukraine, 1,321 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 2-3	-31.45	ln-unit (ng/mL) increase
Woods et al. 2017, 4183148	HOME (2003-2006), United States, 384 mother-infant pairs	Medium	Deficient	Cohort (Prospective)	Trimester 2-3	-5.5	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	-34.6	Parity (0)
						-113.5	Parity (>=1)
						-31.36	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Low	Deficient	Cohort (Prospective)	At birth	122.5	Tertile 2
						81.4	Tertile 3
Li et al., 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	-47.3	ln-unit (ng/mL) increase
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	-1.3	ln-unit (ng/mL) increase
Xu et al., 2019, 5381338	Cross-sectional study (2016-2017), China, 98 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	91.5	ln-unit (ng/mL) increase
Starling et al., 2017, 3858473	Healthy Start cohort (2009-2014), United States, 628 mother-infant pairs	Low	Deficient	Cohort (Prospective)	Trimester 2-3	0.4	Quartile 2
						11.5	ln-unit (ng/mL) increase
Workman et al., 2019, 5387046	Canadian Healthy Infant Longitudinal Development (CHILD) Study (2010-2012), Canada, 414 mother-infant pairs	Low	Deficient	Cohort (Prospective)	Trimester 2-3	-33	ln-unit (ng/mL) increase
Callan et al., 2016, 3858524	AMETS (2008-2011), Australia, 98 mother-infant pairs	Low	Deficient	Cross-sectional	Trimester 3	4	ln-unit (ng/mL) increase
Gao et al., 2019, 5387135	Affiliated Hospital of Capital Medical University (2015-2016), China, 132 mother-infant pairs	Low	Adequate	Cohort (Prospective)	Trimester 3	29.9	Tertile 2
						91.6	Tertile 3

Figure 3-29. Overall study population mean birth weight results for 22 PFDA epidemiological studies.[a–d] (Results can be viewed by clicking the HAWC link.)

BWT = birth weight.

[a]Studies are sorted first by overall study confidence level then by exposure window examined.

[b]Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c]The results displayed here for mean BWT among 587 overall population participants in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared with a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[d]Xu et al. (2019a) results are truncated for the 91.5 g increase; the complete 95% CI ranges from −136.5 to 319.6 g.

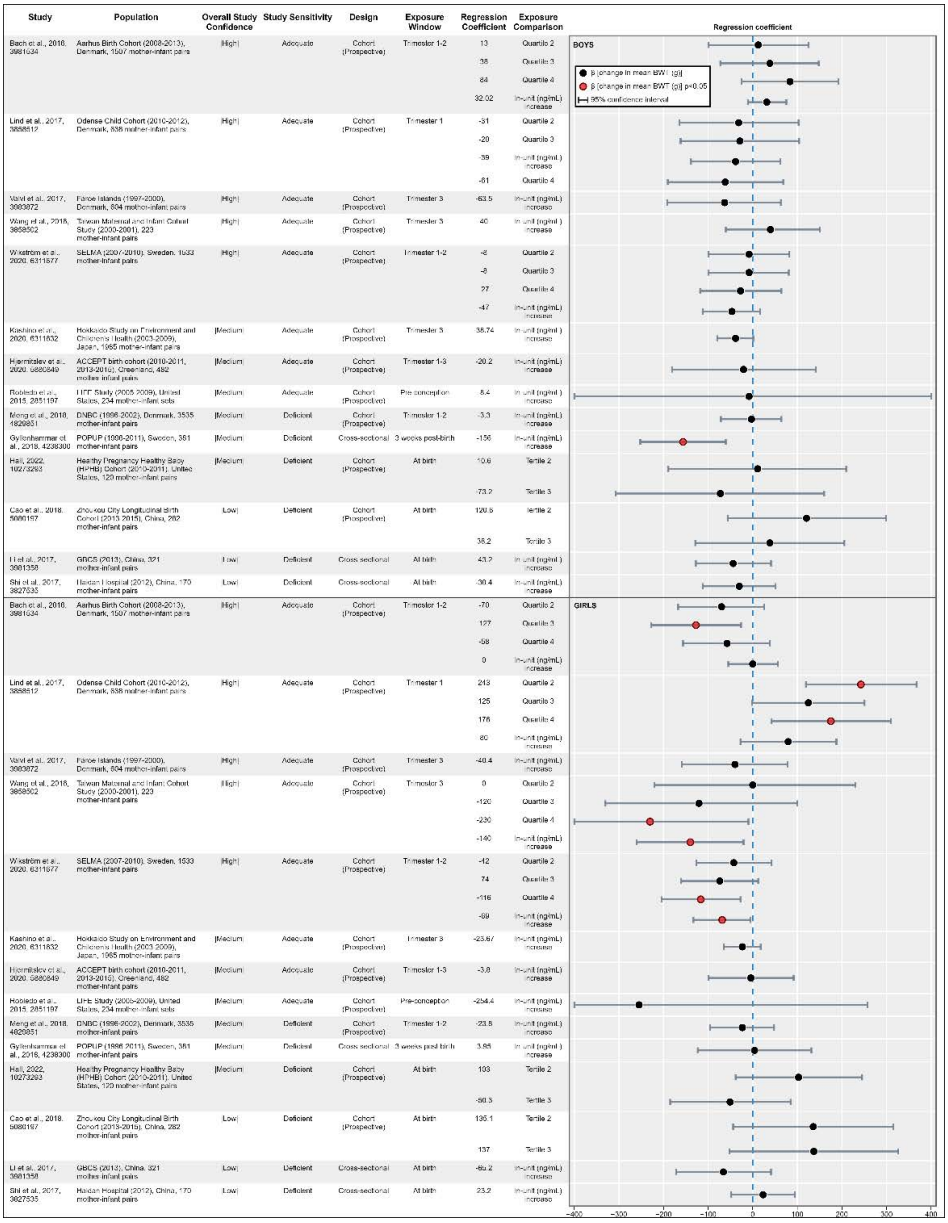


Figure 3-30. Sex-specific male infants only mean birth weight results for 14 PFDA epidemiological studies.[a–e] (Results can be viewed by clicking the HAWC link.)

BWT = birth weight.

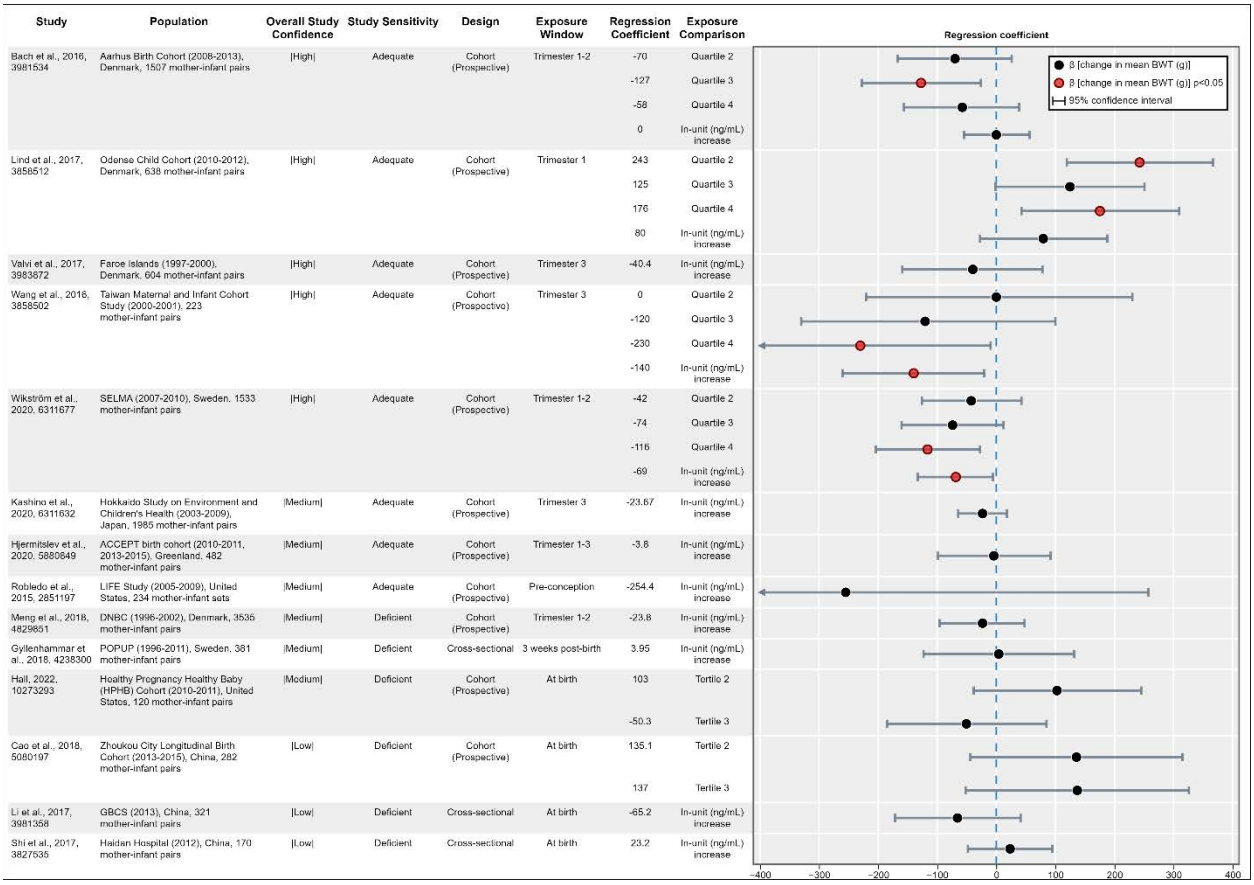
[a]Studies are sorted first by overall study confidence level then by exposure window examined.

[b]Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c]The results displayed here for mean BWT in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared with a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[d](Robledo et al., 2015) regression coefficients for maternal serum PFDA are displayed. The complete 95% CI for the male 8.4 g difference ranges from −434.3 to 417.6 g.

[e]For evaluation of patterns of results, studies that collected biomarker samples concurrently or after birth were considered cross-sectional analyses (e.g., Hall et al. (2022)).



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Bach et al., 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 1-2	-70	Quartile 2
						-127	Quartile 3
						-58	Quartile 4
						0	ln-unit (ng/mL) increase
Lind et al., 2017, 3858512	Odense Child Cohort (2010-2012), Denmark, 638 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 1	243	Quartile 2
						125	Quartile 3
						176	Quartile 4
						80	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	-40.4	ln-unit (ng/mL) increase
Wang et al., 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001), 223 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	0	Quartile 2
						-120	Quartile 3
						-230	Quartile 4
						-140	ln-unit (ng/mL) increase
Wikström et al., 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 1-2	-42	Quartile 2
						-74	Quartile 3
						-116	Quartile 4
						-69	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 3	-23.67	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 1-3	-3.8	ln-unit (ng/mL) increase
Robledo et al., 2015, 2851197	LIFE Study (2005-2009), United States, 234 mother-infant sets	[Medium]	Adequate	Cohort (Prospective)	Pre-conception	-254.4	ln-unit (ng/mL) increase
Meng et al., 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	[Medium]	Deficient	Cohort (Prospective)	Trimester 1-2	-23.8	ln-unit (ng/mL) increase
Gyllenhammar et al., 2018, 4238300	POPUP (1996-2011), Sweden, 381 mother-infant pairs	[Medium]	Deficient	Cross-sectional	3 weeks post-birth	3.95	ln-unit (ng/mL) increase
Hall, 2022, 10273293	Healthy Pregnancy Healthy Baby (HPHB) Cohort (2010-2011), United States, 120 mother-infant pairs	[Medium]	Deficient	Cohort (Prospective)	At birth	103	Tertile 2
						-50.3	Tertile 3
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	[Low]	Deficient	Cohort (Prospective)	At birth	135.1	Tertile 2
						137	Tertile 3
Li et al., 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	[Low]	Deficient	Cross-sectional	At birth	-65.2	ln-unit (ng/mL) increase
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	[Low]	Deficient	Cross-sectional	At birth	23.2	ln-unit (ng/mL) increase

Figure 3-31. Sex-specific female infants only mean birth weight results for 14 PFDA epidemiological studies.[a–f] (Results can be viewed by clicking the HAWC link.)

BWT = birth weight.

[a]Studies are sorted first by overall study confidence level then by exposure window examined.

[b]Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c]The results displayed here for mean BWT in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared with a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[d](Robledo et al., 2015) regression coefficients for maternal serum PFDA are displayed. The complete 95% CI for the female −254.4 g difference ranges from −766.7 to 258.1 g.

[e](Wang et al., 2016) quartile results are truncated; the complete 95% CI for the −230 g difference (Q4) ranges from −440 to −10 g.

[f]For evaluation of patterns of results, studies that collected biomarker samples concurrently or after birth were considered cross-sectional analyses (e.g., Hall et al. (2022)).

Small for gestational age and low birth weight

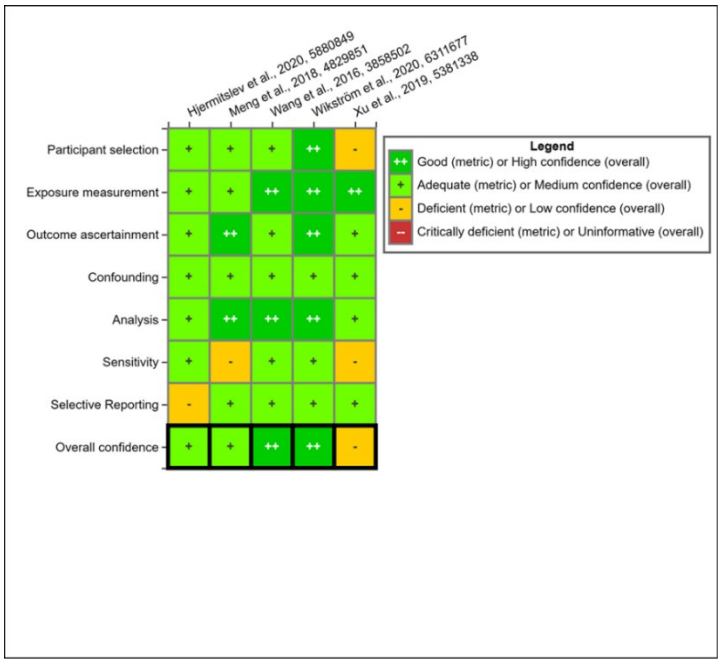


Figure 3-32. Evaluation results for epidemiological studies assessing effects of PFDA exposure on low birth weight/small for gestational age. Results can be viewed by clicking the HAWC link.

Five epidemiological studies included here examined associations between PFDA exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints) (Wikström et al., 2020; Xu et al., 2019a; Wang et al., 2016) or LBW (Hjermitslev et al., 2020; Meng et al., 2018). Two studies were *high* confidence (Wikström et al., 2020; Wang et al., 2016), two were *medium* confidence (Hjermitslev et al., 2020; Meng et al., 2018), and one study was *low* confidence (Xu et al., 2019a). Three of these studies had *adequate* study sensitivity (Hjermitslev et al., 2020; Wikström et al., 2020; Wang et al., 2016) while two were deficient (Xu et al., 2019a; Meng et al., 2018) (see Figure 3-32).

Two (Wikström et al., 2020; Wang et al., 2016) of three epidemiological studies showed some increased risk of SGA, while one study was null (Xu et al., 2019a) (see Figure 3-33). The *high* confidence study by Wang et al. (2016) reported a statistically significant increased OR (3.14; 95% CI: 1.07, 9.19) for SGA per each ln-unit PFDA increase among females. Increased risks were not detected among males (OR = 0.71; 95% CI: 0.33, 1.52). The *medium* confidence study by (Wikström et al., 2020) showed that PFDA was associated with SGA based on a continuous measure (OR = 1.46; 95% CI: 1.06, 2.01 per each ln-unit increase), as well as categorical exposures (Q4: OR = 1.50; 95% CI: 0.94, 2.38 compared with Q1 referent). Results were stronger among females (OR = 1.62; 95% CI: 0.98, 2.67) than males (OR = 1.36; 95% CI: 0.90, 2.07) per each ln-unit increase.

Two studies reported relatively small ORs that were not statistically significant between PFDA and risk of LBW, while another study showed an 80% increased risk of very LBW (VLBW) per

each ln-unit increase. The *medium* confidence study by Meng et al. (2018) reported a larger risk (OR = 1.8; 95% CI: 0.9, 4.0 per each ln-unit increase) for a VLBW (i.e., <2,260 g) measure compared with the typical LBW definition of <2,500 g (OR = 1.3; 95% CI: 0.7, 2.15). There was also no evidence of increased risk across PFDA quartiles or an exposure-response relationship, but the study may have been impacted by sparse cell bias. A nonsignificant increased odds (OR = 1.15; 95% CI: 0.57, 2.33) was reported in the *medium* confidence study by Hjermitslev et al. (2020) per each PFDA ln-unit increase.

SGA/LBW summary

Although they were not always statistically significant, three (Wikström et al., 2020; Meng et al., 2018; Wang et al., 2016) of the five studies examining SGA, LBW, or VLBW showed some increased risks with increasing PFDA exposures (see Figure 3-33). There was no evidence of an exposure-response relationship based on categorical data in one SGA and one LBW study. The relative risks reported in the two LBW studies based on either categorical or continuous exposures (per each unit increase) were consistent in magnitude (OR range: 1.2–1.3), while a larger risk was found (1.8) for the VLBW endpoint. SGA results were more variable based on sex-specific findings but both studies showed larger risks among females. Two of the three studies with stronger results were based on early biomarker sampling.

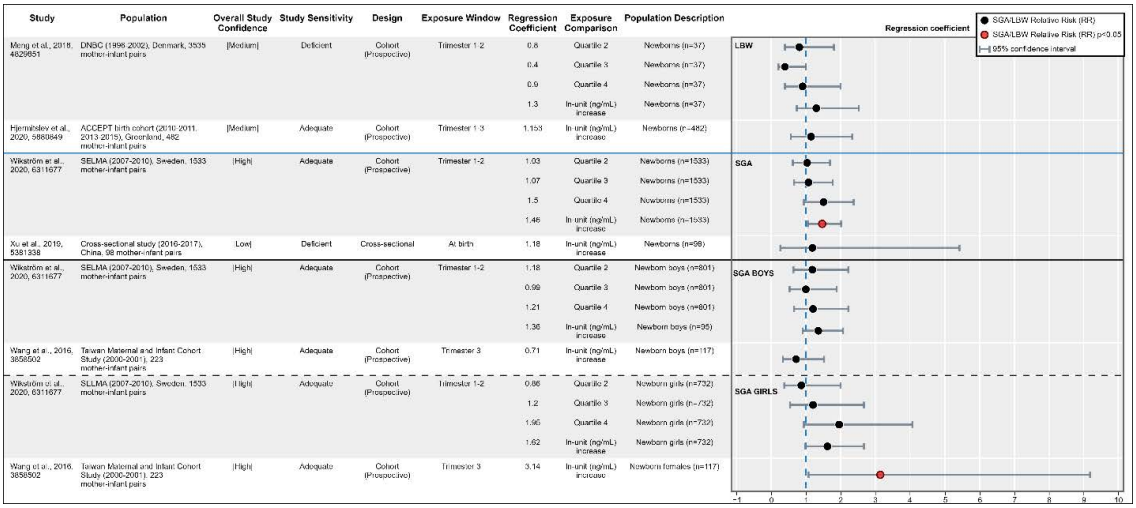


Figure 3-33. Dichotomous fetal growth restriction (small for gestational age and low birth weight) results for 5 PFDA epidemiological studies.[a–d] Results can be viewed at the HAWC link.

SGA = small for gestational age; LBW = low birth weight.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]LBW overall population data above blue reference line.
[c]Overall population SGA data above black reference line; sex-stratified SGA data below reference line.
[d]Sex-stratified SGA; boys above dotted line, girls below.

Birth length

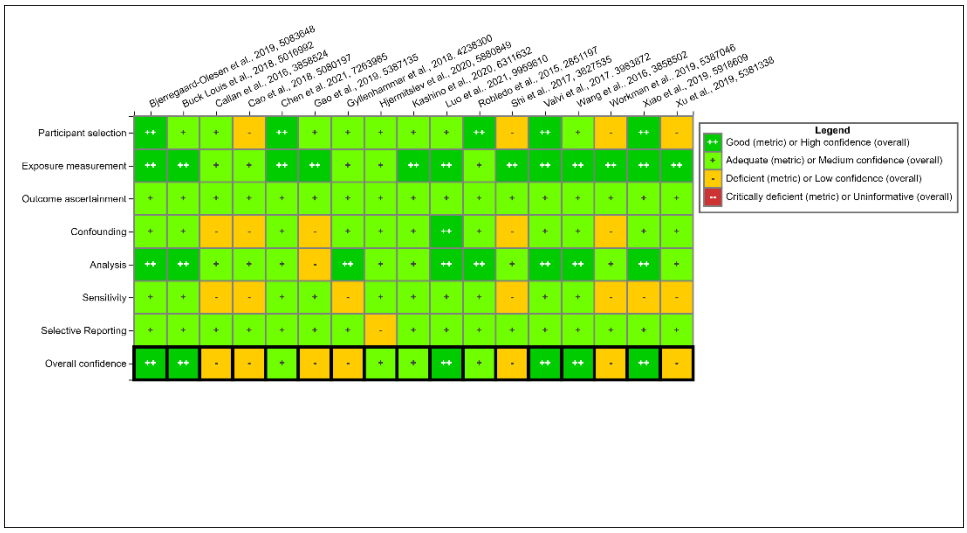


Figure 3-34. Evaluation results for epidemiological studies assessing the effects of PFDA exposure on birth length. Refer to HAWC Human Birth Length for details on the study evaluation review.

Seventeen studies examined the relationship between PFDA exposures and mean or standardized birth length measures including 15 studies that examined changes in the overall population and 10 that examined sex-specific changes (see Figure 3-34). Two of these 10 reported sex-specific analyses only (Wang et al., 2016; Robledo et al., 2015). Most of the studies reported mean birth length differences in relation to PFDA exposures, but two reported standardized birth length measures across the sexes only (Gyllenhammar et al., 2018) or in both sexes as well as the overall population (Xiao et al., 2019).

Six of the 17 studies examining birth length measures in relation to PFDA were classified as *high* confidence (Luo et al., 2021; Xiao et al., 2019; Buck Louis et al., 2018; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016), four were *medium* confidence (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Robledo et al., 2015), and seven were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019b; Cao et al., 2018; Gyllenhammar et al., 2018; Shi et al., 2017; Callan et al., 2016) (see Figure 3-34). All but 1 of the 10 *medium* and *high* confidence studies were considered to have adequate study sensitivity, whereas the remaining 6 *low* confidence studies were classified as deficient.

Overall population results

The majority of studies did not show inverse associations between PFDA and birth length. Four (one *high* and three *low* confidence) studies (Bjerregaard-Olesen et al., 2019; Xu et al., 2019b; Cao et al., 2018; Callan et al., 2016) of the 15 studies examining the overall population reported increased birth length in relation to PFDA, while six studies were null (Hjermitslev et al., 2020; Kashino et al., 2020; Gao et al., 2019; Buck Louis et al., 2018; Shi et al., 2017; Valvi et al., 2017) (see

Figure 3-35). Five of the 15 studies reported reduced mean or standardized birth length in the overall population including 3 of 9 *medium* and *high* confidence studies. The *high* confidence study by Buck Louis et al. (2018) did not show an association between mean birth length and PFDA in the overall population, but they did report that each standard deviation increase in PFDA was associated with reductions in upper arm length (β = −0.09 cm; 95% CI: −0.14, −0.04). These were largely due to associations detected among White (β = −0.21 cm; 95% CI: −0.31, −0.11) and Asian neonates (β = −0.15 cm; 95% CI: −0.25, −0.05). They also reported reductions in upper thigh length (β = −0.14 cm; 95% CI: −0.21, −0.07) in the NICHD cohort with the largest associations detected among White (β = −0.32 cm; 95% CI: −0.45, −0.19) and Asian neonates (β = −0.18 cm; 95% CI: −0.30, −0.06).

Five (two *high*, one *medium*, and two *low* confidence) of the 15 studies reported reduced birth length in the overall population including both studies examining standardized birth length measures. The *high* confidence study by Xiao et al. (2019) reported similar birth length z-scores in the overall population (β = −0.49; 95% CI: −1.00, 0.01), girls (β = −0.46; 95% CI: −1.07, 0.14), and boys (β = −0.53; 95% CI: −1.17, 0.10). A small but precise deficit of 0.19 cm (95% CI: −0.36, 0.02 per each ln-unit) was reported in the *low* confidence study by Gyllenhammar et al. (2018) for their standardized birth length measures. The *high* confidence study by Luo et al. (2021) showed a nonsignificant mean birth length deficit (β = −0.23 cm; 95% CI: −0.64, 0.19) per each ln-unit PFDA increase. The *medium* confidence study by Chen et al. (2021) detected a statistically significant birth length deficit (β = −0.27 cm; 95% CI: −0.53, −0.01 per each ln-unit increase), while the *low* confidence study by Workman et al. (2019) reported a nonsignificant birth length deficit (β = −0.3 cm; 95% CI: −0.8, 0.2 per each ln-unit increase).

Among these five studies (two *high*, one *medium*, and two *low* confidence) showing some evidence of birth length deficits, there was limited evidence of exposure-response relationships with only study (Chen et al., 2021) examining categorical data showing deficits in quartile 4 only (−0.46 cm; 95% CI: −0.91, −0.01). There was a preponderance (four of five studies) of birth length reductions in the overall population from studies based on later sampled biomarkers, which may be indicative of an impact of pregnancy hemodynamics. Study sensitivity did not seem to explain null results, as five of these six studies had adequate ratings.

Sex-specific results

Among the 10 studies with sex-specific results, seven different ones (4 *high*, 3 *medium* confidence) showed some evidence of birth length deficits in relation to PFDA. This included four studies each in girls and in boys (see Figure 3-36). Only the *high* confidence study by Xiao et al. (2019) noted above found reduced standardized birth length measures in both girls and boys. Three studies in girls were null (Hjermitslev et al., 2020; Kashino et al., 2020; Robledo et al., 2015) and two (Cao et al., 2018; Valvi et al., 2017) showed increases in birth length with increasing PFDA exposures. Four studies (Chen et al., 2021; Cao et al., 2018; Shi et al., 2017; Wang et al., 2016) in

boys were null and two (Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019) showed slight nonsignificant increases in birth length.

In addition to the *high* confidence study by Xiao et al. (2019), three other studies showed some evidence of smaller birth length among boys. The *medium* confidence study by Robledo et al. (2015) reported a large but nonstatistically significant reduction among boys (β = −1.15 cm; 95% CI: −3.65, 0.96 per each ln-unit based on maternal serum measures). Smaller birth length deficits per each PFDA ln-unit increase were detected in the *high* confidence study by Valvi et al. (2017) (β = −0.23 cm; 95% CI: −0.68, 0.22) and the *medium* confidence study by Kashino et al. (2020) (β = −0.16 cm; 95% CI: −0.38, 0.07).

Including the Xiao et al. (2019) data above, four of the 10 studies in females reported some birth length reductions. The *high* confidence study by Wang et al. (2016) reported nonstatistically significant deficits among girls in quartile 4 (β = −0.75 cm; 95% CI: −2.09, 0.59) and for each PFDA ln-unit increase (β = −0.47 cm; 95% CI: −1.23, 0.30). Birth length deficits similar in magnitude (β = −0.44 cm; 95% CI: −0.79, −0.09 per each ln-unit PFDA increase) were detected among girls in the *medium* confidence study by Chen et al. (2021). Smaller birth length changes were detected among girls (β = −0.22 cm; 95% CI: −0.86, 0.43) in the Aarhus Birth Cohort Bjerregaard-Olesen et al. (2019) study.

Among the 10 studies in total, 7 different ones reported some evidence of sex-specific associations between PFDA and reduced birth length, including 4 studies each in girls and in boys. Few patterns were evident across study characteristics and study sensitivity did not appear to be an explanatory factor for null studies. Sample timing also did not appear to be a strong determinant of the sex-specific study results as four of the seven different studies reporting reductions were based on later biomarker sampling.

Birth length summary

Although 10 different studies of 16 in total showed some evidence of birth length deficits in relation to PFDA exposures, the majority of studies across each group examined (overall population, boys, girls, or confidence level) did not show inverse associations. For example, only three of nine *medium* and *high* confidence studies examining the overall population detected inverse associations. Four (2 *high* and 2 *medium* confidence) of 10 studies each in boys and girls (3 *high* and 1 *medium* confidence) reported birth length deficits in relation to PFDA. An additional *high* confidence study was null in the overall population but showed some reductions among different ethnic groups.

Five (2 *high*, 1 *medium,* and 2 *low* confidence) of the 14 studies examining the overall population reported birth length deficits including 3 that reported mean birth weight deficits consistent in magnitude (range: 0.23 to 0.30 per each ln-unit increase). Birth length changes were more variable in the seven studies (four *high*, three *medium* confidence) that reported sex-specific

deficits. Although three studies reported mean birth length reductions ~0.20 cm, the remaining sex-specific studies ranged from −0.44 to −1.15 cm per each ln-unit PFDA increase.

Although some of these studies reported large differences in birth length, there was no direct evidence of exposure-response relationships in the few studies with categorical data. However, the Wang et al. (2016) analysis in girls did show some large gradients in birth length among the upper two quartiles. The Chen et al. (2021) study also showed larger deficits in quartile 4 relative to quartile 1. Few patterns were evident across study characteristics, and study sensitivity did not appear to be an explanatory factor for null studies. Seven of 10 different studies reporting some birth length reductions in the overall population (4 of 5) and across sexes (4 of 7) were based on later biomarker samples. This observation may be indicative of potential bias because of the effect of pregnancy hemodynamics and adds some uncertainty.

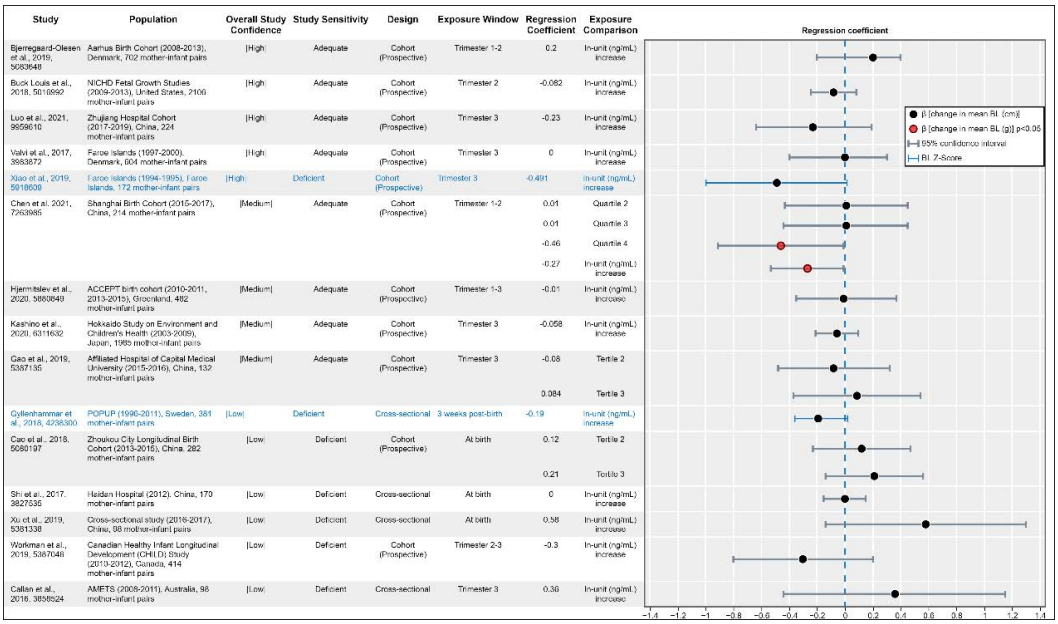
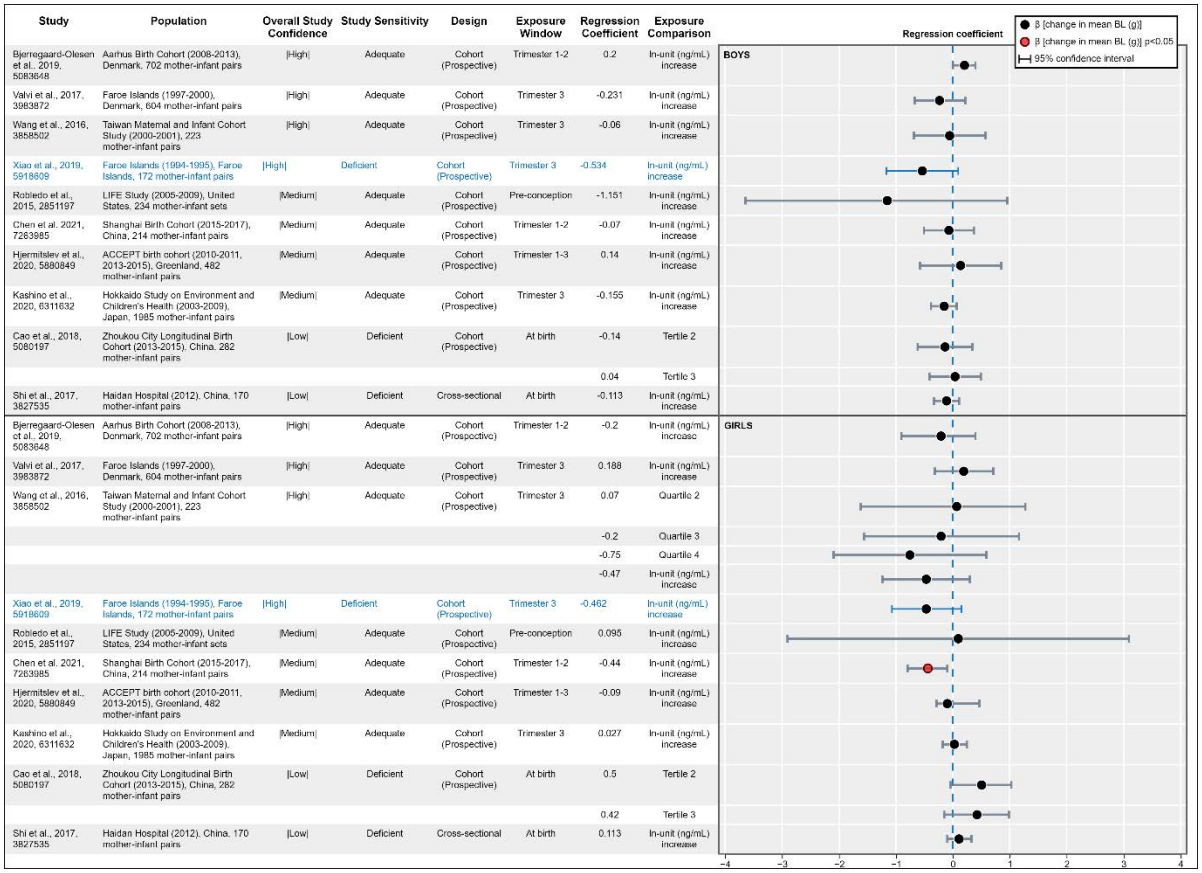


Figure 3-35. Overall study population mean birth length results for 15 PFDA epidemiological studies.[a,b] (Results can be viewed by clicking the HAWC link.)

BL = birth length.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]Xiao et al. (2019) and Gyllenhammar et al. (2018) reported birth length z-score data.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Bjerregaard-Olesen et al., 2019, 5083648	Aarhus Birth Cohort (2008-2013), Denmark, 702 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 1-2	0.2	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	-0.231	ln-unit (ng/mL) increase
Wang et al., 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001), 223 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	-0.06	ln-unit (ng/mL) increase
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), Faroe Islands, 172 mother-infant pairs	[High]	Deficient	Cohort (Prospective)	Trimester 3	-0.534	ln-unit (ng/mL) increase
Robledo et al., 2015, 2851197	LIFE Study (2005-2009), United States, 234 mother-infant sets	[Medium]	Adequate	Cohort (Prospective)	Pre-conception	-1.151	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 1-2	-0.07	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 1-3	0.14	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 3	-0.155	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	[Low]	Deficient	Cohort (Prospective)	At birth	-0.14	Tertile 2
						0.04	Tertile 3
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	[Low]	Deficient	Cross-sectional	At birth	-0.113	ln-unit (ng/mL) increase
Bjerregaard-Olesen et al., 2019, 5083648	Aarhus Birth Cohort (2008-2013), Denmark, 702 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 1-2	-0.2	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	0.188	ln-unit (ng/mL) increase
Wang et al., 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001), 223 mother-infant pairs	[High]	Adequate	Cohort (Prospective)	Trimester 3	0.07	Quartile 2
						-0.2	Quartile 3
						-0.75	Quartile 4
						-0.47	ln-unit (ng/mL) increase
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), Faroe Islands, 172 mother-infant pairs	[High]	Deficient	Cohort (Prospective)	Trimester 3	-0.462	ln-unit (ng/mL) increase
Robledo et al., 2015, 2851197	LIFE Study (2005-2009), United States, 234 mother-infant sets	[Medium]	Adequate	Cohort (Prospective)	Pre-conception	0.095	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 1-2	-0.44	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 1-3	-0.09	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	[Medium]	Adequate	Cohort (Prospective)	Trimester 3	0.027	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	[Low]	Deficient	Cohort (Prospective)	At birth	0.5	Tertile 2
						0.42	Tertile 3
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	[Low]	Deficient	Cross-sectional	At birth	0.113	ln-unit (ng/mL) increase

Figure 3-36. Sex-stratified birth length results for 10 PFDA epidemiological studies.[a,b] Results can be viewed by clicking the HAWC link.

BL= birth length.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]Xiao et al. (2019) reported birth length z-score data.

Head circumference

Fourteen studies examined PFDA levels in relation to head circumference including five *high* (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Valvi et al., 2017; Wang et al., 2016) and five *medium* confidence studies (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Lind et al., 2017a; Robledo et al., 2015) (see Figure 3-37). The four *low* confidence studies (Workman et al., 2019; Xu et al., 2019b; Gyllenhammar et al., 2018; Callan et al., 2016) as well as (Xiao et al., 2019) were considered deficient in the study sensitivity domain largely due to low exposure levels and/or narrow contrasts. The remaining nine *medium* and *high* confidence studies had adequate ratings in the sensitivity domain.

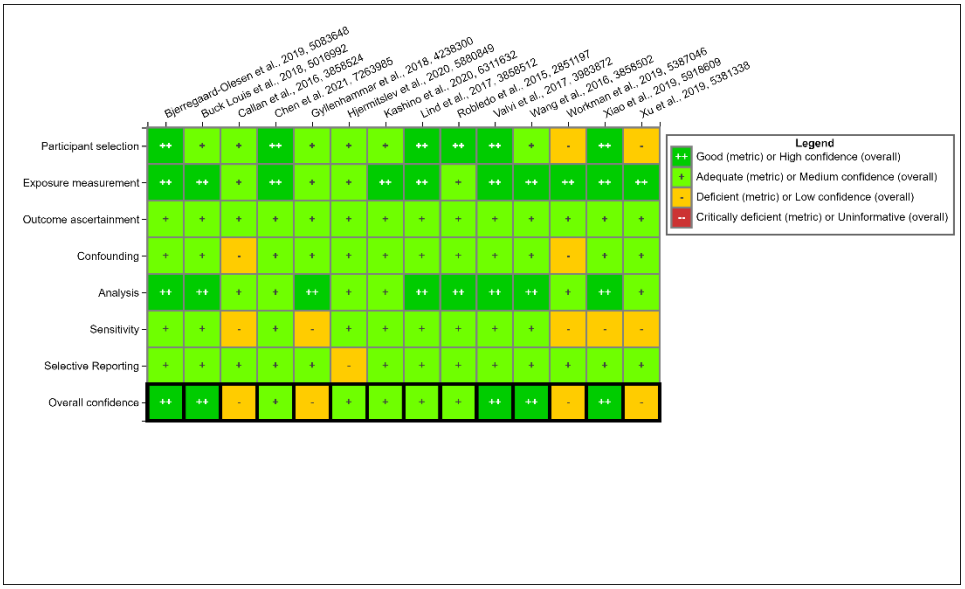


Figure 3-37.Evaluation results for epidemiological studies assessing the effects of PFDA exposure on head circumference. Refer to HAWC Human Head Circumference for details on the study evaluation review**.**

One study provided standardized head circumference data (Xiao et al., 2019), while the other 13 included in Figures 3-38 and 3-39 are based on mean head circumference differences. Eight studies examined sex-specific results in both boys and girls (Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Lind et al., 2017a; Valvi et al., 2017; Wang et al., 2016; Robledo et al., 2015), including three with sex-specific data only (Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015). Eleven studies reported head circumference results in the overall population (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Workman et al., 2019; Xiao et al., 2019; Xu et al., 2019b; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Valvi et al., 2017; Callan et al., 2016).

Head circumference-overall population results

Only 2 of 11 studies in the overall population showed inverse associations between head circumference and PFDA exposures. The *medium* confidence study by Hjermitslev et al. (2020) reported a nonsignificant decreased head circumference in the overall population (β = −0.15 cm; 95% CI: −0.37, 0.07 per each ln-unit increase). Slightly smaller but precise head circumference deficits were reported in the *medium* confidence study by Kashino et al. (2020) (β = −0.10 cm; 95% CI: −0.24, 0.003 per each ln-unit PFDA increase). In contrast, nonsignificant increased head circumference (β range: 0.16 to 0.21 cm) in the overall population was reported in relation to PFDA in three studies (Chen et al., 2021; Workman et al., 2019; Valvi et al., 2017). No associations were reported between PFDA exposures and mean or standardized head circumference measures in 6 of the 11 studies based on the overall population, including three studies each with *high* (Bjerregaard-

Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018) and *low* confidence (Xu et al., 2019b; Gyllenhammar et al., 2018; Callan et al., 2016) (see Figure 3-38).

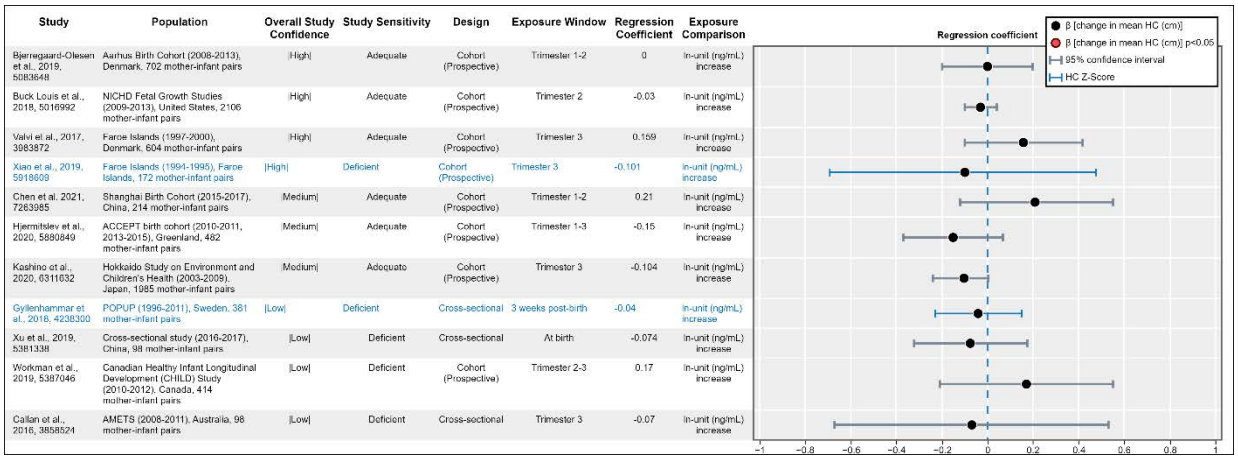


Figure 3-38. Overall population head circumference results in 11 epidemiological studies.[a,b] Refer to the HAWC link.

HC = head circumference.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]Xiao et al. (2019) reported head circumference z-score data.

Head circumference-sex-specific results

Among the eight studies (Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Lind et al., 2017a; Valvi et al., 2017; Wang et al., 2016; Robledo et al., 2015) reporting sex-specific results in both male and female neonates, three studies in girls and one in boys reported head circumference reductions with increasing PFDA exposures (see Figure 3-39). The Lind et al. (2017a) study reported an exposure-response relationship based on PFDA quartiles (range: −0.1. to −0.3 cm) in boys that was larger than that seen for their continuous results scaled to each ln-unit increase (β = −0.10 cm; 95% CI: −0.5, 0.3). The *high* confidence study by Wang et al. (2016) detected a nonsignificant decrease (β = −0.37 cm; 95% CI: −0.85, 0.10 per each ln-unit increase) in mean head circumference only among girls. The *medium* confidence study by Robledo et al. (2015) reported larger but particularly imprecise head circumference reductions for girls (β = −0.62 cm; 95% CI: −2.4, 1.2 per each ln-unit increase). The *high* confidence study by Bjerregaard-Olesen et al. (2019) showed a smaller nonsignificant result (β = −0.22 cm; 95% CI: −0.65, 0.22 per each ln-unit increase). In contrast, one *high* (Valvi et al., 2017); β = 0.51 cm; 95% CI: 0.13, 0.90) and one *medium* (Lind et al., 2017a); β = 0.3 cm; 95% CI: −0.1, 0.7) confidence study each reported increased birth length for female neonates for each ln-unit PFDA increase, as did the Bjerregaard-Olesen et al. (2019) study (β = 0.19 cm; 95% CI: −0.19, 0.38 per each ln-unit increase) in males. Null associations were reported per each ln-unit increase in five studies in boys (Hjermitslev et al., 2020; Kashino et al., 2020; Xiao et al., 2019; Valvi et al., 2017; Wang et al., 2016) and three in girls (Hjermitslev et al., 2020; Kashino et al., 2020; Xiao et al., 2019).

Four of eight available studies reported some head circumference reductions among boys or girls including three that were based on early biomarker samples. In addition to the Lind et al. (2017a) study result noted in boys above, four null studies examining different head circumference measures in relation to continuous exposures reported nonsignificant and imprecise deficits of approximately −0.1 cm per each unit increase for either or both sexes (Hjermitslev et al., 2020; Kashino et al., 2020; Valvi et al., 2017; Wang et al., 2016).

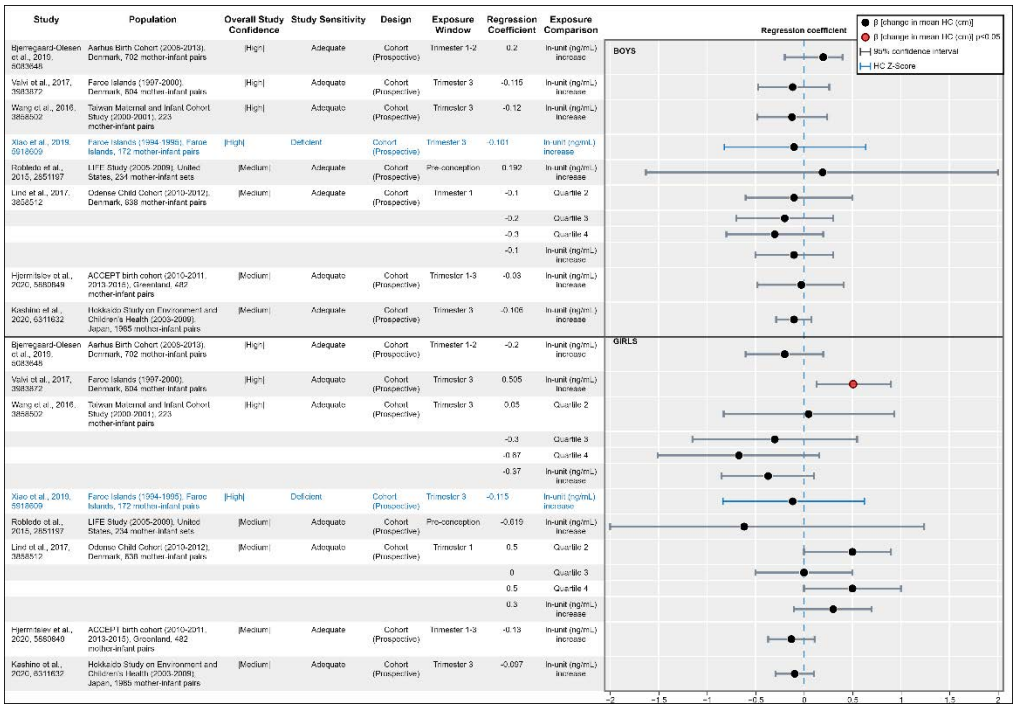


Figure 3-39. Sex-stratified head circumference results in eight PFDA epidemiological studies.[a,b] Refer to the HAWC link.

HC = head circumference.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]Xiao et al. (2019) reported head circumference z-score data.

Head circumference summary

There was limited evidence of associations between PFDA and head circumference with 6 (2 *high* and 4 *medium* confidence) of 14 studies reporting reductions in head circumference in the overall population or either or both sexes. These reductions were reported in the majority (6 of 10) of the *high* and *medium* confidence studies but were predominately due to sex-specific findings. For example, limited evidence was found in the overall population with only 2 of 11 studies including 2 of 7 *medium* and *high* confidence studies. Four of eight sex-specific studies reported some head circumference reductions with three of these occurring among female neonates. Four of these six studies that reported some head circumference reductions in the overall population or either sex was based on early biomarker sampling during or prior to pregnancy. In contrast to the null sex-

specific studies for which only one of five studies had deficient study sensitivity, nearly all (five of six) null studies in the overall population were rated as deficient. Narrow exposure contrasts in many studies of PFDA, likely limited statistical power and may have precluded the ability to detect statistically significant associations that are small in magnitude. Overall, there was limited evidence of associations between PFDA and head circumference with more evidence seen among sex-specific analyses than the overall population.

Fetal growth restriction summary

Eighteen of the 28 studies examining different BWT measures in relation to PFDA measures in the overall population or either or both sexes, reported some evidence of associations. This included 11 different studies (and 9 of 14 *medium* and *high* confidence) of 22 examining mean BWT in the overall population. There was considerable variability in BWT deficits (β range: −29 to −101 g per ln-unit increases) in the overall population, with seven studies ranging from 31 to 59 g deficits per each ln-unit increase. These deficits were seen despite low exposure levels and contrasts in many studies (see Table 3-19). For example, among the nine *medium* and *high* studies reporting it, the PFDA IQR in the overall study populations ranged from 0.07 to 0.37 ng/mL and the median levels ranged from 0.11 to 0.55 ng/mL. Few studies examined nonlinear relationships between PFDA and mean BWT. The lone study that reported deficits across categories demonstrated an exposure-response relationship for mean BWT, while one of two studies showed based on standardized BWT measures. Twelve of the 13 studies reporting sex-specific results showed some evidence of BWT deficits in either or both sexes. However, there was not a clear sex-specific effect of PFDA. Eight studies each in girls and boys showed some reductions and only four studies showed deficits in both sexes.

Although there was no evidence on an exposure-response relationships in the few studies with categorical data, the majority of studies reporting results for either SGA, LBW, or VLBW showed some increased risks with increasing PFDA exposures. Relative risks generally were fairly modest in magnitude ranging from 1.2 to 1.8, with more variable and larger risks for SGA results denoted among females.

Results were more mixed for birth length and head circumference. Ten of 16 studies in total reported some decreased birth length results in relation to increasing PFDA exposures. These studies included 5 (2 *high*, 1 *medium,* and 2 *low* confidence) of the 14 studies that reported birth length deficits fairly consistent in magnitude in the overall population (range: 0.13 to 0.30 per each unit increase) as well as 3 of 9 *medium*/*high* confidence studies. In comparison with the overall population results, birth length changes were more variable in the 10 studies that examined stratified results by sex. Four out 10 studies each in boys (2 *high* and 2 *medium* confidence) and girls (3 *high* and 1 *medium* confidence) birth length deficits in relation to PFDA. The four studies that showed birth length deficits in girls were generally more consistent in magnitude; one study reported mean birth length reductions of 0.20 cm and, the three others ranged from −0.44 to

−0.75 cm per each ln-unit increase. There was no direct evidence of exposure-response relationships in the few birth length studies with categorical data; but one analysis in girls did show some large gradients in birth length among the upper two quartiles.

Six (2 *high*; 4 *medium*) of 14 PFDA studies in total reported reductions in head circumference in the overall population (2 of 11 studies) or either sex (4 of 8 studies). Although these 6 studies showing some reductions accounted for more than half of the 10 *high* and *medium* confidence studies examined here, these findings were largely driven by sex-specific analyses. Therefore, overall, the head circumference results were less consistent but were comparable in magnitude to those seen for birth length. The one analysis in boys that reported head circumference reductions did show an exposure-response relationship. Although not monotonic across all quartiles, another study in girls also showed large gradients in head circumference among the upper two quartiles.

Few explanatory factors were consistently identified by general study characteristics across the fetal growth restriction endpoints including exposure levels, study sensitivity, and sex differences. The limited exposure contrasts in many of the studies may have precluded sufficient statistical power to detect associations small in magnitude and, especially for analyses stratified by sex. One source of uncertainty related to the BWT findings was the predominance of inverse associations reported in studies with later biomarker sampling, which may be indicative of potential bias due to pregnancy hemodynamics. For example, there was a definitive pattern by sampling timing as only 9 of the 11 studies reporting BWT deficits in the overall population had biomarkers based on later sampling during or after pregnancy. The majority of sex-specific studies with inverse associations for BWT were also based on later biomarker samples. The opposite was seen for studies of head circumference with three of four studies in the overall population or either sex based on early samples. Overall, there was fairly consistent evidence of an association between PFDA and different BWT-related measures although this was more mixed for other endpoints. The patterns by sample timing were not consistent across endpoints, but the dearth of birth weight and length results in the overall study populations based on early or prepregnancy measures might be indicative of potential bias due to the impact due to pregnancy hemodynamics on PFDA levels. Thus, there is considerable uncertainty in the fetal growth restriction evidence given that some sample timing differences may explain some of the reported deficits examined here.

Table 3-19. Summary of 33 studies (from 35 publications) of PFDA exposure in relation to fetal and postnatal growth restriction measures sorted by overall confidence[a]

Author (year)	Study location/ years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
***High* confidence studies**								
Wang et al. (2016)	Taiwan, 2000–2001	223	0.46/0.56–Boys 0.43/0.48–Girls (0.16–1.57)	↑ **SGA (Girls)*** Ø (Boys)	**– Girls*[b]** Ø Boys	– Girls Ø Boys	– Girls Ø Boys	**– Wt Girls*** + Wt Boys **– Ht Girls*** – Ht Boys
Bjerregaard-Olesen et al. (2019); Bach et al. (2016)	Denmark, 2008–2013	1,533	0.30/0.20 (<LOD–2.87)		Ø All – Girls + Boys	+ All – Girls + Boys	Ø All – Girls + Boys	
Lind et al. (2017a)	Denmark, 2010–2012	636	0.30/0.10 (0.1–1.8)		**+ Girls*** – Boys		+ Girls Ø Boys [b]	
Valvi et al. (2017)	Faroe Islands, 1997–2000	604	0.28/0.16 (0.22–0.38)[c]		– All – Girls – Boys	Ø All	+ All	
Buck Louis et al. (2018)	USA, 2009–2013	2,106	0.25/0.26 (0.16–0.42)[c]		Ø All	– All		
Gardener et al. (2021)	USA, 2009–2013	354	0.2/0.2 (LOD–2.6)		+ All			
Luo et al. (2021)	China, 2017–2019	224	0.50/0.28 (N/A)		– All	– All		
Wikström et al. (2020)	Sweden, 2007–2010	1,533	0.26/0.15 (N/A)	↑ SGA (All)* ↑ SGA (Girls) ↑ SGA (Boys)	**– All*[b]** – Girls – Boys			
Xiao et al. (2019)	Faroe Islands, 1994–1995	140	N/A/N/A (0.1, 0.9)		– All – Girls – Boys		Ø All Ø Girls Ø Boys	
Yao et al. (2021)	China, 2010–2013	369	0.55/0.37 (0.09–3.77)		– All			

Author (year)	Study location/ years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Gao et al. (2022)	China, 2013–2016	1,350	1.82/1.44 (0.21–26.6)					Ø RWG-Wt (All)* ↓RWG-Wt (Girls) ↑ RWG-Wt (Boys) ↑ RWG-Ht (All)* ↑ RWG-Ht (Girls) ↑ RWG-Ht (Boys)
Starling et al. (2019)	USA, 2009–2014	1,410	0.1/0.1 (N/A)					Ø-Wt ↑RWG-Wt + Adiposity (All/Boys/Girls)
Zhang et al. (2022)	China, 2013–2016	2,395	1.72/1.38 (0.21–27.8)					Ø All Ø Girls Ø Boys
***Medium* confidence studies**								
Robledo et al. (2015)	MI/TX, USA, 2005–2009	234	0.45–Boys[d] 0.40–Girls[d] (N/A)		– Girls Ø Boys	Ø Girls – Boys	– Girls Ø Boys	
Lenters et al. (2016)	Ukraine/ Poland/ Greenland, 2002–2004	1,321	0.16–0.40 (0.07–1.18) range across three countries		– All			
Gyllenhammar et al. (2018); Swedish Environmental Protection Agency (2017)[e]	Sweden, 1996–2001	381/587	0.24/0.14 (LOD–1.1)		**– All*** Ø Girls **– Boys***	– All	Ø All	Ø Wt, Ht
Woods et al. (2017)	OH, USA, 2003–2006	272	0.20/0.10 (0.2–0.3)[f]		Ø All			

Author (year)	Study location/ years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Meng et al. (2018)	Denmark, 1996–2002	2,120	0.20/0.10 (N/A)	↑ LBW (All) ↑ VLBW (All)	– All – Girls Ø Boys			
Kwon et al. (2016)	S. Korea, 2006–2010	268	0.11/0.07 (0.04–0.41)		**– All***			
Chen et al. (2021)	China, 2013–2015	214	1.73/1.47 (N/A)		Ø All	– All* **– Girls*** Ø Boys		
Gao et al. (2019)	China, 2015–2016	132	0.47 (LOD–3.15)		+ All	Ø All		
Hall et al. (2022)					Ø Girls – Boys			
Hjermitslev et al. (2020)	Greenland, 2010–2011; 2013–2015	266	0.71/N/A (0.12–7.84)	↑ LBW (All)	– All Ø Girls – Boys	Ø All	– All Ø Girls Ø Boys	
Kashino et al. (2020)	Japan, 2003–2009	1591	0.6/0.5 (LOD–2.4)		– All – Girls – Boys	Ø All	– All Ø Girls Ø Boys	
***Low* confidence studies**								
Xu et al. (2019b)	China, 2016–2017	98	0.21/0.15 (0.1–0.58)[f]	Ø SGA	+ All	+ All		
Li et al. (2017)	China, 2013	321	0.15/0.16 (LOD–2.12)		– All – Girls – Boys			
Lee et al. (2018)	South Korea, 2012–2013	361	0.37/0.36 (0.04–1.25)					**– Wt***, – Ht[b]
Callan et al. (2016)	W. Australia 2003–2004	98	0.12/N/A (0.03–0.39)		Ø All	Ø All	Ø All	
Cao et al. (2018)	China, 2013–2015	337	0.10/0.09 (0.04–0.22)[g]		+ All + Girls Ø Boys	Ø All + Girls Ø Boys	Ø All – Girls Ø Boys	+ Wt Girls – Wt Boys Ø Ht Girls Ø Ht Boys

Author (year)	Study location/ years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Starling et al. (2017) [h]	CO, USA, 2009–2014	598	0.10/0.10[c] (LOD–3.5)		Ø All			
Shi et al. (2017)	China, 2012	170	0.08/0.10 (LOD–0.60)		Ø All + Girls − Boys	Ø All Ø Girls − Boys		Ø All Ø Girls Ø Boys
Jensen et al. (2020a)	Denmark, 2010–2012	589	0.26/N/A (N/A)					+ Adiposity (All)
Workman et al. (2019)	Canada, 2010–2011	414	0.13/N/A (LOD–1.4)		− All	− All	+ All	

[*]Statistically significant findings based on $p < 0.05$.

Symbols: Ø: null association; + : positive association; − : negative association; ↑: increased odds ratio; ↓: decreased odds ratio.

LOD = limit of detection; N/A: not available; All = overall population of boys and girls; IQR = interquartile range; HC = head circumference; SGA = small for gestational age; LBW = low birth weight; VLBW = very low birth weight; Ht = height; Wt = weight; RWG = rapid weight gain.

[a]Overall confidence descriptor is for the birth weight endpoints when studies included prenatal and postnatal growth measures; four other studies had only postnatal data Gao et al. (2022); Zhang et al. (2022); Starling et al. (2019); Lee et al. (2018).

[b]Exposure-response relationships detected for categorical data.

[c]IQR calculated by subtracting the 25th percentile from the 75%; the 25th percentile estimated here as 0 given it was below the detection limit.

[d]Robledo et al. (2015) regression coefficients for maternal serum PFDA are displayed.

[e]Swedish Environmental Protection Agency (2017) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort compared with a smaller sample size of 381 in the 2018 publication by Gyllenhammar et al. (2018).

[f]Fifth–95th percentiles.

[g]Tenth–90th percentiles.

Note: "Developmental effects" indicated by increased odds ratio (↑) for dichotomous outcomes, (+) for adiposity/body mass index and waist circumference, and negative associations (−) for the other outcomes.

[h]This study based on the ECHO cohort study is detailed in other publications (Chang et al., 2022; Eick et al., 2020; Sagiv et al., 2018); the population analyzed here also overlaps with a more recent pooled analysis by (Padula et al., 2023).

Growth restriction – postnatal growth (infancy and early childhood up to 2 years of age)

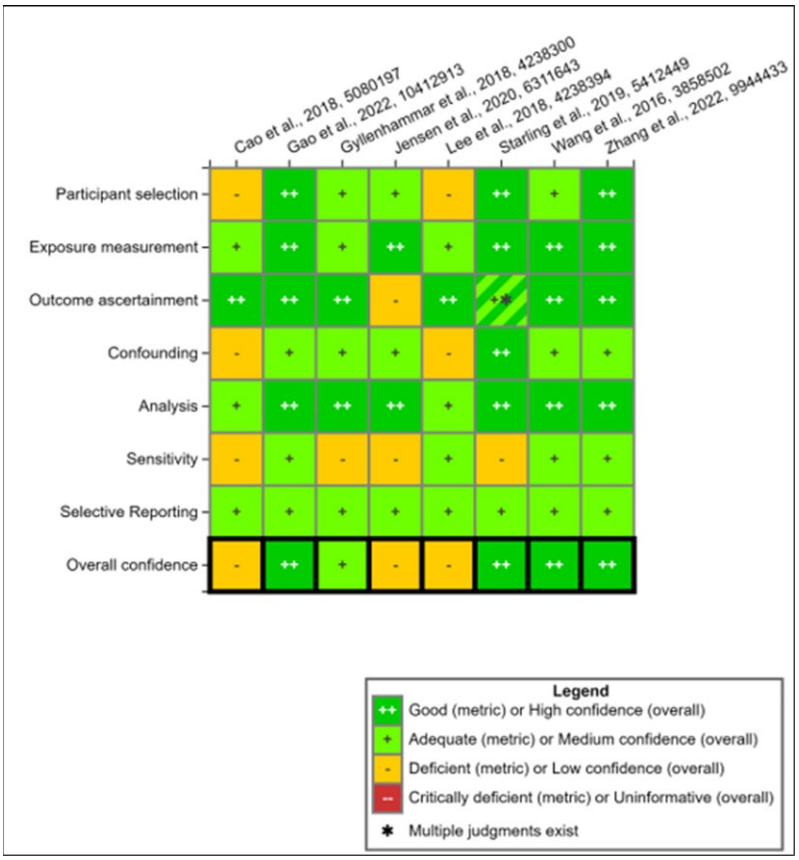


Figure 3-40. Evaluation results for epidemiological studies assessing the effects of PFDA exposure on postnatal growth.[a,b] Refer to HAWC Human Postnatal Growth for details on the study evaluation review.

[a]In Gyllenhammar et al. (2018), the outcomes height, weight, and body mass index are rated as Good, while the outcome head circumference is rated as Adequate.

[b]In Starling et al. (2019), the outcome weight-for-age z-score (at 5 months) rated as Good, while the outcomes length-for-age z-score and adiposity/fat mass at 5 months were rated as Adequate.

Eight studies were identified that assessed postnatal growth in relation to PFDA (see Figure 3-40) with each of these examining some measures of childhood weight and/or height in relation to PFDA. Four studies were considered *high* (Gao et al., 2022; Zhang et al., 2022; Starling et al., 2019; Wang et al., 2016), one was *medium* (Gyllenhammar et al., 2018) and three were *low* confidence (Jensen et al., 2020a; Cao et al., 2018; Lee et al., 2018). Of the eight postnatal growth studies, four each had adequate (Gao et al., 2022; Zhang et al., 2022; Lee et al., 2018; Wang et al., 2016) and deficient (Jensen et al., 2020a; Starling et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018) study sensitivity ratings largely owing to small exposure contrasts. Although there was some overlap across studies, limited serial measures during infancy as well as inconsistent age at examinations and analyses may limit some comparisons and preclude the ability to fully evaluate

consistency across studies. For example, (Zhang et al., 2022) examined growth up to 12 months and (Starling et al., 2019) took measurements at 5 months only. Both (Wang et al., 2016) and (Lee et al., 2018) examined postnatal growth at 2 years, whereas (Cao et al., 2018) analyses were based on a mean of 19 months in participants. (Gyllenhammar et al., 2018) had serial measures of postnatal growth at 3, 6, 12 and 18 months. (Jensen et al., 2020a) examined different adiposity measures at 3 and 18 months, whereas (Gao et al., 2022) examined growth trajectory based on serial measurements at five periods within the first 2 years (at birth, 42 days, 6 months, 12 months, and 24 months).

Postnatal weight

Postnatal weight: overall population

In the overall population, five postnatal studies (two *high*, one *medium*, and two *low* confidence) examined PFDA in relation to either standardized (Zhang et al., 2022; Starling et al., 2019; Gyllenhammar et al., 2018) or mean weight measures in two *low* confidence studies (Cao et al., 2018; Lee et al., 2018). All three standardized weight studies reported null associations including (Gyllenhammar et al., 2018) for PFDA exposures and standard deviation scores (SDS) for weight measured at 3 to 18 months (see Figure 3-41). Similar to findings from (Zhang et al., 2022) examining growth up to 12 months, (Starling et al., 2019) also detected no difference in the overall population at 5 months for either weight-for-age and weight-for-length z-scores across PFDA tertiles and for each ln-unit increase.

Two *low* confidence studies examining mean weight differences in the overall population during early childhood showed some deficits related to upper PFDA exposure (see Figure 3-42) around 2 years of age. (Cao et al., 2018) reported a nonsignificant and imprecise postnatal weight change (β = −130 g; 95% CI: −579, 319) in the overall population (mean age of examination of mean of 19 months) for tertile 3 (relative to tertile 1), but the opposite was seen for tertile 2. Despite their limited exposure contrast, (Lee et al., 2018) reported a nonsignificant mean weight decrease at 2 years for each ln-unit PFDA increase (β = −140 g; 95% CI: −310, 30) in the overall population. They also detected lower mean weight at 2 years across PFDA quartiles in a monotonic fashion (e.g., β range: −200 to −390 g) including a statistically significant weight reduction (β = −390 g; 95% CI: −770, −10) in quartile 4 (relative to quartile 1). As noted above, both studies were based on measurements in children around 2 years of age.

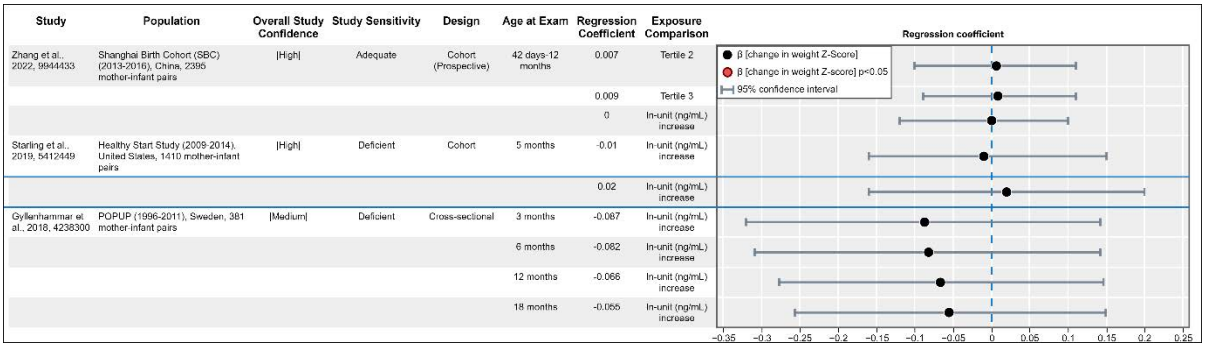


Figure 3-41. PFDA and postnatal growth-standardized weight measures in the overall population.[a,b] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at outcome.
[b]Above the first blue line is weight-for-age z-score; between the two blue lines depicts weight-for-length z-scores; below the last blue line reflects standardized postnatal weight data.

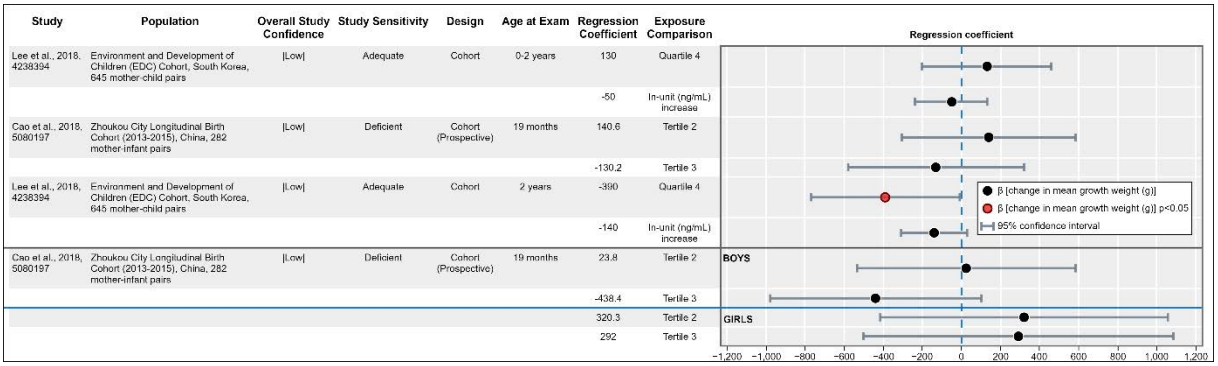


Figure 3-42. PFDA and postnatal growth – mean weight (in grams) results from two epidemiological studies.[a-c] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at outcome.
[b]Overall population data above the black reference line; sex-stratified data below.
[c]Sex-stratified: male infant data above the blue line; females below.

Postnatal weight: sex-specific

Four studies (three *high* and one *low* confidence) included PFDA sex-specific analyses with one (Cao et al., 2018) reporting mean weight changes and three reporting standardized weight measures (Zhang et al., 2022; Starling et al., 2019; Wang et al., 2016) (see Figure 3-43). Two of the four studies showed detected deficits in relation to PFDA albeit not consistent across sexes. The *low* confidence (Cao et al., 2018) study detected imprecise contrasting changes in postnatal weight, with nonsignificant decreases in the highest tertile for boys (β = −438 g; 95% CI: −980, 103) but increases among girls (β = 292 g; 95% CI: −501, 1,085). Two of the sex-standardized weight studies reported null results for boys and girls based either on weight-for-age and weight-for-length standardized measures (see Figure 3-43). Starling et al. (2019) reported no difference in either sex at 5 months for weight-for-age and weight-for-length z-scores across PFDA tertiles or for each ln-

unit increase, as did Zhang et al. (2022) across PFDA tertiles for postnatal growth up to 12 months. In contrast, Wang et al. (2016) detected statistically significant reductions among females only for average childhood weight z-scores (β = −0.32; 95% CI: −0.63, 0) (data not plotted). No association was seen for age 2 for weight z-score in either sex (see Figure 3-43), and the largest weight z-scores were among females detected at birth and at age 11 (data not plotted).

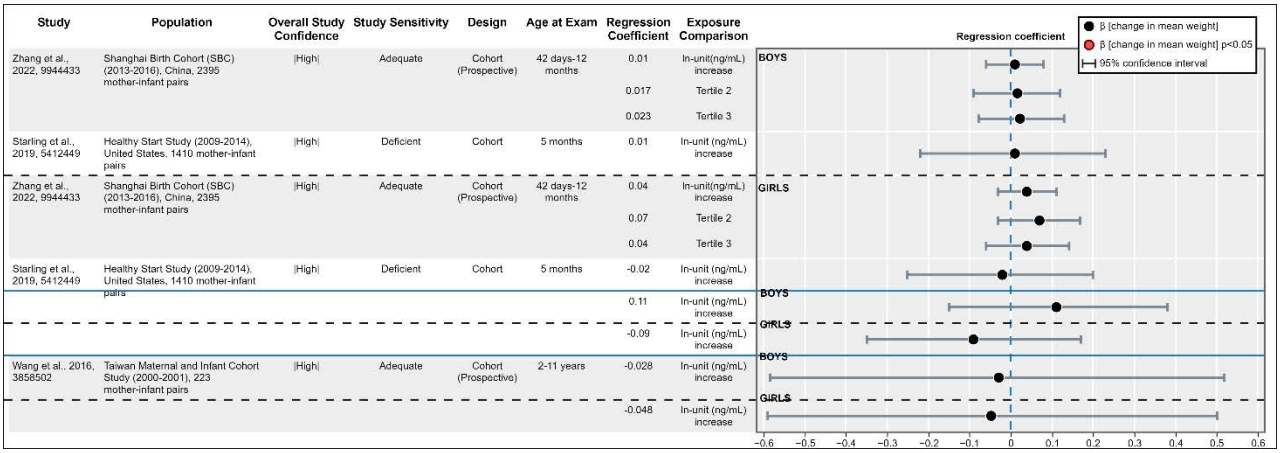


Figure 3-43. PFDA and postnatal growth – standardized weight (sex-stratified; boys above dashed line, girls below).[a,b] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at exam.
[b]Weight-for-age z-score above the blue line; weight-for-length z-score between the two blue lines; weight z-score below the last blue line.

Postnatal height

Postnatal height: overall population

Four studies (one *high,* one *medium,* and two *low* confidence) examined mean differences or standardized postnatal height scores in the overall population (Zhang et al., 2022; Cao et al., 2018; Gyllenhammar et al., 2018; Lee et al., 2018) with only one study reporting height reductions in relation to PFDA. The *medium* confidence by (Gyllenhammar et al., 2018) and the *high* confidence study by (Zhang et al., 2022) were null for standardized height measures in the overall population (see Figure 3-44). The *low* confidence study by (Cao et al., 2018) reported larger mean postnatal height increases across higher PFDA tertiles (β range: 1.27 to 1.56 cm) in the overall population (see Figure 3-45). Despite a limited exposure contrast, the *low* confidence study by Lee et al. (2018) reported lower mean height at 2 years (β = −0.44 cm; 95% CI: −0.77, −0.10). They reported lower mean height in a monotonic fashion with the largest statistically significant weight difference detected in quartile 4 (β = −1.11 cm; 95% CI: −1.86, −0.36).

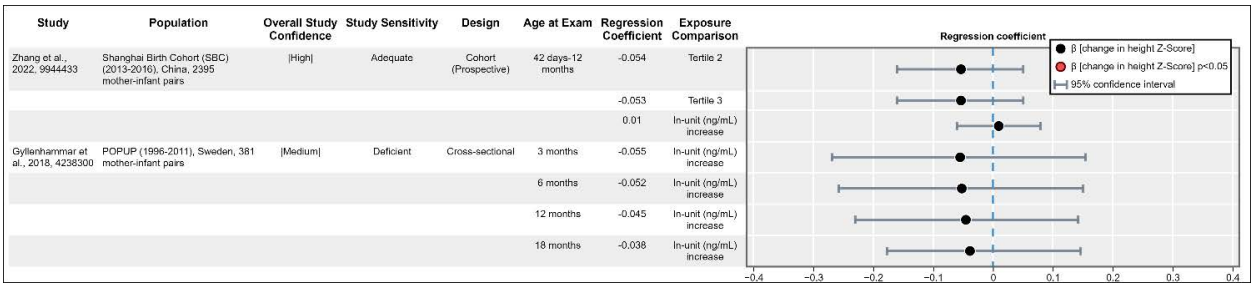


Figure 3-44. PFDA and postnatal growth – standardized height measures in the overall population.[a] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at exam.

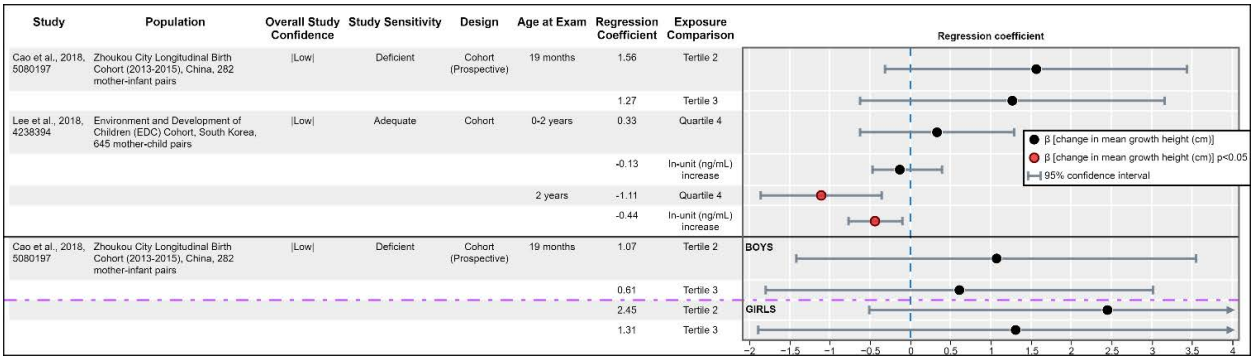


Figure 3-45. PFDA and postnatal growth – mean height (in centimeters).[a,b] Refer to the HAWC link.

[a]Sex-stratified data are located below the solid black line; boys are above the purple dotted line and girls are below.

[b]Cao et al. (2018) female results have upper bounds that have been truncated; the upper bounds are 5.41 for Tertile 2 and 4.5 for Tertile 3.

Postnatal height: sex-specific

Three studies (two *high* and one *low* confidence) examined height in relation to PFDA across sexes including one (Cao et al., 2018) examining mean differences and two studies examining standardized measures (Zhang et al., 2022; Wang et al., 2016). The *low* confidence study by (Cao et al., 2018) reported larger postnatal mean height increases among females (β range: 1.31 to 2.45 cm) than males (β range: 0.61 to 1.07 cm) across PFDA tertiles. The *high* confidence study by Zhang et al. (2022) reported null associations for both sexes based on continuous and categorical PFDA exposures (see Figure 3-46). In contrast, the *high* confidence study by Wang et al. (2016) detected statistically significant reductions among females only for childhood height z-scores averaged from birth, 2, 5, 8, and 11 years (β = −0.52; 95% CI: −0.80, −0.24) (data not plotted). Smaller height z-scores were found for all periods for both male and females but was only statistically significant for females at ages 2 and 11 (data not plotted). For example, they reported

larger height z-score reductions (β = −0.61; 95% CI: −1.02, −0.23 per each ln-unit PFDA increase) at age 2 among females than in males (β = −0.17; 95% CI: −0.63, 0.30).

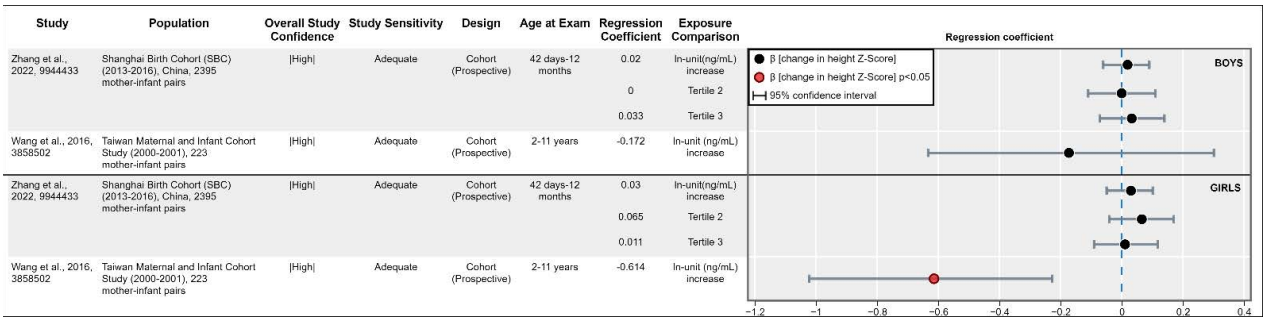


Figure 3-46. PFDA and postnatal growth – standardized height measures (sex-stratified; boys above reference line, girls below).[a,b] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at exam.
[b]Boys above reference line, girls below.

Postnatal head circumference

Three studies (one *high, medium,* and *low* confidence study each) examined postnatal standardized head circumference including two studies (Zhang et al., 2022; Gyllenhammar et al., 2018) that reported standardized results only in the overall population (see Figure 3-47) and one (Cao et al., 2018) that examined mean head circumference data in the overall population as well as across sexes (see Figure 3-48). None of the three studies examining head circumference showed much evidence of decreases in head circumference with increasing PFDA exposures. Null results were detected in Zhang et al. (2022) for postnatal head circumference-for-age z-score up to 12 months of age per each ln-unit increase and across PFDA tertiles and for Gyllenhammar et al. (2018) head circumference SDS measures were based on four different time points (3, 6, 12, and 18 months). In the overall population, Cao et al. (2018) detected a sizeable mean head circumference increase in PFDA tertile 2 (0.50 cm; 95% CI: −0.44, 1.44) but was null in tertile 3 (data not plotted). Results were null for boys, whereas contrasting results were seen for tertile 3 (β = −0.69 cm; 95% CI: −2.26, 0.88) and tertile 2 (β = 0.67 cm; 95% CI: −0.79, 2.13) among girls.

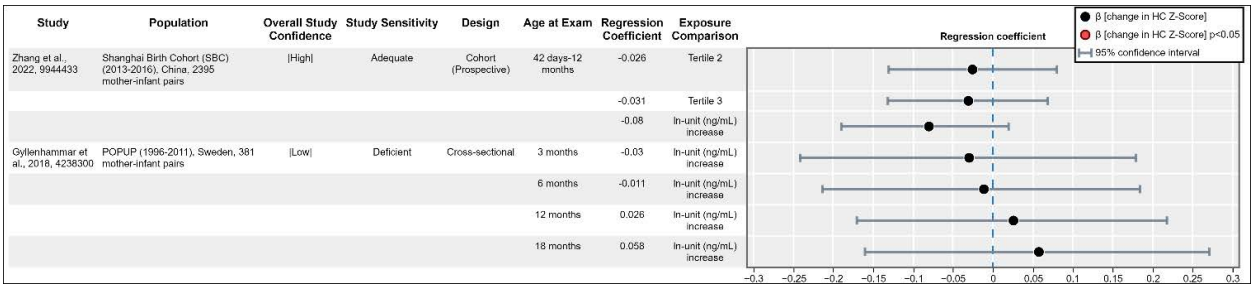


Figure 3-47. PFDA and postnatal growth-standardized head circumference in the overall population.[a] Refer to the HAWC link.

HC = head circumference.
[a]Studies are sorted first by overall study confidence level then by age at exam.

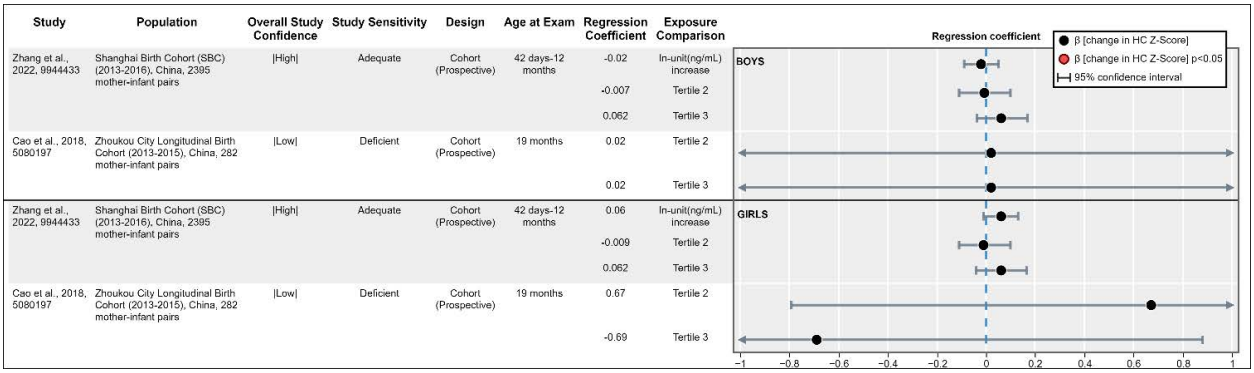


Figure 3-48. PFDA and postnatal growth head circumference (sex-stratified; boys above reference line, girls below).[a–c] Refer to the HAWC link.

HC = head circumference.
[a]Studies are sorted first by overall study confidence level then by age at exam.
[b]Zhang et al. (2022) reported standardized results based on head circumference z-scores, while Cao et al. (2018) reported mean head circumference data (in cm).
[c]Cao et al. (2018) upper and lower bounds have been truncated. For boys, for Tertile 2 the bounds are [−1.23, 1.27] and for Tertile 3 the bounds are [−1.19, 1.24]. For girls, for Tertile 2 the bounds are [−0.79, 2.13] and for Tertile 3 the bounds are [−2.26, 0.88].

Adiposity measures (waist circumference/body mass index/ponderal index)

Three studies (two *high* and one *low* confidence) examined postnatal adiposity measures including % fat mass increase as well as standardized waist circumference, BMI, and ponderal index measures. Two studies detected increased adiposity relative to PFDA exposures, while one study (Zhang et al., 2022) reported null associations for BMI-for-age z-score per each ln-unit PFDA increase in the overall population and across sexes. Jensen et al. (2020a) showed null associations for PFDA and waist circumference SDS in the overall population (see Figure 3-49) and across both sexes (data not plotted). However, they did report increased adiposity measures in the overall

population including body mass index SDS (β = 0.42; 95% CI: 0.01, 0.84 per each ln-unit increase) with stronger associations among females (β = 0.58; 95% CI; −0.03, 1.19 per each ln-unit increase). Similarly, a statistically significant association with larger ponderal index SDS (β = 0.60; 95% CI: 0.18, 1.02 per each ln-unit increase) was detected in the overall population and was driven by associations in females (β = 1.02; 95% CI: 0.40, 1.64 per each ln-unit increase). Starling et al. (2019) reported a slight nonsignificant increase in infant adiposity at 5 months of age for each ln-unit increase in PFDA (β = 0.59% fat mass increase; 95% CI: −0.27, 1.44) with larger increases among males (β = 0.79% fat mass increase; 95% CI: −0.46, 2.04) compared with females (β = 0.44% fat mass increase; 95% CI: −0.82, 1.69). The opposite was seen in their categorical analyses dichotomized at the median with more adiposity in females (β = 0.70% fat mass increase; 95% CI: −0.78, 2.17) and males (β = 0.23% fat mass increase; 95% CI: −1.39, 1.85).

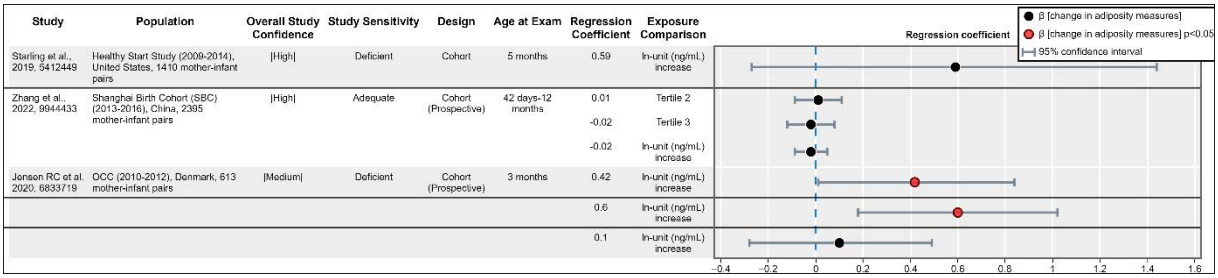


Figure 3-49. PFDA and postnatal growth measures-body mass index, adiposity, ponderal index, waist circumference (overall population).[a–d] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at exam.
[b]Solid black lines divide the outcomes examined here: adiposity, body mass index, ponderal index, and waist circumference (ordered top to bottom).
[c]Zhang et al. (2022) reported standardized body mass index data.
[d]Units: Fat mass increase % for Starling et al. (2019); not applicable for unitless standardized measures depicted for Jensen et al. (2020a) and Zhang et al. (2022).

Rapid weight gain

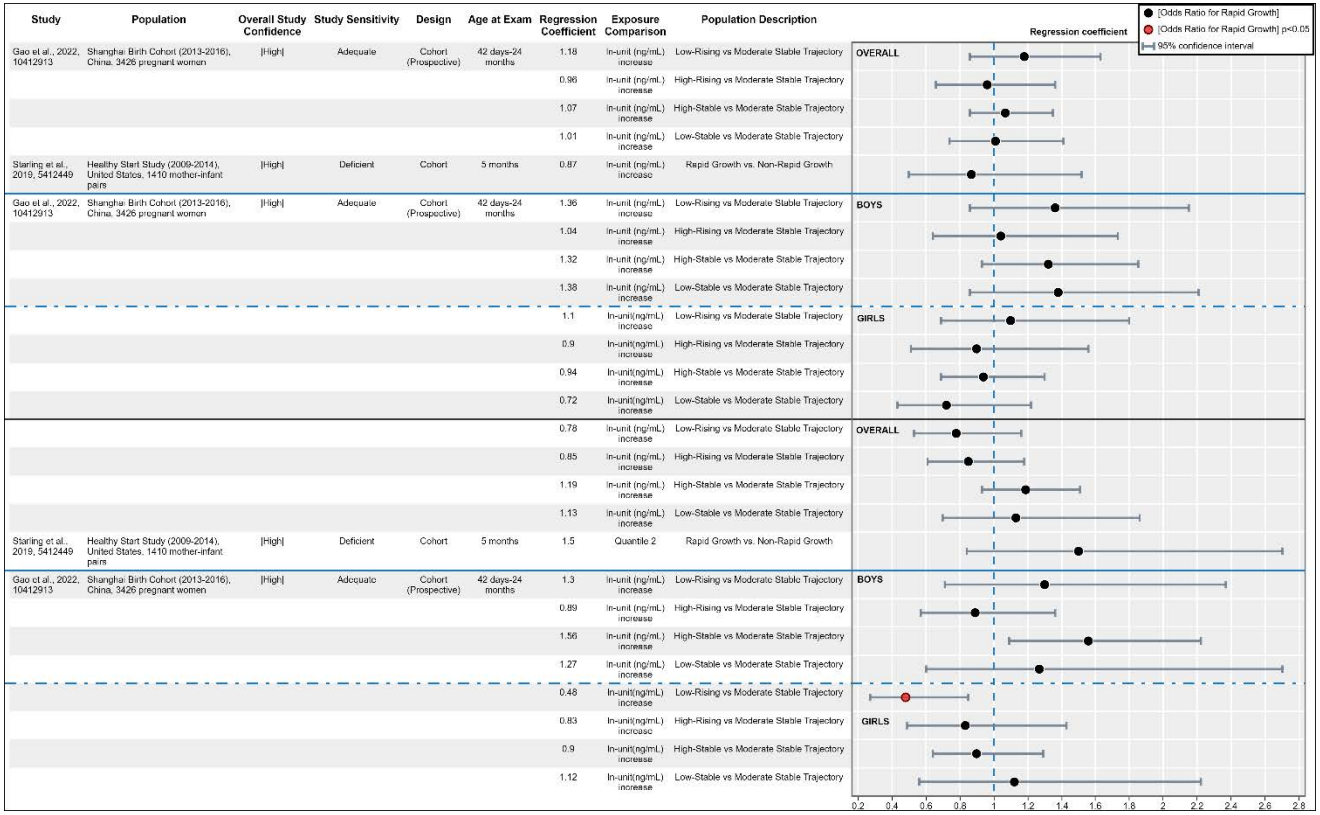
Two *high* confidence studies (Gao et al., 2022; Starling et al., 2019) examined different rapid weight gain measures in relation to PFDA. In the Healthy Start study, Starling et al. (2019) examined different rapid weight gain measures in relation to PFDA for the overall population and both sexes. In the Shanghai Birth Cohort, Gao et al. (2022) examined various measures of growth trajectories in the overall population as well as some sex-specific analyses.

Starling et al. (2019) reported null associations for rapid weight gain measures based on *weight-for-age* z-score (OR = 0.87; 95% CI: 0.50, 1.52 per each ln-unit PFDA increase) and a *weight-for-age* standard deviation growth rate between birth and 5-month follow-up (see Figure 3-50). They did, however, report a nonsignificant increase in rapid weight gain derived from *weight-for-length z-score* (OR = 1.50; 95% CI: 0.84, 2.70) for categorical exposures above the median (0.2–3.5 ng/mL relative to the referent up to 0.1 ng/mL). In the overall population, Gao et al. (2022)

reported null associations between PFDA and their *weight-for-age* and *weight-for-length z-score* endpoints across all trajectory designations. According to the *weight-for-length z-score*, the low-rising participants (e.g., growth trajectory starts with a low value followed by an increased trend afterward) versus moderate-stable referent group (e.g., growth trajectory starts with a moderate value followed by stable growth afterward) had a nonsignificant OR for the overall population (0.78; 95% CI: 0.53, 1.16 ln-unit PFDA increase). Results were contrasting in females (OR = 0.48; 95% CI: 0.27, 0.8 per each ln-unit PFDA increase) and males (OR = 1.30; 95% CI: 0.71, 2.37 per each ln-unit PFDA increase).

Gao et al. (2022) reported a nonsignificant increased risk (OR = 1.54; 95% CI: 0.85, 2.82 per each ln-unit PFDA increase) for *length-for-age z-score* among those participants that were considered high-rising versus the moderate-stable group with comparable risks detected among male and females (OR range: 1.73–1.83). In a weighted quantile sum mixture model, they also detected higher odds (OR = 1.59; 95% CI: 0.90, 2.82 per each ln-unit PFDA increase) among the high-rising group (vs. moderate-stable) based on *length-for-age z-scores*, with PFDA having the highest weight among the PFAS mixtures. Gao et al. (2022) reported nonsignificant inverse associations comparable in magnitude based on *head-circumference-for-age* z-score for high-rising versus moderate-stable (OR = 0.66; 95% CI: 0.38, 1.12 per each ln-unit PFDA increase) and low-stable versus moderate-stable participants (OR = 0.67; 95% CI: 0.49, 0.93 per each ln-unit PFDA increase). They detected a statistically significant inverse association (OR = 0.51; 95% CI: 0.27, 0.99 per each ln-unit PFDA increase) for low-rising versus moderate-stable groups in the single-PFAS model. They reported a lower risk in the weighted quantile sum model (OR = 0.37; 95% CI: 0.18, 0.72), with PFDA having the highest weight among the PFAS mixtures. In general, the low- and high-rising groups examined by Gao et al. (2022) may be at most risk for metabolic syndrome, as evidenced by changes in obesity and other health effects later in life. However, results were not consistent in the overall population or across sexes for these different rapid growth measures. Therefore, there is no compelling evidence of increased postnatal weight gain among those that may represent low birth weight individuals with rapid weight gain trajectories (i.e., low-rising group).

Mixed results within and across these two studies suggest the support is limited for accelerated growth during infancy being related to PFDA. Although there was some evidence of increased risks occurring in the high-rising trajectory group, which may be indicative of rapid weight gain for those that experienced fetal growth restriction, however, the evidence is scant and inconsistent to draw many conclusions in the overall population or across sexes.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Age at Exam	Regression Coefficient	Exposure Comparison	Population Description
Gao et al., 2022, 10412913	Shanghai Birth Cohort (2013-2016), China, 3426 pregnant women	[High]	Adequate	Cohort (Prospective)	42 days-24 months	1.18	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.96	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.07	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.01	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
Starling et al., 2019, 5412449	Healthy Start Study (2009-2014), United States, 1410 mother-infant pairs	[High]	Deficient	Cohort	5 months	0.87	ln-unit (ng/mL) increase	Rapid Growth vs. Non-Rapid Growth
Gao et al., 2022, 10412913	Shanghai Birth Cohort (2013-2016), China, 3426 pregnant women	[High]	Adequate	Cohort (Prospective)	42 days-24 months	1.36	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						1.04	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.32	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.38	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						1.1	ln-unit(ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.9	ln-unit(ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						0.94	ln-unit(ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						0.72	ln-unit(ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						0.78	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.85	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.19	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.13	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
Starling et al., 2019, 5412449	Healthy Start Study (2009-2014), United States, 1410 mother-infant pairs	[High]	Deficient	Cohort	5 months	1.5	Quantile 2	Rapid Growth vs. Non-Rapid Growth
Gao et al., 2022, 10412913	Shanghai Birth Cohort (2013-2016), China, 3426 pregnant women	[High]	Adequate	Cohort (Prospective)	42 days-24 months	1.3	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.89	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.56	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.27	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						0.48	ln-unit(ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.83	ln-unit(ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						0.9	ln-unit(ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.12	ln-unit(ng/mL) increase	Low-Stable vs Moderate Stable Trajectory

Figure 3-50. PFDA and postnatal growth rapid growth (overall population) and sex-specific (in grams).[a–e] Refer to the HAWC link.

[a]Studies are sorted first by overall study confidence level then by age at exam.
[b]Weight-for-age z-score data above the black reference line; weight-for-length below.
[c]Overall population data above the blue line; sex-stratified data below.
[d]Sex-Stratified data: male infants above the blue dash-dotted line; females below.
[e]Quantile 2 in Starling et al. (2019) represents dichotomized exposure at median (quantile 1 referent: LOD–0.1 ng/mL; quantile 2: 0.2–3.5 ng/mL).

Postnatal growth summary

Overall, there were mixed results within and across the eight available postnatal PFDA studies of early childhood. For example, two (one *high* and one *low* confidence) of five different studies measuring height and three (one *high* and two *low* confidence) of six different studies measuring weight reported some deficits in relation to PFDA. Interestingly, there were more consistent results seen in three studies (Cao et al., 2018; Lee et al., 2018; Wang et al., 2016) that examined postnatal growth measures at age 2. For example, both studies showing some postnatal height deficits in either the overall population or across sexes were based on participants examined at 2 years of age. There was no evidence of associations between PFDA exposures and early childhood head circumference, but two (one *high* and one *low* confidence) of three studies showed some suggestion of increased postnatal adiposity. Only two studies examined rapid weight gain in relation to PFDA and were fairly inconsistent within and across studies based on different weight and length measurements.

Only three of the eight total studies reported categorical data, which may inform examination of nonlinearity or exposure-response relationships. Only one of these three studies showed any evidence of any monotonic deficits across PFDA categories. There were a fairly small number of studies across each common endpoint; thus, a lack of patterns across study characteristics (except age at examination) was not unexpected. For example, although there were no studies with good ratings for study sensitivity, this factor did not appear to be explanatory for the null studies. However, limited exposure contrasts and statistical power may have hampered the ability to detect associations small in magnitude especially among the sexes.

In summary, although the evidence was mixed across various postnatal measures and different examination windows, with only minimal evidence of exposure-response relationships to support the continuous exposure scaled results. One challenge in evaluating consistency across heterogeneous studies includes disparate periods of follow-up and assessment (e.g., childhood age at examination). Despite the mixed evidence shown here, there was some suggestion of more consistency in studies that examined postnatal growth measures around 2 years of age. This may reflect the challenges that exist to detect associations in children that experience fetal growth restriction and subsequent rapid growth periods. Although, there was limited information and evidence of rapid weight associations among the two studies that considered this. Overall, the evidence for postnatal associations is *slight* largely due to the early childhood weight and adiposity results along with inconsistency across the other measures.

Anogenital distance

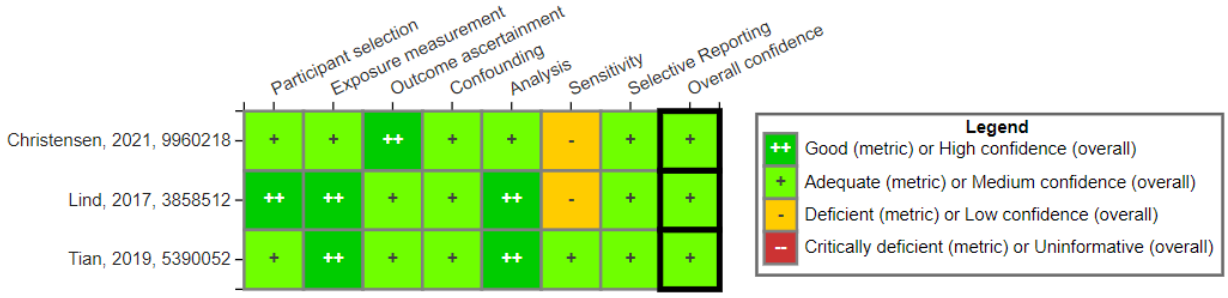


Figure 3-51. Evaluation results for epidemiological studies assessing effects of PFDA exposure on anogenital distance. Refer to the interactive HAWC Human AGD link for additional details**.**

Three *medium* confidence birth cohorts (see Figure 3-51) in Denmark (Lind et al., 2017a), China (Tian et al., 2019) and the Faroe Islands (Christensen et al., 2021) examined the association between PFDA exposure and AGD at 3 months of age. All three studies examined boys while Lind et al. (2017a) and Christensen et al. (2021) also included girls.

Among boys, Tian et al. (2019) reported smaller AGD at birth with higher PFDA exposure (ASD β = −0.58 mm; 95% CI: −1.11, −0.06; APD β = −0.63 mm; 95% CI: −1.24, −0.01). Decrements were also observed at 6 months ($p > 0.05$), but not at 12 months, which may be due to greater heterogeneity in size as children develop. A positive association was observed in Christensen et al. (2021) (Q2 β vs. Q1 = 1.4 mm; 95% CI: 0.4, 2.5; Q3 β = 1.0 mm; 95% CI: 0.0, 2.1; Q4 β = 1.3 mm; 95% CI: 0.3, 2.4). No associations were observed in Lind et al. (2017a). Exposure levels were considerably higher in Tian et al. (2019) (median 2.1 vs. 0.2 and 0.3 ng/mL), but this does not explain the inconsistent direction of association across studies.

For girls, there was an inverse association with PFDA for one of the two AGD measures (AGD_{AC}, measured from the center of anus to the top of clitoris) reported in Lind et al. (2017a). They reported an association based on continuous exposure (β = −1.3 mm, 95% CI: −2.8, 0.2) and across upper two PFDA exposure quartiles in a nonmonotonic fashion (Q2 vs. Q1: β = 0.4 mm; 95% CI: −1.3, 2.0; Q3: β = −0.7 mm; 95% CI: −2.4, 0.9; Q4: −1.7; 95% CI: −3.6, 0.1, p-trend = 0.04). An association was also observed in quartile 4 in the other AGD measure (AGD_{AF}, measured from center of anus to posterior fourchette), although it was not statistically significant (Q4 β = −1.0 mm; 95% CI: −2.4; 0.4). No associations were observed in Christensen et al. (2021).

AGD is a marker of androgen exposure, and thus an inverse association in AGD would be expected to correspond with a decrease in testosterone. This was not observed in the single *low* confidence study of testosterone in neonates (see Male and Female Reproductive Effects); however, there is considerable uncertainty in the reproductive hormones evidence base. Thus, this lack of coherence does not decrease confidence in the AGD findings. Reduced AGD is associated with clinically relevant outcomes in males, including cryptorchidism, hypospadias, and lower semen

quality and testosterone levels (Thankamony et al., 2016), but adversity of reduced AGD is less established in females. As noted above in the few studies of birth defects, EPA did not identify any epidemiological studies that examined PFDA in relation to congenital genitourinary defects, such as cryptorchidism and hypospadias. Overall, the evidence for AGD is *indeterminate* given the mixed results and limited information for various AGD measures across the sexes.

Gestational duration endpoints

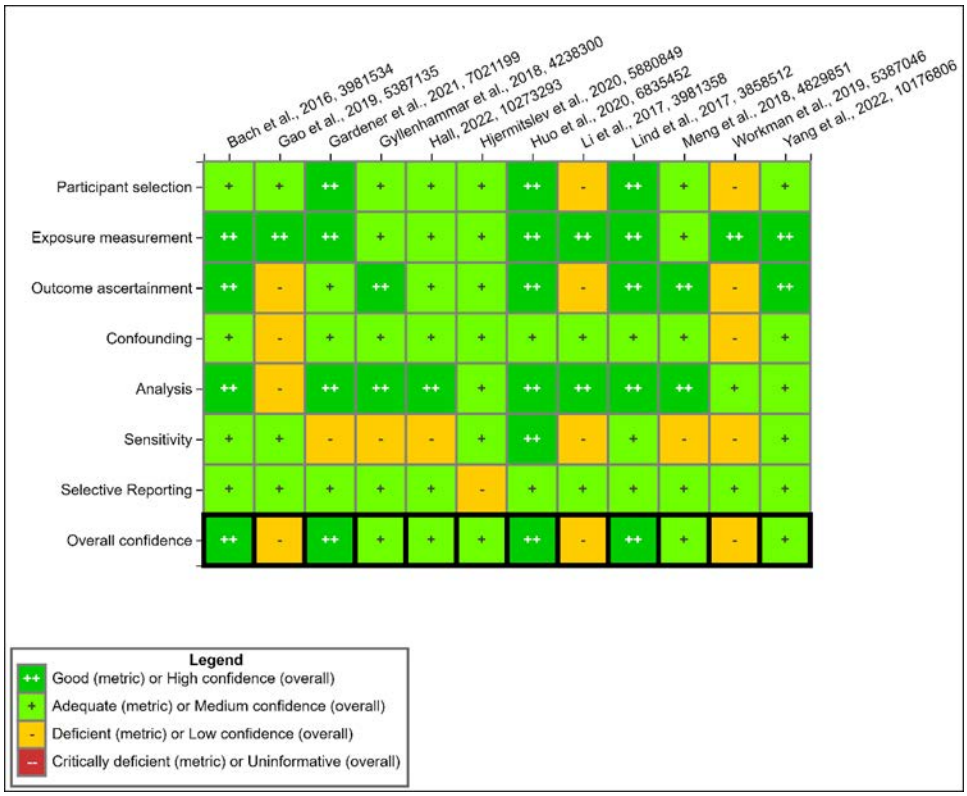


Figure 3-52. Evaluation results for epidemiological studies assessing effects of PFDA exposure on gestational duration. Refer to HAWC Human Gestational Duration for details on the study evaluation review.

As shown in Figure 3-52, 12 epidemiological studies examined PFDA in relation to changes in gestational duration measures (i.e., gestational age or PTB). All 12 examined gestational age measurements, while 6 included preterm birth. Four studies were *high* confidence (Gardener et al., 2021; Huo et al., 2020; Lind et al., 2017a; Bach et al., 2016), five were *medium* (Hall et al., 2022; Yang et al., 2022a; Hjermitslev et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018), and three studies were *low* owing largely to very limited exposure contrasts (Gao et al., 2019; Workman et al., 2019; Li et al., 2017). One study had good sensitivity (Huo et al., 2020), while five were adequate (Yang et al., 2022a; Hjermitslev et al., 2020; Gao et al., 2019; Lind et al., 2017a; Bach et al., 2016) and six were deficient (Hall et al., 2022; Gardener et al., 2021; Workman et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Li et al., 2017). Ten of the 12 studies were prospective cohort or

nested case-control studies (Hall et al., 2022; Yang et al., 2022a; Gardener et al., 2021; Hjermitslev et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Meng et al., 2018; Lind et al., 2017a; Bach et al., 2016), and two were cross-sectional (Gyllenhammar et al., 2018; Li et al., 2017). For examination of consistency and between-study heterogeneity, the type of statistical analyses was examined in addition to the type of study design. As part of this review, cross-sectional analyses were considered for any study that used maternal serum/plasma, umbilical cord, or placental postpartum PFDA measures in relation to gestational duration even if the data were derived from prospective cohort or nested case-control studies (Hall et al., 2022; Yang et al., 2022a).

The epidemiological studies had maternal exposure measures that were sampled either during the first trimester (Lind et al., 2017a), two (Huo et al., 2020), three (Gardener et al., 2021; Gao et al., 2019) across multiple trimesters (Hjermitslev et al., 2020; Meng et al., 2018; Bach et al., 2016), or were based on postpartum maternal or infant biomarker samples (Hall et al., 2022; Yang et al., 2022a; Gyllenhammar et al., 2018; Li et al., 2017). All five of the cross-sectional studies/analyses had late pregnancy or postpartum sampling (defined here as from the second trimester exclusive onward). Four (Hjermitslev et al., 2020; Meng et al., 2018; Lind et al., 2017a; Bach et al., 2016) of the prospective cohort studies had early biomarker sampling (defined here as having at least some first trimester maternal sampling), while the remaining two (Gardener et al., 2021; Workman et al., 2019) relied on late sampling.

Preterm birth

Six studies examined PFDA and preterm birth including three studies each being *high* (Gardener et al., 2021; Huo et al., 2020; Bach et al., 2016) and *medium* confidence (Yang et al., 2022a; Hjermitslev et al., 2020; Meng et al., 2018) (see Figure 3-53). Three studies showed some evidence of increased risk of PTB with increasing PFDA exposures including two studies with early biomarker sampling. Null associations for PTB were reported in the *medium* confidence study by Yang et al. (2022a) and *high* confidence study by Bach et al. (2016), while a nonsignificant inverse association (OR = 0.65; 95% CI: 0.24, 1.79 per each PFDA ln-unit increase) was reported in the *medium* confidence study by Hjermitslev et al. (2020).

Although there was no evidence of an exposure-response relationship, the *high* confidence study by Gardener et al. (2021) reported that participants in PFDA exposure quartile 4 had greater odds of PTB (OR = 1.82; 95% CI: 0.54, 6.19) relative to quartile 1. The *medium* confidence study by Meng et al. (2018) also reported an increased OR similar in magnitude (OR = 1.6; 95% CI: 0.8, 3.0) for PTB based on PFDA quartile 4 exposures but found no evidence of monotonicity or increased risk in the other quartiles. They also detected a larger statistically significant result for each ln-unit increase (OR = 2.2; 95% CI: 1.3, 3.8). Associations between PFDA and different PTB measures (including overall and different subtypes) were at or just below the null value based on continuous exposures in the *high* confidence study by Huo et al. (2020). Similar patterns emerged across PFDA exposure tertiles, albeit nonsignificant ORs with an exposure-

response relationship were suggested for clinically indicated PTBs (T2 OR = 1.11; 95% CI: 0.50, 2.48; T3 OR = 1.30; 95% CI: 0.59, 2.89). This result seemed to be largely driven by results in female neonates (OR = 1.38; 95% CI: 0.61, 3.11 per each ln-unit PFDA increase) (sex-specific data not shown on forest plots below).

Preterm birth summary

Three (two *high* and one *medium* confidence) of six studies showed increased odds of PTB with increasing PFDA exposures with risks ranging from 1.3 to 2.2. Although the number of studies was small, two of these three studies showing increased risks were based on late biomarker samples. No other patterns were evident by study confidence or other characteristics. For example, study sensitivity did not seem to be an explanatory factor among the null studies. One of the four studies with categorical data showed evidence of exposure-response relationships.

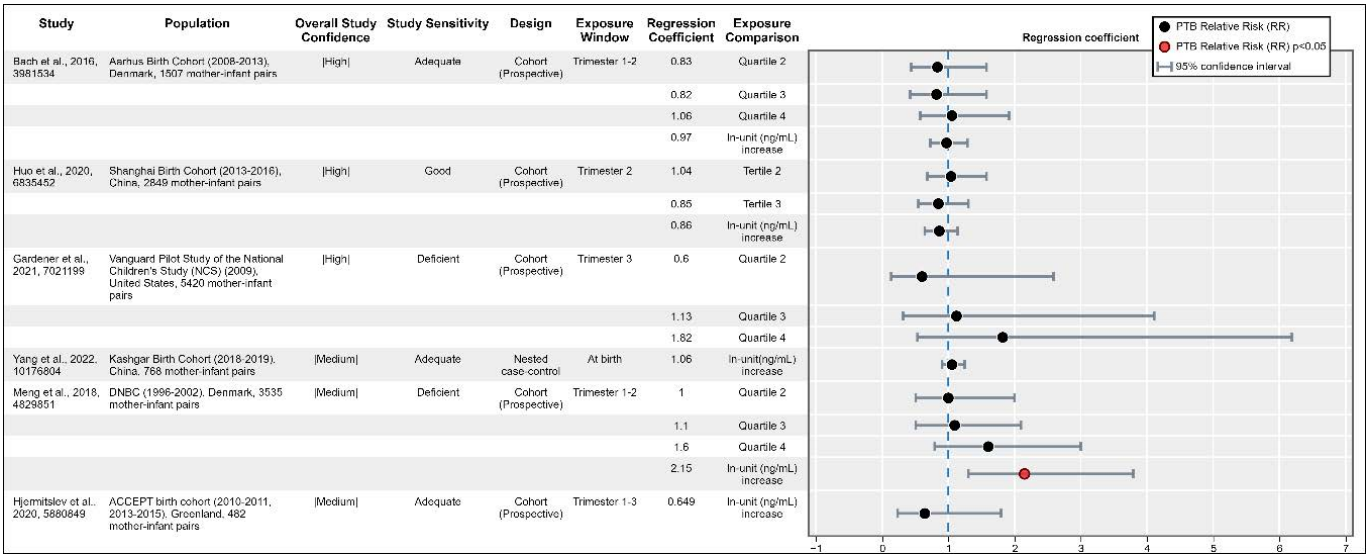


Figure 3-53. Preterm birth forest plot-six studies based on the overall population.[a,b] Refer to the HAWC link.

PTB = preterm birth.

[a]Studies are sorted first by overall study confidence level then by exposure window examined.

[b]For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses [e.g., (Yang et al., 2022a)].

Gestational age

Twelve studies examined PFDA in relation to changes in gestational age. Two of these studies reported only sex-specific data (Hall et al., 2022; Lind et al., 2017a), and three studies reported both sex-specific and overall population results (Hjermitslev et al., 2020; Meng et al., 2018; Li et al., 2017).

Overall population results

Six of the 10 studies based on the overall population were null including the *high* confidence studies by Bach et al. (2016) and Huo et al. (2020), the *medium* confidence study by Hjermitslev et al. (2020), and the *low* confidence studies by Gao et al. (2019); Workman et al. (2019); Li et al. (2017) (see Figure 3-54). No patterns were seen by study sensitivity among these null studies as four of the six had adequate or good domain ratings.

Four studies in the overall population (one *high* and three *medium* confidence) showed some evidence of lower gestational age relative to PFDA in the overall population. The *high* confidence study by Gardener et al. (2021) showed decreased gestational age in only PFDA quartile 4 with no exposure-response relationship evident (Q4 β = −0.26 weeks vs. Q1). Although it was null for term births, there was an inverse association between gestational age and each PFDA (β = −0.72 weeks; 95% CI: −3.39, 1.97 per each ln-unit increase) among preterm births in the *medium* confidence study by Yang et al. (2022a). Two other *medium* confidence studies reported only slight nonsignificant deficits (−0.12) per each ln-unit increase (Gyllenhammar et al., 2018; Meng et al., 2018), but the latter showed larger deficits in both exposure quartile 3 and 4 (β range: −0.20 to −0.50 weeks, respectively). Three of these four studies reporting lower gestational age were based on later biomarker sampling.

Sex-specific results

Two of the five studies in male neonates reported some gestational age deficits compared with just one study in girls (see Figure 3-55). The *medium* confidence study by Hjermitslev et al. (2020) and the *low* confidence study by Li et al. (2017) reported null findings for both boys and girls. The *high* confidence study by Lind et al. (2017a) showed minimal evidence of associations in the upper quartiles for either sex, although they reported an imprecise gestational age reduction of −0.21 weeks (95% CI: −0.66, 0.24) among girls that was incongruous with their categorical data. The *medium* confidence study by Hall et al. (2022) reported nonsignificant deficits in the upper tertile for boys (β = −0.26 weeks; 95% CI: −0.77, 0.27). The *medium* confidence study by Meng et al. (2018) detected a statistically significant decrease for boys per each ln-unit increase (β = −0.25 weeks; 95% CI: −0.43, −0.04) that was similar in magnitude.

Gestational age summary

Overall, there was mixed evidence of associations between PFDA and gestational duration endpoints. Only 6 of the 12 PFDA (two *high* and four *medium* confidence) studies showed some evidence of associations with gestational age in either the overall population, term/preterm subsets, or either sex; this included 4 of the 7 available *high* and *medium* confidence studies. Four of the six studies that showed some gestational age deficits were based on later biomarker sampling, which might be indicative of an impact of pregnancy hemodynamics. Four of these studies had deficient study sensitivity ratings, which may explain why some results were not statistically significant or why some differences were not discernible especially among the sex-specific analyses.

No patterns were observed by study sensitivity among the six different null studies. There was limited evidence to draw further conclusions from the three sex-specific findings given that only two of five studies among boys and one study in girls detected any evidence of gestational age differences in relation to PFDA.

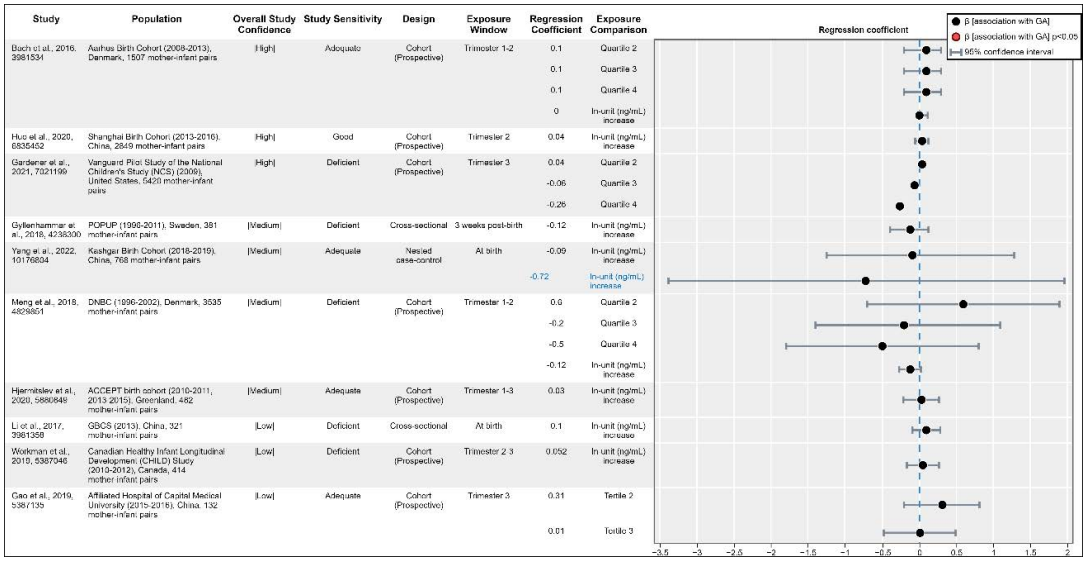


Figure 3-54. Overall population results for 10 gestational age studies.[a–d] Refer to the HAWC link.

GA = gestational age.

[a]Studies are sorted first by overall study confidence level then by exposure window examined.

[b]For Yang et al. (2022b), the −0.72 per ln-unit increase value is reported in the preterm birth population and the −0.09 per IQR increase value is in the term birth population.

[c]Gardener gestational age differences estimated from digitization of their Figure 4; 95% CIs were not estimable.

[d]For evaluation of patterns of results, studies that collected biomarker samples concurrently or after birth were considered cross-sectional analyses [e.g., (Yang et al., 2022a)].

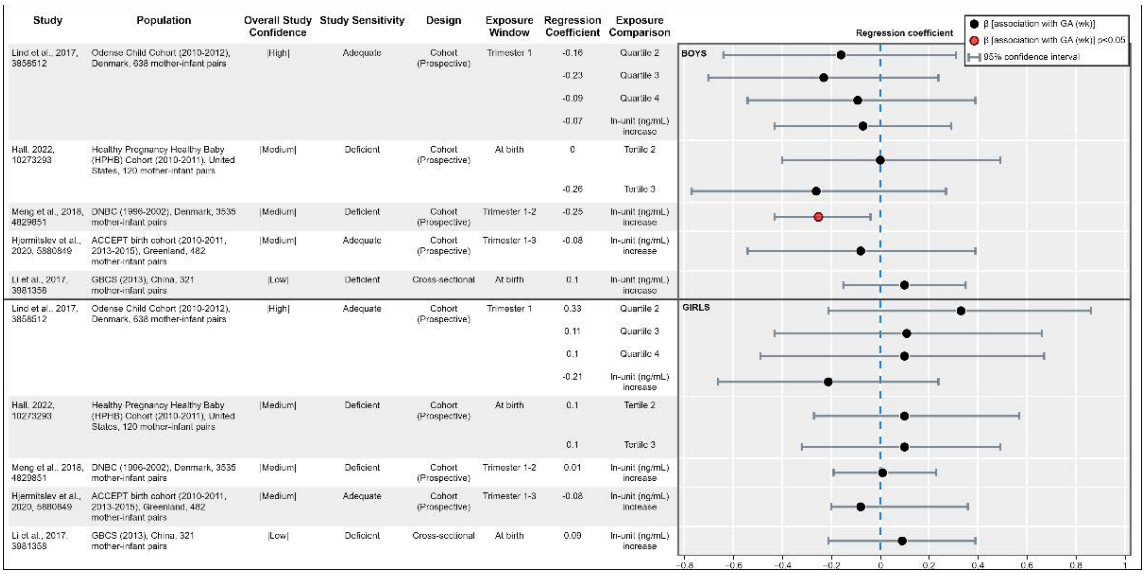


Figure 3-55. Sex-stratified results for five gestational age studies.[a,b] Refer to the HAWC link.

GA = gestational age.
[a]Studies are sorted first by overall study confidence level then by exposure window examined.
[b]For evaluation of patterns of results, studies that collected biomarker samples concurrently or after birth were considered cross-sectional analyses [e.g., Hall et al. (2022)].

Gestational duration summary

Five (2 *high* and 3 *medium* studies) different studies of 12 showed some associations between PFDA exposures and different gestational duration measures with comparable levels of evidence in preterm birth and gestational age (see Table 3-20). This included five *high* and *medium* studies of nine in total and none of the *low* confidence studies. Five of these seven studies were based on later biomarker sampling, which might be indicative of an impact of pregnancy hemodynamics. Study sensitivity was limited in some studies and could explain some of the null results and lack of statistical significance especially in the sex-stratified analyses. Few other patterns were evident across sex or different study characteristics.

Table 3-20. Summary of 12 studies of PFDA exposure and gestational duration measures

Author	Study location/years	n	Exposure median/IQR (range) in ng/mL	Study sensitivity domain judgment	PTB	GA
***High* confidence studies**						
Bach et al. (2016)	Denmark, 2008–2013	1,507	0.30/0.20 (LOD–2.87)	Adequate	Ø All	Ø All
Gardener et al. (2021)	USA, 2009–2013	354	0.2/0.2 (LOD–2.6)	Deficient	–↑ All	– All
Huo et al. (2020)	China, 2013–2016	2,849	1.69/1.38 (N/A)	Good	↑ All ↑ Girls Ø Boys	Ø All
Lind et al. (2017a)	Denmark, 2010–2012	636	0.30/0.10 (0.1–1.8)	Adequate		Ø Girls Ø Boys
***Medium* confidence studies**						
Gyllenhammar et al. (2018); Swedish Environmental Protection Agency (2017)[a]	Sweden, 1996–2001	381	0.24/0.14 (LOD–1.1)	Deficient		– All
Hall et al. (2022)	USA, 2010–2011	120	0.06/N/A (LOD–0.3)	Deficient		– Boys Girls
Hjermitslev et al. (2020)	Greenland, 2010–2011; 2013–2015	266	0.71/N/A (0.12–7.84)	Adequate	↓ All	Ø All Ø Girls Ø Boys
Meng et al. (2018)	Denmark, 1996–2002	2,132	0.20/0.10 (N/A)	Deficient	↑ **All***	– All **– Boys*** Ø Girls
Yang et al. (2022a)	China, 2018–2019	768	0.035–cases; 0.027–controls (range: 0.003–0.359)	Adequate	Ø All	– All

Author	Study location/years	n	Exposure median/IQR (range) in ng/mL	Study sensitivity domain judgment	PTB	GA
***Low* confidence studies**						
Li et al. (2017)	China, 2013	321	0.15/0.16 (ND–2.12)	Deficient		Ø All Ø Girls Ø Boys
Gao et al. (2019)	China, 2015–2016	132	0.47 (LOD–3.15)	Adequate		Ø All
Workman et al. (2019)	Canada, 2010–2011	414	0.13/N/A (LOD–1.4)	Deficient		Ø All

*$p < 0.05$; Ø: no association; +: positive association; −: negative association; ↑: increased odds ratio; ↓: decreased odds ratio.
IQR = interquartile range; PTB = preterm birth; GA = gestational age.
Note: "Adverse effects" are indicated by both increased ORs (↑) for dichotomous outcomes and negative associations (−) for the other outcomes.
[a]Swedish Environmental Protection Agency (2017) and Gyllenhammar et al. (2018) results are included here (both analyzed the POPUP cohort).

Birth defects

Two studies examined birth defects in relation to PFDA exposures with one each having adequate and deficient study sensitivity. The *medium* confidence congenital heart defect study by Ou et al. (2021) showed increased risks for PFDA ≥0.53 ng/mL (vs. <0.53 ng/mL) for every defect group examined including septal defects (OR = 2.33; 95% CI: 1.00, 5.45), conotruncal defects (OR = 2.58; 95% CI: 0.92, 7.25), and all heart defects combined (OR = 1.83; 95% CI: 1.07, 3.12). The *low* confidence Cao et al. (2018) study showed minimal evidence of associations between PFDA exposures and all birth defects (OR = 1.37; 95% CI: 0.60, 3.08). There is considerable uncertainty in interpreting results for broad birth defect groupings, which decreases study sensitivity given the etiological heterogeneity across different birth defects. Outside of increased risk of heart defects noted in the *medium* confidence study, there was limited evidence of associations between PFDA exposures and birth defects in the two available epidemiological studies. However, there is insufficient data for any specific birth defects to draw further conclusions.

Fetal loss-spontaneous abortion

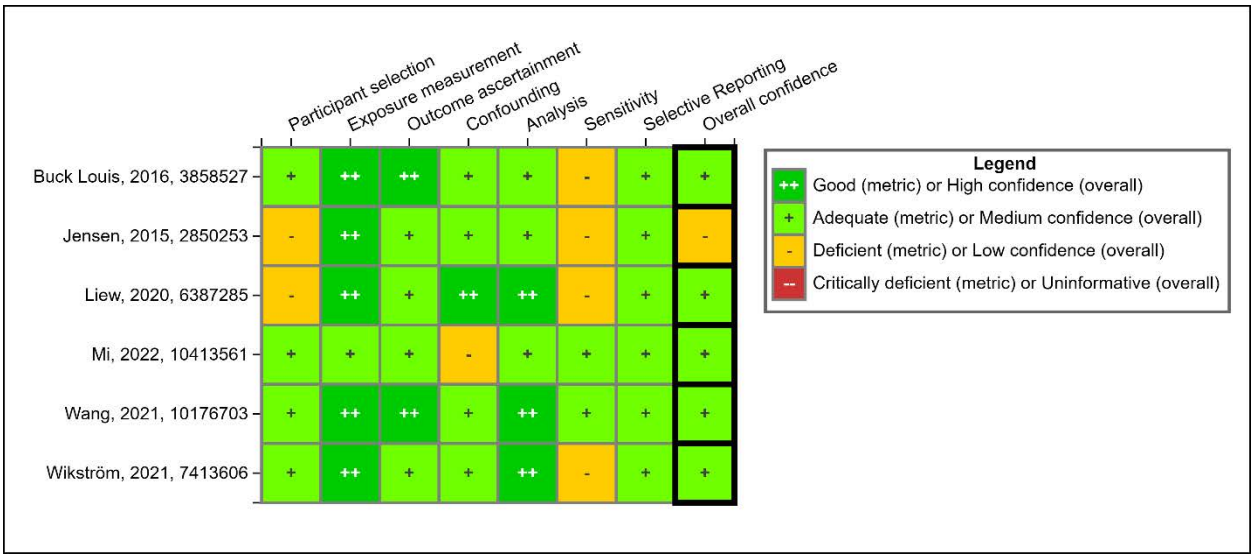


Figure 3-56. Evaluation results for epidemiological studies assessing effects of PFDA exposure on spontaneous abortion. Refer to HAWC Human Spontaneous Abortion for details on the study evaluation review.

Six (five *medium* and one *low* confidence) epidemiological studies (Mi et al., 2022; Wang et al., 2021; Wikström et al., 2021; Liew et al., 2020; Louis et al., 2016; Jensen et al., 2015) reported on the relationship between PFDA exposure and spontaneous abortion, which is defined as pregnancy loss occurring before 20–22 weeks gestation. This period can be further divided into preclinical/early loss (occurring before implantation or before a pregnancy is clinically recognized) and clinical loss (occurring from 5 to 28 weeks gestation). The study evaluations of the available

studies are summarized in Figure 3-56. Two *medium* confidence studies were prospective cohorts with high ascertainment of early losses, one included couples trying to conceive who were followed through delivery (Louis et al., 2016) and one included women undergoing in vitro fertilization (Wang et al., 2021). Three additional *medium* confidence studies assigned pregnant women from existing cohorts as controls and enrolled cases with first trimester losses (Wikström et al., 2021), throughout pregnancy (Mi et al., 2022), or identified cases via medical registry (Liew et al., 2020). the lone *low* confidence study by Jensen et al. (2015) was based on a cohort of pregnant women enrolled at 8–16 weeks gestation and was deficient for participant selection due to the high risk of incomplete case ascertainment (i.e., due to not including early losses and potential for loss to follow-up). Studies that miss early fetal losses have the potential to bias the results toward the null or even in a protective direction if there is a true effect, however, are considered unlikely to result in a spurious positive association. This potential also existed in Liew et al. (2020), but this study was not downgraded to *low* confidence as loss to follow-up was not a concern.

The results of the studies on spontaneous abortion are summarized in Table 3-21. Three of six studies showed some evidence of increased risk of spontaneous abortion. This included two studies (one *medium* and one *low* confidence) that reported strong positive associations between PFDA exposure and spontaneous abortion with large effect sizes (OR range: 2.7–5.0) and statistical significant results (Mi et al., 2022; Jensen et al., 2015). In addition, another *medium* confidence study by Liew et al. (2020) reported a smaller (OR = 1.3; 95% CI: 0.7, 2.2) but not statistically significant positive association, while another *medium* confidence study (Wikström et al., 2021) was largely null. Two *medium* confidence studies, which were the only studies able to consider preclinical losses, reported inverse (nonsignificant) associations (Wang et al., 2021; Louis et al., 2016) (RR range: 0.67 to 0.68). It is unlikely that the limitations identified in the *low* confidence study would explain the observed positive associations, as bias in Jensen et al. (2015) is expected to be toward or past the null. The evidence was mixed for exposure-response relationships among the three studies with categorical exposure data. While one study (Jensen et al., 2015) did show monotonic increased relative risks across tertiles (OR range: 1.9–2.7), a similar pattern was seen in the study showing inverse associations (Louis et al., 2016). The remaining study was null for quartile 2 but did show some evidence of nonsignificant risks increasing in the upper two quartiles (OR range: 1.1–1.3)

Overall, two of the five *medium* confidence studies (and three of six studies in total) reported evidence of associations with spontaneous abortion. However, there is considerable uncertainty due to inconsistency and mixed findings across *medium* confidence studies. It is possible that this uncertainty is related to the inclusion of preclinical loss, but it is not clear based on available evidence.

Table 3-21. Associations between PFDA and spontaneous abortion in six epidemiological studies

Reference, study confidence	Population	Median exposure (25th, 75th) in ng/mL	Spontaneous abortion types included	Effect estimate description	Effect estimate (95% CI)
Liew et al. (2020), *medium*	Case-control nested within pregnancy cohort, Denmark; 438 women	0.2 (0.1–0.2)	Clinical, 12–22 wk	OR (95% CI) for quartiles vs. Q1	Q2: 1.0 (0.6, 1.7) Q3: 1.1 (0.7, 1.9) Q4: 1.3 (0.7, 2.2)
Wikström et al. (2021), *medium*	Case-control nested within pregnancy cohort, Sweden; 1,529 women	0.3 (0.2–0.3)	Clinical, first trimester	OR (95% CI) for doubling of exposure	1.10 (0.81, 1.53)
Jensen et al. (2015), *low*	Pregnancy cohort, Denmark; 392 women	0.3 (0.2–0.6)	Clinical, post enrollment at 8–16 wk	OR (95% CI) for tertiles vs. T1	T2: 1.9 (0.9, 3.8) **T3: 2.7 (1.3, 5.4)***
Louis et al. (2016), *medium*	Preconception cohort, U.S.; 344 women	0.4 (0.2–0.6)	Total	HR (95% CI) for tertiles vs. T1	T2: 0.83 (0.49, 1.40) T3: 0.68 (0.41, 1.14)
Wang et al. (2021), *medium*	Preconception cohort of women undergoing first in vitro fertilization cycle, China, 305 women	0.5 (0.3–0.7)	Preclinical	RR (95% CI) for log-unit increase	0.67 (0.16, 2.73)
Mi et al. (2022), *medium*	Case-control nested within pregnancy cohort, China; 88 women	0.8	Clinical (9–12 wk)	OR (95% CI) for above vs. below median	**5.00 (1.53, 16.33)***

OR = odds ratio; HR = hazard ratio; RR = relative risk; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.
*Denotes statistical significance at $p < 0.05$.

Animal Studies

One toxicity study evaluated effects of PFDA on offspring (Harris and Birnbaum, 1989). This gavage study in mice examined maternal health, fetal survival, growth, and morphological development in two experiments covering different developmental windows. The two respective experiments consisted of gavage administration of 0–32.0 mg/kg-day on GD 10–13 to examine the developmental window related to cleft palate and hydronephrosis and gavage administration of 0–12.8 mg/kg-day on GD 6–15 to examine the entire developmental window related to the major period of organogenesis. The dams were necropsied on GD 18; the fetuses were removed from the uterus and examined. The Harris and Birnbaum (1989) study was evaluated as *high* confidence for most endpoints examined in both experiments (see Figure 3-57). Concerns were noted for fetal body weight measures as the study failed to report fetal body weights by sex, which impacted the results presentation domain and lowered the overall confidence of this endpoint to *medium*.

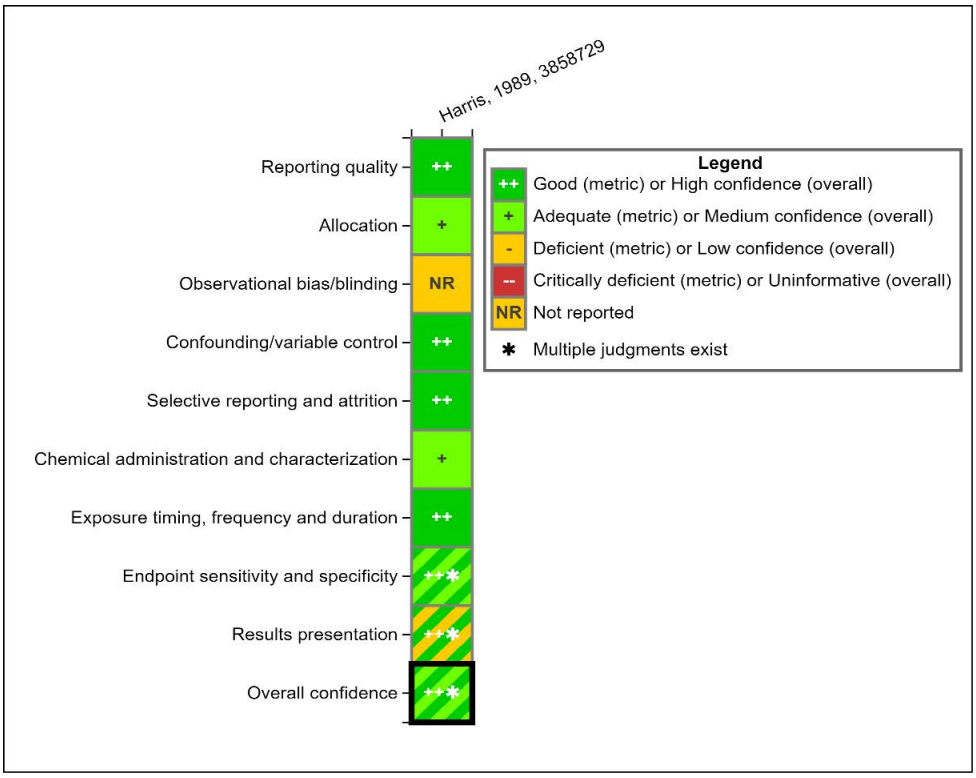


Figure 3-57. Evaluation results for an animal study assessing effects of PFDA exposure on development. Refer to HAWC for details on the study evaluation review.

Fetal growth

Fetal body weights were measured at GD 18 for each experiment (GD 10–13 or GD 6–15). Both experiments reported a significant trend in fetal body weight with decreases ≥5% being observed at ≥0.5 mg/kg-day (9.6%–44%) for the GD 10–13 experiment and ≥3 mg/kg-day (6%–50%) for the GD 6–15 experiment (see Figure 3-58 and Table 3-22). The changes in fetal body weight were of large magnitude and occurred at doses not associated with maternal toxicity. In the GD 10–13 experiment, changes in fetal body weight were ~10% at doses ranging from 0.5 to 4 mg/kg-day and were >40% at the highest dose (32 mg/kg-day). In the GD 6–15 experiment, changes in fetal body weight were 23% at 6.4 mg/kg-day and as large as 50% at the highest dose (12.8 mg/kg-day). It should be noted that the magnitude of fetal body weight changes was actually higher in the shorter duration study (GD 10–13 vs. GD 6–15) at comparable doses. For example, decreases in fetal body weight were 4% and 10% at 0.25 and 1 mg/kg-day in the shorter (GD 10–13) experiment versus 1% and 4% at 0.3 and 1 mg/kg-day in the longer (GD 6-15) experiment. Although this dose-response is not expected, the reductions in fetal body weight observed in both experiments are still considered to be adverse.

Endpoint Name	Study Name	Outcome Confidence	Study Design	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
Fetal Body Weight	Harris, 1989, 3858729	High confidence	Gestational Oral (GD 10-13)	F1 Mouse, C57BL/6n (♂♀)	significant	litter	0
							0.25
							0.5
							1
							2
							4
							8
							16
							32
			Gestational Oral (GD 6-15)	F1 Mouse, C57BL/6n (♂♀)	significant	litter	0
							0.03
							0.1
							0.3
							1
							3
							6.4
							12.8

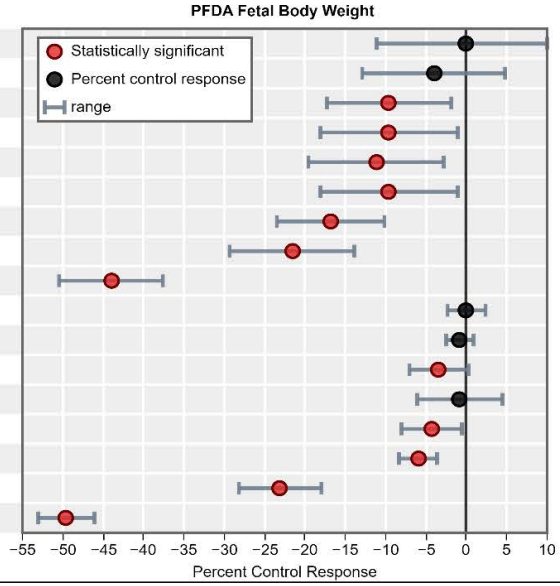


Figure 3-58. PFDA fetal body weight after gestational exposure. (Results can be viewed by clicking the HAWC link.)

Table 3-22. Percent changes relative to controls in fetal body weight in a developmental mouse study after PFDA exposure (Harris and Birnbaum, 1989)

Endpoint	Dose (mg/kg-d)							
	0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Decreased fetal body weight for the GD 10–13 experiment	−4	**−10**	**−10**	**−11**	**−10**	**−17**	**−22**	**−44**
	Dose (mg/kg-d)							
Endpoint	**0.03**	**0.1**	**0.3**	**1**	**3**	**6.4**	**12.8**	
Decreased fetal body weight for the GD 6–15 experiment	−1	**−3**	−1	**−4**	**−6**	**−23**	**−50**	

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

Maternal health

In the Harris and Birnbaum (1989) study, the health of the dams was assessed during both experiments through examination of body weight, liver weight, and survival. Both exposure durations resulted in a significant trend in body weight change (defined as final body weight – gravid uterus weight + empty uterus weight – initial body weight) for the dams with statistically significant decreases in the two highest dose groups of both experiments. Body weight gain was markedly decreased (−149% change from controls) in the 12.8 mg/kg-day group of the GD 6–15 experiment (see Figure 3-59). A significant trend was also reported for increased liver weight in both the GD 10–13 and GD 6–15 experiments; refer to Section 3.2.1 for more detail on this effect. Maternal deaths were not observed in the GD 10–13 experiment, but three dams died in the high-dose group (12.8 mg/kg-day) of the GD 6–15 experiment. This result is consistent with the overt toxicity of PFDA at high doses (refer to Section 3.2.10 for more details).

Fetal viability

In the Harris and Birnbaum (1989) study, endpoints related to fetal viability were measured at GD 18 for each experiment (i.e., groups dosed on GD 10–13 or GD 6–15). In both experiments, there was no difference in total implantations per litter between the control and treated groups indicating that the pregnancy rate was similar prior to exposure. However, following exposure, an increase in percent resorptions per litter (defined as [total number of resorptions and dead fetuses/number of total implantation sites] × 100) was observed in the high-dose groups of both experiments (170% and 344% for the GD 10–13 and GD 6–15 experiments, respectively) with statistical significance reported for the GD 6–15 experiment (see Figure 3-59). A reduction in the number of live fetuses per litter was also reported in high-dose groups of both experiments (32% and 36% for the GD 10–13 and GD 6–15 experiments, respectively) with statistical significance reported for the GD 6–15 experiment. Additionally, there was an increase in the number of dams that experienced total resorption in the high-dose groups of both experiments (4/12 dams vs. 0/13

in controls for the GD 10–13 experiment; 3/10 dams vs. 0/12 in controls for the GD 6–15 experiment) although the number of litters with resorptions were not different between control and treated groups (see Figure 3-59). Although these data might suggest an effect of maternal exposure on fetal viability as increased resorptions and decreased number of live fetuses are indicative of developmental toxicity per EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), effects on these endpoints were observed at doses also associated with significant maternal toxicity.

Morphological development

In the Harris and Birnbaum (1989) study, morphological development was examined in GD 18 fetuses for both the GD 10–13 and GD 6–15 experiments. This included external evaluation of all fetuses, soft tissue evaluation of 50% of the litters in each dose group (using Bouin's fixation and Wilson's free-hand sectioning technique), and skeletal evaluation of the remaining 50% of the litters in each dose group (using alizarin red S staining of ossified bone). In the GD 6–15 experiment, PFDA exposure caused significant dose-related trends for multiple skeletal variations (i.e., absence of fifth sternebrae, delay in braincase ossification, and delay in phalanges ossification) (see Figure 3-59). The fetal incidence of delayed braincase ossification was significantly increased at ≥0.03 mg/kg-day with the incidence rates ranging from 26% to 100%; it is unclear exactly which cranial bones are included in "braincase ossification." The number of fetuses with absence of the fifth sternebrae and delayed phalanges ossification was significantly increased at ≥6.4 mg/kg-day ranging from 15% to 35%. The statistical analyses of the skeletal variations data were performed independently by EPA and not included in the original study. Litter incidence and individual fetus per litter data were not reported for these effects. Data for skeletal variations were reported as fetal incidence whereas data for individual fetus per litter is the preferred unit of analysis for these effects. Absence of the fifth sternebrae and delayed phalanges ossification were associated with significant reductions in mean fetal weight and occurred at the same doses as maternal toxicity (i.e., decreased maternal body weight gain [92%–149%] at ≥6.4 mg/kg-day and mortality at 12.8 mg/kg-day). Whereas skeletal variations were significantly increased, the GD 6–15 experiment reported no soft tissue or skeletal malformations. Per EPA's *Guidelines for Developmental Toxicity Risk Assessment*, a malformation is defined as "as a permanent structural change that may adversely affect survival, development, or function," while a variation "is used to indicate a divergence beyond the usual range of structural constitution that may not adversely affect survival or health." Furthermore, skeletal variations are commonly associated with maternal toxicity (Carney and Kimmel, 2007) as was observed for the absence of the fifth sternebrae and delayed phalanges ossification in mice exposed to PFDA. Given the considerations above, including a lack of malformations and/or that some skeletal variations were observed at the same doses as maternal toxicity, the biological adversity for PFDA-induced skeletal variations is considered unlikely. Thus, the greatest level of concern is interpreted for the delayed brain ossification, although the significance of this variation (in terms of later biological consequences) is unclear.

Effect	Outcome Confidence	Experiment Name	Endpoint Name	Animal Description	Trend Test Result
Maternal Body Weight	High confidence	Gestational Oral (GD 10-13)	Maternal Body Weight Gain, Corrected	P0 Mouse, C57BL/6n (♀)	significant
		Gestational Oral (GD 6-15)	Maternal Body Weight Gain, Corrected	P0 Mouse, C57BL/6n (♀)	significant
Fetal survival	High confidence	Gestational Oral (GD 10-13)	Live Fetuses per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percent Resorptions per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with 100% Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Implantations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percentage Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
		Gestational Oral (GD 6-15)	Percent Resorptions per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with 100% Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Live Fetuses per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Implantations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percentage Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
Fetal growth	Medium confidence	Gestational Oral (GD 10-13)	Fetal Body Weight	F1 Mouse, C57BL/6n (♂♀)	significant
		Gestational Oral (GD 6-15)	Fetal Body Weight	F1 Mouse, C57BL/6n (♂♀)	significant
Morphological Development	Medium confidence	Gestational Oral (GD 10-13)	External Malformations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Fetuses with Cleft Palate	F1 Mouse, C57BL/6n (♂♀)	not reported
			Fetuses with Hydronephrotic Kidneys	F1 Mouse, C57BL/6n (♂♀)	not reported
		Gestational Oral (GD 6-15)	Delay in Braincase Ossification	F1 Mouse, C57BL/6n (♂♀)	significant
			Absence of Fifth Sternabrae	F1 Mouse, C57BL/6n (♂♀)	significant
			Delay in Phalanges Ossification	F1 Mouse, C57BL/6n (♂♀)	significant
	High confidence	Gestational Oral (GD 6-15)	Number of Fetuses with Skeletal Defects	F1 Mouse, C57BL/6n (♂♀)	not reported
	Medium confidence	Gestational Oral (GD 6-15)	Misaligned Sternabrae	F1 Mouse, C57BL/6n (♂♀)	not reported
			Delay in Sternal Ossification	F1 Mouse, C57BL/6n (♂♀)	not reported
			Delay in Supraoccipital Ossification	F1 Mouse, C57BL/6n (♂♀)	not reported
			External Malformations, Variations or Cleft Palates per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Visceral Malformations or Variation	F1 Mouse, C57BL/6n (♂♀)	not reported

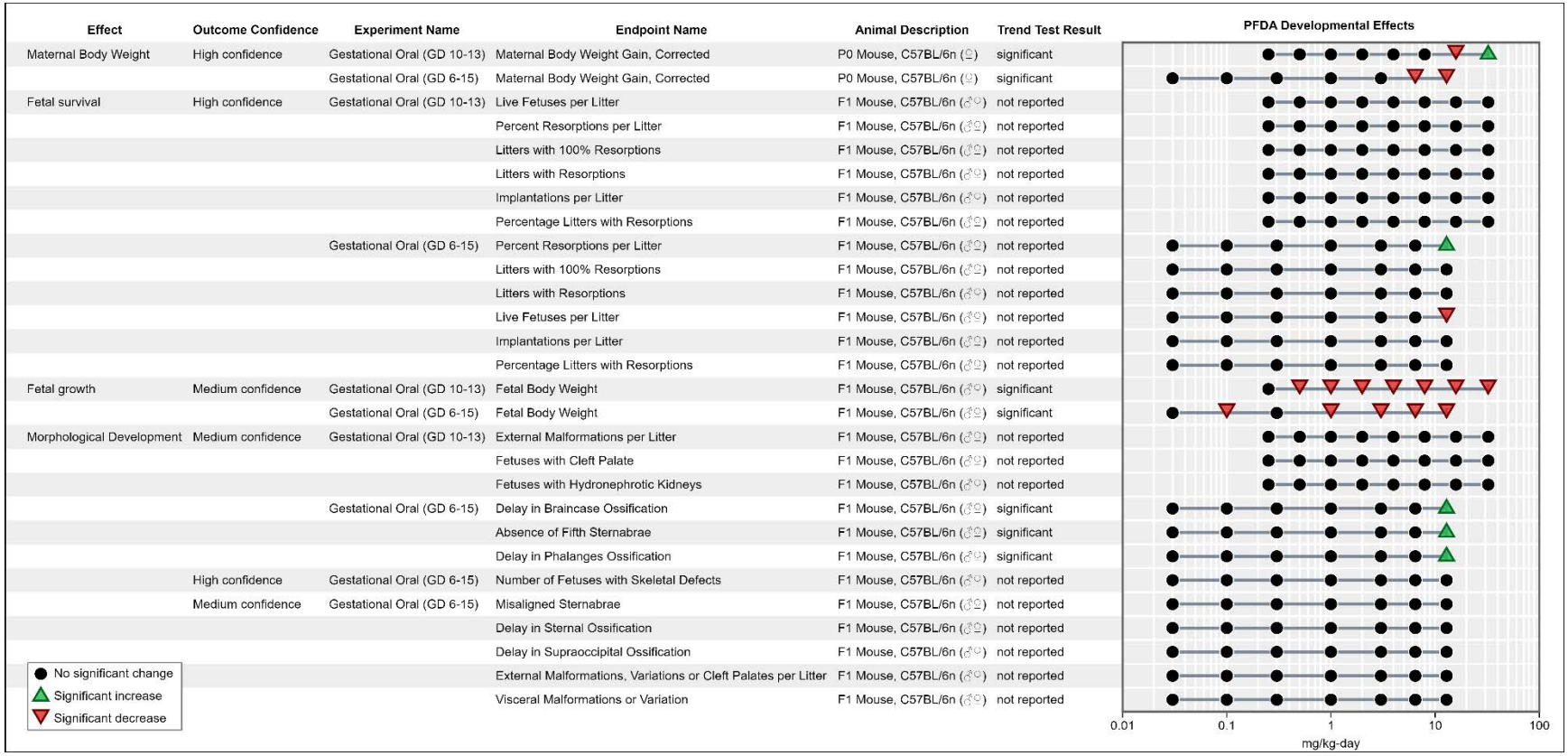


Figure 3-59. PFDA developmental effects. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

In support for PFDA-induced developmental effects in humans and mice, (Truong et al., 2022) reported that PFDA caused morphological effects in embryonic zebrafish from a developmental toxicity screening study. Of the 139 PFAS tested, PFDA was determined to be the most potent for the induction of teratogenic effects. Similar results were reported in an additional study using zebrafish (Ulhaq et al., 2013). Ulhaq et al. (2013) reported that spinal curvature was a common malformation observed in zebrafish embryos exposed to PFDA and of the seven PFAS tested, PFDA was the second most potent for the induction of developmental toxicity.

Evidence Integration

On the basis of more than 45 different epidemiological studies discussed in this review, the evidence of an association between PFDA exposure and developmental effects in humans is considered *slight* but was supported by the *moderate* evidence in animals. The epidemiological evidence was strongest and most consistent for fetal growth restriction and in particular for birth-weight-related measures, which were assessed by the most accurate growth restriction measures available. Of 28 in total, 18 different studies showed some deficits for the overall population or for either or both sexes across various birth weight measures. For example, 11 of 22 PFDA studies in the overall population reported some birth weight deficits, which included 9 of 14 *medium* and *high* confidence studies. Although data were more mixed, there appeared to be some coherence across these and other prenatal growth measures with different postnatal growth parameters. For example, there was some consistency across two (one *high* and one *low* confidence) of the three postnatal weight studies with a common examination window (~2 years of age). The evidence for other endpoints was not as strong or consistent, including 10 of 17 birth length studies that showed some associations. The degree of consistency across the observational epidemiological studies varied depending on the developmental endpoints examined, with more mixed findings for non-BWT measures. In addition, the evidence of inverse associations between PFDA exposure and birth weight and birth length was less compelling when based on early or pre pregnancy measures of PFDA. This might be indicative of potential bias due to the impact of pregnancy hemodynamics on PFDA levels. Thus, despite the reasonably consistent evidence of an association between PFDA and different BWT-related measures, and more mixed findings for some other endpoints, there is considerable uncertainty given that sample timing differences may explain at least some of the reported fetal growth restriction deficits.

Across the outcomes, this set of developmental studies was of good quality and generally had a low risk of bias, as 34 of the 45 studies across the six primary endpoints [fetal growth restriction (including both birth weight and length measures), gestational duration, postnatal growth, anogenital distance, birth defects, and spontaneous abortions] were either *medium* or *high* overall confidence. Several studies demonstrated sufficient sensitivity to detect associations in the overall population and across subgroups. However, many studies lacked power to detect statistical

interactions or differences across populations, especially those based on stratified analyses. This often results from low exposure levels with limited contrasts in many of the study populations, which may have diminished the sensitivity of some studies to detect associations. As such, any null findings for studies with endpoints, which lacked sensitivity should not be interpreted as supporting a lack of effect. In addition to the outcomes discussed in this section, pubertal development is discussed in the reproductive sections (see Sections 3.2.4 and 3.2.5) but could also be a developmental effect. The evidence for both males and females was based on one *medium* confidence study and was weak, but study sensitivity was again a concern.

As noted above, fetal growth restriction endpoints provided the strongest evidence for adverse developmental effects among the available studies. In considering the dose dependence of the birth weight decreases, only one of four *medium* or *high* confidence studies with categorical PFDA exposure data showed an exposure-response relationship. In addition, 9 of 14 *medium* or *high* confidence studies of the overall population as well as 9 of 14 sex-specific results showing adverse results based on continuous exposure also offer support for a biological gradient. Exposure-response relationships were less evident for other endpoints that were examined.

It can be challenging to identify patterns across heterogenous epidemiologic studies and study populations in the current database given the low exposure levels and/or limited and variable exposure contrasts. Examining birth weight differences in human populations is also challenging, since it can be difficult to differentiate pathological deficits versus natural biological variation. There was considerable variability in BWT deficits (β range: –29 to –101 g per ln-unit increases) in the overall population, with seven studies ranging from 31 to 59 g deficits per each ln-unit increase. The clinical significance of these changes may not be immediately evident, but effects of this magnitude can increase the number of infants at higher risk for other comorbidities and mortality especially during the first year of life. These population-level changes may have a large public health impact when these mean birth weight deficits shift the birth weight distribution to include more infants in the low birth-weight category. Additionally, decreased birth weight has been associated with long-term adverse health outcomes (Osmond and Barker, 2000).

Supporting the human evidence, the large and dose-dependent effects on fetal body weight observed across two independent experiments reported in the lone mouse study by Harris and Birnbaum (1989) (medium confidence for this endpoint) are without evidence to the contrary and thus provided *moderate* evidence coherent with the findings in humans. Following gestational PFDA exposure, decreases in fetal body weight with a significant trend were consistently observed in both experiments at ≥0.5 mg/kg-day, including doses (0.5–4 mg/kg-day) well below those that produced maternal toxicity. The changes in fetal body weight were also large in magnitude with the percent changes of up to 10% at the lower doses and ranging as high as 44%–50% at the highest doses tested in both experiments. The rodent data for decreased fetal body weight are coherent with data from the human studies in which the strongest and most consistent evidence was for fetal growth restriction. Although an increased fetal incidence of several skeletal variations (i.e., delayed

braincase and phalanges ossification and absence of fifth sternebrae) was observed, the delays in brain ossification, which started at ≥0.03 mg/kg-day, well below doses eliciting maternal toxicity, were most notable. This change is potentially indicative of delayed development (which would be coherent with the PFDA-induced changes on fetal body weight); however, the significance of this variation (in terms of future adverse consequences), is unknown, and malformations, which are known to be adverse, were not observed. On a related note, PFDA was reported to be teratogenic in embryonic zebrafish (Truong et al., 2022; Ulhaq et al., 2013). Statistically significant changes were also reported for fetal viability in mice (i.e., increased % of resorptions per litter and reduced number of live fetuses per litter) at the highest dose tested in the GD 6–15 experiment (Harris and Birnbaum, 1989); however, effects on fetal viability were observed at the same doses as significant maternal toxicity, preventing the ability to draw conclusions at these doses.

A notable data gap exists, as animal studies evaluating the effect of PFDA on postnatal development were not identified. Although data were limited and not entirely consistent, some effects of PFDA on postnatal growth were observed in humans. Additionally, effects on postnatal development (e.g., delayed eye opening; reduced postnatal growth) have been observed in rodents exposed to other PFAS such as PFOA, PFBS, PFBA. Overall, the information for postnatal developmental effects is limited, introducing uncertainty on whether more sensitive developmental effects of PFDA might occur. An additional data gap is the lack of data to inform the potential mechanisms for PFDA-induced fetal growth restriction effects.

Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause developmental toxicity in humans given sufficient exposure conditions[13] (see Table 3-23). This conclusion is based primarily on findings of dose-dependent decreases in fetal weight in the only available toxicological study, with mice gestationally exposed to PFDA doses ≥0.5 mg/kg-day and supported by evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, primarily with median PFDA values ranging from 0.11 to 0.46 ng/mL. The conclusion is further supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length).

[13]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-23. Evidence profile table for PFDA exposure and developmental effects

Evidence stream summary and interpretation					Inferences and summary judgment
Evidence from studies of exposed humans-fetal growth restriction (see Section 3.2.3: Human Studies)					⊕⊕⊙ ***Evidence indicates (likely)***
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
<u>Fetal growth restriction</u> (mean birth weight/z-scores; small for gestational age/low birth weight) Eight *high*, 10 *medium*, and 10 *low* confidence studies	• Eighteen of the 28 studies reported some inverse associations between PFDA exposures and standardized or mean birth weight measures including 17 of 26 studies of mean birth weight • Eleven of 22 studies showed evidence of mean birth weight deficits in the overall population, including 9 of 14 *medium* or *high* confidence studies • Nine of 14 studies in boys and girls reported some birth weight deficits including 8 of 11 *medium* and *high* confidence studies in girls and 7 of 11 in boys; 4 studies reported deficits in both sexes. • Three of 5 studies of small for gestational age or low birth weight reported increased risks in the overall population; fairly consistent in magnitude (OR range: 1.2–1.8)	• Consistent decreases across different populations and with variable study sensitivity • Most of the evidence among *high* and *medium* confidence studies (e.g., 9 of 14 *medium* or *high* confidence studies showed BWT deficits) • Dose-dependent (evidence of linear relationships) in many studies examining continuous measures • Moderate or large magnitude of effect in many studies (typically > −30 g per each ln-unit) • Although some variability is anticipated for observational studies of heterogenous populations, exposure levels/sources, and design/analysis elements, coherence	• Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth weight deficits, especially based on late biomarker sampling defined at trimester 2 or later, e.g., 9 of 11 studies in the overall population and 6 of 9 studies in girls and 5 of 9 in boys • Uncertainty of potential confounding in some studies due to some highly correlated PFAS like PFNA, although an evaluation of this possibility concludes that it would not fully explain the observed PFDA associations (see Appendix F) • One of 4 *medium* or *high* confidence studies with categorical data showed exposure-response relationships in overall population as	⊕⊙⊙ *Slight* Based on consistent evidence for birth weight reductions, the most sensitive endpoint, with coherence across some other developmental endpoints (e.g., preterm birth, postnatal growth, and other fetal growth measures such as birth length, small for gestational age and low birth weight); more mixed for other endpoints like head circumference and gestational duration.	*Primary basis*: Slight human evidence for fetal and postnatal growth restriction supported by coherent *moderate* evidence in animals and for some other developmental endpoints in humans. *Human relevance*: Evidence in animals is presumed relevant to humans. *Cross-stream coherence*: Impaired fetal growth was observed in both humans and mice. *Susceptible populations and lifestages*: Based on evidence of impaired fetal growth from human and animal studies, early lifestages may be at higher risk. *Other inferences*: No specific factors are noted.

Evidence stream summary and interpretation					Inferences and summary judgment
		with findings for related outcomes, most notably for birth length and postnatal growth measures	well as in girls for standardized and mean BWT measures • Imprecision of some effect estimates		
Fetal growth restriction (birth length) Six *high*, 4 *medium,* and 7 *low* confidence studies	• Ten of 17 studies in total including 5 (2 *high*, 1 *medium,* and 2 *low* confidence) of 15 examining the overall population reported some birth length deficits (including 3 of the 10 total *medium* or *high* confidence studies) • Seven (4 *high* and 3 *medium* confidence) of 10 sex-specific studies reported some birth length deficits; 4 studies each in boys and girls	• Overall population results were similar in magnitude despite between-study sources of heterogeneity including different exposure contrasts • Sex-specific deficits were often larger and more variable than the overall population	• Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth length deficits based on later biomarker sampling, e.g., 4 of 5 studies in overall population and 4 of 7 sex-specific studies		
Fetal growth restriction (head circumference) Five *high*, 5 *medium*, and 4 *low* confidence studies	• Five (2 *high*; 3 *medium* confidence) of 14 studies reported smaller head circumference including 2 of 11 in overall population; and 3 of 7 sex-specific studies	• Five of the 10 *high* and *medium* confidence studies reported smaller head circumference in the overall population or either sex • Two of the 6 studies with adequate sensitivity reported some head circumference deficits across sexes	• Limited evidence of associations especially in the overall population for which five of the six null studies had deficient study sensitivity		

Evidence stream summary and interpretation					Inferences and summary judgment
Evidence from studies of exposed humans-congenital anomalies (see Section 3.2.3: Human Studies)					
Congenital anomalies (i.e., birth defects) One *medium*, and 1 *low* confidence studies	• Most of the evidence was limited to one congenital heart study (OR range: 1.8–2.6)	• Results were consistent in magnitude across different heart defects in the *medium* confidence study (OR range: 1.8–2.6)	• The *low* confidence study examined all birth defects together and lacks specificity to add to weight of evidence and likely decreases study sensitivity if there is etiologic heterogeneity across defects	⊕⊙⊙ *Slight* The lone (*medium* confidence) epidemiologic study examining specific defects showed consistent associations for heart defects.	
Evidence from studies of exposed humans-anogenital distance (see Section 3.2.3: Human Studies)					
Anogenital distance Three *medium* confidence studies	• Inverse association between PFDA exposure and anogenital distance (AGD) in one of three *medium* confidence studies in boys and one of two studies in girls	• Adverse association in boys observed in 1 *medium* confidence study	• Unclear adversity of AGD decreases in girls • Although some variability is anticipated for observational studies of heterogenous populations, exposure levels/sources, and design/analysis elements, unexplained inconsistency	⊙⊙⊙ *Indeterminate* Based on inconsistent results across *medium* confidence studies	
Evidence from studies of exposed humans-gestational duration (see Section 3.2.3: Human Studies)					
Gestational duration (preterm birth) Three *high* and 3 *medium* confidence studies	• Three (2 *high* and 1 *medium* confidence) of 6 preterm birth studies reported increased risk; 6 studies had deficient study sensitivity; 5 adequate, and 1 good	• Risks fairly consistent in magnitude (OR range: 1.3–2.2).	• Some uncertainty due to potential impact of pregnancy hemodynamics as 2 of 3 studies based on later biomarker sampling • Potential confounding by PFAS including highly correlated PFNA; limited evidence for PFNA suggests would	⊕⊙⊙ *Slight* Mixed evidence and uncertainty due to the potential impact of hemodynamic changes among studies with gestational duration deficits	

Evidence stream summary and interpretation					Inferences and summary judgment
			not likely fully explain PFDA associations		
Gestational duration (gestational age) Four *high*, 5 *medium*, and 3 *low* confidence studies	• Six of 12 studies reported lower gestational age; 4 of these 6 had deficient study sensitivity	• No factors noted	• Unexplained inconsistency, although this may be partially due to poor sensitivity • Substantial uncertainty due to the potential impact of hemodynamic changes among 4 of 6 studies showing gestational age deficits, especially based on late sampling (defined as trimester 2 or later) • Outcome may be prone to some measurement error		
Evidence from studies of exposed humans-postnatal growth (see Section 3.2.3: Human Studies)					
Postnatal growth Four *high*, 1 *medium* and 3 *low* confidence studies	• Three (1 *high* and 2 *low* confidence) of 6 studies showed postnatal weight deficits; with limited sensitivity in some studies (3 adequate; 3 deficient) • Two (1 *high* and 1 *low* confidence) of 5 studies showed postnatal height deficits; with limited sensitivity in some studies (3 adequate; 2 deficient) • Two (1 *high* and 1 *low* confidence) of 3 studies showed increased	• *Consistency* across 2 of the 3 weight studies with a common examination window (2 yr of age), including one *high* and one *low* confidence study	• Potential confounding across PFAS for some endpoints • Unknown critical window(s) for childhood growth endpoints; assumption was in utero period is most relevant	⊕⊙⊙ *Slight* Mixed results across different measures, with limited study sensitivity in some studies. Results were more consistent when a homogenous population considered (~2 yr of age).	

Evidence stream summary and interpretation					Inferences and summary judgment
	adiposity; with limited sensitivity in some studies (1 adequate; 2 deficient) • Both *high* confidence studies showed minimal and mixed rapid weight gain results; with limited sensitivity in some studies (1 adequate; 1 deficient)				
Evidence from studies of exposed humans-spontaneous abortion (see Section 3.2.3: Human Studies)					
<u>Spontaneous abortion</u> Five *medium* and 1 *low* confidence studies	• Two *medium* and one *low* confidence studies reported increased odds of spontaneous abortion while 2 *medium* confidence study reported an inverse association.	• Large effect size in two studies (OR >2)	• Unexplained inconsistency across *medium* confidence studies • Potential confounding across PFAS	⊕⊙⊙ *Slight* Based on inconsistent evidence across studies	
Evidence from in vivo animal studies (see Section 3.2.3: Animal Studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
<u>Fetal growth</u> One *medium* confidence study (2 independent experiments)	• Fetal body weight was reduced at ≥0.5 mg/kg-d in the GD 10–13 experiment (maternal body weight decreased at ≥16.0 mg/kg-d). • Fetal body weight was reduced at ≥1.0 mg/kg-d in the GD 6–15 experiment (maternal body weight decreased at ≥6.4 mg/kg-d, with mortality at higher doses).	• *Consistency* across the *medium* confidence GD 10–13 and GD 6–15 experiments • *Dose-response* gradient observed within experiments and exposure duration gradient observed across experiments • Large magnitude of effects (up to 50%)	• No factors noted	⊕⊕⊙ *Moderate* Based primarily on decreased fetal growth at ≥0.5 mg/kg-d in two independent experiments from a single study in mice. The reliability and biological significance of other, potentially related, findings from this study are unclear.	

Evidence stream summary and interpretation					Inferences and summary judgment
Fetal viability One *high* confidence study	• A treatment-related increase in the percentage of resorptions per litter was reported at 12.8 mg/kg-d in dams treated from GD 6–15. • The number of live fetuses per litter was reduced at 12.8 mg/kg-d in dams treated from GD 6–15.	• Coherence of effects on percentage of resorptions and number of live fetuses in a *high* confidence study	• Substantial concern for potential confounding as decreased fetal viability occurred at the same dose as maternal mortality.		
Morphological development One *medium* confidence study (two independent experiments)	• Increased fetal incidences of skeletal variations (i.e., absence of fifth sternebrae at ≥6.4 mg/kg-d • Delayed ossification of the phalanges at ≥6.4 mg/kg-d • Delayed braincase ossification at ≥0.03 mg/kg-d).	• Dose-response gradient for skeletal and braincase ossification variations • Consistent increase in variations across two *medium* confidence experiments	• Unclear biological relevance of variations as no malformations were reported. • Potential confounding of skeletal and phalanges ossification variations at doses causing overt toxicity.		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Other evidence	*Interpretation*: PFDA causes developmental toxicity in embryonic zebrafish. Key findings: • Of the 139 PFAS chemicals tested, PFDA was the most potent for the induction of teratogenic effects in zebrafish (Truong et al., 2022) • Of the 7 PFAS chemicals tested, PFDA was the second most potent for the induction of developmental effects in zebrafish. Spinal curvature, a malformation, was commonly reported in zebrafish embryos exposed to PFDA (Ulhaq et al., 2013). *Limitations*: A comprehensive list of tested/observed developmental endpoints was not provided.			The findings in zebrafish provide some support for the biological plausibility of the developmental effects in humans and animals.	

3.2.4. Male Reproductive Effects

Human Studies

Nine epidemiological studies examined the association between PFDA exposure and male reproductive effects. The outcomes included in these studies were semen parameters, reproductive hormones, timing of pubertal development, and anogenital distance. The studies are described below.

Semen evaluations

Semen concentration and sperm motility and morphology were considered the core endpoints for the assessment of semen parameters. Key issues for the assessment of semen parameters involve sample collection and sample analysis. Samples should be collected after an abstinence period of 2–7 days, and analysis should take place within 2 hours of collection and follow guidelines established by the World Health Organization (WHO, 2010). While exposure would be measured ideally during the period of spermatogenesis rather than concurrent with the outcome, a cross-sectional design is considered adequate because the period of spermatogenesis in humans is fairly short (74 days plus 12 days of maturation) (Sigman et al., 1997), the half-life of PFDA is long, and there is no concern for reverse causality with this outcome because it is not expected the semen quality would influence PFDA concentrations in blood.

Four cross-sectional studies examined the relationship between PFDA and semen quality. Given the considerations noted above, three were evaluated as *medium* confidence overall (see Figure 3-60), although one of these was considered uninformative for the core endpoint sperm motility due to the overnight delay between collection and analysis (Buck Louis et al., 2015). One study analyzed male partners from a preconception cohort in the U.S. (Buck Louis et al., 2015), one study enrolled young adult men whose mothers were enrolled in a national pregnancy cohort (Petersen et al., 2022), and one enrolled healthy young man being considered for military service (Joensen et al., 2013). The remaining study was *low* confidence because of multiple identified deficiencies and was focused on men seeking infertility assessment (Huang et al., 2019a). All four studies analyzed PFDA in serum and used appropriate methods, and, thus, exposure misclassification is expected to be minimal.

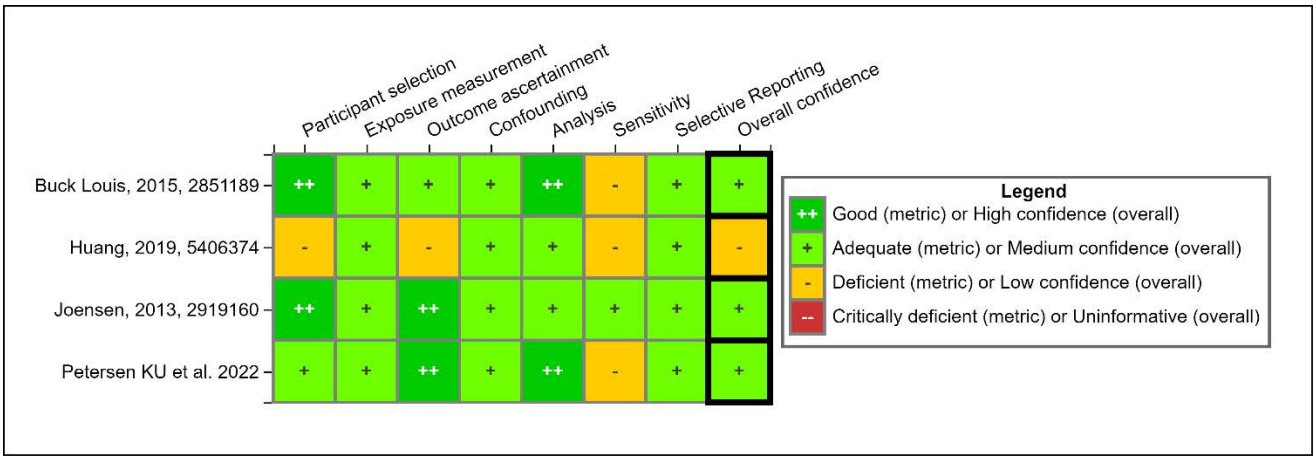


Figure 3-60. Evaluation results for epidemiological studies assessing effects of PFDA exposure on semen parameters. Refer to HAWC Human Semen Parameters for details on the study evaluation review.

The results for the association between PFDA exposure and semen quality are presented in Table 3-24. The studies analyzed the outcomes differently, so the effect estimates are not directly comparable. None of the results were statistically significant, but there was a suggestion of a decrease in motility with increased exposure in Joensen et al. (2013) and in concentration in Huang et al. (2019a), but not in Petersen et al. (2022). Because the methods used to assess motility were considered critically deficient in Buck Louis et al. (2015), it was not possible to evaluate its consistency with the other *medium* confidence studies. For concentration and morphology, there was no clear decrease in the *medium* confidence studies. However, PFDA levels in both studies were lower than levels of other measured PFAS (≤0.5 ng/mL) and the exposure contrasts were narrow, which introduces concerns regarding sensitivity (i.e., lack of ability to detect an association if present).

Table 3-24. Associations between serum PFDA and semen parameters in epidemiological studies

Reference; study confidence	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Concentration (× 10^6/mL)	Motility (% motile)	Morphology (% normal)
Huang et al. (2019a); *low*	Cross-sectional study of men seeking infertility assessment (2009–2010); 57 men	0.0 (range 0.0–1.2)	β (95% CI) for 1 ln-unit increase in serum PFDA	−21.59 (−77.91, 34.73)	5.96 (−11.58, 23.50)	−0.02 (−0.10, 0.07)

Reference; study confidence	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Concentration ($\times 10^6$/mL)	Motility (% motile)	Morphology (% normal)
Petersen et al. (2022), *medium*	Cross-sectional analysis within cohort of general population men (2017-2019), Denmark; 1,041 men (18–20 yr)	0.2 (5th–95th: 0.1–0.3)	% difference (95% CI) for tertiles of PFDA vs. T1	T2: 3 (−9, 17) T3: −3 (−15, 11)	T2: −1 (−6, 6) T3: −3 (−9, 3)	T2: −1 (−11, 10) T3: 2 (−8, 13)
Joensen et al. (2013); *medium*	Cross-sectional study of men evaluated for military service (2008–2009), Denmark; 247 men (18–22 yr)	0.4 (0.3–0.5)	β (95% CI) for 1-unit increase in serum PFDA	Cubic root transformed 0.22 (−0.76, 1.19)	Square transformed −1343 (−2759, 73.69)	Square root transformed −0.097 (−0.88, 0.69)
Buck Louis et al. (2015); *medium*	Cross-sectional analysis within preconception cohort (2005–2009), U.S.; 462 men	0.5 (0.3–0.6)	β (95% CI) for 1 ln-unit increase in serum PFDA	−1.06 (−30.5, 28.3)	Uninformative	5.80 (−1.31, 12.9)

*$p < 0.05$.

Reproductive hormones

Testosterone and estradiol were considered the primary endpoints for male reproductive hormones. Progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG) were also reviewed when available. Key issues for the evaluation of these studies were sample collection and processing (see Figure 3-61). For testosterone, LH, and FSH, blood sample collection should be in the morning because of diurnal variation; if not possible, time of collection should be accounted for in the analysis. If there is no consideration of time of collection for these hormones, the study is classified as *deficient* for outcome ascertainment and *low* confidence overall. A cross-sectional design was considered appropriate for this outcome since levels of these hormones are capable of being rapidly upregulated or downregulated and they are not expected to directly bind to or otherwise interact with circulating PFAS.

Seven studies (eight publications) examined the relationship between PFDA and reproductive hormones. Three studies were *medium* confidence cross-sectional studies in adults, including Joensen et al. (2013) and Petersen et al. (2022), which were cross-sectional studies of young adult men described above. An analysis of NHANES data in adult men (Xie et al., 2021) was also *medium* confidence for estradiol but *low* confidence for testosterone due to potential outcome misclassification as previously described. A cross-sectional study in adolescents (aged 13–15 years) (reported in Zhou et al. (2016) and Zhou et al. (2017b)) was *low* confidence because of concerns for

confounding (e.g., pubertal indicators were not considered). Three studies, one a birth cohort in Denmark (Jensen et al., 2020b) and two cross-sectional studies in China (Liu et al., 2020b; Yao et al., 2019) examined associations in infants. Yao et al. (2019) and Yao et al. (2019) were *low* confidence because time of day of sample collections was not accounted for (both studies) and potential concerns for confounding (Yao et al., 2019). Liu et al. (2020b) was *medium* confidence because of less concern for diurnal variation of the included hormone (progesterone).

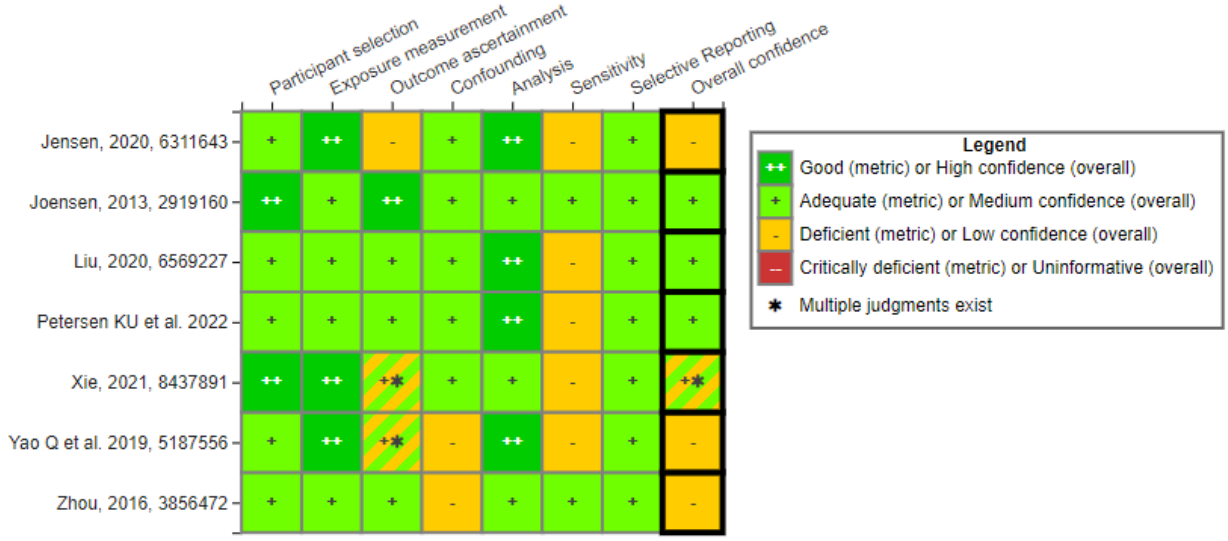


Figure 3-61. Evaluation results for epidemiological studies assessing effects of PFDA exposure on male reproductive hormones. Refer to HAWC Human Male Reproductive Hormones for details on the study evaluation review.

*Outcome-specific ratings differed for this domain.

Given the differences in populations (adults, adolescents, newborns), evaluation of consistency across studies is not straightforward. For testosterone, inverse associations between PFDA exposure and testosterone levels were observed in two studies. Among the two *medium* confidence studies for this outcome, Joensen et al. (2013) observed a decrease in log-transformed testosterone with higher PFDA exposure in adult men, although this was not statistically significant (β (95% CI) = −0.17 (−0.41, 0.07)). Petersen et al. (2022) reported no association with exposure. Also in adults, but *low* confidence for testosterone, Xie et al. (2021) found positive associations between PFDA exposure and free and total testosterone (statistically significant for free testosterone, with exposure gradient observed across quartiles). In adolescent boys, the *low* confidence study by Zhou et al. (2016) reported an inverse association (β (95% CI) = −0.26 (−0.41, −0.10)). In infants, one study (Jensen et al., 2020b) reported a positive association between PFDA exposure and testosterone (β = 0.37, 95% CI: −0.11, 0.84, p = 0.1), whereas no association was observed in Yao et al. (2019). Given that the inverse associations were observed only in the studies with highest exposure concentrations in the participants, it is possible that the observed

inconsistency is due to nonmonotonicity of the effect of PFDA exposure on testosterone, but the data are insufficient to determine whether this is likely, so the inconsistency decreases certainty.

For estradiol in adults in Joensen et al. (2013), there was also a decrease with higher PFDA exposure (β (95% CI) = −0.22 (−0.48, 0.002)), but this was not observed in the other two studies in adults (Petersen et al., 2022; Xie et al., 2021), in adolescents in Zhou et al. (2016), or in infants in Yao et al. (2019). Joensen et al. (2013) also examined several other reproductive hormones and SHBG in young men and found no evidence of association with PFDA exposure for SHBG, luteinizing hormone, or inhibin-B, but did report a positive association with FSH (β (95% CI) = 0.42 (−0.005, 0.85)). The increase in FSH would be consistent with an increase in gonadotropin production as a compensatory response to a decrease in testosterone. However, Petersen et al. (2022) found no association with FSH, LH, or SHBG. In (Jensen et al., 2020b), inverse associations, although not statistically significant, were observed with DHEA, DHEAS, and androstenedione. Liu et al. (2020b) found no association with progesterone.

Pubertal development

Pubertal development is primarily assessed using established criteria, such as Tanner stage ratings. For boys, Tanner staging involves evaluation of the development of genitalia (scrotum appearance, testes, and penile size) and pubic hair. Stage 1 represents prepubertal development; stage 2, the onset of pubertal development; and stage 5 represents full sexual maturity. Two *medium* confidence birth cohorts in Denmark (Ernst et al., 2019) and the United States (Carwile et al., 2021) examined timing of pubertal development with PFDA exposure (Figure 3-62). Ernst et al. (2019) used maternal exposure measured in blood and prospectively identified pubertal onset with follow-up checks every 6 months. In boys, they reported that there was no clear pattern of association between PFDA exposure and Tanner stages of genital development or pubic hair, or other markers of pubertal development such as axillary hair, acne, voice break, or first nocturnal ejaculation when exposure was analyzed in tertiles. For each outcome, the mean age of onset was later in the middle (0.16–0.21 ng/mL) versus the lowest (0.08–0.15 ng/mL) tertile, but earlier in the highest tertile (0.22–0.9 ng/mL). This pattern was also observed with a combined puberty indicator outcome, with boys in the middle tertile reaching the indicator 4.59 months later (95% CI: −0.93, 10.11) and the highest tertile 2.83 months earlier (95% CI: −8.43, 2.77) than the lowest tertile. Carwile et al. (2021) used exposure measured during mid-childhood (median 8 years) with follow-up to early adolescence (median 13 years). Using a pubertal development score based on parental responses to scales of multiple pubertal markers (voice deepening, body hair growth, facial hair growth, acne, and growth spurt), they reported no association with PFDA exposure. This was consistent with their findings for older age at peak heigh velocity (used as a proxy for pubertal development). Exposure contrast was narrow in both studies (median 0.2 ng/mL, 10th–90th percentile 0.1–0.3 in Ernst et al. (2019), 0.3, 25th–75th percentile 0.2–0.5 in Carwile et al. (2021)), which may have reduced study sensitivity.

Figure 3-62. Evaluation results for epidemiological studies assessing effects of PFDA exposure on male pubertal development. Refer to HAWC Human Male Pubertal Development for details on the study evaluation review.

Summary of human studies

Overall, there is inconsistent evidence for male reproductive effects of PFDA exposure. One *medium* confidence study in adult men found reduced sperm motility and testosterone (Joensen et al., 2013) and one *low* confidence study also found an inverse association in adolescents (Zhou et al., 2016). This observation is coherent with an inverse association with anogenital distance in one *medium* confidence study (Tian et al., 2019) (see Section 3.2.3). However, the other available studies did not report consistent findings for semen parameters and reproductive hormones. No clear association was observed with estradiol or pubertal development.

Animal Studies

Only one animal toxicity study evaluated male reproductive effects after PFDA exposure (NTP, 2018). This study examined the following endpoints after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) in 7- to 8-week-old male SD rats: sperm evaluations, histopathology, hormone levels, and organ weights. The endpoints evaluated by NTP (2018) are considered reliable measures for assessing male reproductive toxicity (Creasy and Chapin, 2018; Creasy et al., 2012; Sellers et al., 2007; U.S. EPA, 1996b). The NTP (2018) study was evaluated as *high* confidence for most endpoints examined with no notable concerns in any of the study evaluation domains (see Figure 3-63). Concerns for potential insensitivity were identified for sperm measures as the exposure duration (28 days) used for this experiment was insufficient to fully detect potential effects on sperm development, resulting in a *low* confidence rating; this potential bias is toward the null. In rats, spermatogenesis takes ~8 weeks for germ cells to mature from spermatogonia to spermatozoa (Creasy and Chapin, 2018).

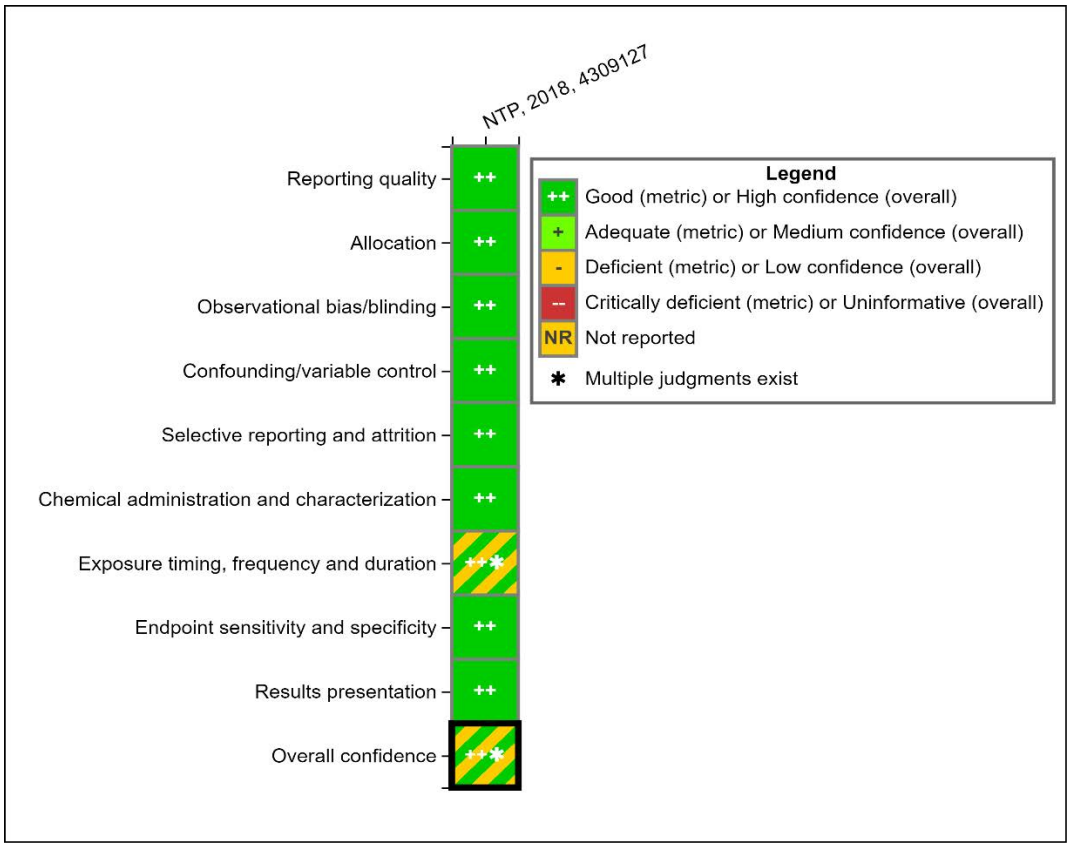


Figure 3-63. Evaluation results for an animal study assessing effects of PFDA exposure on male reproduction. Refer to HAWC for details on the study evaluation review.

Sperm evaluations

Testicular and epididymal sperm counts and testicular sperm motility were only measured for the three highest dose groups (0.625, 1.25 and 2.5 mg/kg-day) (see Figure 3-64). Testicular sperm counts are indicative of changes in sperm production in the testis, while epididymal counts indicate both changes in testicular sperm production and storage of sperm in the epididymis; therefore, both measures are considered informative for evaluating effects on sperm parameters (Creasy and Chapin, 2018; Creasy et al., 2012). Testicular sperm counts (absolute and relative to organ weight) decreased in a dose-dependent manner at 0.625 and 1.25 mg/kg-day (−10% and −19%–21% change compared with controls, respectively) but not at the highest dose group (2.5 mg/kg-day). As such, a clear trend for testicular sperm counts could not be established. A significant trend was reported for cauda epididymal sperm counts with decreases of 11%–30% compared with controls across 0.625–2.5 mg/kg-day. NTP (2018) also reported sperm counts normalized to cauda epididymis weight and observed no treatment-related effects (data not shown in Figure 3-64). However, this measure is not considered a sensitive as sperm contributes to epididymal weight and reporting findings as a ratio may mask reductions in sperm number (U.S. EPA, 1996b). A nonstatistically significant decrease in testicular sperm motility of 11% compared

with controls was reported at 2.5 mg/kg-day, but there was no clear dose-response effect. In summary, the dose-related decreases in sperm counts in the epididymis suggest that PFDA can affect sperm parameters at doses ≥0.625 mg/kg-day after 28-day exposure.

The findings on sperm measures from NTP (2018) are interpreted with caution as sensitivity concerns for these outcomes are based on the exposure duration used in this study, which did not capture the entire process of spermatogenesis (~8 weeks in rats) (Creasy and Chapin, 2018).

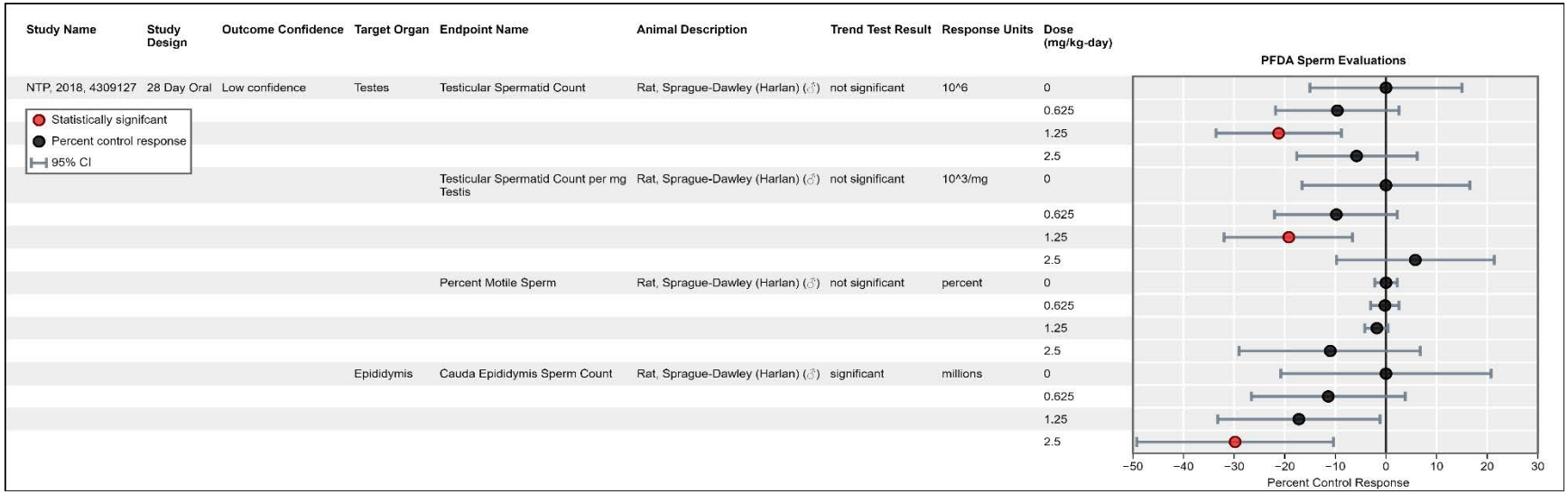


Figure 3-64. Effects on sperm evaluations following exposure to PFDA in short-term oral studies in animals.
(Results can be viewed by clicking the HAWC link.)

Histopathology

Testicular and epididymal lesions were reported in the 28-day rat study by NTP (2018). The testes were examined in all dose groups for histopathological responses (see Figure 3-65). Minimal to mild atrophy of the interstitial (Leydig) cells was observed in nearly all the rats exposed to the two highest PFDA dose groups (8/10 and 10/10 for 1.25 and 2.5 mg/kg-day, respectively) but not in the controls. Leydig cell atrophy is a response coherent with reduced sperm production (Creasy and Chapin, 2018; Creasy et al., 2012) and indicative of reduced androgen levels, which were also observed in this study (see synthesis of reproductive hormones in this section). Mild degeneration of the germinal epithelium and spermatid retention within the seminiferous tubules was also increased in 4/10 rats from the high-dose group; control group incidence was 1/10 and 0/10, respectively. The epididymis was examined in the three highest dose groups (0.625, 12.5, and 2.5 mg/kg-day) (see Figure 3-65). Only the highest dose group (2.5 mg/kg-day) displayed mild duct germ cell exfoliation in 4/10 rats examined compared with 1/10 rats in the control group and a single marked case of hypospermia (1/10 rats) not observed in the controls. Sperm granuloma was found in 1/10 rats in the controls but not in the exposed animals (data not shown in Figure 3-65). NTP (2018) did not observe any histopathological effects on the preputial gland, seminal vesicle, and prostate when examining animals in the control and high-dose groups. In summary, there is consistent evidence of histopathological observations indicative of mild degenerative changes in the testes and epididymis at doses ≥1.25 mg/kg-day after 28-day exposure. Note that these doses are associated with significant body weight changes (see "Evidence Integration" section below for a discussion on potential confounding due to co-occurring systemic toxicity at doses causing some PFDA-induced male reproductive effects).

Study Name	Study Design	Outcome Confidence	Target Organ	Endpoint Name	Animal Description	Trend Test Result	Incidence	Dose (mg/kg-day)
NTP, 2018, 4309127	28 Day Oral	High confidence	Testes	Germinal Epithelium Degeneration	Rat, Sprague-Dawley (Harlan) (♂)	significant	1/10 (10.0%)	0
							0/10 (0.0%)	0.156
								0.312
								0.625
								1.25
							4/10 (40.0%)	2.5
				Interstitial Cell Atrophy	Rat, Sprague-Dawley (Harlan) (♂)	significant	0/10 (0.0%)	0
								0.156
								0.312
								0.625
							8/10 (80.0%)	1.25
							10/10 (100.0%)	2.5
				Seminiferous Tubule Spermatid Retention	Rat, Sprague-Dawley (Harlan) (♂)	significant	0/10 (0.0%)	0
								0.156
								0.312
								0.625
								1.25
							4/10 (40.0%)	2.5
			Epididymis	Exfoliated Germ Cell, Epididymal Duct	Rat, Sprague-Dawley (Harlan) (♂)	significant	1/10 (10.0%)	0
							0/10 (0.0%)	0.625
								1.25
							4/10 (40.0%)	2.5
		Low confidence	Epididymis	Hypospermia	Rat, Sprague-Dawley (Harlan) (♂)	not significant	0/10 (0.0%)	0
								0.625
								1.25
							1/10 (10.0%)	2.5

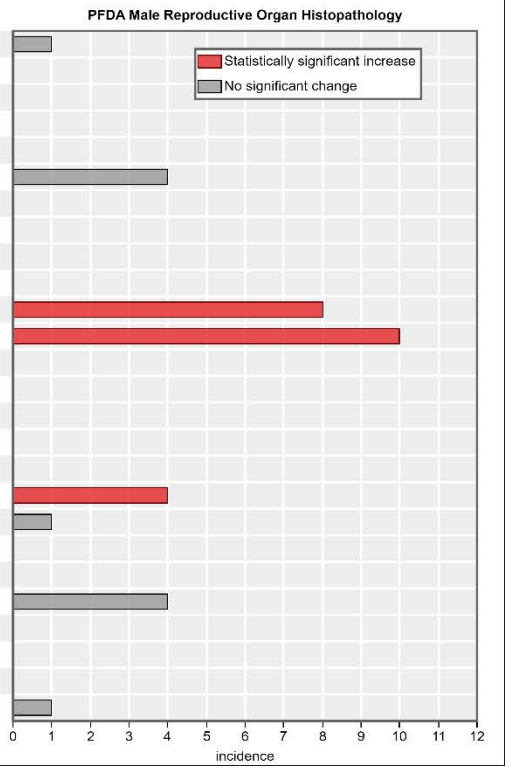


Figure 3-65. Effects on male reproductive organ histopathology following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Reproductive hormones

NTP (2018) evaluated serum testosterone in all dose groups at study termination (see Figure 3-66). A significant trend was reported with 25%, 64%, and 75% decreases in serum testosterone when compared with controls for the 0.625, 1.25, and 2.5 mg/kg-day dose groups, respectively. Testosterone is essential for the development and maturation of the male reproductive system, and it also plays a role in maintaining spermatogenesis and reproductive functions in adults (Toor and Sikka, 2017). The changes in serum testosterone levels at doses ≥0.625 mg/kg-day are concordant with the reductions in sperm counts and Leydig cell damage in adult male rats exposed to PFDA for 28 days (see syntheses on sperm evaluations and histopathology in this section).

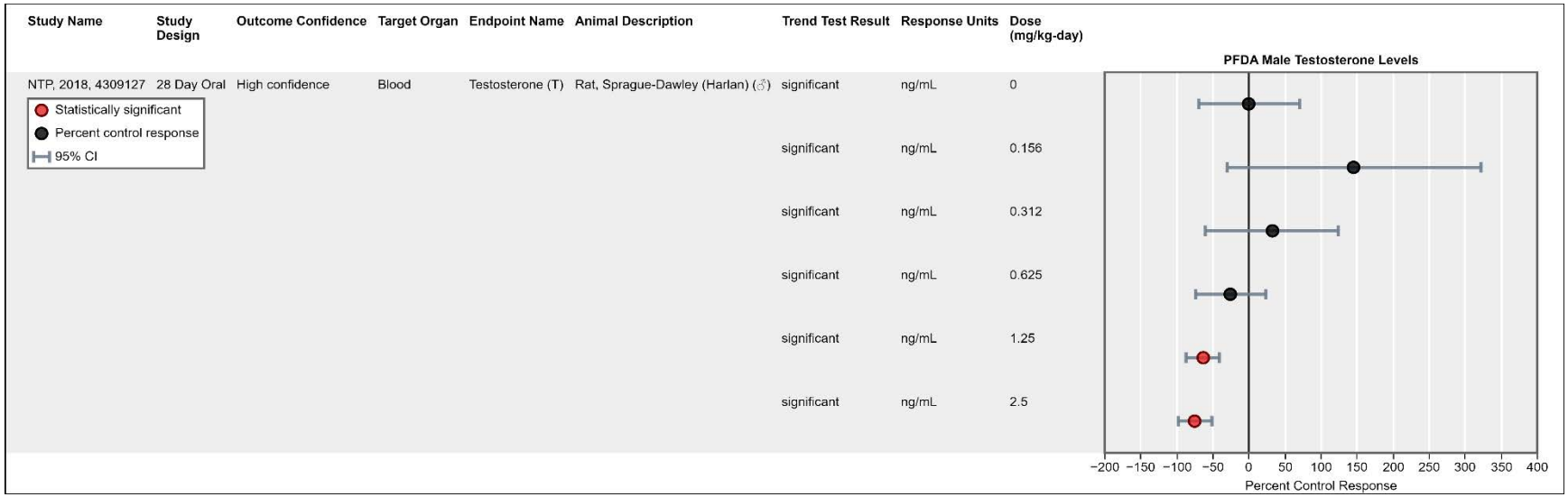


Figure 3-66. Effects on serum testosterone levels following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Organ weight

The right testis was measured at study termination in all dose groups, while epididymis weights (both whole and the cauda segments) were evaluated in the three highest dose groups (0.625, 12.5, and 2.5 mg/kg-day) (NTP, 2018) (see Figure 3-67). Absolute weights are the preferred measure for testis and epididymis as these organs appeared to be conserved even with body weight changes (Creasy and Chapin, 2018; U.S. EPA, 1996b). A decreasing trend ($p < 0.01$) in absolute testis weight was reported across the doses, reaching a −13% change compared with controls at 2.5 mg/kg-day. Absolute epididymis weights for whole and cauda segments also showed a decreasing trend ($p < 0.01$) and reported −10% to −11% and −23% to −25% change relative to controls for the 1.25 and 2.5 mg/kg-day dose groups, respectively. Decreases in epididymis weight, particularly in the cauda segment, may reflect reductions in sperm counts (Creasy and Chapin, 2018; Evans and Ganjam, 2011), which was observed to occur at similar doses (see synthesis on sperm evaluations in this section). Overall, the data show consistent dose-related decreases in organ weights in the testis and epididymis at ≥0.625 mg/kg-day after short-term exposure to PFDA.

Study Name	Study Design	Outcome Confidence	Target Organ	Endpoint Name	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
NTP, 2018, 4309127	28 Day Oral	High confidence	Testes	Right Testis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.156
								0.312
								0.625
								1.25
								2.5
			Epididymis	Cauda Epididymis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.625
								1.25
								2.5
				Epididymis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.625
								1.25
								2.5

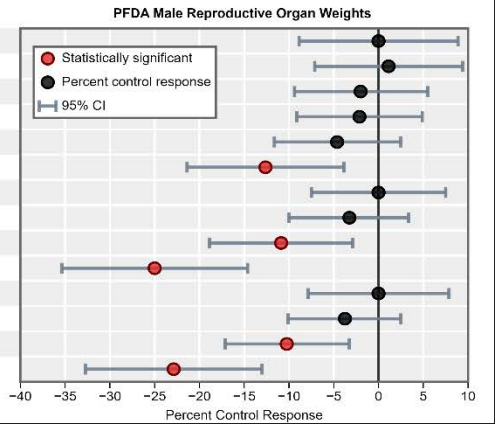


Figure 3-67. Effects on male reproductive organ weights following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

Several studies have evaluated the potential mechanisms by which PFDA exposure may lead to male reproductive effects. Experimental studies have investigated PFDA-induced effects on Leydig cell steroidogenesis, androgen (AR) and estrogen (ER) receptor functions, aromatase activity and androgen metabolism and excretion, and the potential impact of indirect systemic toxicity on the male reproductive effects of this chemical.

In vitro cell culture studies have evaluated PFDA-induced effects on Leydig cell functions and steroidogenesis. Leydig cells are the primary site of testosterone synthesis (Creasy and Chapin, 2018). Cholesterol uptake by the mitochondria in Leydig cells is a critical step in human chorionic gonadotropin (hCG)-induced testosterone production (Scott et al., 2009). In both immortalized mouse (MA-10) Leydig cells and primary rat Leydig cells, exposure to PFDA significantly decreased mitochondrial cholesterol uptake and hCG-stimulated testosterone synthesis (Boujrad et al., 2000). The PFDA exposure levels affecting hormone synthesis in MA-10 cells did not lead to increased cytotoxicity measured as DNA damage, protein synthesis, and mitochondrial integrity (Boujrad et al., 2000). In contrast, PFDA showed a lack of activity in HTS assays from the EPA's ToxCast and Tox21 database evaluating steroid hormone biosynthesis, including glucocorticoids, androgens, estrogens, and progestogens in adrenal gland H295R cells (U.S. EPA (2019b); refer to Appendix E.2 for more details on the HTS results).

The in vitro observations of PFDA-induced effects on Leydig cell functions are consistent with both the 28-day gavage study in rats by NTP (2018) discussed above and high-dose, i.p. injection studies that exposed rodents (predominantly rats) to single PFDA doses ranging from 20 to 400 mg/kg and evaluated effects on histopathology, androgen levels, and androgen-responsive reproductive organ weights after observational periods of 7 to 28 days (Bookstaff et al., 1990; Van Rafelghem et al., 1987b; Olson and Andersen, 1983). The i.p. injections studies reported decreases in serum testosterone and 5-α-dihydrotestosterone levels (Bookstaff et al., 1990), altered testicular testosterone production (Bookstaff et al., 1990), and reduced androgen-responsive reproductive organ weights in rats (Bookstaff et al., 1990; Olson and Andersen, 1983). Furthermore, these studies reported that PFDA exposure was associated with increased incidence of histopathological effects considered indicative of androgen disruption and spermatogenic disturbance (Creasy and Chapin, 2018; Creasy et al., 2012). Effects observed in rats include increased seminal vesicle and prostatic acini atrophy and reduced seminal vesicle epithelial cell height, (Bookstaff et al., 1990), and while mice appeared to be resistant to seminiferous tubule degeneration, rats, hamsters, and guinea pigs were responsive to this PFDA-induced effect (Van Rafelghem et al., 1987b).

Another mechanism by which PFDA could alter male reproductive function is via increased hepatic metabolism and excretion of androgens or metabolic precursors such as cholesterol. Bookstaff et al. (1990) performed an experiment in which castrated SD rats were supplemented with testosterone via sustained release capsules and then treated with vehicle or PFDA. They observed that acute PFDA exposure (20–80 mg/kg, i.p.) had no effect on serum testosterone levels

when the source of this hormone was the capsule rather than the testes. These findings suggest that PFDA does not impact hepatic androgen metabolism and excretion, and that decreases in serum testosterone levels observed after exposure are likely caused by a disruption in steroidogenesis in the testis. This argument is supported by the reductions in testosterone secretion in response to hCG stimulation in testicular tissue harvested from PFDA-exposed rats evaluated in the same study (Bookstaff et al., 1990) and inhibition of hGC-mediated steroidogenesis in cell culture rodent models using immortalized and primary Leydig cells described above (Boujrad et al., 2000).

Overall, the findings from available in vivo and cell culture studies provide support for an effect of PFDA exposure on Leydig cell functions ultimately resulting in reduced steroidogenesis.

Separately, PFDA-induced effects on AR and ER functions and on aromatase activity have been evaluated in in vitro cell culture studies and HTS assays from the EPA's ToxCast and Tox21 platforms ((https://comptox.epa.gov/dashboard),U.S. EPA (2022a); data retrieved on November 03, 2022; refer to Appendix E.2 for more details on the HTS results). AR and ER are known to regulate male reproductive functions (Wan et al., 2013; Wilson et al., 2008) and aromatase is a key enzyme in the conversion of androgens to estrogens, which is important for sexual development and differentiation (Sweeney et al., 2015; Hotchkiss et al., 2008; Jones et al., 2006). Disruption of AR transactivation has been demonstrated in Chinese hamster ovary cells (CHO-K1) (Kjeldsen and Bonefeld-Jørgensen, 2013) and androgen-sensitive TARM-Luc cells (McComb et al., 2019) at PFDA concentrations that did not induce cytotoxicity. No significant effects on ER transactivation were observed in human breast adenocarcinoma MCF-7 cells with PFDA exposure alone (Li et al., 2020b; Kjeldsen and Bonefeld-Jørgensen, 2013) but in combination with 17β-estradiol, PFDA displayed antiestrogenic activity measured by inhibition of ER transactivation and downregulation of ER-responsive genes at noncytotoxic concentrations (Li et al., 2020b). In HTS assays profiling AR and ER functions across multiple endpoints and in vitro test models, PFDA displayed low activity for these receptors at concentrations closely associated with cytotoxicity (see Table E-3 in Appendix E.2). PFDA was active in 2 of 17 AR assays (displaying binding activity in rat prostrate tissue and induction of cell proliferation in human prostate carcinoma 22Rv1 cells) and in 2 of 21 assays profiling the ERα (1 of 2 independent assays measuring transcriptional activity in HepG2 cells and an antagonist transactivation assays in human embryonic kidney HEK293T cells). Consistent with the HTS results, the ToxCast model predictions suggest that PFDA is inactive for both AR/ER agonist and antagonist activities (see Table E-4 in Appendix E.2). Lastly, PFDA exposure decreased aromatase activity in the human choriocarcinoma JEG-3 cell line under conditions of cytotoxicity (Kjeldsen and Bonefeld-Jørgensen, 2013) but no activity in a HTS assay measuring aromatase inhibition in human breast cancer MCF-7 cells (see Table E-5 in Appendix E). Taken together, findings from in vitro cell culture studies and HTS assays do not provide consistent and reliable evidence for potential effects of PFDA on AR or ER functions, or on aromatase activity. However, for the most part, these in vitro cell models are not derived from the male reproductive system and

variability in the cellular/tissue environment may lead to differences in hormone receptor/enzyme functions (Leehy et al., 2016; Abdel-Hafiz and Horwitz, 2014).

In addition to the mechanisms described above, PFDA-induced wasting syndrome (see Section 3.2.10) may indirectly affect the male reproductive system because severe decreases in body weight are known to alter reproductive functions (Creasy and Chapin, 2018; U.S. EPA, 1996b). Decreased body weight and food consumption were observed in acute, i.p. injection studies at doses ≥40 mg/kg and lethality were reported in some studies at doses ≥50 mg/kg (Bookstaff et al., 1990; Van Rafelghem et al., 1987b; Olson and Andersen, 1983). Bookstaff et al. (1990) addressed the impact of PFDA-induced changes in body weight on male reproductive endpoints by adding pair-fed control rats that were weight-matched to each PFDA treatment groups. The authors observed that single exposure to 20, 40, or 80 mg/kg of PFDA via i.p. injection significantly decreased serum testosterone and DHT, testicular testosterone production, seminal vesicle and prostate weights, and seminal vesicle epithelial cell height. In pair-fed control animals, there were no significant responses in the male reproductive system except in the group matched to the highest PFDA dose (80 mg/kg), which was associated with large reductions in food intake (44%) and body weight (72%) and observed responses were attenuated compared with PFDA exposure. These results indicate that PFDA-induced effects at the low and medium doses were direct reproductive system effects and not secondary to chemical-induced systemic effects. The body weight changes (−21% to −38%) in male rats observed in the 28-day gavage study at 1.25–2.5 mg/kg-day are not associated with confounding effects from severe body weight reductions (72%) reported in supplemental studies tailored to examine that potential linkage.

Overall, the available evidence from in vivo and cell culture studies provides evidence of a biologically plausible mechanism for PFDA-induced adverse responses in the male reproductive system by disruption of steroidogenesis in Leydig cells, which in turn could impair reproductive functions and spermatogenesis. Specifically, it appears that PFDA exposure can disrupt androgen production in Leydig cells, which may lead to downstream histopathological effects, organ weight changes, and decreased spermatogenesis. Disruptions in androgen levels/production is a known pathway for chemical-induced alterations in spermatogenesis (Toor and Sikka, 2017; Sharpe, 2010). This support for biological plausibility is derived from studies in exposed animals and in vitro animal models; studies informing the relatability of these data to exposed humans are currently unavailable.

Evidence Integration

The evidence of an association between PFDA exposure and male reproductive effects in humans is limited to two *medium* (Tian et al., 2019; Joensen et al., 2013) and one *low* confidence study (Zhou et al., 2016), with findings suggesting potential decreases in testosterone, decreased sperm motility, and anogenital distance (see Section 3.2.3) with higher PFDA exposure. There are concerns over inconsistency and imprecision, thus, the evidence is considered *indeterminate*.

The available evidence from a 28-day gavage study in rats and supportive data from i.p. injection and cell culture studies in rodents provided *moderate* evidence of male reproductive toxicity in animals with PFDA exposure. The 28-day rat study showed coherent effects across several relevant endpoints, including sperm evaluations, histopathology, hormone levels, and organ weights (NTP, 2018), with most effects observed at doses below those shown to cause overt toxicity. Adverse histopathological changes were observed at doses associated with body weight decrements of potential concern. The study methods were considered *high* confidence for all endpoints other than sperm evaluations, which were considered potentially insensitive due to an inadequate exposure duration (i.e., biased toward the null; confidence is reduced specifically in the interpreted reliability of null findings [i.e., sperm motility]). A consistent pattern of decreased testicular and epididymal sperm counts occurred at ≥0.625 mg/kg-day, but only the effects in the epididymis were dose related. Dose-related decreases in serum testosterone levels and testicular and epididymal weights were also reported in rats at ≥0.625 mg/kg-day. The reduction in sperm counts, serum testosterone levels and organ weights are coherent with the mild degenerative changes found in testes and epididymis at similar doses, particularly Leydig cell atrophy, which is associated with androgen deficiency and decreased spermatogenesis (Creasy et al., 2012). Consistent effects on serum androgen levels, male reproductive organ weights, and histopathology were observed in rodents exposed to high doses of PFDA (≥20 mg/kg) in single, i.p. injection studies. The adverse effects observed in the in vivo oral and i.p. exposure studies are biologically consistent with a potential mechanism for PFDA-induced reproductive effects in which alterations in Leydig cell functions result in decreased steroidogenesis and androgen levels (see synthesis on mechanistic studies and supplemental information above for more details).

Limitations of the animal evidence base include the availability of only a single, short-term oral exposure study in a single species, and uncertainties regarding the potential impact of systemic toxicity, particularly with regard to the observed histopathological effects. Significant reductions in body weight were reported in the highest dose groups in the 28-day gavage study (21% at 1.25 mg/kg-day and 38% at 2.5 mg/kg-day; see Section 3.2.10 for more details) (NTP, 2018). However, concern for nonspecific effects on the male reproductive system is attenuated by the observed dose-related effects (i.e., sperm counts, testosterone levels, and organ weights) at a lower PFDA dose, not associated with body weight changes (0.625 mg/kg-day). Likewise, an i.p. injection study that examined potential effects of PFDA-induced "wasting syndrome" using pair-fed control rats observed androgenic deficiency and male reproductive toxicity at 20 and 40 mg/kg that were independent from severe body weight depression at the highest dose (72% at 80 mg/kg) (Bookstaff et al., 1990). With respect to in vitro evidence, a general lack of in vitro models derived from the male reproductive system and of models restricted to rodents, limits the ability of the available evidence to inform potential pathways involved in PFDA-induced male reproductive toxicity and to elucidate conserved mechanisms across species, including humans. Nonetheless, the mechanistic information from acute i.p. and in vitro animal studies is both consistent and coherent with the oral

exposure study evidence and, therefore, provides support for the biological plausibility of the phenotypic responses. In the absence of information to the contrary and given the conserved role of androgen-dependent pathways in male reproductive functions across species (including humans), the available evidence is considered to be relevant to humans. This assumption is based on *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996a).

A potentially susceptible population for PFDA-induced male reproductive effects are young individuals exposed during critical developmental lifestages (e.g., the masculinization programming, which occurs prior to the differentiation of androgen-sensitive tissues and determines penis size and anogenital distance (Dent et al., 2015)), although no such studies were available in the current animal evidence base and few epidemiological studies examining pubertal development and anogenital distance were available. Androgens play a critical role in the normal development of the male reproductive system and disruptions caused by exposures to reproductive toxicants during gestation and early postnatal lifestages can lead to agenesis of the male reproductive system and/or infertility (Foster and Gray, 2013; Sharpe, 2010; Scott et al., 2009).

Taken together, available ***evidence indicates*** that PFDA is likely to cause male reproductive effects in humans under sufficient exposure conditions[14] (see Table 3-25). This conclusion is based primarily on a constellation of coherent evidence from a *high* confidence study in animals exposed to 0.625–2.5 mg/kg-day for 28 days, with some support for biological plausibility provided by mechanistic evidence from i.p. and cell culture models. Although no direct information on the human relevance of the animal evidence is available, many aspects of the male reproductive system are conserved across species, and the limited sensitivity in human studies may explain the lack of associations observed. Uncertainties in the database of PFDA-induced male reproductive toxicity includes the absence of subchronic, chronic, developmental, or multigenerational studies testing these outcomes in animals (which, overall, are anticipated to be more sensitive than the available short-term study design), and a general lack of adequate epidemiological or toxicological studies evaluating the potential for effects of early-life PFDA exposure on male reproductive system development.

[14]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-25. Evidence profile table for PFDA exposure and male reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.4: Human Studies)					⊕⊕⊙ ***Evidence indicates (likely)***
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Semen evaluations Three *medium* and 1 *low* confidence cross-sectional studies (1 is *uninformative* for motility)	• Decreased motility with increased exposure in Joensen et al. (2013). • No clear decrease in concentration or morphology in three medium confidence studies, but sensitivity is low.	• Large effect size for motility in medium confidence study	• Unexplained inconsistency in medium confidence studies for motility • Imprecision	⊕⊙⊙⊙ *Indeterminate* Coherent results in semen motility and testosterone across a *medium* and a *low* confidence study; inconsistency and imprecision add uncertainty.	*Primary basis:* Single, short-term study (*high* confidence) in rats, generally at ≥0.625 mg/kg-d PFDA *Human relevance:* Effects in rats are presumed relevant to humans based on the conserved role of androgen-dependent pathways in male reproductive functions across species. *Cross-stream coherence:*
Reproductive hormones For estradiol: 2 *medium* and 1 *low* confidence cross-sectional studies For testosterone: 1 *medium* and 3 *low* confidence studies	• Decreased testosterone in one of three studies of adults (one of two medium confidence) and one low confidence study of adolescents. No inverse association observed in two studies of infants.	• No factors noted	• Unexplained inconsistency in medium confidence studies • Imprecision		

Evidence stream summary and interpretation					Evidence integration summary judgment
<u>Pubertal development</u> Two *medium* confidence cohort studies	• In one study, for several indicators of puberty, mean age of onset was later in middle vs. lowest tertile of exposure, but earlier in the highest tertile. The other study reported no association with timing of puberty.	• No factors noted	• Unexplained inconsistency		N/A, human evidence is *indeterminate*. *Susceptible populations and lifestages:* Based on the potential for exposure to cause impaired androgen function, males exposed during critical windows of androgen-dependent development may be susceptible. *Other inferences:* Mechanistic evidence from rodent i.p. studies and cell culture models suggest that male reproductive toxicity is a primary target for PFDA (likely through disruption of Leydig cells and steroidogenesis), even at doses associated with significant body weight decreases.
Evidence from in vivo animal studies (see Section 3.2.4: Animal Studies)					
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Sperm evaluations</u> One *low* confidence study (due to	• Decreases in testicular and epididymal sperm counts at ≥0.625 mg/kg-d	• Consistent effects for decreased sperm count across tissues	• Lack of expected dose-response for testicular sperm counts	⊕⊕⊙ *Moderate*	

Evidence stream summary and interpretation					Evidence integration summary judgment
insensitivity) in rats exposed for 28 d	• No effects on sperm motility • *Low* confidence (due to the potential insensitivity of a short exposure duration) is mitigated by consistent effects	• Dose-response gradient for epididymal sperm counts		Coherent effects across sperm counts, serum testosterone levels and male reproductive histopathology and organ weights in a single, *high* confidence study; some concerns about insensitivity due to short-term exposure.	
<u>Histopathology</u> One *high* confidence study in rats exposed for 28 d	• Mild degenerative lesions in testes and epididymis at ≥1.25 mg/kg-d	• Consistent pattern of lesions across tissues • Leydig cell atrophy is coherent with decreased sperm counts and testosterone levels • *High* confidence study	• Potential confounding by body weight decreases, although this concern is mitigated by findings from supplemental mechanistic studies.		
<u>Reproductive hormones</u> One *high* confidence study in rats for 28 d	• Decreases in serum testosterone levels at ≥0.625 mg/kg-d	• Dose-response gradient • *High* confidence study	• No factors noted		
<u>Organ weight</u> One *high* confidence study in rats for 28 d	• Decreases in testis and epididymis weights at ≥0.625 mg/kg-d	• Consistent effects across tissues • Coherence with sperm counts histopathology and testosterone levels • Dose-response gradient • *High* confidence study	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways (or other information)	**Summary of key findings, interpretation, and limitations**			**Evidence stream judgment**	
<u>Leydig cell androgen function</u>	*Key findings and interpretation:* • Impaired Leydig cell mitochondrial cholesterol uptake and testosterone synthesis in two vitro rodent models.			Evidence of altered Leydig cell function and decreased androgen production	

Evidence stream summary and interpretation			Evidence integration summary judgment
	• Altered testosterone secretion in rat testes and altered androgen levels, reproductive organ weights and histopathology in rodent species after acute, i.p. injection consistent with evidence of reduced steroidogenesis. *Limitations*: few studies; in animal models only; acute, i.p. exposure at high doses associated with systemic toxicity	provide support for the biological plausibility of the male reproductive effects of PFDA.	
Reproductive hormone signaling	*Key findings and interpretation*: • Effects in a minority of in vitro studies/assays relating to the AR (receptor binding, transactivation and cell proliferation) and ER pathways (transactivation), and in one study on aromatase. • ToxCast model predictions suggests that PFDA is inactive for AR/ER agonist and antagonist activities. *Limitations*: Mixed results across studies; some effects at cytotoxic levels; models generally not in male reproductive tissues.		
Other mechanisms	*Key findings and interpretation*: • Generally, lack of support for potential role of hepatic androgen metabolism or indirect systemic toxicity in PFDA-induced male reproductive effects in rodent studies *Limitations*: acute i.p. exposure; high dose; few studies.		

3.2.5. Female Reproductive Effects

Human Studies

Studies of possible female reproductive effects of PFDA are available for reproductive hormones, fecundity (i.e., time to pregnancy), menstrual cycle characteristics, and endometriosis. In addition, studies were available for spontaneous abortion and preterm birth, which could be driven by either female reproductive or developmental toxicity. These outcomes are reviewed in Section 3.2.3 in this Toxicological Review but are also included in the consideration of coherence across outcomes for female reproductive effects. The study evaluations for these outcomes are summarized in Figure 3-68.

Figure 3-68. Evaluation results for epidemiological studies assessing effects of PFDA exposure on female reproduction. Refer to HAWC Human Female Reproductive Effects for details on the study evaluation review.

Reproductive hormones

Reproductive hormones examined in the evaluated studies include testosterone, estradiol/estrogen, insulin like growth factor 1 (IGF-1), FSH, LH, progesterone, prolactin, and inhibin-B, as well as SHBG. Key issues for the evaluation of these studies were sample collection and processing. For testosterone, LH, FSH, and prolactin, blood sample collection should be in the morning because of diurnal variation; if not possible, time of collection must be accounted for in the analysis. If there is no consideration of time of collection for these hormones, the study is classified as deficient for outcome ascertainment and *low* confidence overall. The timing of PFDA exposure relevant for influencing reproductive hormones is unclear and dependent on several factors, and thus all exposure windows with available data were considered relevant for these endpoints of interest, particularly given the long half-life of PFDA. Cross-sectional studies were included as levels of these hormones are capable of being rapidly upregulated or downregulated and they are not expected to directly bind to or otherwise interact with circulating PFAS.

Ten studies (Timmermann et al., 2022; Yang et al., 2022b; Xie et al., 2021; Jensen et al., 2020b; Liu et al., 2020b; Yao et al., 2019; Zhang et al., 2018a; McCoy et al., 2017; Zhou et al., 2016; Barrett et al., 2015) reported on associations between PFDA exposure and female reproductive hormones. Four studies were *medium* confidence, including cross-sectional studies of healthy adults in Norway (Barrett et al., 2015) and the United States (Xie et al., 2021) (latter is *low* confidence for testosterone), a cross-sectional study of newborns in China (Liu et al., 2020b), and a pregnancy cohort in China (Yang et al., 2022b). Most of the remaining six studies were *low* confidence. In adults, these studies included an analysis of women with premature ovarian insufficiency in China (Zhang et al., 2018a) and a cohort of pregnant women in Denmark (Timmermann et al., 2022). In children and adolescents, there was a cohort of adolescents in Taiwan (Zhou et al., 2016) and two studies in infants, a cohort in Denmark (Jensen et al., 2020b), and a cross-sectional study in China (Yao et al., 2019). Lastly, McCoy et al. (2017) was considered *uninformative* because of multiple deficiencies in study evaluation.

For estrogen, one study, a cohort in pregnant women with follow-up across pregnancy (Yang et al., 2022b), examined estrone (E_1), estradiol (E_2), and estriol (E_3) and reported an inverse association between PFDA (median 0.8 ng/mL) and estrone (β [95% CI]: −0.12 (−0.24, −0.01)). Associations with estradiol and estriol were in the same direction but not statistically significant. The remaining studies examined only estradiol. In general population adults, an inverse, although nonmonotonic, association (β [95% CI] vs. Q1 for Q2: −78.64 [−310.37, 153.09]; Q3: −183.04 [−353.51, −12.56]; Q4: =117.92 [−285.64, 49.70]) was also reported in (Xie et al., 2021) (median 0.1 ng/mL). Associations varied by age group, with inverse associations in adolescents and 12- to 49-year-olds, but a positive association in women 50 years of age and older. No association with PFDA was reported with follicular estradiol in Barrett et al. (2015) (mean PFDA 0.3 ng/mL), or with blood estradiol in Zhang et al. (2018a) (median PFDA 0.4 ng/mL), Zhou et al. (2016) (median PFDA 1.0 ng/mL), or cord blood estradiol in Yao et al. (2019) (median PFDA 0.2 ng/mL).

For testosterone, as Barrett et al. (2015) did not examine associations with this hormone, all of the available evidence is *low* confidence. None of the four available studies reported a statistically significant association between PFDA and testosterone (Xie et al., 2021; Yao et al., 2019; Zhang et al., 2018a; Zhou et al., 2016), and the direction of association was not consistent across studies (positive association in Yao et al. (2019) and Xie et al. (2021), inverse association in the other two studies.

For other reproductive hormones, Barrett et al. (2015) also examined luteal phase progesterone, finding a positive association with PFDA (0.472 (−0.043, 0.987)). Liu et al. (2020b) examined progesterone in newborns and found no association with PFDA. Zhang et al. (2018a) examined FSH, LH, and prolactin and also found no association with PFDA. Jensen et al. (2020b) reported inverse associations between PFDA and DHEA ($p < 0.05$), DHEAS, androstenedione, and 17-OHP ($p > 0.05$). Lastly, Timmermann et al. (2022) found a positive although imprecise association with prolactin during pregnancy (3.3% difference (95% CI: −0.4, 7.2) per doubling of PFDA concentrations).

Overall, the findings in reproductive hormones are primarily null, with a few inconsistent associations observed. However, because of low exposure levels in most studies and the availability of a small number of studies per population type (adult women, adolescents, newborns) and reproductive hormones, the evidence is difficult to interpret.

Fecundity

Six epidemiological studies reported on the association between PFDA exposure and fecundity. Fecundity is the biological capacity to reproduce. Time to pregnancy, defined as the number of calendar months or menstrual cycles from the time of cessation of contraception to detection of pregnancy, is a primary outcome measure used to study fecundity. There are challenges in studying this outcome as it is ideal to enroll women at the point when contraception is discontinued, but this is generally limited to women trying to get pregnant who may not be representative of the general population. An alternative approach is to enroll pregnant women and ask for their recall of time to pregnancy, but this is subject to selection bias that is due to excluding women who are unable to conceive and are thus potentially most affected. Two studies were preconception cohorts and considered *medium* confidence (Lum et al., 2017; Vestergaard et al., 2012), and two were pregnancy cohorts and considered *low* confidence (Bach et al., 2018; Bach et al., 2015) because of the potential for selection bias described above. Another fecundity-specific consideration is the potential for confounding in parous women because of factors related to previous pregnancies (Bach et al., 2018). In addition to the studies of time to pregnancy, two studies examined women undergoing infertility treatment; one *medium* confidence cohort examined successful pregnancies using in vitro fertilization (IVF) (Wang et al., 2021) and one *low* confidence cross-sectional study compared PFAS concentrations in women with different types of infertility (with male factor infertility as the control group) and associations with fertilization rate

(Kim et al., 2020c). A summary of the study evaluations is presented in Figure 3-68 and additional details can be obtained from HAWC.

The results for the association between PFDA exposure and time to pregnancy are presented in Table 3-26. A fecundability ratio less than 1 indicates a decrease in fecundity/increase in time to pregnancy. One study (Bach et al., 2018) reported longer time to pregnancy with higher exposure in the fourth quartile, but only in parous women, which despite adjustment for interpregnancy interval, may be more likely to be confounded. None of the other available studies reported a decrease in fecundity/increase in time to pregnancy with higher exposure, although this observed lack of association could be due to poor study sensitivity resulting from low exposure levels. In addition to the time to pregnancy results, two studies (Bach et al., 2015; Vestergaard et al., 2012) also analyzed infertility as an outcome and found no increase with higher exposure. Similarly, Wang et al. (2021) reported no increase in negative hcG test or clinical pregnancy failure following IVF with higher PFDA exposure (associations indicated less pregnancy failure and test negativity with higher exposure). Kim et al. (2020c) found no association between different infertility factors (endometriosis, PCOS, genital tract infections, or idiopathic) compared with male factor infertility. However, Kim et al. (2020c) did report an inverse, although imprecise, association between PFDA exposure and fertilization rate (β = −60.83, 95% CI: −129.25, 7.59).

Table 3-26. Associations between PFDA and time to pregnancy in epidemiological studies

Reference, study confidence	Population	Median exposure (IQR) or as specified	Comparison for effect estimate	Fecundability ratio (FR) (95% CI)
Vestergaard et al. (2012), *medium*	Preconception cohort (1992–1995), Denmark; 222 nulliparous women	0.1 (0.1, 0.1)[a]	log-unit increase	1.15 (0.89, 1.49)
			Above median vs. below	1.40 (0.96, 2.03)
Bach et al. (2018), *low*	Danish National Birth Cohort subsample (1996–2002), Denmark, 638 nulliparous women and 613 parous women	0.2 (0.1–0.2)	Quartiles vs. Q1	Nulliparous Q2: 1.13 (0.89, 1.43) Q3: 1.02 (0.82, 1.28) Q4: 1.11 (0.89, 1.39) Parous[b] Q2: 0.92 (0.68, 1.26) Q3: 0.95 (0.71, 1.28) Q4: 0.86 (0.65, 1.15)
Bach et al. (2015), *low*	Aarhus pregnancy cohort (2008–2013), Denmark; 1,372 nulliparous women	0.3 (0.2–0.4)	0.1 ng/mL increase	1.00 (0.97, 1.03)
			Quartiles vs. Q1	Q2: 1.08 (0.91, 1.28) Q3: 0.98 (0.83, 1.16) Q4: 1.08 (0.91, 1.28)

Reference, study confidence	Population	Median exposure (IQR) or as specified	Comparison for effect estimate	Fecundability ratio (FR) (95% CI)
Lum et al. (2017), *medium*	LIFE preconception cohort (2005–2009), U.S.; 401 women	0.4 (0.2–0.6)	Tertiles vs. T1	T2: 0.7 (0.5, 1.1) T3: 0.9 (0.6, 1.3)

*$p < 0.05$.

[a]Participants with pregnancy.

[b]These results were based on a model that corrected PFAS exposure based on an interpregnancy interval of median length. An alternate model for which interpregnancy interval was included as a covariate was statistically significant in Q4. A model with no adjustment for interpregnancy interval was not significant but had a monotonic decrease across quartiles (fecundability ratios of 0.92, 0.87, 0.78).

Pubertal development

Pubertal development is primarily assessed using established criteria, such as Tanner stage ratings. In girls, Tanner staging involves evaluation of the development of breasts and pubic hair. Stage 1 represents prepubertal development; stage 2, the onset of pubertal development; and stage 5 represents full sexual maturity. Age at menarche and age at peak height velocity (i.e., the age at which a child experiences the largest increase in height) can also be used as measures of pubertal development. Three studies, including two *medium* confidence cohorts in Denmark (Ernst et al., 2019) and the United States (Carwile et al., 2021) and one *low* confidence cross-sectional study (Wise et al., 2022) examined timing of pubertal development with PFDA exposure.

Carwile et al. (2021) used exposure measured during mid-childhood (median 8 years) with follow-up to early adolescence (median 13 years). Using a pubertal development score based on parental responses to scales of multiple pubertal markers (breast development, body hair growth, acne, growth spurt, and menarche), they reported less pubertal development in early adolescence with higher exposure (β (95%) per doubling of exposure: −0.11 (−0.18, −0.03)). This was consistent with their findings for older age at peak height velocity (0.23 (0.11, 0.35)) and older age at menarche (HR (95% CI) per doubling of exposure: 0.91 (0.77, 1.06)). Ernst et al. (2019) used maternal exposure measured in blood and prospectively identified pubertal onset with follow-up checks every 6 months. In girls, age at Tanner stages 2 and 3 for breast development was lower with higher exposure, consistent with Carwile et al. (2021), although not statistically significant. No association was observed for Tanner stages 4 and 5. No clear patterns for associations were observed with pubic hair development, axillary hair, or age at menarche. Results for the second and third tertiles were discordant for some outcomes (lower age at menarche and axillary hair development in second tertile, higher in third). Looking at a combined puberty indicator outcome, there was lower age at puberty (not significant) in the second tertile and no difference in the third tertile compared with the first. Wise et al. (2022) did not report a clear association with age at menarche (age was higher in both the first and third tertiles compared with the second), but this study was *low* confidence because of concerns for lack of temporality between exposure and outcome misclassification due to recall of age at menarche among adult women. Sensitivity was a

concern for all three studies, as exposure contrast was narrow. Exposure levels and contrast were slightly higher in Carwile et al. (2021) than in the other studies (IQR 0.4 ng/mL vs. 10th–90th percentile difference of 0.2 ng/mL in Ernst et al. (2019), so it is possible that this better sensitivity is a basis for the clearer associations in the former study.

Menstrual cycle characteristics

Four epidemiological studies reported on the association between PFDA exposure and menstrual cycle characteristics. Two were cohorts, one a preconception cohort already described for fecundity (Lum et al., 2017), and one a pregnancy cohort (Singer et al., 2018). Two studies were cross-sectional, one of participants in a preconception cohort (Zhou et al., 2017a) and one of general population Black women of reproductive age (Wise et al., 2022). For any outcome related to menstruation, there is potential for reverse causation because menstruation is one of the mechanisms by which PFAS are removed from the body (Wong et al., 2014; Zhang et al., 2013b). This potential bias could be away from the null with irregular and longer cycles. Thus, all four studies were considered *low* confidence. No associations were reported between menstrual cycle length or irregularity and PFDA exposure, but because of limited sensitivity related to exposure contrasts and *low* confidence in the studies, these findings are difficult to interpret.

Endometriosis

Two epidemiological studies reported on the association between PFDA exposure and endometriosis (Wang et al., 2017; Louis et al., 2012). Both studies were cross-sectional, which decreases confidence for this chronic outcome due to the inability to establish temporality and the likely lack of measurement in the relevant etiologic window. There is potential for reverse causality as described above since endometriosis can influence the menstrual cycle and could act in a protective direction as endometriosis can be associated with heavier and more frequent bleeding, which could increase elimination of PFDA from the body. Parity and related factors, such as time since last child, have also been suggested as sources of reverse causality for this association because a longer interpregnancy interval could allow more accumulation of PFAS levels (Wang et al., 2017); however, it was not a major concern in this set of studies as one study adjusted for parity and the other performed a sensitivity analysis with only women without a history of pregnancy. Nonetheless, because of the concern related to menstrual cycle irregularity association with endometriosis, all the studies were classified as *low* confidence; one of them—which included two groups of women, one scheduled for surgery (laparoscopy or laparotomy) and the other identified through a population database who underwent pelvic MRI to identify endometriosis (Louis et al., 2012)—is considered higher quality within that classification. The remaining study was additionally deficient for outcome ascertainment, specifically a case definition including only endometriosis-related infertility among surgically confirmed cases (Wang et al., 2017), which is likely to include less severe or asymptomatic cases among the controls. The *low* confidence study with good outcome ascertainment (Louis et al., 2012) reported higher odds of endometriosis with

higher exposure in the operative sample (OR = 2.95, 95% CI: 0.72, 12.1), but lower odds in the population sample (OR = 0.06, 95% CI: 0.00, 12.3), although both estimates were imprecise. The *low* confidence study by Wang et al. (2017) reported lower odds of endometriosis-related infertility with higher exposure (OR (95% CI) for T2 vs. T1: 0.93 (95% CI: 0.51, 1.70), T3 vs T1: 0.74 (95% CI: 0.40, 1.35)). It is difficult to reconcile the differing results considering the low number of studies, all of which were *low* confidence, and the potential for reverse causality for this outcome.

Premature ovarian insufficiency

One *low* confidence study, a case-control study in China, examined the association between PFDA exposure and premature ovarian insufficiency (POI) (Zhang et al., 2018b). In this study, POI was defined as an elevated FSH level greater than 25 IU/L on two occasions more than 4 weeks apart and oligo/amenorrhea for at least 4 months. Because this definition is closely tied to menstruation, there are concerns for reverse causality as with the previous two outcomes, which would be expected to be biased away from the null as there is reduced bleeding/elimination of PFDA from the body. The study reported higher odds of POI (not statistically significant) with higher PFDA exposure (OR (95% CI) for T2 vs. T1: 1.03 (0.54, 1.96), T3 vs. T1: 1.36 (0.71, 2.60)), but given the lack of additional evidence and concerns for reverse causality, there is considerable uncertainty in these results.

Breastfeeding duration

Three *medium* confidence birth cohorts examined duration of breastfeeding in relation to exposure to PFDA measured during gestation. Five additional cross-sectional or case-control studies without prospective measurement of exposure that reported analyses predicting PFNA concentrations based on past breastfeeding duration were considered supplemental evidence because of the high probability of reverse causation due to lactation being an elimination route (Kim et al., 2020b; Pirard et al., 2020; Ammitzbøll et al., 2019; Lee et al., 2018; Harris et al., 2017). The results of the three included studies are summarized in Table 3-27. One study reported an inverse association with breastfeeding duration (Timmermann et al., 2017b), while another study reported lower likelihood of cessation of breastfeeding by 3 or 6 months with higher exposure (Rosen et al., 2018), and the third reported no association. The inconsistency across studies reduces certainty in an association with breastfeeding duration.

Table 3-27. Associations between PFDA and breastfeeding duration in epidemiological studies

Reference, confidence	Population	Median exposure (IQR)	Form and units of effect estimate	Endpoint	Effect estimate
Risk of cessation of breastfeeding (>1 indicates earlier cessation)					

Reference, confidence	Population	Median exposure (IQR)	Form and units of effect estimate	Endpoint	Effect estimate
Rosen et al. (2018), *medium*	Norwegian Mother and Child Study (1999–2008), Norway, 1,716 women	0.1 (0.04–0.2)	HR (95% CI) for IQR change	Cessation of any breastfeeding by 3 mo	**0.73 (0.62, 0.86)***
				Cessation of any breastfeeding by 6 mo	**0.82 (0.72, 0.92)***
Timmermann et al. (2022), *medium*	Odense Child Cohort (2010–2012), Denmark, 932 women	0.3 (0.2–0.4)	HR (95% CI) for doubling	Cessation of any breastfeeding	0.98 (0.90, 1.08)
				Cessation of exclusive breastfeeding	0.95 (0.87, 1.03)
Continuous duration of breastfeeding (<0 indicates earlier cessation)					
Timmermann et al. (2017b), *medium*	Two birth cohorts in Faroe Islands (1997–2009), Denmark, 1,092 women	0.3 (0.2–0.4)	Difference in months (95% CI) for doubling	Duration of any breastfeeding	**−0.8 (−1.4, −0.3)***
				Duration of exclusive breastfeeding	−0.2 (−0.4, 0.0)

*$p < 0.05$.
IQR = interquartile range.

Animal Studies

A single study in the database of toxicity studies for PFDA evaluated female reproductive effects (NTP, 2018). The study examined the following endpoints after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) in adult female rats: organ weights, histopathology, hormone levels, and estrous cycles. The NTP (2018) study was evaluated as *high* confidence for all endpoints examined (see Figure 3-69). Although there is only a 28-day study available, the duration of the study is sufficient for assessing female reproductive toxicity given that significant effects on estrous cyclicity were observed as early as day 21 of the 28-day study and the mean estrous cyclicity length is reported to be 4.4 days among multiple substrains of SD rats (Marty et al., 2009).

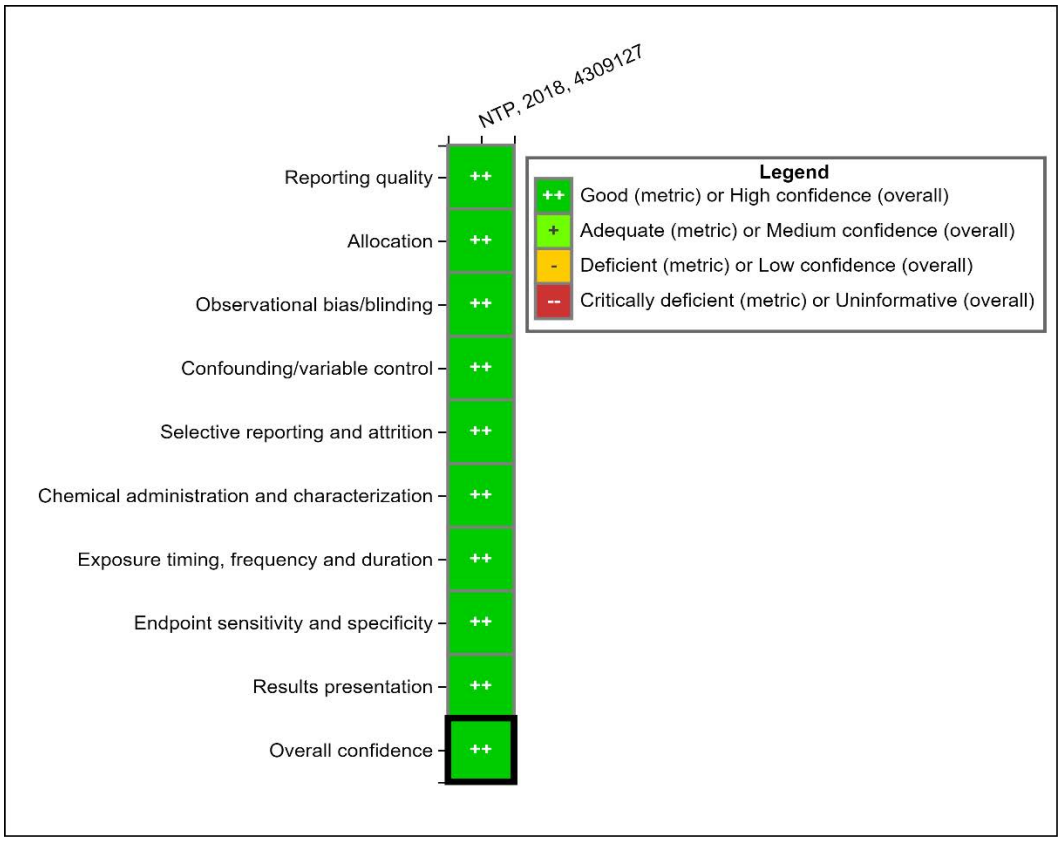


Figure 3-69. Evaluation results for an animal study assessing effects of PFDA on female reproduction. Refer to HAWC for details on the study evaluation review.

Estrous cycle

Female rats from the three highest dose groups (0.625, 1.25, and 2.5 mg/kg-day) were evaluated for changes in the estrous cycle that were due to PFDA exposure, compared with controls. To examine this endpoint, vaginal smears were performed for 16 consecutive days before animals were necropsied. Changes in the percent of time spent in each estrous stage (proestrus, estrus, metestrus, diestrus) were affected by exposure (see Figure 3-70 and Table 3-28). Specifically, for proestrus, the percentage of time spent increased by 103% and 123% at 0.625 and 1.25 mg/kg-day, respectively, but then decreased by 81% at 2.5 mg/kg-day. For metestrus, the percentage of time spent was increased by 23% at 0.625 mg/kg-day but then decreased by 100% at ≥1.25 mg/kg-day. A significant trend test was observed for the percentage of time spent in estrus with statistically significant decreases (42%–84%) at ≥1.25 mg/kg-day (see Figure 3-70 and Table 3-28). Correspondingly, a significant trend test was observed for the percentage of time spent in diestrus with statistically significant increases (27%–63%) at ≥1.25 mg/kg-day (see Figure 3-70 and Table 3-28). Estrous cyclicity was disrupted and all female rats remained in a continuous state of diestrus at 2.5 mg/kg-day starting on day 21 (day 9 of the 16 days in which vaginal cytology was assessed). The sustained state of diestrus suggests that these animals may have been infertile (U.S. EPA, 1996a), although this was not specifically evaluated. Although decreased body weight in

female rats was observed at the same doses (body weight decreases were 12%–36% at ≥1.25 mg/kg-day; refer to Section 3.2.10 for more details) as effects on estrous cyclicity, it is unclear if these effects are related and the effect on female reproductive function is disproportionately more severe and concerning than the changes in body weight. Although body weight has been shown to fluctuate during the different estrous stages and weight loss has been shown to correlate with disrupted estrous cyclicity in rats (Tropp and Markus, 2001), it is not possible to determine if the decreases in body weight in female rats might be responsible for the effects on estrous cyclicity observed in the NTP (2018) study. Furthermore, even though no changes were observed on other stages of the estrous cycle (i.e., proestrus and metestrus), the effects of PFDA on estrus and diestrus are still considered biologically relevant given the potential influence that the lack of cyclicity may have on fertility, regardless of whether the observed decrease in body weight may have partially contributed to these changes. Changes in cycle length and the number of cycles during the study were not affected in the 0.625 and 1.25 mg/kg-day groups. Data for cycle length and number of cycles could not be determined for the 2.5 mg/kg-day group because estrous cyclicity was disrupted in all female rats at this dose and all animals remained in a state of continuous diestrus starting at day 21 until sacrifice.

Table 3-28. Percent changes relative to controls in time spent in each estrous stage (proestrus, estrus, metestrus, diestrus) in female SD rats exposed to PFDA exposure for 28 days (NTP, 2018)

Endpoint	Dose (mg/kg-d)		
	0.625	1.25	2.5
% of estrous cycle in diestrus	10	**27**	**63**
% of estrous cycle in estrus	−22	**−42**	**−84**
% of estrous cycle in metestrus	23	−100	−100
% of estrous cycle in proestrus	103	123	−81

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

Hormone levels

Testosterone was measured in all dose groups at study termination; it is unclear from the study description if the study authors controlled for fasting or time of necropsy. A significant trend test was observed with statistically significant increases reported at ≥0.312 mg/kg-day (see Figure 3-70). Increases were monotonic and varied from 30% to 348% change from controls; levels of circulating testosterone were increased more than twofold at 1.25 mg/kg-day. Other sex hormones (e.g., estradiol) were not measured in this study. The biological relevance of increased testosterone to the development of PFDA-induced female reproductive toxicity is not entirely clear. Specifically, the association of increased testosterone and altered estrous cycling (e.g., prolonged diestrus) requires further investigation. However, studies have shown that high levels of androgens

(e.g., testosterone) can cause irregular menstruation cycles in women. For example, Van Anders and Watson (2006) reported an association between high levels of testosterone and increased menstrual cycle length in healthy premenopausal women. Such findings could suggest possible coherence between increased testosterone levels and prolonged diestrus observed in PFDA-treated female rats from the NTP, 2018, 4309127 study given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006).

Histopathology

Histological examination of the clitoral gland, ovaries, uterus, and mammary glands were performed at study termination. Histopathology was examined for the ovaries at all doses; all other reproductive tissues were examined only in the control and high-dose groups. Histological changes due to PFDA treatment were not reported for any tissue examined including the uterus (see Figure 3-70) even though PFDA effects on estrous cyclicity and uterine weight were reported.

Organ weights

Uterine weights were measured in all dose groups at study termination. A significant trend test was observed for both absolute and relative weights with the two highest dose groups reaching statistically significant decreases for both measures (see Figure 3-70). Decreases reached −64% and −44% change from controls for absolute and relative weights, respectively. Other organs related to the female reproductive system were not measured. It should be noted that comparisons of uterine weights were not made in rats that were in the same estrous stage. As noted below, many studies in rats have shown that uterus weight decreases during diestrus. Therefore, it is unclear whether the reductions in uterus weight are a direct effect of PFDA or rather a secondary effect due to prolonged diestrus owing to PFDA exposure.

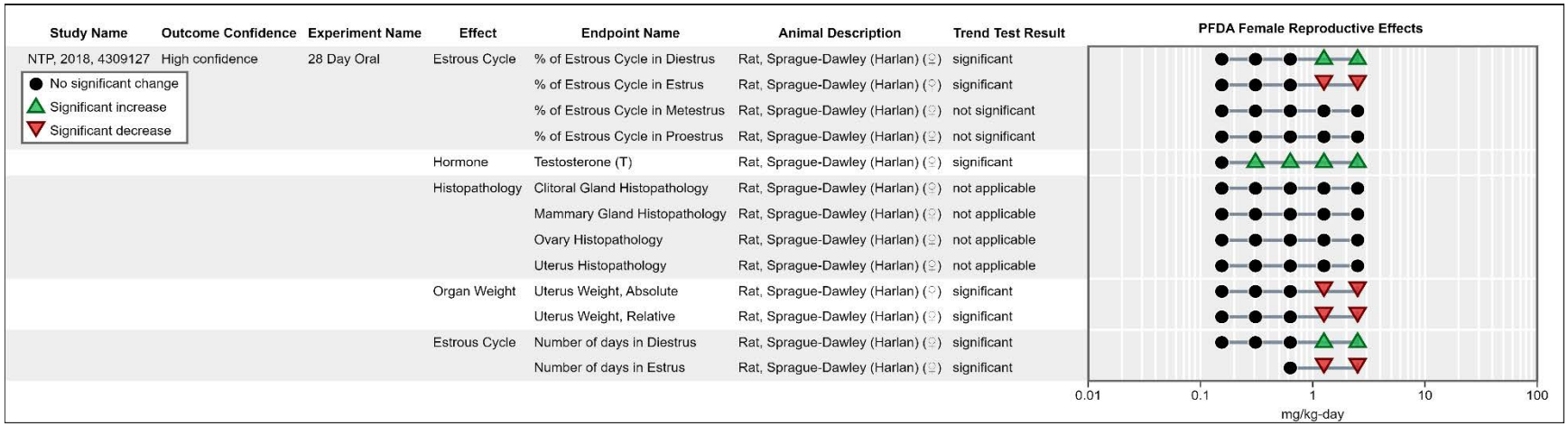


Figure 3-70. PFDA female reproductive effects. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

As discussed in the male reproductive section (see Section 3.2.4), PFDA-induced effects on AR and ER functions and on aromatase activity have been evaluated in in vitro cell culture studies and HTS assays from ToxCast and Tox21. Findings from in vitro cell culture studies and HTS assays do not provide consistent evidence for potential effects of PFDA on AR or ER functions, or on aromatase activity. Additional in vivo and/or cell culture studies are necessary to address inconsistencies in the available in vitro data and determine whether these pathways might be disrupted by PFDA exposure. In an in vitro study, PFDA inhibited progesterone production in mouse Leydig tumor cells, which the study authors postulated was due to oxidative stress (Zhao et al., 2017). It is not possible to corroborate this effect with data from the lone reproductive study in rats (NTP, 2018) given that progesterone was not measured in the (NTP, 2018) study. In the NTP (2018) study, Wyeth-14,643 (a PPARα agonist) was shown to cause effects on estrous cyclicity similar to those reported for PFDA. However, mechanistic studies that investigate the role of PPARα in PFDA-altered estrous cyclicity are not available.

Evidence Integration

There is *indeterminate* evidence of an association between PFDA exposure and female reproductive effects in human studies, although the *low* confidence studies that were available had concerns for study sensitivity, which reduces the ability to interpret the observed null findings. A significant inverse association between PFDA and anogenital distance in girls was observed in one study (see Section 3.2.3), which is relevant to female reproductive toxicity. The biological relevance of this effect on anogenital distance is unclear given that an increase in this measure is considered adverse in girls rather than a decrease per EPA's *Guidelines for Reproductive Toxicity Risk Assessment*. Furthermore, the available reproductive hormone evidence for PFDA does not support an association. Previous studies have shown an association between increased testosterone and increased anogenital distance in women (Mira-Escolano et al., 2014), however, the *human evidence* is *inadequate* for examining PFDA-induced effects on testosterone in women. Whereas increased testosterone was observed in female rats in the NTP (2018) study, the study authors did not measure anogenital distance given that there was no developmental exposure in the study. The increased testosterone observed in female rats is considered relevant to humans and given the known association between increased testosterone and anogenital distance in women, an increase in anogenital distance rather than a decrease would be expected in women exposed to PFDA. Overall, there is little biological understanding of how hormonal perturbation or other biological processes might result in a decrease in anogenital distance owing to PFDA exposure.

In addition to the outcomes described in this section, there is potential for two of the outcomes described in the developmental section (refer to Section 3.2.3 for more details), preterm birth and spontaneous abortion, to be related to female reproductive toxicity. The evidence for these outcomes was inconsistent. Given that most of the evidence for female reproductive effects

was null or inconsistent, there is little clear indication of an association. However, the exposure levels in most of the study populations were low, which resulted in low sensitivity to detecting an effect, and thus these findings should not be interpreted as supporting a lack of effect.

The available data from a 28-day gavage study in rats provided *moderate* evidence that PFDA exposure may cause female reproductive toxicity (see Table 3-29). The evidence is sparse. The data are from a single animal study that did not evaluate fertility, pregnancy outcomes, multiple hormone levels (only testosterone was measured), or markers of reproductive development. PFDA was observed to cause effects on the following female reproductive parameters: organ weight (i.e., decreased uterine weights at ≥1.25 mg/kg-day), hormone levels (i.e., increased testosterone levels at ≥0.312 mg/kg-day), and estrous cycle (i.e., percentage of time spent in estrus and diestrus at ≥1.25 mg/kg-day). One factor increasing the strength of the evidence is the severity of the effect on estrous cyclicity; specifically, that PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested (2.5 mg/kg-day), which could be indicative of reductions or delays in fertility. However, some caution in the interpretation of the higher dose effects is warranted given the significant decreases in body weight, particularly at 2.5 mg/kg-day (36% decrease). Support for the adversity and concerning nature of prolonged diestrus and its association with infertility is provided by the following text in EPA's *Guidelines for Reproductive Toxicity Risk Assessment*:

- "Persistent diestrus indicates temporary or permanent cessation of follicular development and ovulation, and thus at least temporary infertility."
- "Pseudopregnancy is another altered endocrine state reflected by persistent diestrus."
- "Significant evidence that the estrous cycle (or menstrual cycle in primates) has been disrupted should be considered an adverse effect."
- "The greatest confidence for identification of a reproductive hazard should be placed on significant adverse effects on sexual behavior, fertility or development, or other endpoints that are directly related to reproductive function such as menstrual (estrous) cycle normality, sperm evaluations, reproductive histopathology, reproductive organ weights, and reproductive endocrinology."

Furthermore, prolonged diestrus is commonly reported in rodent models of impaired fertility (Li et al., 2017; Caldwell et al., 2014; Miller and Takahashi, 2014; Mayer and Boehm, 2011) and continuous diestrus is observed during reproductive senescence in aged female rats (Lefevre and Mcclintock, 1988). There was also possible coherence between increased testosterone levels and increased percentage of time spent in diestrus at ≥1.25 mg/kg-day. As stated above, high levels of testosterone have been shown to increase menstrual cycle length in women (Van Anders and Watson, 2006). There was also coherence between decreased uterus weight and increased percentage of time spent in diestrus at ≥1.25 mg/kg-day. Previous studies have shown that

decreased uterus weight in rats is commonly observed during diestrus (Westwood, 2008; Vasilenko et al., 1981; Walaas, 1952; Boettiger, 1946). In addition to prolonged diestrus, PFDA decreased the percentage of time spent in estrus (NTP, 2018), which could indirectly cause infertility given that rodents are sexually receptive only during estrus (Goldman et al., 2007). The severe, PFDA-induced decreased time spent in estrus is expected to result in decreased opportunities for mating in the rats, and therefore reductions or delays in fertility. Unfortunately, no multigenerational studies of PFDA were available to inform this hypothesis.

In this study, PFDA did not cause histopathological changes in female reproductive tissues. Given the short-term duration of the lone animal study, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer study duration or during a sensitive developmental window (e.g., in utero or pregnancy). The short-term duration of the lone animal study does not reduce confidence in the database for PFDA-induced female reproductive effects given that biologically relevant effects (e.g., prolonged diestrus) were still observed.

Taken together, the available ***evidence indicates*** that PFDA is likely to cause female reproductive toxicity in humans under sufficient exposure conditions[15] (see Table 3-29). This conclusion is based primarily on evidence from a *high* confidence study in rats exposed to doses ranging from 1.25 to 2.5 mg/kg-day PFDA for 28 days. These findings are interpreted as relevant to humans in the absence of evidence to the contrary. This assumption is based on *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996). Specifically, the PFDA-induced disruption of estrous cyclicity observed in female rats from the NTP study (NTP, 2018) and its implications for infertility can be considered relevant to humans given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006). Given the sparse evidence base (i.e., one short-term animal study and largely *low* confidence or null human studies) and the lack of understanding for how PFDA exposure causes the observed reproductive effects or whether they might progress with longer exposures, further studies that could inform this conclusion include those that examine the effect of PFDA on female fertility and pregnancy outcomes in exposed animals from subchronic, chronic, developmental, or multigenerational studies, as well as in vivo or cell culture mechanistic studies.

[15]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-29. Evidence profile table for PFDA exposure and female reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.5: Human Studies)					⊕⊕⊙ ***Evidence indicates (likely)***
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
Reproductive hormones Four *medium* and 5 *low* confidence studies	• Inverse association between PFDA exposure and estrogen observed in 2 studies. Most studies reported no association with female reproductive hormones, but sensitivity was limited in most studies	• No factors noted	• No factors noted	⊙⊙⊙ *Indeterminate* Within and across outcomes, findings were mixed, null, and/or of *low* confidence. Interpretation of the lack of an association for most outcomes in these studies is complicated by poor sensitivity for observing effects due to *low* exposure levels.	*Primary basis*: Evidence from a high confidence study in rats showing biologically coherent effects on uterus weight and the estrous cycle after oral exposure to PFDA at ≥1.25 mg/kg-d for 28 d. *Human relevance*: Evidence in animals is presumed relevant to humans given that mechanisms regulating female reproduction are similar between rats and humans. *Cross-stream coherence*: N/A, human evidence is *indeterminate*. *Susceptible populations and lifestages*: Based on altered estrous cyclicity data in animals, females of
Fecundity Three *medium* and 3 *low* confidence studies	• One study reported longer time to pregnancy with higher PFDA exposure, but only in parous women. No association observed in other studies, but sensitivity was limited.	• No factors noted	• Unexplained inconsistency, although a lack of association in some studies may be attributable to limited sensitivity		
Pubertal development Two *medium* and 1 *low* confidence cohort studies	• One study reported later age at pubertal onset based on pubertal development score, age at peak height velocity, and age at menarche. Two other studies reported no clear association	• Coherence of related effects in one study	• Unexplained inconsistency		
Menstrual cycle Four *low* confidence studies	• No association observed between PFDA exposure and menstrual cycle characteristics, but sensitivity was limited.	• No factors noted	• Potential for reverse causality		
Endometriosis Two *low* confidence studies	• Higher odds of endometriosis with higher PFDA exposure in women scheduled for laparoscopy or laparotomy in one study, but lower odds of endometriosis in a population-based sample in the same study and a *low* confidence study.	• No factors noted	• Unexplained inconsistency across *low* confidence studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
			• Potential for reverse causality		reproductive age may be at higher risk. *Other inferences*: No specific factors are noted.
<u>Breastfeeding duration</u> Three *medium* confidence studies	• One study reported an inverse association with breastfeeding duration, while one study reported lower likelihood of cessation of breastfeeding and one study reported no association.	• No factors noted	• Unexplained inconsistency		
Evidence from in vivo animal studies (see Section 3.2.5: Animal Studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
<u>Estrous cycle</u> One *high* confidence study	• The percentage of time spent in estrus was significantly decreased at ≥1.25 mg/kg-d. • The percentage of time spent in diestrus was significantly increased at ≥1.25 mg/kg-d. • Estrous cyclicity was disrupted at 2.5 mg/kg-d and all female rats in this dose group remained in a continuous state of diestrus by Day 21.	• Large magnitude of effect and concerning severity • In a *high* confidence study • Dose-response gradient for effects on the percentage of time spent in estrus and diestrus. • Coherence with reduced uterus weight • Possible coherence with increased testosterone levels	• Lack of expected coherence for histopathology, although possibly explained by short exposure duration • Potential confounding by body weight decreases.	⊕⊕⊙ *Moderate* Based on multiple, coherent changes in female reproductive endpoints, most notably that PFDA induced a continuous phase of diestrus, which could be indicative of infertility, in 100% of rats at 2.5 mg/kg-d.	
<u>Organ weight</u> One *high* confidence study	• Decreased absolute and relative uterine weights at ≥1.25 mg/kg-d.	• Dose-response gradient in a *high* confidence study	• Potential confounding by body weight decreases (mitigated some by comparable effects on absolute and relative weights)		
<u>Hormone levels</u>	• Increased testosterone levels at ≥0.312 mg/kg-d.	• Dose-response gradient in a *high* confidence study			

Evidence stream summary and interpretation					Evidence integration summary judgment
One *high* confidence study					
Histopathology One *high* confidence study	• No PFDA-induced histopathological changes were observed for the clitoral gland, ovaries, uterus, and mammary glands.	• No factors noted	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**	**Evidence stream judgment**			
Hormone levels	*Interpretation:* PFDA inhibits progesterone production. *Key findings:* • PFDA reduced progesterone production in mouse Leydig tumor cells. The study authors suggested that oxidative stress may be a possible mechanism. *Limitations:* Single study available, lack of evidence examining effects on other sex hormones.	• Evidence of decreased progesterone production provides limited support for the biological plausibility of the female reproductive effects of PFDA. It is not possible to corroborate this effect with data from the lone reproductive study in rats (NTP, 2018) progesterone was not measured in the (NTP, 2018) study.			

3.2.6. Cardiometabolic Effects

Methodological Considerations

Cardiometabolic risk refers to the likelihood of developing diabetes, heart disease, or stroke. Contributors to this risk include a combination of metabolic dysfunctions mainly characterized by insulin resistance, dyslipidemia, hypertension, and adiposity.

Human Studies

Twenty-two epidemiological studies reported on the relationship between PFDA exposure and cardiometabolic effects, including serum lipids (12 studies), blood pressure (5 studies), atherosclerosis (2 studies), cardiovascular disease (2 studies), ventricular geometry (1 study), diabetes and insulin resistance (11 studies), adiposity and weight gain (6 studies), and metabolic syndrome (2 studies).

Serum lipids

Cholesterol as found in low-density lipoprotein (LDL) is one of the major controllable risk factors for cardiovascular disease including coronary heart disease, myocardial infarction, and stroke. Cholesterol levels are typically measured in the blood. Twenty-three studies (28 publications) reported on the association between PFDA exposure and serum lipids (e.g., total cholesterol, lipoprotein complexes, and triglycerides). Multiple outcome-specific considerations for study evaluation were influential on the ratings. First, for outcome ascertainment, collection of blood during a fasting state is preferred for all blood lipid measurements (NIH, 2020; Nigam, 2011) but lack of fasting was considered deficient for triglycerides and LDL cholesterol (which is typically calculated using levels of triglycerides, as well as total cholesterol and HDL, using the Friedewald equation). This is because triglyceride levels remain elevated for several hours after a meal (Nigam, 2011). Self-reported high cholesterol was also considered deficient due to the high likelihood of misclassifying cases as controls (Natarajan et al., 2002). Both of these issues are likely to result in nondifferential outcome misclassification and to generally bias results toward the null. It was also considered important to account for factors that meaningfully influence serum lipids, most notably use of cholesterol lowering medications and pregnancy. Studies that did not consider these factors by exclusion, stratification, or adjustment were considered deficient for the participant selection domain. All the available studies analyzed PFDA in serum or plasma and serum lipids using standard, appropriate methods. As described in Section 3.2.8 on endocrine effects, reverse causation was considered but is unlikely to significantly bias the results because PFAS, including PFDA, do not preferentially bind to serum lipids, so exposure measurement was adequate for this outcome across all studies.

A summary of the study evaluations is presented in Figure 3-71, and additional details can be obtained from HAWC. Three studies were excluded from further analysis due to critical

deficiencies in at least one domain. Most studies (14) were classified as *medium* confidence, although five of these were classified as *low* confidence for triglycerides and LDL cholesterol due to lack of fasting as described above (Blomberg et al., 2021; Jensen et al., 2020a; Yang et al., 2020; Zeng et al., 2015; Starling et al., 2014b). Six studies were classified as *low* confidence (Varshavsky et al., 2021; Khalil et al., 2020; Lin et al., 2020b; Koshy et al., 2017; Christensen et al., 2016; Fu et al., 2014) for all lipid endpoints. For the majority of studies, sensitivity to detect an effect was a concern due to limited exposure contrast, and thus null associations are interpreted with caution. Potential for confounding across PFAS was considered within individual study evaluations and synthesized across studies.

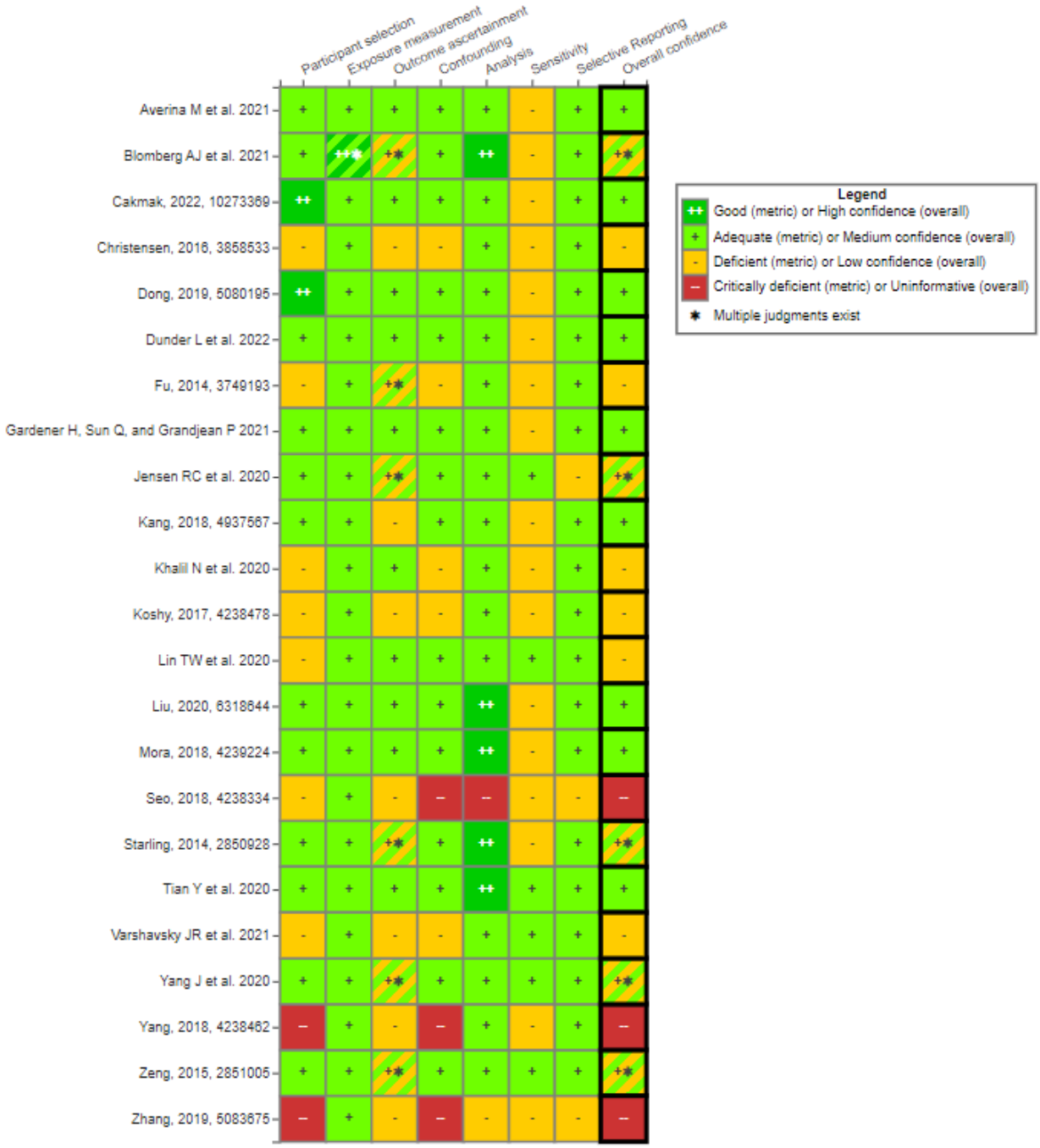


Figure 3-71. Evaluation results for epidemiological studies assessing effects of PFDA exposure on serum lipids. Refer to HAWC Human Serum Lipids for details on the study evaluation review.

Multiple publications of the same study: Dong et al. (2019) (on figure) includes Christensen et al. (2019) and Jain and Ducatman (2019a). Liu et al. (2020a) (on figure) includes Liu et al. (2020a).

The results for the association between PFDA exposure and blood lipids among the *medium* confidence studies are presented in Table 3-30. Of the 14 *medium* confidence studies, 4 were in

general population adults, 3 were in pregnant women, and 7 were in adolescents and children. In adults, the majority of studies reported higher total cholesterol with higher exposure, including four in general population adults (Cakmak et al., 2022; Dunder et al., 2022; Liu et al., 2020a; Dong et al., 2019) and two in pregnant women (Gardener et al., 2021; Starling et al., 2014a). Statistical significance was found in three studies (Cakmak et al., 2022; Dunder et al., 2022; Gardener et al., 2021) and an exposure-response gradient was found in both studies that examined categorical exposure (Gardener et al., 2021; Liu et al., 2020a). Results in children were less consistent. Four studies reported statistically significant positive associations in at least one analysis (Averina et al., 2021; Blomberg et al., 2021; Jensen et al., 2020a; Mora et al., 2018), but other studies reported inverse (Tian et al., 2021; Kang et al., 2018; Zeng et al., 2015) or null associations. In addition to the continuous serum lipids measurements, one study (Averina et al., 2021) examined dyslipidemia as a dichotomous outcome (defined as total cholesterol ≥5.17 mmol/L). The authors reported increased odds of lipidemia with higher exposure (OR [95% CI] vs. quartile 1: Q2: 2.34 [1.08, 5.05]; Q3: 2.19 [1.01, 4.74]; Q4: 2.36 [1.08, 5.16]). Results for triglycerides were not available for all studies, but a positive association was observed in two studies in adults (Cakmak et al., 2022; Dunder et al., 2022) and one study in pregnant women (Gardener et al., 2021), while the other one study in adults and two studies in pregnant women showed no association. An inverse association was observed in Mora et al. (2018) in children; the direction of this association was not coherent with the reported positive associations for total and LDL cholesterol in the same cohort, which increases uncertainty. Other studies in children indicated no association with triglycerides.

Looking at the *low* confidence studies in adults (Varshavsky et al., 2021; Khalil et al., 2020; Lin et al., 2020b; Christensen et al., 2016; Fu et al., 2014) and adolescents (Koshy et al., 2017), four reported increases in total cholesterol (Lin et al., 2020b; Koshy et al., 2017; Fu et al., 2014) or unspecified high cholesterol (Christensen et al., 2016) with increased exposure, with one being statistically significant (Koshy et al., 2017). Two studies (Varshavsky et al., 2021; Khalil et al., 2020) reported inverse results. The results of all the *low* confidence studies were interpreted with caution because of serious limitations.

Overall, evidence for the association between PFDA exposure and serum lipids is inconsistent, and this inconsistency cannot be easily explained by study confidence level or the participant-demographics. This may be partly explained by narrow exposure contrasts, which may have reduced sensitivity and impaired the ability of some studies to observe an effect. However, the strongest associations were observed in studies (Dong et al., 2019; Mora et al., 2018; Starling et al., 2014a) with low PFDA exposure levels (median <0.5 ng/mL),which could be an indication that sensitivity in this body of evidence is adequate or could be due to residual confounding, such as by other PFAS or the demographics of the study population. There is some support for the PFAS scenario, as PFDA was highly correlated with PFNA (0.7) and moderately correlated with PFOS and PFOA (0.4) in both Starling et al. (2014a) and Dong et al. (2019), and positive associations were stronger for PFOA in Starling et al. (2014a) and for PFNA, PFOS, and PFOA in Dong et al. (2019).

Conversely, in Mora et al. (2018), PFDA was highly correlated with PFOA (0.7) and moderately correlated with PFOS (0.6) and PFNA (0.5), but the observed positive associations were strongest in PFDA, and thus are unlikely to be completely explained by confounding. Given available data, there is not enough evidence to state conclusively whether confounding contributed to these results.

Table 3-30. Associations between PFDA and blood lipids in *medium* confidence epidemiological studies

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
General population, adults						
Dong et al. (2019)	Cross-sectional study, U.S. (NHANES 2003–2014); 8,950 adults (20–80 yr)	0.2	β (95% CI) for 1-unit increase[a]	6.6 (−8.5, 21.7)	10.7 (−8.5, 29.9)	NR
Cakmak et al. (2022)	Cross-sectional study, Canada (CHMS 2007–2017); 6,045 participants	0.2 (GM)	% change for increase equivalent to GM[b]	**2.8 (0.2, 5.3)***	**10.7 (5.5, 16.1)***	**7.0 (1.0, 13.2)***
Dunder et al. (2022)	Cohort (2001–2004), Sweden; 864 older adults (70–80 yr)	0.3 (0.2–0.4)	β (95% CI) for change in exposure and outcome over 10 yr[b]	**0.23 (0.14, 0.32)***	**0.12 (0.03, 0.20)***	**0.08 (0.04, 0.12)***
Liu et al. (2020a)	Cross-sectional analysis from randomized clinical trial of weight loss; 326 overweight adults	0.4 (0.2–0.5)	Means ± SE for tertiles[a]	T1: 183.1 ± 7.9 T2: 186.6 ± 7.5 T3: 192.1 ± 7.6 p = 0.2	NR	T1: 138.9 ± 11.3 T2: 119.7 ± 10.7 T3: 129.3 ± 10.8 p = 0.3

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
Pregnant women						
Starling et al. (2014a)	Cross-sectional analysis from birth cohort (2003–2004), Norway; 891 women	0.09 (<LOQ–0.2)[c]	β (95% CI) for ln-unit increase[a]	1.8 (−2.1, 5.8)	0.2 (−3.3, 3.7)[d]	−0.03 (−0.07, 0.01)[d]
Gardener et al. (2021)	Pregnancy cohort (2009), U.S., 433 women	0.2 (0.1–0.3)	Means ± CI for quartiles[b]	Positive association with exposure-response gradient*	NR	Positive association with exposure-response gradient*
Yang et al. (2020)	Pregnancy cohort (2013–2014), China, 436 women	1.0 (0.6–1.7)	β (95% CI) for ln-unit increase[b]	−0.03 (−0.09, 0.04)	**−0.05 (−0.10, −0.01)***	0.06 (−0.02, 0.14)
Adolescents and children						
Kang et al. (2018)	Cross-sectional study (2012–2014), Korea, 150 children (3–18 yr)	0.06 (0.04, 0.1)	β (95% CI) for ln- unit increase[a]	−3.3 (−7.8, 0.8)	−1.9 (−5.7, 2.0)	−0.04 (−0.1, 0.03)
Blomberg et al. (2021) (additional results with different timing of exposure and outcome measurement are available in the publication)	Birth cohort (2007–2009), Faroe Islands, 459 children (followed to 9 yr)	0.09 (0.07, 0.1)	β (95% CI) for doubling[b] PFAS and lipids at birth	Overall −0.03 (−0.11, 0.05) Girls 0.05 (−0.07, 0.16) Boys −0.1 (−0.21, 0.00)	Overall −0.02 (−0.07, 0.03) Girls −0.02 (−0.05, 0.09) Boys −0.06 (−0.12, 0.01)	Overall 2.2 (−4.1, 8.8) Girls 7.3 (−2.3, 18) Boys −1.9 (−9.9, 6.8)
			PFAS at birth and lipids at 18 mo	Overall −0.1 (−0.26, 0.06) Girls −0.02 (−0.26, 0.22) Boys −0.17 (−0.39, 0.05)	Overall −0.09 (−0.22, 0.03) Girls −0.03 (−0.22, 0.15) Boys −0.15 (−0.32, 0.02)	Overall 0.33 (−7.9, 9.3) Girls −2.8 (−15, 11) Boys 2.8 (−8.2, 15)
			PFAS and lipids at 9 yr	**Overall 0.19 (0.07, 0.32)* Girls**	**Overall 0.12 (0.02, 0.22)* Girls**	Overall −0.16 (−7.6, 7.9) Girls 2.9 (−8.4, 16)

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
				0.2 (0.01, 0.39)* **Boys 0.19 (0.02, 0.36)***	**0.19 (0.04, 0.33)*** Boys 0.07 (−0.06, 0.2)	Boys −2.5 (−12, 8.2)
Averina et al. (2021)	Cross-sectional study (2010–2011), Norway; 940 children ~16 yr)	Girls 0.3 Boys 0.2 (GMs)	β (95% CI) for log increase[b]	**0.35 (0.12, 0.57)***	**0.34 (0.14, 0.54)***	0.01 (−0.15, 0.17)
Jensen et al. (2020a)	Birth cohort (2010–2012), Denmark; 612 children (followed to 18 mo)	0.3 (5th–95th: 0.2–0.5)	β (95% CI) for 1 unit increase[b]	3 mo −0.23 (−0.90, 0.43) 18 mo **1.06 (0.08, 2.03)***	3 mo −0.05 (−0.73, 0.62) 18 mo 0.64 (−0.43, 1.71)	3 mo −0.21 (−0.88, 0.47) 18 mo 0.92 (−0.11, 1.95)
Mora et al. (2018)	Birth cohort (1999–2002), U.S.; 682 children (7–8 yr)	0.3 (0.2–0.5)	β (95% CI) for IQR increase[a]	**6.8 (3.6, 10.1) *** similar for boys and girls	**3.2 (0.6, 5.8) *** similar for boys and girls	−3.6 (−8.2, 1.0) similar for boys and girls
Zeng et al. (2015)	Cross-sectional analysis (2009–2010), Taiwan; 225 adolescents (12–15 yr)	1.0 (range <LOQ[e]–5.0) (boys)	β (95% CI) for 1-unit increase[a]	−1.3 (−9.0, 6.4)	−0.6 (−6.5, 5.4)[d]	0.6 (−10.0, 11.1)[d]
Tian et al. (2021)	Birth cohort (2012), China; 306 newborns	2.2 (1.4–3.3) in cord blood	β (95% CI) for ln-unit increase[a]	**−0.12 (−0.19, −0.05)***	−0.09 (−0.18, 0.01)	**−0.09 (−0.18, −0.01)***

*$p < 0.05$.
U: uninformative; NR: not reported.
Not all results (e.g., subgroup analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation. Only *medium* confidence studies underwent data extraction.
[a]Lipids were measured in mg/dL.
[b]Lipids were reported in mmol/L.
[c]30% below the LOQ.
[d]*Low* confidence endpoint within *medium* confidence study.
[e]Less than 6% below the LOQ.

Other risk factors for cardiovascular disease

Ten studies reported on the association between PFDA exposure and other risk factors for cardiovascular disease, including blood pressure in the general population (six studies),

hypertensive disorders and blood pressure during pregnancy (four studies), atherosclerosis (two studies), and ventricular geometry (one study). The study evaluations for these outcomes are summarized in Figure 3-72.

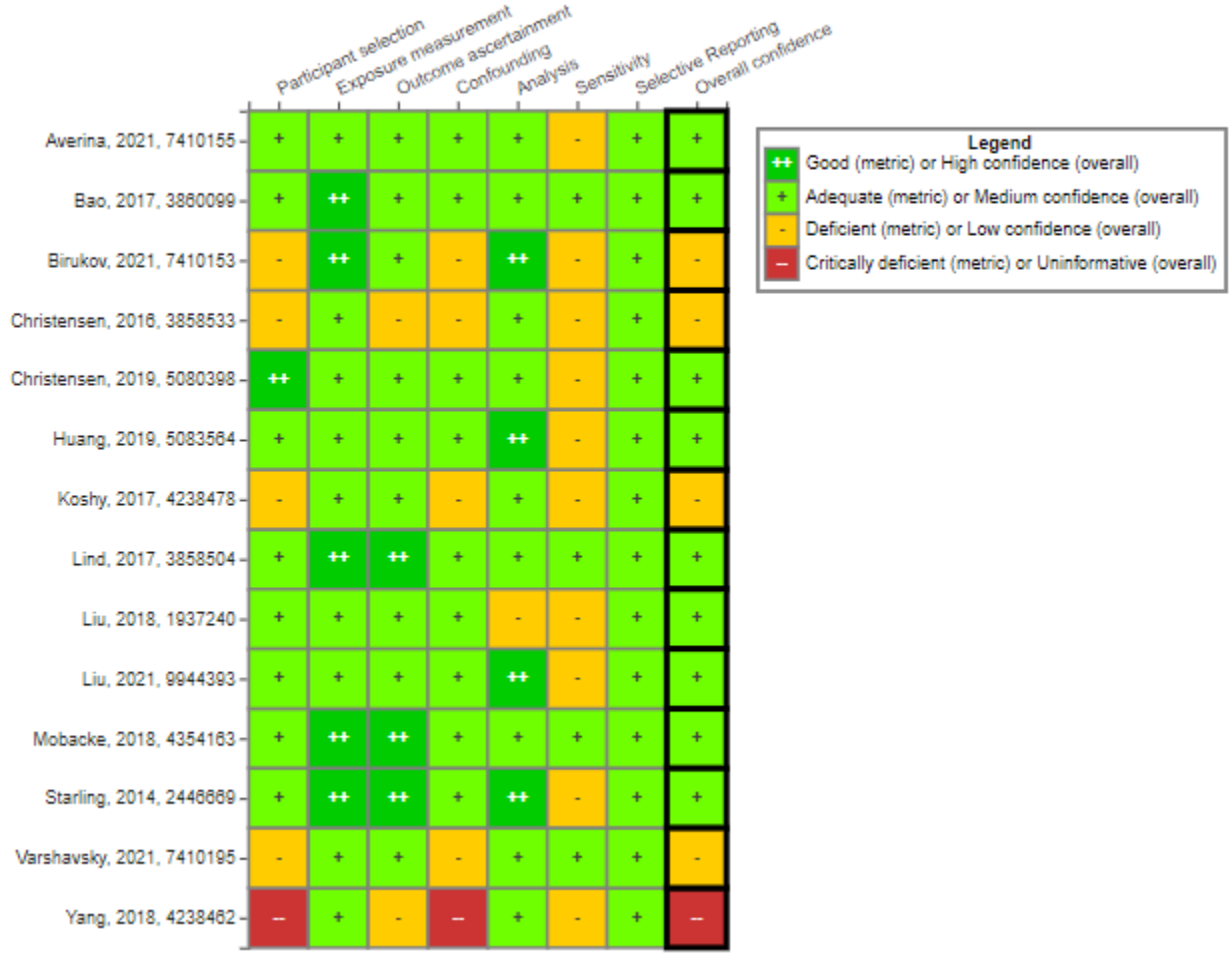


Figure 3-72. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cardiovascular risk factors other than serum lipids. Refer to HAWC Human Other Cardiovascular Risk Factors for details on the study evaluation review.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2020b) and Jain (2020a).

For blood pressure, one study of blood pressure (Yang et al., 2018) was excluded from further analysis due to critical deficiencies in participant selection in confounding. One *medium* confidence cross-sectional study (NHANES) reported higher blood pressure with higher PFDA exposure in two publications (Jain, 2020a; Christensen et al., 2019), but the association was nonsignificant and not monotonic across quartiles (OR [95% CI] for Q2 vs. Q1: 1.1 (0.7, 1.6), Q3: 1.3 (0.7, 2.2), Q4: 1.1 (0.6, 1.9) in (Christensen et al., 2019)) and the other four studies, including three

medium confidence studies, reported no increase in adults (Liu et al., 2018; Bao et al., 2017; Christensen et al., 2016) or adolescents (Averina et al., 2021).

Of four studies of hypertensive disorders of pregnancy (see Table 3-31), one medium and one *low* confidence study reported positive associations with gestational hypertension Birukov et al. (2021); (Liu et al., 2021a), although neither was statistically significant and Birukov et al. (2021) did not report a positive association with preeclampsia. The other two *medium* confidence studies reported no increase in the odds of preeclampsia (Huang et al., 2019b; Starling et al., 2014a) or gestational hypertension (Huang et al., 2019b). Associations were in the inverse direction in both studies, but neither was statistically significant. In addition, one *low* confidence study (Varshavsky et al., 2021) reported positive associations with continuous blood pressure (both systolic and diastolic) during mid-gestation.

Table 3-31. Associations between PFDA and hypertensive disorders of pregnancy in epidemiological studies

Reference, study confidence	Population	Median exposure in ng/mL (IQR)	Effect estimate	Gestational hypertension	Preeclampsia
Starling et al. (2014a), *medium*	Nested case-control study within cohort in Norway; 1,046 women	0.1	HR (95% CI) for above vs. below median	NR	0.81 (0.63, 1.05)
Huang et al. (2019b), *medium*	Cross-sectional study in China; 674 women at delivery	0.4 (0.2–0.5)	OR (95% CI) for tertiles vs. T1	T2: 1.26 (0.48, 3.31) T3: 0.63 (0.20, 2.00)	T2: 1.16 (0.38, 3.53) T3: 1.00 (0.31, 3.19)
Liu et al. (2021a), *medium*	Nested case-control study within cohort in China; 544 women	0.4 (0.3–0.7)	OR (95% CI) for tertiles vs. T1	T2: 1.24 (0.74, 2.06) T3: 1.48 (0.89, 2.45)	NR
Birukov et al. (2021), *low*	Cohort in Denmark; 1,436 women	0.6 (0.5–0.9)	HR (95% CI) for doubling of exposure	1.35 (0.86, 2.11)	0.93 (0.71, 1.22)

IQR = interquartile range; NR = not reported; HR = hazard ratio; OR = odds ratio; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

For atherosclerosis, there was a nonsignificant increase in the echogenicity of the intima-media complex (a measure of the structural composition of the arterial wall that is an indicator of early change in the carotid artery) and in the number of carotid arteries with atherosclerotic plaques only in women in one *medium* confidence study (Lind et al., 2017b), but no association with atherosclerosis in the *low* confidence study, which did not stratify by sex (Koshy et al., 2017). In the single *medium* confidence study of ventricular geometry (Mobacke et al., 2018), there was a small

but statistically significant decrease in relative wall thickness (RWT) (β = −0.02, 95% CI: −0.04, −0.01) and increase in left ventricular end-diastolic volume (β = 0.95, 95% CI: 0.11, 1.79). There is some inconsistency in the literature about the adversity of decreased RWT, with some studies indicating increased RWT is associated with hypertension (Li et al., 2001) and concentric left ventricular geometry (de Simone et al., 2005), and others indicating decreased RWT is associated with abnormal left ventricular geometry (Hashem et al., 2015) and ventricular tachyarrhythmia (Biton et al., 2016). In either case, it is difficult to interpret these results without additional studies.

Overall, there is limited evidence of an association between PFDA exposure and cardiovascular risk factors. One *low* confidence study reported a positive association with blood pressure, and *medium* confidence studies reported associations with atherosclerosis and ventricular geometry, but no association was observed in *medium* confidence studies of blood pressure.

Cardiovascular disease

Three studies examined cardiovascular disease and its association with PFDA exposure in adults. All reported on coronary heart disease (Huang et al., 2018; Christensen et al., 2016; Mattsson et al., 2015), while one additionally examined total cardiovascular disease, congestive heart failure, angina pectoris, myocardial infarction (heart attack), and stroke (Huang et al., 2018). Two studies were *medium* confidence (see Figure 3-73), including a case-control study nested within a prospective cohort of farmers and other rural residents in Sweden (Mattsson et al., 2015), while the other (Huang et al., 2018) was based on NHANES, a nationally representative cross-sectional survey in the United States. The third study was *low* confidence and based on a survey of male anglers in Wisconsin (Christensen et al., 2016). The timing of exposure measurement in all three studies was considered adequate, although the prospective measurement in Mattsson et al. (2015) may be more likely to capture the relevant etiologic period of these chronic outcomes. Exposure levels in the *medium* confidence studies were similar (median = 0.2 ng/mL), and slightly higher in the *low* confidence study (median = 0.5 ng/mL).

For coronary heart disease, Huang et al. (2018) reported significantly higher odds with higher exposure (see Table 3-32). Christensen et al. (2016) also reported higher odds, although not statistically significant, while Mattsson et al. (2015) reported no increase. For other outcomes, Huang et al. (2018) reported higher odds of total cardiovascular disease, angina pectoris, and myocardial infarction, and stroke, although these were not statistically significant and only myocardial infarction and angina pectoris had monotonic gradients across the quartiles (angina pectoris Q2 vs. Q1: 1.16 (0.67, 1.99), Q3: 1.21 (0.75, 1.95), Q4: 1.23 (0.68, 2.24); myocardial infarction Q2: 0.99 (0.65, 1.49), Q3: 1.32 (0.90, 1.92), Q4: 1.38 (0.83, 2.28)). There is not a clear explanation for the differing results in the *medium* confidence studies; both had similar exposure levels (median 0.2 ng/mL). The populations in Mattsson et al. (2015) and Christensen et al. (2016) are fairly homogeneous (farmers/rural residents in Sweden and male anglers in Wisconsin, respectively), in contrast to the nationally representative sample in Huang et al. (2018). It is

possible that the prospective exposure measurement in Mattsson et al. (2015) played a role (vs. cross-sectional measurement in Huang et al. (2018) and Christensen et al. (2016)), and the lack of additional prospective studies makes this difficult to interpret. Given that the timing of exposure measurement in Mattsson et al. (2015) is more likely to be during the relevant etiologic window, the lack of association in that study contributes to considerable uncertainty in this body of evidence.

Figure 3-73. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cardiovascular disease. Refer to HAWC Human Cardiovascular Disease for details on the study evaluation review.

Table 3-32. Associations between PFDA and coronary heart disease in epidemiological studies

Reference, study confidence	Population	Median exposure in ng/mL (IQR)	Coronary heart disease OR (95% CI)
Mattsson et al. (2015), *medium*	Nested case-control study of farmers and rural residents in Sweden, exposure measured 1990–1991 and 2002–2003, cases identified through 2009, N = 462	0.2 (0.1)	Q2: 0.87 (0.49, 1.60) Q3: 1.13 (0.66, 1.94) Q4: 0.92 (0.53, 1.60)
Huang et al. (2018), *medium*	Cross-sectional study of general population in U.S. (NHANES), N = 10,859	0.2 (0.2–0.4)	Q2: 1.50 (0.97, 2.32) Q3: 1.17 (0.77, 1.79) **Q4: 1.84 (1.26, 2.69) ***
Christensen et al. (2016), *low*	Cross-sectional study of male anglers in U.S., N = 154	0.5 (0.3–0.9)	1.12 (0.49, 2.18)

*$p < 0.05$.
IQR = interquartile range; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4.

Diabetes and insulin resistance

Twenty-one studies (23 publications) reported on the relationship between PFDA exposure and diabetes, insulin resistance, fasting blood glucose, or gestational diabetes. A summary of the study evaluations is presented in Figure 3-74, and additional details can be obtained from HAWC.

Figure 3-74. Evaluation results for epidemiological studies assessing effects of PFDA exposure on diabetes and insulin resistance. Refer to HAWC Human Diabetes and Insulin Resistance for details on the study evaluation review.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2021) and Jain (2020a).

For diabetes, because of concerns for reverse causality resulting from metabolic and behavioral changes following a diabetes diagnosis, the optimal epidemiological studies would be

longitudinal cohort studies with repeated measurements before onset. Two *medium* confidence studies evaluated PFDA exposure and incident diabetes (Charles et al., 2020; Sun et al., 2018). Sun et al. (2018), a nested case-control study, found that at the highest tertile of PFDA exposure (range: 0.2–1.95 ng/mL), there was a nonstatistically significant inverse (i.e., "protective") association seen with diabetes (OR = 0.7, 95% CI: 0.5, 1.1). Charles et al. (2020), also a nested case-control study, reported results that differed based on the selected control group; an inverse association was observed with controls matched for birth year and year of blood collection, controlling for BMI (OR = 0.89, 95% CI: 0.55, 1.44), while a positive association was observed with controls additionally matched for BMI (OR = 1.52, 95% CI = 0.76, 3.07), although neither was statistically significant.

For insulin resistance and blood glucose, there were several outcome-specific considerations for study evaluation that were influential on the ratings. Homeostatic model assessment (HOMA) is a method for assessing insulin resistance and β-cell function from fasting glucose and insulin measured in the plasma (Matthews et al., 1985). The HOMA of insulin resistance (HOMA-IR) is often used in studies evaluating future risk for diabetes and was considered a primary outcome for this review along with fasting blood glucose. Measures of insulin resistance and blood glucose, including HOMA-IR, are not interpretable in the presence of diabetes, particularly if diabetes is treated with hypoglycemic medication since the treatment will affect insulin production and secretion. Studies that did not consider diabetes status and use of diabetes medications by exclusion, stratification, or adjustment were thus considered deficient for participant selection. For the timing of the exposure measurement, unlike the criteria described for diabetes, exposure and outcome can be assessed concurrently as insulin resistance and blood glucose can represent short-term responses, and establishing temporality was not deemed a major concern.

Sixteen studies examined associations between PFDA exposure and insulin resistance or fasting blood glucose. Nine studies examined associations in adolescents and adults, five studies in pregnant women, and two studies in children. Four studies in adults did not consider diabetes status of participants and were thus considered *low* confidence (Khalil et al., 2020; Lin et al., 2020b; Liu et al., 2018; Koshy et al., 2017). The remaining 12 studies were *medium* confidence (Cakmak et al., 2022; Gardener et al., 2021; Goodrich et al., 2021; Valvi et al., 2021; Yu et al., 2021; Duan et al., 2020; Ren et al., 2020; Christensen et al., 2019; Jensen et al., 2018; Kang et al., 2018; Wang et al., 2018b; Fleisch et al., 2017).

Results of the insulin resistance and fasting blood glucose are presented in Table 3-33. In all studies of insulin resistance, the results were generally null, and in the *low* confidence study by Fleisch et al. (2017), an inverse association was observed. In the studies of fasting blood glucose, there was again no clear positive association observed. It is possible that the null associations could be due to poor sensitivity from narrow exposure contrasts in most of the studies, but a minority of studies had higher exposure levels with corresponding greater contrast and also found no association. Additionally, null, and even inverse associations could be due to outcome

misclassification resulting from inclusion of participants with diabetes in some studies. However, based on the current evidence, there is no indication that PFDA exposure is associated with greater insulin resistance or higher fasting blood glucose levels.

Table 3-33. Associations between PFDA and insulin resistance in epidemiological studies

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
General population, adolescents, and adults							
Goodrich et al. (2021)	*Medium*	Cohort and cross-sectional study of adolescents in U.S.; 310 in cohort and 137 in cross-sectional	NR (due to high proportion below the LOD)	NR	NR[a]	"Not associated"	"Not associated"
Koshy et al. (2017)	*Low*	World Trade Center Health Registry (WTCHR) who resided in NYC and were born between Sept. 11, 1993, and Sept. 10, 2001; U.S.; 402 adolescents	Control 0.1 (0.2) WTCHR 0.1 (0.1)	ln-unit change	Beta coefficient (95% CI)[b]	NR	−0.04 (−0.11, 0.03) [#]
Christensen et al. (2019)	*Medium*	Cross-sectional study in U.S. (NHANES 2007–2014); 2975 individuals aged 20 yr and older	0.2 (0.1–0.4)	Quartiles	Odds ratio (95% CI) for glucose ≥100 mg/dL	Q2: 0.9 (0.7, 1.3) Q3: 1.1 (0.7, 1.7) Q4: 0.9 (0.6, 1.5)	NR
Cakmak et al. (2022)	*Medium*	Cross-sectional study in Canada (CHMS 2007–2017); 3,356–6,024 individuals 12 yr and older	GM 0.2	Change equivalent to GM	Percent change[b]	−0.3 (−1.4, 0.8)	5.3 (−3.5, 15.0)
Valvi et al. (2017)	*Medum*	Birth cohort in Faroe Islands; 699 young adults	0.2 (0.2–0.3)	Log_2 change	β (95% CI)	Glucose AUC Exposure at 7 yr 0.0 (−0.01, 0.02) Similar with exposure at 14, 22, 28 yr and in men and women	Exposure at 7 yr 0.03 (−0.03, 0.10) Similar with exposure at 14, 22, 28 yr and in men and women
Khalil et al. (2020)	*Low*	Cross-sectional study of firefighters in U.S.; 38 men	0.3 (0.2–0.3)	Log-unit change	β (95% CI)[a]	No association (estimates reported on figure)	NR
Liu et al. (2018)	*Low*	Cross-sectional analysis in weight loss clinical trial in U.S.; 621 adults (30–70 yr)	Male 0.4 (0.3–0.5) Female 0.4 (0.3–0.6)	n/a	Spearman correlation[b]	0.08	0.05

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
Lin et al. (2020b)	*Low*	Cross-sectional study of older adults living near a high contamination area in Taiwan; 397 adults (55–75 yr)	Median (range) 1.7 (0.6–27)	Quartiles	β (95% CI)[a]	Women Q2: −4.83 (−13.34, 3.68) Q3: −4.33 (−12.91, 4.26) Q4: −5.72 (14.37, 2.94) Men Q2: −5.38 (−19.68, 8.92) Q3: 2.67 (−11.7, 17.05) Q4: 3.9 (−11.1, 18.9)	NR
Duan et al. (2020)	*Medium*	Cross-sectional study in China; 294 adults	2.1 (1.0–4.1)	1% increase	Percent change[b]	0.009 (−0.002, 0.020)	NR
Pregnant women							
Gardener et al. (2021)	*Medium*	Pregnancy cohort in U.S.; 433 pregnant women	0.2 (0.1–0.3)	Quartiles	Means (95% CI)	NR	Insulin: No association (estimates reported on figure)
Jensen et al. (2018)	*Medium*	Birth cohort in Denmark; 649 pregnant women (15–49 yr)	0.3 (0.2–0.5)	Twofold change	% Change (95% CI)[b]	−1.3 (−3.6, 1.0)	−1.5 (−13.5, 12.1)
Wang et al. (2018b)	*Medium*	1:2 matched case control of pregnant women in China; 84 cases and 168 noncases	Controls 0.3 (0.2–0.4) Cases 0.3 (0.2–0.4)	Dichotomous exposure (tertiles of outcome)	Odds ratio (95% CI) vs. Low glucose (Low: 3.20–4.74 mmol/L; Medium: 4.75–5.04; High: 5.05–6.84)	Medium vs. Lowest FBG 1.3 (0.7–2.4) Highest vs. Lowest FBG 1.0 (0.5–1.8)	NR
(Yu et al., 2021)	*Medium*	Pregnancy cohort in China; 2,747 pregnant women	1.7 (1.4)	Log-unit change	β (95% CI)[b]	0.01 (−0.02, 0.04) 1 hr post glucose tolerance test 0.12 (0.01, 0.22) 2 hr post 0.08 (−0.002, 0.17)	NR

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
Ren et al. (2020)	*Medium*	Pregnancy cohort in China; 856 pregnant women	2.0 (1.3–3.2)	ln-unit change	OR (95% CI) for high glucose (≥4.6 mmol/L; 8.3 mmol/L for 1 hr post test)	1.24 (0.87, 1.76) 1 hr post glucose tolerance test 1.61 (1.10, 2.44)	NR
Children							
Fleisch et al. (2017)	*Medium*	Birth cohort in U.S.; 665 mother-child pairs (median 7.7 yr)	GM (IQR) Mid-childhood 0.3 (0.2, 0.5)	Quartiles	Beta coefficient (95% CI)[a]	NR	Mid-childhood Q2: −7.1 (−22.1, 10.6) **Q3: −31.3 (−42.8, −17.5) *** **Q4: −21.5 (−34.0, −6.7)***
Kang et al. (2018)	*Medium*	Cross-sectional study in South Korea; 150 children (3–18 yr)	0.06 (0.04–0.1)	ln-unit change	Beta coefficient (95% CI)[a]	−0.2 (−1.3, 0.9)	NR

**p*-value or *p*-trend < 0.05.
IQR = interquartile range; NR = not reported; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4.
# HOMA-IR was log-transformed.
Note: Not all results (e.g., subgroup analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation.
[a]Blood glucose was measured in mg/dL.
[b]Blood glucose was reported in mmol/L.

Six studies reported on the association between PFDA exposure and gestational diabetes (Liu et al., 2019b; Rahman et al., 2019; Wang et al., 2018b; Valvi et al., 2017; Zhang et al., 2015). Four studies were *medium* confidence, one was *low* confidence, and one (Liu et al., 2019b) was *uninformative* due to lack of control for confounding in single-pollutant models. The three *medium* confidence studies were inconsistent, with one (Valvi et al., 2017) reporting higher odds of gestational diabetes with higher exposure (OR for doubling of exposure: 1.2 (0.7, 2.0)), but the association was not statistically significant and nonmonotonic (OR for tertile 2: 2.0 (0.9, 4.1), tertile 3: 1.0 (0.5, 2.3)). Two *medium* confidence studies reported close to null association with gestational diabetes and PFDA exposure (OR: 1.02 (0.86, 1.20) in the overall cohort in Rahman et al. (2019), OR 0.95 (0.78, 1.16) in Yu et al. (2021)), and the other *medium* confidence study Wang et al. (2018b) reported a nonstatistically significant inverse association (OR: 0.85 (0.30–2.92)). The *low* confidence study (Zhang et al., 2015) reported no association (OR: 1.0 (0.7–1.5)).

Overall, for diabetes and insulin resistance, there were no clear associations with higher PFDA exposures. Results were generally null or in the inverse direction. While it is possible that a positive association with these outcomes exists but was obscured by poor sensitivity and/or bias, there is no clear explanation for the inconsistency based on study confidence, design, or population.

Adiposity

Thirteen studies reported on the association between PFDA exposure and obesity or related outcomes. Two studies were excluded due to critical deficiencies in participant selection (Yang et al., 2018) and confounding (Zhao et al., 2022; Yang et al., 2018). Of the 11 remaining studies, four were cohorts that examined early-life exposure to PFDA and adiposity at 18 months (Karlsen et al., 2017), at 4–8 years of age (Bloom et al., 2022), at 5 years of age (Chen et al., 2019; Karlsen et al., 2017), and at 13 years of age (Janis et al., 2021); one was a clinical trial of weight loss diets in adults that examined weight change (Liu et al., 2018); and one was a cohort of adults living near a uranium processing site (Blake et al., 2018). All of these were classified as *medium* confidence. Five studies (three in adults and two in children) were cross-sectional (Lind et al., 2022; Wise et al., 2022; Thomsen et al., 2021; Domazet et al., 2020; Christensen et al., 2019) and were *low* confidence due to the potential for reverse causation resulting from metabolic changes in obese individuals. The evaluations are summarized in Figure 3-75.

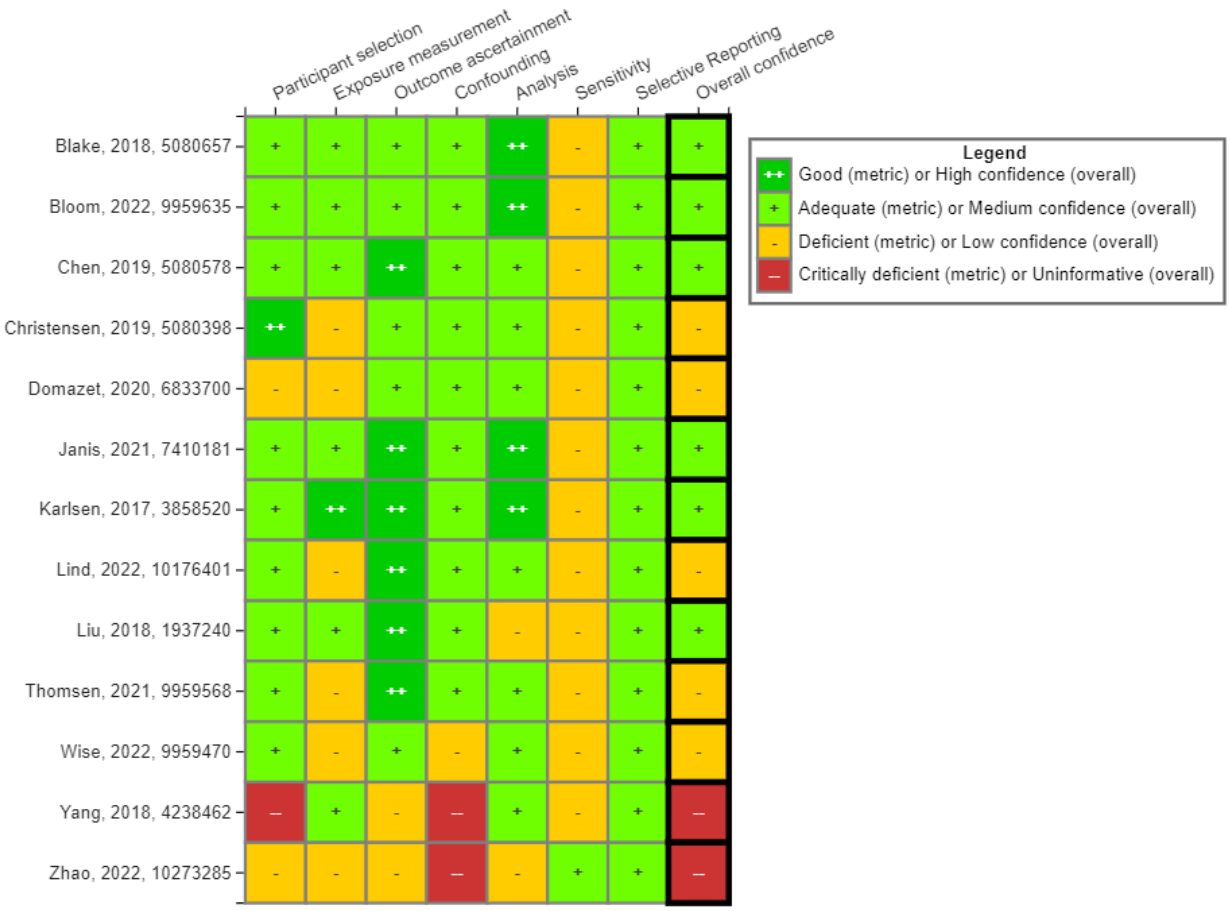


Figure 3-75. Evaluation results for epidemiological studies assessing effects of PFDA exposure on adiposity. Refer to HAWC Human Adiposity for details on the study evaluation review.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2020a).

The available studies look at several different outcomes and populations, so are generally not directly comparable (see Table 3-34). In the five studies in adults, one *medium* confidence study reported higher BMI with higher exposure (Blake et al., 2018) and the other *medium* confidence study reported greater weight gain following a weight loss trial (Liu et al., 2018), with only the latter being statistically significant. Of the three *low* confidence cross-sectional studies, two reported statistically significant inverse associations with BMI in women (Lind et al., 2022; Wise et al., 2022), while the third also reported an inverse, although not statistically significant, association with waist circumference. In children, one *medium* confidence birth cohort (Karlsen et al., 2017) reported a slightly higher proportion of overweight participants with higher exposure at 18 months when maternal exposure was modeled as a continuous variable (RR = 1.14, 95% CI: 0.91, 1.43), but this was not statistically significant and not monotonic when modeled in tertiles (RR T2 vs. T1 = 0.90 (95% CI: 0.71, 1.15), T3 vs. T1 = 1.03 (95% CI: 0.82, 1.31)) or in follow-up of the children

at 5 years. However, a cross-sectional analysis by Karlsen et al. (2017) in this population at 5 years indicated lower BMI and incidence of children who were overweight with higher exposure. A second *medium* confidence birth cohort study reported nonsignificant inverse associations in girls and nonsignificant positive associations in boys at 5 years (Chen et al., 2019). The other two *medium* confidence cohort studies, including a birth cohort with exposure measurement in gestation and follow-up to 4–8 years (Bloom et al., 2022) and a cohort with exposure measurement in mid-childhood (age 8) and follow-up to age 13 (Janis et al., 2021) were null overall with regard to BMI and fat mass. The two *low* confidence cross-sectional studies reported inverse associations with fat mass (Domazet et al., 2020) and measures of fat obtained with MRI and Dual X-ray absorptiometry (Thomsen et al., 2021). Overall, there is some limited evidence of an association between PFDA exposure and adiposity in adults in two *medium* confidence studies, but there is considerable *uncertainty*, and this association was not observed in studies of children.

Table 3-34. Associations between PFDA and adiposity in epidemiological studies

Reference, study confidence	Population	Median exposure (IQR) in ng/mL	Effect estimate	BMI	Waist circumference	Other
Adults						
Blake et al. (2018), *medium*	Prospective cohort near a uranium processing site in U.S.; 210 adults	0.1 (0.1, 0.2)	% change (95% CI) for IQR increase in exposure	0.7 (−1.3, 2.7)	NR	NR
Christensen et al. (2019), *low*	NHANES, cross-sectional in U.S.; 2,975 adults (20+ yr)	0.2 (0.1, 0.4)	OR (95% CI) for increased WC (≥102 cm for men, 88 cm for women) by quartiles (ref Q1)	NR	Q2: 0.9 (0.6, 1.2) Q3: 0.9 (0.5, 1.5) Q4: 0.8 (0.5, 1.3)	NR
Liu et al. (2018), *medium*	Clinical trial of weight loss diet in U.S.; 621 adults	Male 0.4 (0.3–0.5) Female 0.4 (0.3–0.6)	Mean difference	NR	NR	Weight gain (kg) following trial T1: 2.5 ± 0.9 T2: 3.1 ± 0.9 T3: 4.2 ± 0.8, *p*-trend: 0.03
Children						
Karlsen et al. (2017), *medium*	Prospective birth cohort in Faroe Islands; 444 children at 18 mo and 371 at 5 yr		β (95% CI) for BMI; Relative risk for overweight	18 mo 0.1 (−0.1, 0.3) 5 yr (−0.04 (−0.2, 0.1)	NR	Overweight 18 mo 1.1 (0.9, 1.4) 5 yr 1.0 (0.6, 1.7)
Chen et al. (2019), *medium*	Prospective birth cohort in China; 404 children at 5 yr	0.4 (range 0.2–2.0)	β (95% CI) for log-unit change	Girls: −0.2 (−0.4, 0.1) Boys: 0.1 (−0.3, 0.5)	Girls: −0.7 (−1.5, 0.1) Boys: 0.2 (−0.8,1.0) (WC measured in cm)	Body fat percentage (%) Girls: −1.1 (−2.3, 0.2) Boys: 1.1 (−0.2, 2.3)
			β (95% CI) for tertiles (ref T1)	Girls T2: −0.1 (−0.7, 0.4) T3: 0.0 (−0.6, 0.5) Boys T2: −0.2 (−0.8, 0.4) T3: 0.2 (−0.5, 0.8)	Girls T2: −0.6 (−2.2, 1.0) T3: −0.5 (−2.2, 1.1) Boys T2: −0.9 (−2.5, 0.7) T3: 0.5 (−1.1, 2.1)	Girls T2: −0.6 (−3.2, 1.9) T3: −1.5 (−4.1, 1.0) Boys T2: 0.5 (−1.5, 2.6) T3: 2.0 (−0.1, 4.1)

NR = not reported; WC = waist circumference.

Metabolic syndrome

The current criteria for clinical diagnosis of metabolic syndrome include the following: larger waist circumference (>35 inches for women, >40 inches for men); elevated triglycerides ≥150 mg/dL (1.7 mmol/L); reduced HDL-C <40 mg/dL (1.0 mmol/L) in males and <50 mg/dL (1.3 mmol/L) in females; elevated blood pressure: systolic ≥130 and/or diastolic ≥85 mm Hg; and elevated fasting glucose ≥100 mg/dL (Alberti et al., 2009). Main considerations are that three abnormal findings of five in the criteria would qualify a person for the metabolic syndrome and that country- or population-specific cut points for waist circumference should be used (Alberti et al., 2009).

Three studies reported on the association between PFDA exposure and metabolic syndrome. One study was *uninformative* due to critical deficiencies in participant selection (Yang et al., 2018). The remaining two studies were cross-sectional, with one (Christensen et al., 2019) being *medium* confidence and one being *low* confidence (Lin et al., 2020b). Christensen et al. (2019) found an exposure-dependent, significant inverse association between PFDA exposure and metabolic syndrome (OR: 0.72; 95% CI: 0.54, 0.97 with ln (PFDA); Q2: 0.93; 95% CI: 0.64, 1.35, Q3: 0.71; 95% CI: 0.43, 1.18, and Q4: 0.56; 95% CI: 0.31, 1.01). Lin et al. (2020b) also reported an inverse association (not statistically significant) in women (OR (95% CI) for quartiles vs. Q1, Q2: 0.68 (0.33, 1.4); Q3: 0.78 (0.38, 1.61); Q4: 0.51 (0.24, 1.08) but reported a positive association (also not statistically significant) in men (Q2: 0.94 (0.31, 2.85); Q3: 1.43 (0.48, 4.22); Q4: 1.9 (0.63, 5.77).

Animal Studies

There is a single study available in experimental animals that evaluated endpoints related to cardiometabolic effects following short-term exposure to PFDA (NTP, 2018). The study exposed female and male SD rats to PFDA doses of 0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day for 28 days via gavage and included endpoints such as serum lipids, histopathology, and organ weights. Confidence in the study was rated as *high* during study evaluation for these endpoints with no outstanding issues regarding risk of bias or sensitivity (see Figure 3-76).

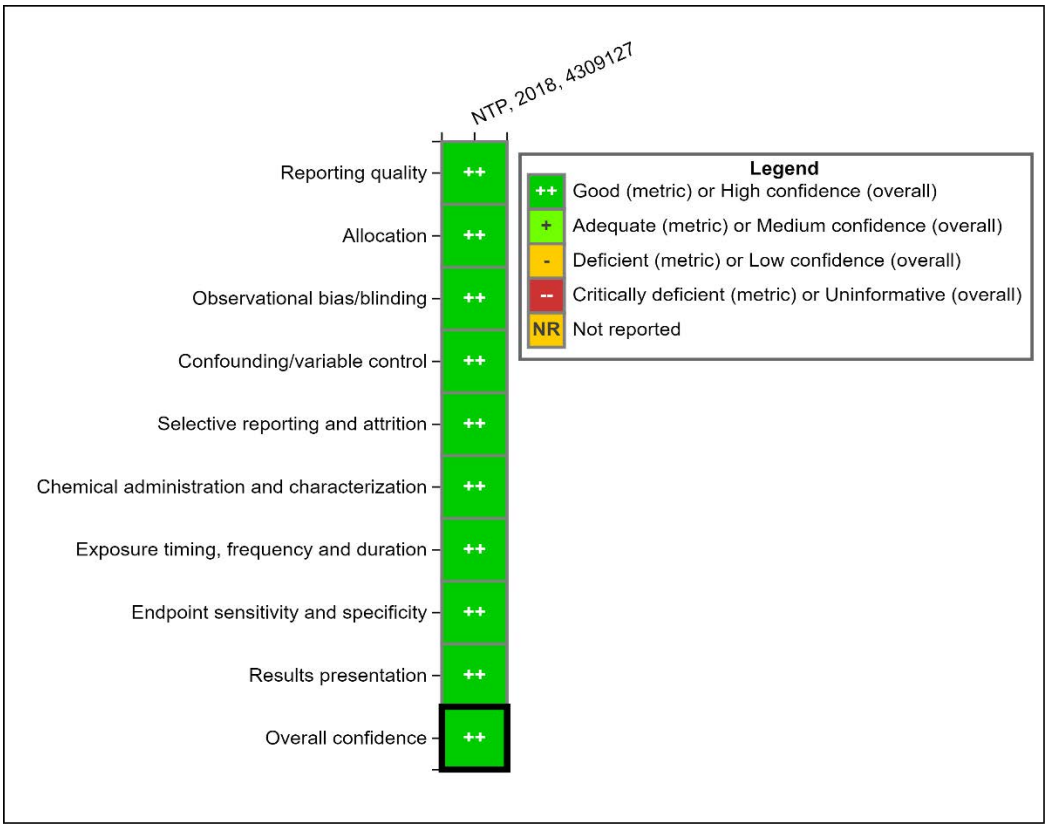


Figure 3-76. Evaluation results for an animal study assessing effects of PFDA exposure on cardiometabolic effects. Refer to HAWC for details on the study evaluation review.

Histopathology

The heart and blood vessel were examined histologically in rats in the control and high-dose groups (2.5 mg/kg-day) at study termination (see Figure 3-77). An increase in the incidence of granulomatous inflammation of the epicardium (2/10 rats; moderate severity) was reported in high-dose females after PFDA exposure. Granulomas are focal, inflammatory tissue responses that arise from a broad range of etiologies, including infectious and noninfectious processes (Boros and Revankar, 2017). This lesion was not observed in exposed males or in the controls. Results for blood vessel histopathology were null. The biological significance of the histopathological observations in females is unknown given the sparse information available.

Serum lipids

Cholesterol is important for maintaining cell membrane integrity and transport and is also used as a precursor for the synthesis of steroid hormones, bile acids, and other substances in the body. Triglycerides are an essential source of energy storage and production. Both cholesterol and triglycerides are routinely evaluated in blood lipid panels as cardiovascular risk measures. Cholesterol and triglyceride levels were measured in rat serum after 28-day exposure (see

Table 3-35 and Figure 3-77). Dose-related decreases in triglyceride levels were reported in male and female rats exposed to PFDA, with the largest changes occurring in males at the highest doses (35% and 52% compared with controls at 1.25 and 2.5 mg/kg-day, respectively). A downward trend ($p < 0.01$) was reported for cholesterol levels in females, reaching 35% compared with controls at 2.5 mg/kg-day. In males, cholesterol decreased 14%–38% compared with controls across 0.156–2.5 mg/kg-day, but the effects did not display a significant trend. The findings should be interpreted with caution given the known species differences in lipid metabolism and blood cholesterol levels between rodents and humans that may impact the evaluation of the human relevance of the observed responses (Getz and Reardon, 2012; Davidson, 2010).

Table 3-35. Percent change relative to controls in serum lipids in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Triglycerides					
Male SD rats	14	−2	−21	**−35**	**−52**
Female SD rats	27	18	−7	−23	−27
Cholesterol					
Male SD rats	**−27**	**−38**	**−27**	−12	−14
Female SD rats	1	−8	0	−9	**−35**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

Organ weight

Terminal absolute and relative heart weights were measured in all exposed animals (see Table 3-36 and Figure 3-77). It is unclear which metric (i.e., absolute, or relative) would be more appropriate to evaluate effects on heart weight in the presence of significant body weight changes (Bailey et al., 2004). As such, both absolute and relative measures were considered herein. Absolute heart weight showed a decreasing trend ($p < 0.01$) in males and females, with 15%–37% decreases compared with controls at doses of 1.25 and 2.5 mg/kg-day. In contrast, changes in relative heart weights did not show a significant trend. The reductions in absolute heart weight coincide with reductions in body weight observed in these animals at the high-dose groups (≥1.25 mg/kg-day) (see Section 3.2.10 for additional details).

Table 3-36. Percent change relative to controls in heart weights in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Absolute heart weight					
Male SD rats	5	−2	2	**−18**	**−37**
Female SD rats	1	1	−2	**−15**	**−36**
Relative heart weight					
Male SD rats	2	−1	6	4	1
Female SD rats	−3	−3	−2	−3	1

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

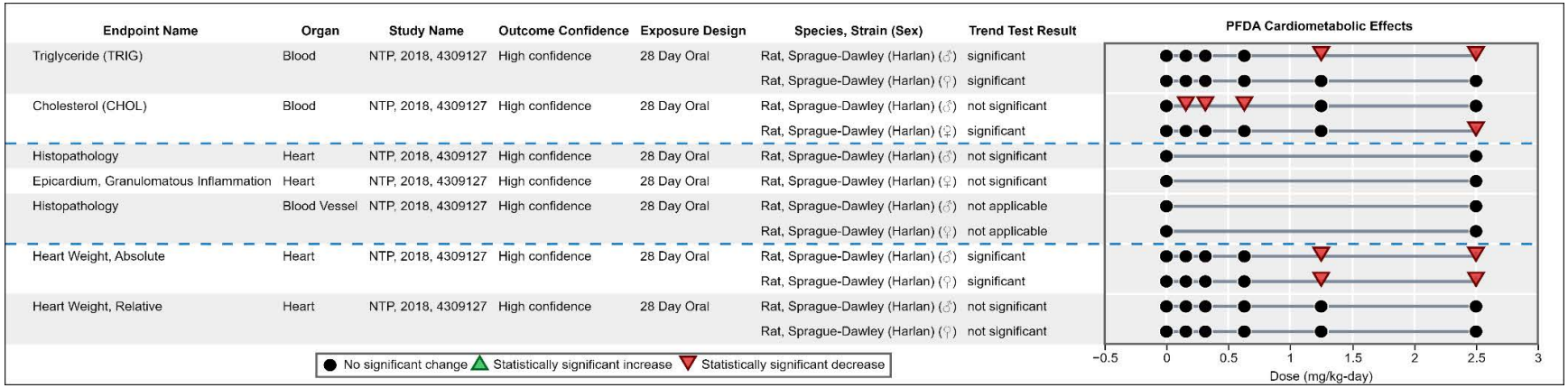


Figure 3-77. Cardiometabolic effects following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.

Evidence Integration

The evidence of an association between PFDA exposure and cardiometabolic effects in humans is *slight*, with an indication of higher serum lipids, adiposity, cardiovascular disease, and possible markers of atherosclerosis with higher PFDA exposure. While most results were imprecise and not statistically significant, exposure contrasts for PFDA in the study populations were relatively narrow, which is interpreted to result in low sensitivity to detecting an effect. However, there is inconsistency across studies for similar outcomes, so there is considerable uncertainty in the evidence. There is no evidence of an association with diabetes, insulin resistance, and metabolic syndrome, but the null results are difficult to interpret due to concerns for sensitivity.

Overall, the animal evidence is *indeterminate* given that the observed changes fail to establish a coherent pattern of adverse cardiometabolic effects in animals following short-term PFDA exposure. The evidence in animals is limited to a *high* confidence study in rats exposed via gavage for 28 days that examined cardiovascular histopathology, serum lipids, and heart weights (NTP, 2018). Dose-related decreases in triglyceride levels occurred in males and females and cholesterol also decreased in a dose-dependent manner in females. However, the biological significance of these responses is unclear. Absolute heart weights decreased in a dose-dependent manner in rats at the highest doses (≥1.25 mg/kg-day) but confidence in the results is reduced by potential confounding with decreased body weights and a lack of corroborative findings from histopathological evaluations or other organ weight measures (relative heart weight was unchanged). A major limitation in the animal toxicity database of this chemical is the lack of studies examining prolonged or chronic oral exposures. In addition, for some cardiometabolic endpoints (i.e., serum lipids), it would be preferred if studies were available in models that are more physiologically relevant to humans given species differences in lipid metabolism between humans and rodents (Getz and Reardon, 2012; Davidson, 2010). In the absence of such studies or mechanistic information on these responses, the human relevance of effects on rodent lipid profiles cannot be determined.

Overall, ***evidence suggests*** that PFDA exposure has the potential to cause cardiometabolic effects in humans under sufficient exposure conditions[16] (see Table 3-37). This conclusion is based on evidence of an association between PFDA exposure and certain cardiometabolic outcomes (serum lipids, adiposity, cardiovascular disease, and atherosclerosis) in a small number of epidemiological studies with median exposure levels from 0.1–0.4 ng/mL; however, issues with inconsistency across studies raise considerable uncertainty. Moreover, evidence in animals is sparse and largely uninterpretable regarding its relevance to humans.

[16]Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

Table 3-37. Evidence profile table for PFDA exposure and cardiometabolic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.7: Human Studies)					⊕⊙⊙ ***Evidence suggests*** *Primary basis:* Some coherent effects in a small number of *medium* confidence epidemiological studies, but data are largely inconsistent. Evidence from a *high* confidence rat study was *indeterminate*. *Human relevance:* The utility of the observed serum lipid effects in rats for informing human health hazard is uncertain given the species differences in lipid metabolism between humans and rodents. *Cross-stream coherence, susceptibility, and other inferences:* No specific factors are noted.
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
<u>Serum lipids</u> Fourteen *medium* and 6 *low* confidence studies	• Five of six *medium* confidence studies in adults (including two in pregnant women) reported higher serum total cholesterol with higher PFDA exposure ($p < 0.05$ in three studies). • In children, results were inconsistent.	• Consistency of direction of association across studies in adults for total cholesterol. • Exposure-response gradient in the only two studies that examined categorical exposure.	• Imprecision in most positive associations • Lack of coherence across measures (total cholesterol and triglycerides) in some studies	⊕⊙⊙ *Slight* Positive associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in some studies, but with the exception of total cholesterol in adults, findings were inconsistent or incoherent across studies. Exposure levels were low, which may explain the lack of association in some studies.	
<u>Other cardiovascular risk factors</u> Nine *medium* and 4 *low* confidence studies	• Studies of blood pressure in the general population were largely null. Three of five studies reported hypertension or a positive association with blood pressure among pregnant women, but there was inconsistency among *medium* confidence studies. • There was a nonsignificant increase in the number of carotid arteries with atherosclerotic plaques in women in one study. • One study reported statistically significant changes in ventricular geometry.	• No factors noted	• Unexplained inconsistency across studies for blood pressure • Imprecision in positive associations observed for blood pressure and atherosclerosis		

Evidence stream summary and interpretation					Evidence integration summary judgment
<u>Cardiovascular disease</u> Two *medium* and 1 *low* confidence studies	• One *medium* and one *low* confidence studies reported higher odds of coronary heart disease (the former being statistically significant), but another *medium* confidence study was null. • Higher odds of angina pectoris, myocardial infarction, and stroke were reported in the single study that examined them.	• No factors noted	• Unexplained inconsistency across medium confidence studies, possibly related to timing of exposure measurement • Imprecision in results of specific cardiovascular conditions		
<u>Diabetes and insulin resistance</u> Fifteen *medium* and 6 *low* confidence studies	• One study reported higher odds of gestational diabetes with higher PFDA exposure, but the association was nonmonotonic and not statistically significant. Other studies reported either null or inverse associations with gestational diabetes. • Two studies of incident diabetes and 16 studies of insulin resistance indicated primarily null associations with PFDA exposure. • Low sensitivity across majority of studies	• No factors noted	• Unexplained inconsistency across studies		
<u>Adiposity</u> Six *medium* and 5 *low* confidence studies	• One study in adults reported an increase in weight gain (significant trend) and one reported	• No factors noted	• Unexplained inconsistency across studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
	higher BMI with higher PFDA exposure, but other studies reported null or inverse associations • Low sensitivity across studies				
Metabolic syndrome Two *medium* confidence studies	• Inverse association between metabolic syndrome and PFDA exposure in two studies (one reported a positive association in men).	• No factors noted	• No factors noted		
Evidence from in vivo animal studies (see Section 3.2.7: Animal Studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
Histopathology One *high* confidence study in rats for 28 d	• No significant effects in heart and blood vessel histopathology in rats up to 2.5 mg/kg-d	• *High* confidence study	• No factors noted	⊙⊙⊙ *Indeterminate* Lack of coherent, adverse effects indicative of cardiometabolic toxicity.	
Serum lipids One *high* confidence study in rats for 28 d	• Decreases in triglyceride (males and females) and cholesterol levels (females only) in rats at ≥1.25 mg/kg-d for 28 d	• Dose-response gradient for most effects • *High* confidence study	• Unclear biological significance of decreases in lipids		
Organ weight One *high* confidence study in rats for 28 d	• Decreases in absolute (but not relative) heart weight in rats at doses ≥1.25 mg/kg-d	• Dose-response gradient for absolute heart weights • *High* confidence study	• Unexplained inconsistency across heart weight measures • Potential confounding by body weight decrease (particularly since only absolute weights affected)		

C = cohort study; CS = cross-sectional study; CC = case-control study.

3.2.7. Neurodevelopmental Effects

Human Studies

Neurodevelopment

Thirteen studies (19 publications) reported on PFDA and neurodevelopmental outcomes in humans. The study evaluations are summarized for Figure 3-78. In the case of multiple publications for the same study population, they were evaluated under one record if the selection procedures for the analysis population were similar but evaluated under different records if selection procedures were significantly different (see figure footnote for details). All but one study (Gump et al., 2011) was *medium* confidence, however all but (Niu et al., 2019) were *deficient* for study sensitivity due to limited exposure contrast. With the exception of Gump et al. (2011), all studies were birth cohorts or case-controls studies nested in cohorts that evaluated maternal exposure to PFDA during pregnancy and/or during childhood. Functionally, there is considerable overlap between different domains of neurodevelopment, but for the purposes of this review, the outcomes were categorized: eight studies (nine publications) examined attention deficient hyperactivity disorder (ADHD), attention , or related behaviors (Dalsager et al., 2021b; Harris et al., 2021; Skogheim et al., 2021; Luo et al., 2020; Vuong et al., 2018; Høyer et al., 2017; Oulhote et al., 2016; Liew et al., 2015; Gump et al., 2011), eight studies (10 publications) examined cognition and summary measures of neurodevelopment (Yao et al., 2022; Harris et al., 2021; Skogheim et al., 2020; Niu et al., 2019; Harris et al., 2018; Liew et al., 2018; Lyall et al., 2018; Vuong et al., 2018; Vuong et al., 2016; Wang et al., 2015), five studies examined autism spectrum disorder (ASD) or social behaviors (Skogheim et al., 2021; Shin et al., 2020; Niu et al., 2019; Lyall et al., 2018; Liew et al., 2015), three examined motor effects (Yao et al., 2022; Niu et al., 2019; Harris et al., 2018), and one examined congenital cerebral palsy (Liew et al., 2014).

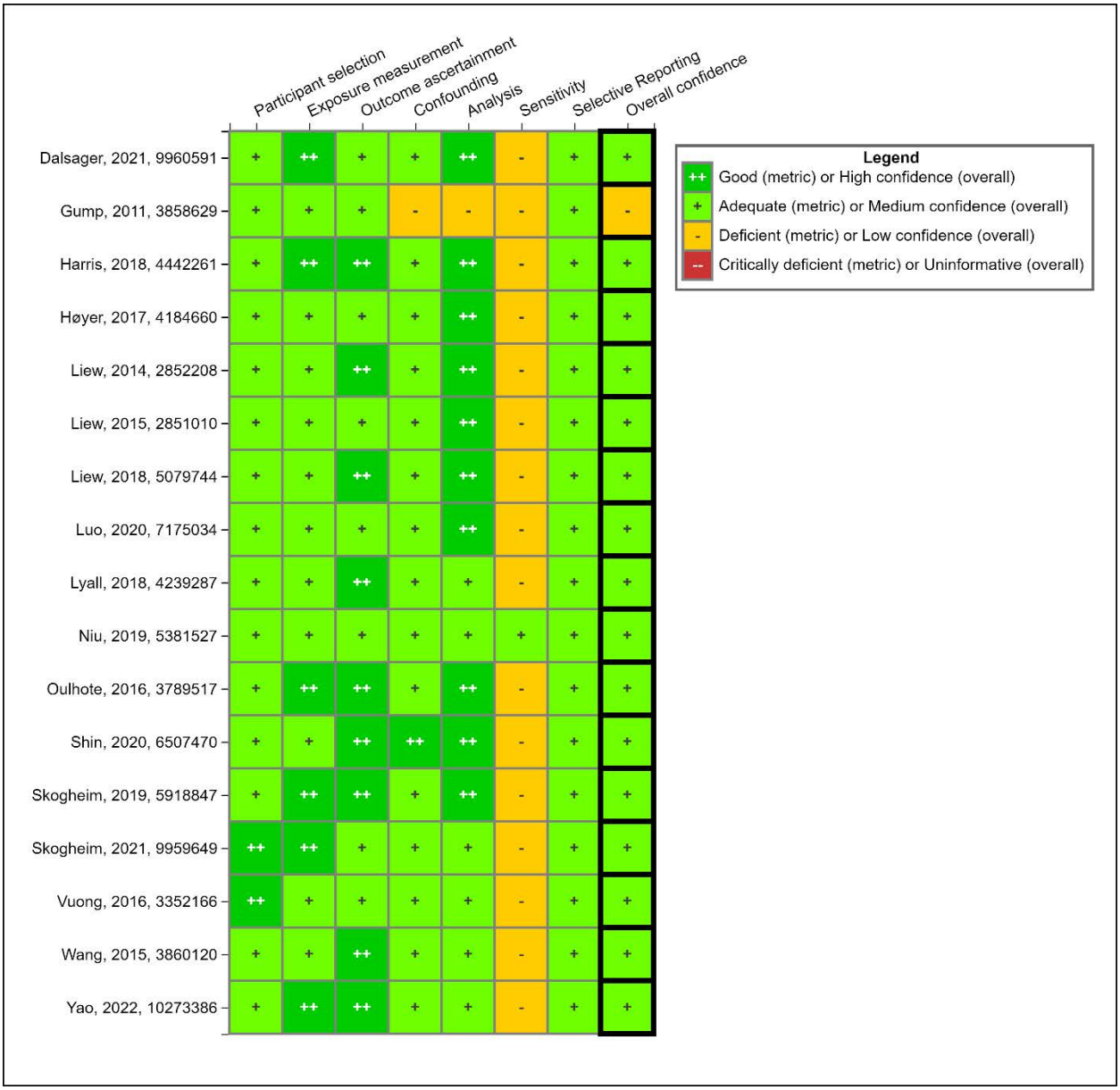


Figure 3-78. Evaluation results for epidemiological studies of PFDA and neurodevelopmental effects. Refer to the HAWC link for details on the study evaluation review.[a–c]

[a]Multiple publications of the same study population: Project Viva – Harris et al. (2018) also includes (Harris et al., 2021); HOME study – Vuong et al. (2016) also includes (Vuong et al., 2018).

[b]Four publications with data from the Danish National Birth Cohort were evaluated separately due to significantly different selection procedures but should not be considered independent: (Liew et al., 2014); (Liew et al., 2015); (Liew et al., 2018); (Luo et al., 2020).

[c]Two publications with data from the Norwegian Mother Father and Child Cohort were evaluated separately due to significantly different selection procedures but should not be considered independent: (Skogheim et al., 2020) and (Skogheim et al., 2021).

Most of the eight studies (reported in nine publications) examining ADHD or related behaviors reported associations with greater difficulties in attention or behavior problems, but there is some inconsistency within and across studies and imprecision in the results. Results for the *medium* confidence studies are displayed in Table 3-38. Notably, the two studies with the most clinically relevant outcome measure (Skogheim et al., 2021; Liew et al., 2015) examined diagnosed ADHD and found no increase in the odds of diagnosis (effect estimates were in the inverse direction). The remaining *medium* confidence studies (including another publication using the same population as Liew et al. (2015), resulting in six studies) examined scores on neurobehavioral assessments including the Strengths and Difficulties Questionnaire (SDQ), the Child Behavior Checklist (CBC), and the Behavior Rating Inventory of Executive Function (BRIEF). With the exception of Luo et al. (2020), which reported inconsistent results across child ages, all of these studies reported associations consistent with greater difficulties in attention or behavior problems with higher PFDA exposure, although effect estimates were small in most studies. This included statistically significant associations in Harris et al. (2021) and Oulhote et al. (2016) with SDQ scores and an exposure-response gradient across categories in Harris et al. (2021) and Høyer et al. (2017). However, in most studies, the confidence intervals were wide. It is possible that the limited study sensitivity could explain the nonsignificant findings, but it would likely not explain the inconsistency with studies of the more apical outcome of ADHD diagnosis, and thus there is uncertainty in the findings overall. Finally, a *low* confidence cross-sectional study examined inter-response time (IRT) at age 9–11 and found statistically significant decreases in IRT, which indicates poor response inhibition (a primary deficit in children with ADHD) as the test is designed to reward longer response times (Gump et al., 2011).

For the other neurodevelopmental outcomes, results were less consistent. In the eight studies of cognition and summary neurodevelopmental scores, Vuong et al. (2018), reported higher odds of "at risk" scores for metacognition and global executive indices at ages 3 and 8 (statistically significant for the global executive composite, OR 2.95, 95% CI: 1.20, 7.23). Nonstatistically significant decreases in IQ or similar scores were reported in two studies (Harris et al., 2018; Wang et al., 2015), but the remaining studies did not report associations with IQ (Liew et al., 2018), executive function (Harris et al., 2021), communication and problem solving (Niu et al., 2019), working memory (Skogheim et al., 2020), adaptive or language developmental quotient (Yao et al., 2022), or intellectual disability (Lyall et al., 2018). Among the five studies of ASD and social behavior, four examined diagnosed ASD; three of these four reported inverse associations (statistically significant in one) (Skogheim et al., 2021; Shin et al., 2020; Liew et al., 2015) and one reported a null finding (Lyall et al., 2018). One study examined personal-social skills and found a positive association with problems, which was statistically significant in girls (Niu et al., 2019). Two of three studies of motor effects reported nonstatistically significant associations with reduced motor performance (Niu et al., 2019; Harris et al., 2018). Lastly, one study of congenital cerebral

palsy found no association with PFDA exposure (Liew et al., 2014). Because of the poor sensitivity of the available studies, it is difficult to interpret the primarily null results for these outcomes.

Table 3-38. Results for *medium* confidence epidemiological studies of PFDA exposure and behavioral and attention effects

Study name, reference(s)	Measured outcome	Exposure measurement timing	Estimate type (adverse directional)	N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
Norwegian Mother, Father, and Child cohort (nested case-control) Skogheim et al. (2021)	Diagnosed ADHD (controls frequency matched for sex and year of birth)	Maternal (second trimester)	OR (↑)	1,801	Q1	0.02–0.13	Ref		
					Q2	0.13–0.17	0.86	0.65	1.13
					Q3	0.17–0.23	0.77	0.59	1.02
					Q4	0.23–1.5	0.61	0.46	0.81
Danish National Birth Cohort Liew et al. (2015) **(nested case-control)** Luo et al. (2020)	Diagnosed ADHD (through ~10.7-yr follow-up, controls frequency matched for sex)	Maternal (first trimester)	RR (↑)	760	Ln-unit increase in exposure	0.2 (0.1–0.2)	**0.76***	0.64	0.91
	Externalizing problems at 7 yr		OR (↑) (odds of elevated score)	2,421	Per doubling of exposure		1.09	0.78	1.53
	Internalizing problems at 7 yr						1.03	0.72	1.47
	Total SDQ score at 7 yr						1.11	0.87	1.43
	Externalizing problems at 11 yr			2,070			0.95	0.70	1.28
	Internalizing problems at 11 yr						0.95	0.72	1.26
	Total SDQ score at 11 yr						0.86	0.68	1.08
Odense child cohort Dalsager et al. (2021b)	ADHD symptom score on CBC at 2.5 and 5 yr	18 mo	IRR (↑) (relative difference in score)	775	Per doubling of exposure	0.2	0.98	0.88	1.09
		Maternal (first trimester)		1,113		0.3	1.02	0.95	1.09
		18 mo	OR (↑) (odds of elevated score)	775	Per doubling of exposure	0.2	1.06	0.78	1.44
		Maternal (first trimester)		1,113		0.3	1.08	0.85	1.37
HOME study		3 yr		208		0.2	1.95	0.83	4.62

Study name, reference(s)	Measured outcome	Exposure measurement timing	Estimate type (adverse directional)	N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
Vuong et al. (2018)	Behavioral regulation index on BRIEF at 8 yr	8 yr	OR (↑) (odds of elevated score)		Ln-unit increase in exposure	0.2	1.70	0.59	4.88
Project Viva Harris et al. (2021)	Externalizing problems at 6–10 yr	Median 7.7 yr	Mean difference (↑)	628	Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.2	−0.5	0.9
					Q3	0.4–0.4	0.3	−0.4	1.0
					Q4	0.5–1.9	0.5	−0.2	1.2
	Internalizing problems at 6–10 yr				Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.2	−0.4	0.7
					Q3	0.4–0.4	0.4	−0.2	0.9
					Q4	0.5–1.9	0.6	0.0	1.1
	Total SDQ score at 6–10 yr				Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.4	−0.6	1.3
					Q3	0.4–0.4	0.7	−0.4	1.7
					Q4	0.5–1.9	**1.1***	0.1	2.1
Faroe Island cohort **(Oulhote et al., 2016)**	Externalizing problems at 7 yr	5 yr	Mean difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	**0.45***	0.02	0.87
		Maternal (32 wk gestation)		539		0.3 (0.2–0.4)	0.26	−0.29	0.81
	Internalizing problems at 7 yr	5 yr	Mean difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	0.27	−0.11	0.65
		Maternal (32 wk gestation)		539		0.3 (0.2–0.4)	0.26	−0.29	0.81
	Total SDQ score at 7 yr	5 yr	Mean difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	**0.72***	0.07	1.38
		Maternal (32 wk gestation)		539		0.3 (0.2–0.4)	−0.01	−0.98	0.96

Study name, reference(s)	Measured outcome	Exposure measurement timing	Estimate type (adverse directional)	N	Group or unit change	Exposure median (IQR) or range (quartiles)	Effect estimate	CI LCL	CI UCL
INUENDO (Biopersistent organochlorines in diet and human fertility) (Høyer et al., 2017)	SDQ hyperactive-ity score at 5–9 yr	Maternal (second trimester median)	Regression coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.13	−0.10	0.36
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.11	−0.22	0.44
					High exposure	2.0–18.8	0.13	−0.27	0.53
	Total SDQ score at 5–9 yr	Maternal (second trimester median)	Regression coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.40	−0.15	0.95
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.07	−0.71	0.85
					High exposure	2.0–18.8	0.65	−0.30	1.61

*$p < 0.05$.

SDQ: Strengths and Difficulties Questionnaire. Externalizing problems calculated from conduct and hyperactivity subscales; internalizing problems calculated from emotional and peer subscales. BRIEF: Behavior Rating Inventory of Executive Function. IQR: interquartile range. CI: confidence interval. LCL: lower confidence limit. UCL: upper confidence limit.

[a]The arrows indicate the direction the effect estimate will be if there is an association between PFDA and reduced behavior. For all the tests included here, higher scores indicate more difficulties/behavior problems/ADHD diagnosis. For ratio measures such as odds ratios (OR), an effect estimates greater than 1 indicates more difficulties/behavior problems, while for regression coefficients and mean differences, an effect estimates greater than 0 indicates more difficulties/behavior problems.

Animal Studies

There are no available animal toxicity studies informing of potential neurodevelopmental effects of PFDA via any relevant exposure route and duration.

Evidence Integration

The evidence for potential neurodevelopmental effects in humans is considered *slight.* Associations between PFDA exposure and outcomes related to attention and behavior were reported in multiple epidemiological studies, although there was inconsistency between these findings and the more clinically relevant measure of ADHD diagnosis. Results for other neurodevelopmental effects were largely inconsistent, although poor sensitivity due to limited exposure contrast may explain the lack of association in some studies. No animal toxicity studies are available. Altogether, based on the available human studies, the ***evidence suggests*** that PFDA exposure might cause neurodevelopmental effects in humans under sufficient exposure conditions[17] (see Table 3-39).

[17]Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

Table 3-39. Evidence profile table for PFDA exposure and neurodevelopmental effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.7: Human Studies)					⊕⊙⊙ ***Evidence suggests*** *Primary basis:* *Slight* evidence of attention and behavior effects in humans. *Human relevance, cross-stream coherence, susceptibility, and other inferences:* Evidence comes from studies in humans at a susceptible lifestage (in utero or childhood exposure).
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
<u>ADHD and related behaviors</u> Seven *medium* and 1 *low* confidence studies	• 5/6 studies examining behavioral issues and/or attention problems reported positive associations but the two studies examining ADHD diagnosis (the most clinically relevant outcome) reported inverse findings.	• Consistency in direction of association for studies of behavior and attention	• Unexplained inconsistency with studies of ADHD diagnosis • Imprecision in most study results	⊕⊙⊙ *Slight* There is some evidence of greater problem behaviors and decreased attention with increasing PFDA exposure but there is remaining uncertainty due to inconsistency and imprecision.	
<u>Other neurodevelopmental effects</u> Fourteen *medium* confidence studies	• Some studies reported decreases in cognition or motor scores, but findings were inconsistent across studies. No association was observed with ASD/social behavior or cerebral palsy.	• No factors noted	• Unexplained inconsistency		

3.2.8. Endocrine Effects

Human Studies

Thyroid effects

Twenty-three studies examined thyroid hormones and PFDA exposure. A summary of the study evaluations is presented in Figure 3-79, and additional details can be obtained from HAWC. Two studies were considered *uninformative* and excluded from further analysis due to critical deficiencies in confounding and analysis (Seo et al., 2018) or serious deficiencies in several domains (Kim et al., 2011). Sixteen studies were classified as *medium* confidence and five studies were classified as *low* confidence (Liu et al., 2021b; Itoh et al., 2019; Zhang et al., 2018a; Ji et al., 2012; Bloom et al., 2010). Of the *medium* confidence studies, five were cross-sectional, nine were prospective cohorts, one was a retrospective cohort, and one was participants from a randomized clinical trial of energy-reduced diets (functionally equivalent to a prospective cohort).

In addition to the general considerations described in Section 1.2.2, there were several outcome-specific considerations for study evaluation that were influential on the ratings. First, for outcome ascertainment, collection of blood during a fasting state and at the same time of day for all participants (or adjustment for time of collection) is ideal for measurement of thyroid hormones to avoid misclassification due to potential diurnal variation (van Kerkhof et al., 2015). Studies that did not consider these factors (e.g., by study design or adjustment) were considered *deficient* for the outcome ascertainment domain, primarily for thyroid-stimulating hormone (TSH), which is more impacted by these issues than thyroxine (T4) or triiodothyronine (T3). However, this was not expected to result in substantial bias, and thus studies were not downgraded in overall study confidence if lack of fasting and consideration of diurnal variation were the primary limitations identified. This possible outcome misclassification was expected to be nondifferential and thus likely a bias toward the null; the domain ratings were used to assess possible sources of inconsistency in the results. For participant selection, it was considered important to account for current thyroid disease and/or use of thyroid medications; studies that did not consider these factors by exclusion or another method were considered *deficient* for the participant selection domain. Concurrent measurement of exposure with the outcome was considered appropriate for this outcome since circulating hormone levels can change quickly in response to a change in exposure and the half-life of PFDA in humans is long. All the available studies analyzed PFDA in serum or plasma using appropriate methods (as described in the protocol). Thyroid hormones were analyzed using standard and well-accepted methods in all studies. Overall, while most studies were considered *medium* confidence, nearly all of them had limitations in outcome ascertainment and/or study sensitivity (primarily due to limited PFDA exposure contrast in the study populations). These issues and other (nondifferential) sources of measurement error are likely to bias the results toward the null, and thus null associations are difficult to interpret. The *low* confidence studies generally had additional concerns such as selection bias or confounding.

Figure 3-79. Study evaluation results for epidemiological studies assessing effects of PFDA exposure on thyroid effects. Refer to HAWC Human Thyroid Effects for details on the study evaluation review.

Multiple publications of single study: Berg et al. (2017) includes Berg et al. (2015). Aimuzi et al. (2019) and Aimuzi et al. (2020) examine the same birth cohort but are considered separately because the populations are different (neonates/cord blood in Aimuzi et al. (2019) and pregnant women in Aimuzi et al. (2020). These studies should not be considered fully independent.

The results for thyroid hormones are summarized in Table 3-40. Seven studies (three *medium* confidence) examined associations with thyroid hormones in general population adults (7 for T4, 3 for T3, 7 for TSH). Results were mixed across studies for each hormone, with results in

both the positive and inverse direction and no clear pattern to explain the inconsistency (e.g., study confidence or population characteristics). Most studies were null, and none were statistically significant. One *medium* (Blake et al., 2018) and one *low* confidence study (Liu et al., 2021b) reported small positive associations with TSH (higher levels with higher exposure), but these differences were imprecise (wide confidence intervals), and among the other *medium* confidence studies, one reported an inverse association (Cakmak et al., 2022) and one was null (Liu et al., 2018). In pregnant women, eight studies (seven *medium* confidence) were available (7 for T4, 6 for T3, 8 for TSH) and results were again primarily null.

Two *medium* confidence studies in children and adolescents similarly found no association. Seven *medium* confidence studies and one *low* confidence study examined associations with thyroid hormones in neonates (all studies reported all three hormones). For T4 (total or free), only one of seven studies reported an association; Liang et al. (2020) reported an inverse association with T4 but not total T4 (tT4) (β (9% CI for ln-unit increase in PFDA: −5.07 (−9,78, −0.37)). For total T3, two of six *medium* confidence studies reported higher T3 with higher PFDA (Liang et al., 2020; Shah-Kulkarni et al., 2016). In contrast, one study reported lower T3 ($p < 0.05$) in boys with maternal thyroid antibody negative but higher T3 in boys with maternal thyroid antibody positive ($p > 0.05$) and girls (Itoh et al., 2019). Three studies reported inverse associations between TSH and PFDA exposure, but in Itoh et al. (2019), this inverse association was observed only in boys with maternal thyroid antibody positive, while in Shah-Kulkarni et al. (2016), the association was observed only in girls and not statistically significant. The association was observed in the overall population in Wang et al. (2014a), but this was also not statistically significant. In addition, one study reported a positive association with TSH Liu et al. (2021b). The remaining studies reported no association (Guo et al., 2021; Aimuzi et al., 2019; Yang et al., 2016a; Berg et al., 2015). It is possible that the lack of consistency was due to differences in the timing of exposure measurement (maternal sampling at median 18 weeks in Berg et al. (2017), second trimester in Itoh et al. (2019), third trimester in Wang et al. (2014a), 1–2 days before delivery in Yang et al. (2016a), and cord blood sampling in Shah-Kulkarni et al. (2016), Aimuzi et al. (2019), Guo et al. (2021); Liu et al. (2021b), but it is not possible to evaluate these differences further because of the lack of multiple studies per sampling period other than cord blood.

Table 3-40. Associations between PFDA and blood lipids in medium confidence epidemiological studies

Reference, study confidence	Population	Exposure measurement timing	Exposure median (IQR) in ng/mL	Effect estimate description	T4	T3	TSH
Adults							
Blake et al. (2018), ***medium***	Cohort of residents living in proximity to a uranium processing site in the U.S.; N = 210	Enrollment (1952) and during follow-up	0.1 (0.07–0.2)	% change (95% CI) per IQR change	Repeated measures model 2.5 (−2.94, 8.25) Latent model 1.19 (−3.08, 5.65)	NR	Repeated measures model 11.0 (−4.45, 28.8) Latent model −4.53 (−17.1, 9.90)
Cakmak et al. (2022), ***medium***	Nationally representative CS in Canada; N = 6,045	Concurrent	GM 0.2	% change per GM increase	0.2 (−1.6, 1.9)	NR	−7.0 (−17.2, 4.4)
Liu et al. (2018), ***medium***	Participants from a 2-yr randomized trial of energy-reduced diets in the U.S.; N = 621	Trial baseline	0.4 (0.3–0.5)	Partial Spearman correlation coefficient	TT4 0.03 FT4 0.06	TT3 −0.01 FT3 0.04	−0.03
Bloom et al. (2010), *low*	CS of licensed anglers and their partners in the U.S.; N = 31	Concurrent	GM 0.2	β (95% CI) per ln-unit change	0.09 (−0.02, 0.21)	NR	0.21 (−0.26, 0.68)
Liu et al. (2021b), *low*	CS of controls from case-control study of thyroid cancer in China, N = 185	Concurrent	0.5 (0.3–0.9)	% change per ln-unit change	TT4 0.65 (−3.51, 4.98) FT4 3.26 (−0.32, 6.96)	TT3 −3.79 (−7.69, 0.27) FT3 −1.41 (−4.27, 1.54)	9.53 (−6.15, 27.92)
Ji et al. (2012), *low*	CS in cohort in Korea, N = 633	Concurrent	0.9 (0.6–1.5)	β (95% CI) per unit change	−0.02 (−0.04, 0.01)	NR	0.07 (−0.05, 0.18)
Zhang et al. (2018b), *low*	CS in case-control study of POI in China (cases only); N = 240	Concurrent	1.7 (1.0–2.6)	β (95% CI) per log-unit change	−1.19 (−2.66, 0.28)	−0.56 (−1.27, 0.16)	0.85 (−0.03, 1.72)
Pregnant women							
Wang et al. (2013), ***medium***	CS analysis in pregnancy cohort in Norway; N = 903	Concurrent (12-37 wk gest)	0.1 (0.04–0.2)	β (95% CI) per unit change	NR	NR	0.06 (−0.46, 0.58)

Reference, study confidence	Population	Exposure measurement timing	Exposure median (IQR) in ng/mL	Effect estimate description	T4	T3	TSH
Inoue et al. (2019), ***medium***	CS in pregnancy cohort in Denmark; N = 1,366	Concurrent (5–19 wk gest)	0.2 (0.1–0.2)	% change (95% CI) per IQR change	0.8 (−0.4, 1.9)	NR	−1.3 (−7.2, 4.9)
Berg et al. (2017), ***medium***	CS in pregnancy cohort in Norway; N = 370	Concurrent (second trimester)	0.2 (0.2–0.3)	β (95% CI) vs. Q1	"No association"	Q2: −0.01 (−0.03, 0.01) Q3: −0.01 (−0.03, 0.01) Q4: −0.02 (−0.04, −0.01)	"No association"
Reardon et al. (2019), ***medium***	Pregnancy cohort in Canada; N = 494	Each trimester	0.3	β (*p*-value) per unit change in repeated measures	−0.01 (0.3)	0.003 (0.7)	0.02 (0.6)
Yang et al. (2016a), ***medium***	CS of mother-infant pairs in China; N = 157	Concurrent (1–2 d before delivery)	0.4 (0.04–2.0)	Spearman correlation coefficient	TT4 −0.01 FT4 −0.09	TT3 −0.08 FT3 −0.09	−0.22*
Wang et al. (2014a), ***medium***	CS within pregnancy cohort in Taiwan; N = 285	Concurrent (third trimester	0.5 (0.1–0.7)	β (95% CI) per unit change	TT4 −0.001 (−0.006, 0.005) FT4 0.047 (−0.028, 0.123)	TT3 0.002 (−0.00, 0.003)*	0.004 (−0.037, 0.045)
Aimuzi et al. (2020), ***medium***	CS within pregnancy cohort in China; N = 1,885	Concurrent (9–16 wk gest)	1.6 (1.1–2.4)	β (95% CI) per ln-unit change	0.05 (−0.03, 0.13)	0.11 (0, 0.21)	−0.03 (−0.11, 0.05)
Itoh et al. (2019), *low*	CS within pregnancy cohort in Japan; N = 701	Concurrent (9–13 wk gest)	0.5 (0.4–0.7)	β (95% CI) per unit change	0.03 (−0.02, 0.08)	0.04 (−0.00, 0.09)	−0.11 (−0.49, 0.26)
Children and adolescents							
Kang et al. (2018), ***medium***	Nationally representative CS in Korea; N = 150	Concurrent (3–18 yr)	0.1 (0.04–0.1)	β (95% CI) per ln-unit change	0.02 (−0.01, 0.04)	NR	−0.09 (−0.31, 0.13)
Kim et al. (2020a), ***medium***	Cohort of children in Korea with follow-up to 6 yr; N = 660	1 yr	0.4 (0.2–0.6)	β (SE) per unit change	0.01 (0.01)	0.00 (0.01)	−0.01 (0.04)
Infants							

Reference, study confidence	Population	Exposure measurement timing	Exposure median (IQR) in ng/mL	Effect estimate description	T4	T3	TSH
Shah-Kulkarni et al. (2016), ***medium***	CS analysis within birth cohort in Korea; N = 279	Concurrent (cord blood)	0.1 (0.1–0.1)	β (95% CI) per unit change	0.13 (−0.18, 0.45)	2.40 (−0.27, 5.09)	−1.01 (−2.65, 0.62)
Berg et al. (2017), ***medium***	CS in pregnancy cohort in Norway; N = 370	Concurrent (second trimester)	0.2 (0.2–0.3)	β (95% CI) per unit change per Q1	"No association"	Q2: −0.01 (−0.03, 0.01) Q3: −0.01 (−0.03, 0.01) Q4: −0.02 (−0.04, −0.01)	"No association"
Aimuzi et al. (2019), ***medium***	CS in pregnancy cohort in China; N = 568	Concurrent (cord blood)	0.4 (0.3–0.6)	β (95% CI) per unit change	0.1 (−0.01, 0.22)	−0.01 (−0.06, 0.03)	−0.05 (−0.08, −0.02)*
Yang et al. (2016a), ***medium***	Pregnancy cohort in China; N = 157	Maternal (1–2 d before delivery)	0.4 (0.04–2.0)	Spearman correlation coefficient	TT4 0.07 FT4 0.14	TT3 0.04 FT3 0.06	−0.04
Wang et al. (2014a), ***medium***	Pregnancy cohort in Taiwan; N = 285	Maternal (third trimester	0.5 (0.1–0.7)	β (95% CI) per unit change	TT4 −0.51 (−1.73, 0.71) FT4 0.02 (−0.12, 0.16)	−0.02 (−0.03, −0.01)*	−3.51 (−7.82, 0.81)
Guo et al. (2021), ***medium***	CS in birth within birth cohort in China; N = 490	Concurrent (cord blood)	0.7 (0.4–1.7)	β (95% CI) per ln-unit change	TT4 −0.01 (−0.03, 0.02) FT4 −0.00 (−0.02, 0.01)	TT3 −0.01 (−0.02, 0.03) FT3 0.00 (−0.02, 0.02)	−0.01 (−0.07, 0.05)
Liang et al. (2020), ***medium***	Birth cohort in China; N = 300	Maternal (12–16 wk gest)	2.2 (1.4–3.3)	β (95% CI) per ln-unit change	TT4 −5.07 (−9.78, −0.37* FT4 −0.10 (−0.45, 0.26)	TT3 0.06 (0.03, 0.09)* FT3 0.08 (0.01, 0.15)*	−0.96 (−1.89, −0.03)*
Itoh et al. (2019), *low*	CS within pregnancy cohort in Japan; N = 701	Maternal (9–13 wk gest)	0.5 (0.4–0.7)	β (95% CI) per unit change	Boys −0.03 (−0.09, 0.02) Girls 0.04 (−0.04, 0.12)	Boys −0.19 (−0.37, −0.01)* Girls 0.26 (0.06, 0.46)	Boys −0.06 (−0.26, 0.15) Girls −0.09 (−0.29, 0.11)

*$p < 0.05$; NR = not reported.
Bold text indicates *medium* confidence study. Rows are sorted by study population, then study confidence, then median exposure.

Overall, the evidence for the association between PFDA exposure and thyroid effects in human studies is inconsistent. A few studies do suggest an association between thyroid hormones and PFDA exposure, but most studies are null, and the direction of association is not consistent across studies. For most studies, the exposure levels were low (median exposure was less than 0.5 ng/mL) and there were narrow exposure contrasts, which along with potential for outcome misclassification in most studies, reduced the study sensitivity and could have impaired the ability of these studies to observe a true effect. However, this poor sensitivity would not explain the observed differences in the direction of association, and thus considerable uncertainty remains.

Animal Studies

Two studies in the database of toxicity studies for PFDA evaluated endocrine effects. One study exposed female SD rats for 28 days (0, 0.125, 0.25, and 0.5 mg/kg-day) and examined the adrenal glands (weight and histopathology) (Frawley et al., 2018). The second study examined the following endpoints in both male and female SD rats after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day): thyroid hormone levels, histopathology, and organ weights (NTP, 2018). Potential PFDA effects on male and female reproductive organs (e.g., testes and ovaries) and reproductive hormones (e.g., testosterone) that also encompass part of the endocrine system are discussed in the "Male Reproductive Effects" and "Female Reproductive Effects" sections.

Thyroid hormone levels

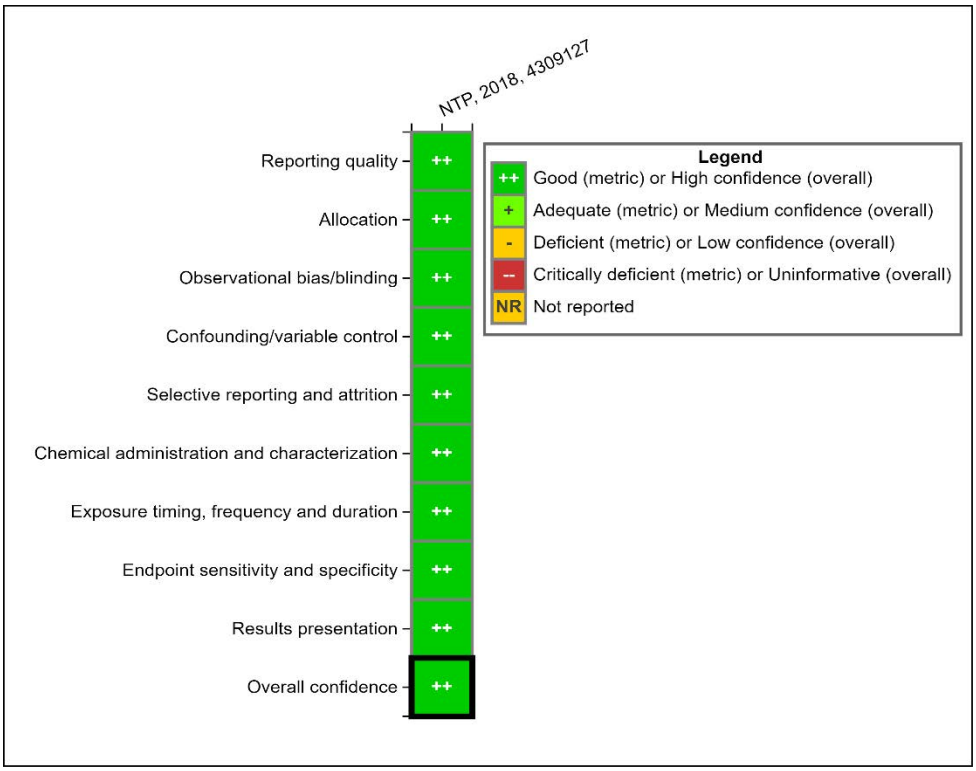


Figure 3-80. Evaluation results for an animal study assessing effects of PFDA exposure on thyroid hormone levels. Refer to HAWC for details on the study evaluation review.

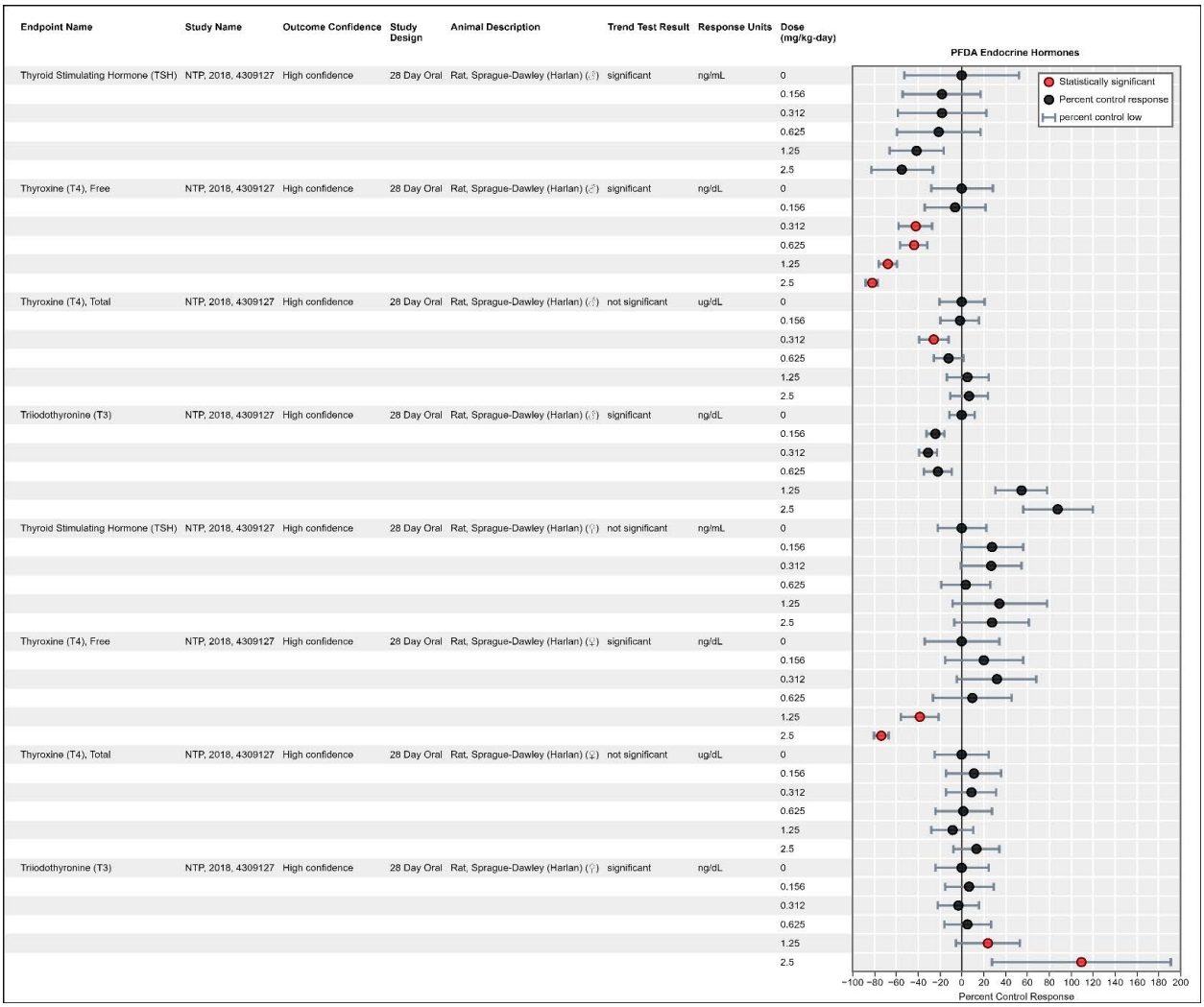
In the NTP (2018) study, which was considered *high* confidence (see Figure 3-80), thyroid hormones were measured in male and female rats exposed to 0–2.5 mg/kg-day for 28 days (see Figure 3-81 and Table 3-41). For TSH, a statistically significant decreasing trend (18% to 55%) was observed in male rats, but a significant decrease compared with controls was not reported at any dose. No statistically significant change for TSH was observed in the female rats but increases ranged from 3% to 35% with the lowest effect occurring at 0.625 mg/kg-day. A statistically significant increasing trend was reported for T3 in male (22% to 88%) and female rats with significant increases (24%–109%) reported at ≥1.25 mg/kg-day for females only. A statistically significant decreasing trend in free thyroxine (fT4) was reported in male and female rats with significant decreases at ≥0.312 mg/kg-day in males (42%–82%) and at ≥1.25 mg/kg-day in females (39%–74%). A statistically significant decrease in tT4 was observed in males only at 0.312 mg/kg-day and was unchanged in females at all doses. fT4 is the preferred measurement over tT4 in adult animals given that the level of tT4 can be dependent on the amount of serum binding proteins while fT4 is available to be used by the body. The effects of PFDA on fT4 and TSH in male and female rats are consistent with secondary hypothyroidism, which is characterized by decreased T4 and decreased or normal levels of TSH (Lewiński and Stasiak, 2017). However, there is

uncertainty in this conclusion given that changes in fT4 and T3 are often expected to occur in the same direction, with T3 being the more active hormone form and formation of T3 contingent on the deiodination of fT4. The potential mechanism and interpretation for an observation of decreasing fT4 with increasing T3 is unknown and unexamined in the PFDA evidence base.

Table 3-41. Percent changes relative to controls in thyroid hormone levels in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Thyroid-stimulating hormone (TSH)					
Female Sprague-Dawley rats	28	27	3	35	27
Male Sprague-Dawley rats	−18	−18	−22	−41	−55
Triiodothyronine (T3)					
Female Sprague-Dawley rats	7	−4	5	**24**	**109**
Male Sprague-Dawley rats	−24	−31	−22	54	88
Free thyroxine (fT4)					
Female Sprague-Dawley rats	20	32	10	**−39**	**−74**
Male Sprague-Dawley rats	−6	**−42**	**−44**	**−68**	**−82**
Total thyroxine (tT4)					
Female Sprague-Dawley rats	11	9	1	−9	13
Male Sprague-Dawley rats	−2	**−26**	−12	5	7

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.



Endpoint Name	Study Name	Outcome Confidence	Study Design	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
Thyroid Stimulating Hormone (TSH)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/mL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Free	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Total	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant	ug/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Triiodothyronine (T3)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroid Stimulating Hormone (TSH)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant	ng/mL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Free	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Total	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant	ug/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Triiodothyronine (T3)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5

Figure 3-81. PFDA thyroid hormone levels after short-term oral exposure.
(Results can be viewed by clicking the HAWC link.)

Histopathology

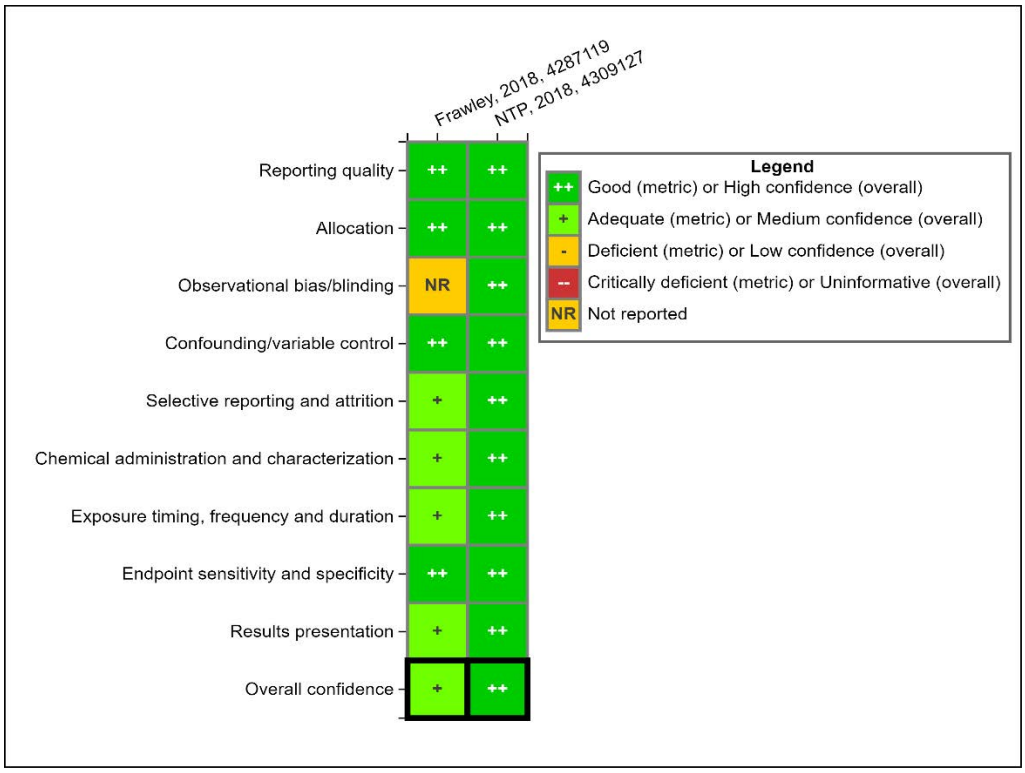
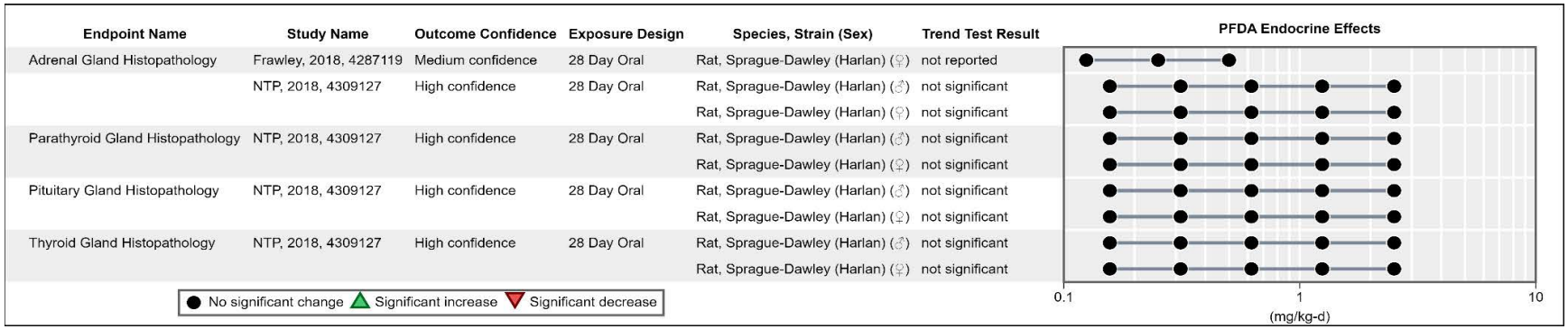


Figure 3-82. Evaluation results for animal studies assessing effects of PFDA exposure on endocrine histopathology. Refer to HAWC for details on the study evaluation review.

Both the NTP (2018) and Frawley et al. (2018) studies performed histopathological examinations to examine PFDA-related effects. The NTP (2018) study was considered *high* confidence while the Frawley et al. (2018) study was evaluated as *medium* confidence due to incomplete reporting of the null data (see Figure 3-82). NTP (2018) performed histopathological examination of the thyroid gland, adrenal cortex and medulla, parathyroid gland, and pituitary gland in both male and female rats (NTP, 2018). Histopathology was examined for the thyroid gland at all doses; all other endocrine tissues were examined only in the control and high-dose (2.5 mg/kg-day) groups. NTP (2018) reported that there were no tissue changes observed in any of the examined organs in either sex (see Figure 3-83). Results from the histopathological examination of the adrenal glands in female rats from the Frawley et al. (2018) were qualitatively reported as being unchanged by PFDA exposure (Frawley et al., 2018).



Endpoint Name	Study Name	Outcome Confidence	Exposure Design	Species, Strain (Sex)	Trend Test Result
Adrenal Gland Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Parathyroid Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Pituitary Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Thyroid Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant

Figure 3-83. PFDA endocrine histopathology. (Results can be viewed by clicking the HAWC link.)

Organ weight

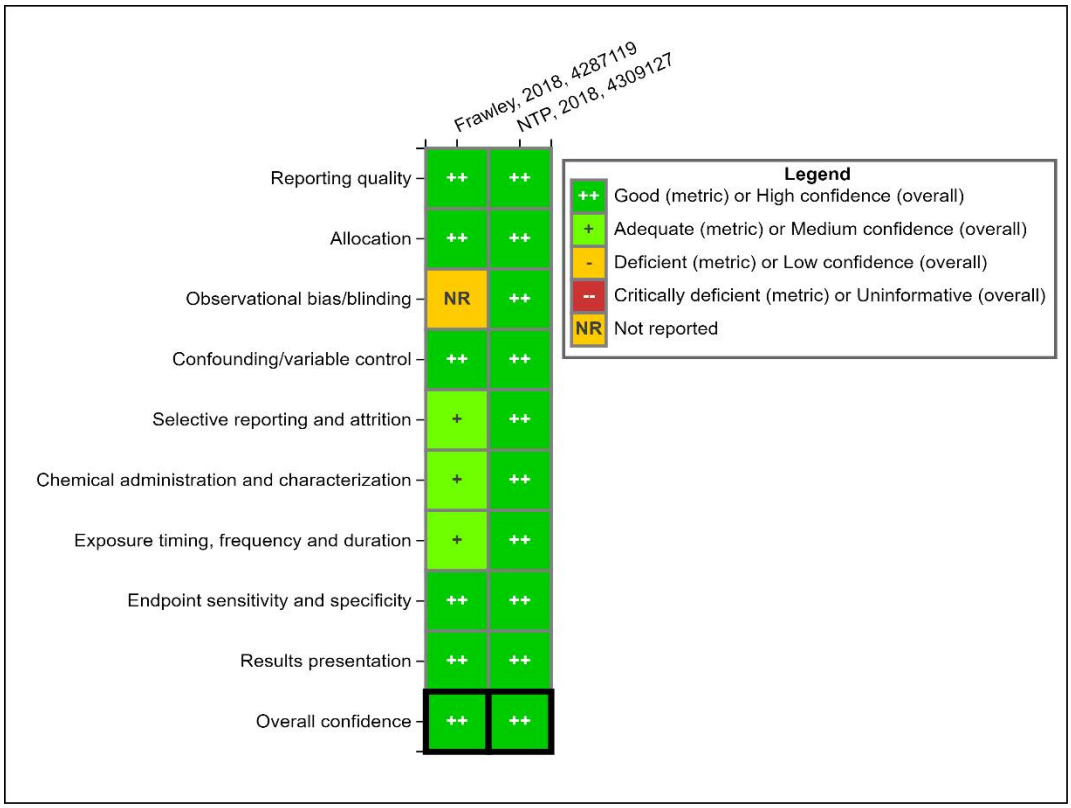


Figure 3-84. Evaluation results for animal studies assessing effects of PFDA exposure on endocrine organ weights. Refer to HAWC for details on the study evaluation review.

Both the NTP (2018) and Frawley et al. (2018) studies evaluated PFDA effects on endocrine organ weights and were considered *high* confidence for this outcome (see Figure 3-84). As indicated above, both studies measured adrenal weights. Only the NTP (2018) study measured thyroid weight; both sexes in rats demonstrated a statistically significant trend in relative thyroid weight with statistically significant increases reported at ≥1.25 mg/kg-day in male rats (43% at both 1.25 and 2.5 mg/kg-day) and at ≥0.312 mg/kg-day in female rats (27%–45%). For absolute thyroid weight in male rats, there was no significant trend, and no significant change was observed at any dose tested. In female rats, there was no significant trend but significant increases (33%–34%) were observed at doses ranging from 0.312 to 1.25 mg/kg-day but not at the highest dose tested (2.5 mg/kg-day). Relative (to body weight) thyroid weight is the preferred measure for this organ particularly in the presence of body weight changes (Bailey et al., 2004). Significant reductions in body weight were observed in the NTP (2018) study at the two highest doses tested (≥1.25 mg/kg-day; refer to the Section 3.2.10 for additional details). For adrenal weight in female rats, no significant changes were observed at doses up to 1.25 mg/kg-day in both studies. A statistically significant decrease (36%) for absolute adrenal gland weight was observed at the

highest dose group (2.5 mg/kg-day) in female rats from the NTP (2018) study; no change was reported for relative adrenal weight in females in this study. A statistically significant decrease (15%–21%) was reported in absolute adrenal gland weight in male rats at all dose groups. Conversely, relative adrenal weight in males was significantly increased (50%) at the highest dose tested (NTP, 2018). The toxicological significance of the adrenal organ-weight changes is unclear; the opposing direction of absolute and relative organ-weight changes suggests a confounding effect of body weight changes (refer to the General toxicity section for more detail on body weight effects) at the same doses. Furthermore, no PFDA-induced histopathological changes on the adrenal gland were observed (see discussion above and Figure 3-83).

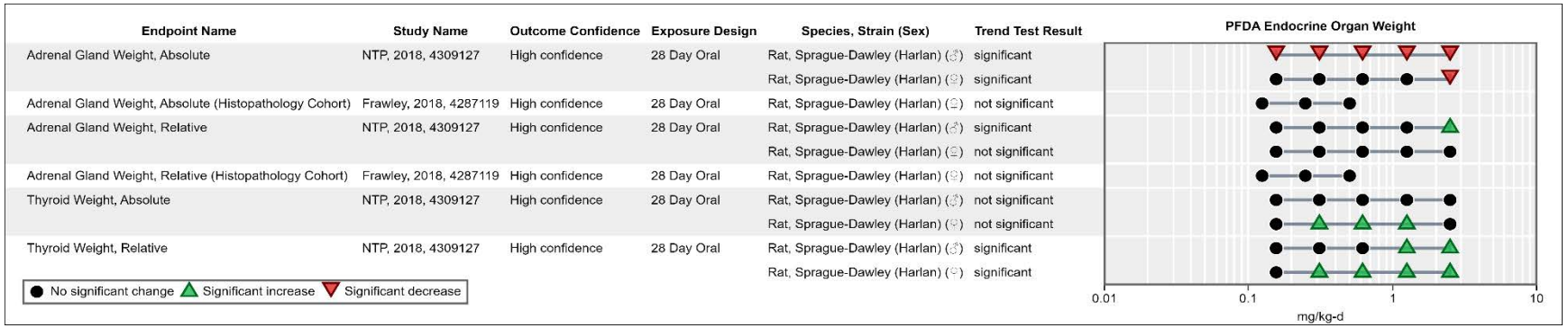


Figure 3-85. PFDA endocrine organ weight. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

In support for PFDA-induced changes on thyroid hormones observed in rats from the 28-day NTP (2018) study, structurally related PFAS (e.g., PFNA; PFOA) have been shown to affect thyroid hormone levels in rodents, specifically, PFNA induced hypothyroxinemia in rodents. Hypothyroxinemia has been defined in humans as a low percentile value of serum fT4 (ranging from the 2.5th percentile to the 10th percentile of fT4), with a TSH level within the normal reference range (Alexander et al., 2017).

Additionally, multiple high-dose, intraperitoneal (i.p.) injection studies have demonstrated that PFDA affects T3 and T4 serum levels. Specifically, decreases in serum T4 have been repeatedly observed in rats exposed to PFDA via i.p. injection at doses ranging from 20 to 80 mg/kg (Gutshall et al., 1989, 1988; Van Rafelghem et al., 1987a; Langley and Pilcher, 1985). Evaluations of PFDA effects on T3 varied among i.p. studies in rats. Langley and Pilcher (1985) observed an initial significant decrease in T3 levels starting at 12 hours after PFDA exposure (75 mg/kg i.p.) compared with pair-fed controls, which remained significantly decreased until day 4 of the study. Following day 4 of the study, there were no significant differences in T3 serum levels between pair-fed and PFDA-exposed animals, while serum T4 levels remained significantly diminished through day 8 of the study compared with the pair-fed controls. Gutshall et al. (1989) also reported significant decreases in T3 at 75 mg/kg i.p. in rats at 12 and 24 hours after PFDA exposure. Conversely, no changes in T3 were observed in rats exposed via i.p. to PFDA at doses up to 80 mg/kg-day (Gutshall et al., 1988; Van Rafelghem et al., 1987a). However, the inconsistencies in PFDA effects on T3 levels could be due to differences in experimental design and the time at which thyroid hormones were measured. In the studies that showed no effect on T3 levels in rats (Gutshall et al., 1988; Van Rafelghem et al., 1987a), thyroid hormones were measured at 7 and 14 days after PFDA treatment compared with the positive studies that showed effects on hormone levels at 12 to 48 hours after exposure.

Under normal physiologic conditions, neurons in the hypothalamus release thyroid releasing hormone (TRH) to stimulate thyrotrophs of the anterior pituitary gland to release TSH. TSH plays several important metabolic functions, including stimulation of the thyroid gland to release T3 and T4. When increased T3 and T4 serum levels reach above a certain blood concentration threshold, secretion of TRH from the hypothalamus is inhibited via a negative feedback loop.

To evaluate whether PFDA altered the ability of the pituitary and thyroid glands of the PFDA-exposed animals to respond to a physiological stimulation, Gutshall et al. (1989) challenged male Wistar rats with 500 µg/kg TRH at 15 or 22 hours after a single, high-dose 75 mg/kg (i.p.) PFDA exposure and found that although the percent response changes in T4 and T3 compared with baseline (i.e., pre-TRH challenge) were similar between the control and PFDA-exposed animals, the absolute values for T4 and T3 in the sera from PFDA-exposed animals was significantly less than that of their control counterparts following TRH stimulation (Gutshall et al., 1989). These data

indicate that PFDA may alter the ability of the glands in the hypothalamic-pituitary-thyroid (HPT) axis to respond to physiological stimulation (Gutshall et al., 1989). Additional studies would help clarify whether this observation is relevant in other species and at lower, more physiologically relevant levels of PFDA exposure. Impaired responsiveness of the HPT axis to hormonal stimulation could explain the results from the 28-day study in rats (NTP, 2018) in which TSH and T4 were both decreased by PFDA exposure in male rats; this mechanistic information does not however provide insight on why T3 was increased in the presence of decreased TSH and T4.

Additionally, the high-dose, i.p. study by Gutshall et al. (1989) showed that PFDA is able to displace T4 from plasma proteins (Gutshall et al., 1989). The fate of the displaced (i.e., free) T4 is unknown, but the authors postulated increased biliary excretion may be a potential route of T4 loss. Using a fluorescence displacement assay, Ren et al. (2016) reported that PFDA binds to transthyretin, a major transport protein for thyroid hormone, with the potential to displace T4 from the transport protein in occupational exposure settings. It is unclear how these mechanistic data, which indicate that PFDA decreases protein binding of T4, support the PFDA-induced effects on thyroid hormone homeostasis observed in rats from the NTP (2018) study. A decrease in protein binding of T4 could result in increased fT4 (unbound form) and a decrease in tT4 (bound form). Conversely, decreased fT4 was observed in rats while tT4 was decreased only at the mid-dose in males and unchanged in female rats exposed to PFDA (NTP, 2018). Evaluation of unsaturated binding capacity of thyroid-binding proteins, measured by T3 uptake analysis showed that T3 uptake was significantly reduced in the 80 mg/kg PFDA-exposed animals compared with the pair-fed controls (Van Rafelghem et al., 1987a). Under in vitro conditions, Ren et al. (2015) reported binding of PFDA to the human thyroid receptor but that PFDA did not exhibit antagonistic or agonistic effects on the thyroid receptor pathway (Ren et al., 2015).

Kelling et al. (1987) sought to determine the effects of PFDA on the thyroid by evaluating the hepatic activities of L-glycerol-3-phosphate dehydrogenase, malic enzyme, and glucose-6-phosphate dehydrogenase, which are enzymes that are sensitive to thyroid status. The activity of these enzymes is increased during hyperthyroidism and decreased during hypothyroidism (Mariash et al., 1980). Similar to the study performed by Langley and Pilcher, SD male rats received a single, high dose i.p. injection of either 20, 40, or 80 mg/kg PFDA and then hepatic subcellular fractions were prepared following euthanasia. These hepatic fractions were then used to assay the activity of L-glycerol-3-phosphate dehydrogenase, lactate dehydrogenase, malic enzyme, and glucose-6-phosphate dehydrogenase. PFDA significantly increased the activity of L-glycerol-3-phosphate dehydrogenase, cytosolic lactate dehydrogenase, and cytosolic malic enzyme compared with their pair-fed and ad libitum controls indicating that the increase of enzyme activity is a direct result of PFDA exposure and not a secondary effect caused by decreased food intake and subsequent loss in body weight (Kelling et al., 1987). Similar effects of PFDA on L-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme in rats were also reported by Gutshall et al. (1989). There was no significant difference in glucose-6-phosphate dehydrogenase

activity, hepatic DNA content or protein content. These data indicate that while evidence such as decreased serum T4 in rats exposed to PFDA is suggestive of a lessened thyroid state, the activation of thyroid-sensitive enzymes is increased in rats exposed to PFDA.

Overall, there is uncertainty in the relevance of the mechanistic studies and supplemental information to the thyroid effects observed in rats from the NTP (2018) study. Specifically, the doses from these studies (20–80 mg/kg) are much higher than those used in the 28-day study (0.156–2.5 mg/kg-day) (NTP, 2018) and have been shown to cause overt systemic toxicity including a "wasting syndrome" (refer to Section 3.2.10), which could confound the interpretation of the mechanistic data. Additionally, the mechanistic studies and supplemental information are shorter duration in which rats were exposed to PFDA via i.p. injection rather than gavage as was done in the NTP (2018) study. Furthermore, a data gap exists because there are no mechanistic studies available that determined the effect of PFDA on the activities of deiodinases, which convert fT4 to T3. Data on how PFDA might affect deiodinase activity could inform the mechanism by which PFDA was observed to decrease fT4 while increasing T3 in rats from the NTP (2018) study.

Evidence Integration

There is *indeterminate* evidence of an association between PFDA exposure and endocrine related effects in studies of exposed humans. The evidence is largely null, but there are concerns for study sensitivity. The observed associations are inconsistent across studies and not coherent across thyroid hormones.

There is *indeterminate* animal evidence of endocrine toxicity; specifically, thyroid effects, with PFDA based on incoherent evidence from a single *high* confidence short-term study in rats (a second short-term study examined adrenal effects). PFDA was shown to cause changes in thyroid hormone levels, some of which may be interpreted as suggestive of secondary hypothyroidism, a phenotype characterized by decreased T4 and decreased or normal levels of TSH (Lewiński and Stasiak, 2017); however, the PFDA data are not entirely coherent with such a hypothesis. Specifically, in the NTP (2018) study, significant trends were reported for decreased TSH and fT4 (but not tT4) in male rats at ≥0.312 mg/kg-day, while significant trends were also reported for increased T3 (the latter findings are not coherent with hypothyroidism). Likewise, in females, increased T3 and decreased fT4 was observed at ≥1.25 mg/kg-day. High-dose PFDA exposure-induced decreases in total T4 were consistently observed in multiple, high-dose i.p. studies in rats. The cause of secondary hypothyroidism is although to be due to impaired responsiveness of the HPT axis (Lewiński and Stasiak, 2017). Consistent with this, PFDA was shown to impair the response of the HPT axis to TRH stimulation in rats from a high-dose i.p. study (Gutshall et al., 1989). These data provide mechanistic insight and biological plausibility for how PFDA could decrease serum levels of T4. Furthermore, there was coherence with increased relative thyroid weight and decreased fT4 serum levels at ≥1.25 mg/kg-day in male and female rats. A previous study observed increased relative thyroid weight in a rat model of methimazole-induced hypothyroidism (Soukup et al., 2001). Also, an enlarged thyroid is a symptom of hypothyroidism in

humans (IQEHC, 2014). In support for PFDA-induced changes on thyroid hormone homeostasis, structurally related PFAS compounds (e.g., PFNA; PFOA) have been shown to effect thyroid hormone levels in rodents. However, several aspects of the available animal data decrease the strength or certainty of the evidence informing thyroid effects, which was only available from a single oral exposure study. Whereas the NTP (2018) study reported changes in fT4 and TSH in rats that may indicate secondary hypothyroidism, there was an increase in T3 that cannot be explained. Furthermore, there are no mechanistic studies that determined the effect of PFDA on deiodinase activity that could offer insight on how PFDA decreased fT4 and TSH while increasing T3. Additionally, while fT4 was decreased in male and female rats from the NTP (2018) study, a consistent decrease in tT4 was not observed. However as noted above, fT4 not tT4 is the preferred measure in adult animals. Whereas there was potential coherence between decreased fT4 and increased thyroid weight in rats, it is unclear how thyroid weight and T3 were increased in the absence of increased TSH or histopathological changes. Regarding the lack of PFDA-induced histopathological changes in endocrine tissues, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer study duration.

Uncertainty is also associated with the mechanistic studies and supplemental information. Specifically, inconsistent results were observed for effects on T3 in rats exposed to PFDA via i.p. injection and results from the protein binding studies (Gutshall et al., 1989) suggest that PFDA decreased protein binding of T4, which could result in increased fT4 and decreased tT4, which is not consistent with the results from the NTP (2018) study. The mechanistic database is also limited in that there are no studies that investigated the effects of PFDA on deiodinase activity. Furthermore, the activities of thyroid-sensitive hepatic enzymes (e.g., L-glycerol-3-phosphate dehydrogenase) were increased in rats exposed to PFDA via the i.p. route suggesting that thyroid activity may not be decreased due to PFDA treatment. In general, the interpretation and relevance of the mechanistic studies and supplemental information to thyroid effects observed in the NTP (2018) study is unclear given that these studies used doses that were much higher (i.e., 20–80 mg/kg-day compared with ≤2.5 mg/kg-day) and associated with overt systemic toxicity. Additionally, the mechanistic studies and supplemental information are of shorter duration and rats were exposed to PFDA via i.p. injection rather than gavage as was done in the NTP (2018) study.

In addition to the uncertainty in the available evidence in adults, due to the sparse evidence base available, concern remains for potential susceptible populations to PFDA-induced endocrine effects in susceptible populations including young individuals exposed during gestation, early childhood, and puberty. Importantly, T3 and T4 levels play critical roles in bone growth and brain development (O'Shaughnessy et al., 2019) at these various lifestages. On a related note, it is important to highlight that the ***evidence indicates*** that PFDA exposure is likely to cause developmental toxicity and the ***evidence suggests*** that PFDA exposure might cause neurodevelopmental effects in humans, respectively, given sufficient exposure condition.[3] However,

at the present time few epidemiological studies and no animal toxicological studies have addressed the potential for PFDA-induced effects in these populations. A primary delineating feature between adult animals and developing offspring is that adults have a considerable reserve thyroid hormone capacity whereas developing offspring do not. Thus, there is an elevated concern regarding the potential for decreases in thyroid hormones during developmental lifestages due to the critical endocrine dependency of in utero and neonatal development.

Taken together, there is ***inadequate evidence*** across human, animal, and mechanistic data to determine whether PFDA exposure would cause endocrine effects in humans (see Table 3-42). This conclusion is based on inconsistent evidence from human studies and from a single high confidence rat study investigating PFDA doses ≤2.5 mg/kg-day that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and T4; increased thyroid weight; no histopathology) that cannot be interpreted based on the currently available evidence base.

Table 3-42. Evidence profile table for PFDA exposure and endocrine effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.6: Human Studies)					⊙⊙⊙ ***Evidence inadequate***
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
Thyroid hormones Sixteen *medium* and 5 *low* confidence studies	• Results from studies of thyroid hormones were inconsistent. Most results were null, but study sensitivity was limited, which hinders interpretation. Positive and inverse associations were observed in a few studies, but there was a lack of *consistency* of direction of association across studies.	• No factors noted	• Unexplained inconsistency • Incoherence in direction of association across hormones	⊙⊙⊙ *Indeterminate* While a subset of studies suggests changes in thyroid hormone levels with higher levels of PFDA, there is considerable uncertainty due to inconsistency across studies and endpoints.	*Primary basis:* Single *high* confidence study in rats showing mixed effects on thyroid hormone levels that cannot be reliably interpreted. *Human relevance:* Given the general conservation of thyroid function across rodents and humans, evidence in animals is presumed relevant to humans in the absence of evidence to the contrary. *Cross-stream coherence:* No factors noted. *Susceptible populations and lifestages:* None identified, as a hazard is not supported by the current evidence. *Other inferences:*
Evidence from in vivo animal studies (see Section 3.2.6: Animal Studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
Thyroid hormones One *high* confidence study	• Significantly decreased trend for TSH in males. • Significant increased trend for T3 in males.	• Consistency for decreased fT4 in male and female rats in a high confidence study. • Dose-response gradient for	• Lack of expected coherence across thyroid measures (the pattern of changes is inconsistent with any currently available	⊙⊙⊙ *Indeterminate* There is mixed evidence from a single *high* confidence rat study that reported largely incoherent effects on thyroid hormone	

Evidence stream summary and interpretation					Evidence integration summary judgment
	• Increased T3 in females at ≥1.25 mg/kg-d. • Decreased fT4 was reported at ≥0.312 mg/kg-d in males and at ≥1.25 mg/kg-d in females. • No change in tT4	decreased TSH (males only), decreased fT4 (males and females), and increased T3 (males and females). • Supportive evidence for decreased fT4 from supplemental (mechanistic and i.p.) studies.	understanding of adverse thyroid-related changes) • Unexplained inconsistency across T4 (free and total) measurements	homeostasis and thyroid structure (i.e., increased T3, decreased TSH and fT4, but not tT4; increased thyroid weight; no histopathology) that cannot be reliably interpreted based on the currently available evidence base.	None
Histopathology One *high* confidence study and 1 *medium* confidence study	• No PFDA-induced histopathological changes were observed for the thyroid gland, adrenal cortex and medulla, parathyroid gland, and pituitary gland.	• No factors noted.	• No factors noted		
Organ weights Two *high* confidence studies	• Decreased absolute adrenal gland weight in males at ≥0.156 mg/kg-d and females at 2.5 mg/kg-d (NTP, 2018). • Increased relative adrenal gland weight in males at 2.5 mg/kg-d (NTP, 2018). • Increased absolute thyroid in females at 0.312 to 1.25 mg/kg-d but not at the	• Consistency for increased relative thyroid weight in male and female rats across two high confidence studies. • Dose-response gradient for decreased absolute adrenal gland weight (males and females), increased relative adrenal gland weight (males only), and increased relative	• No factors noted		

Evidence stream summary and interpretation					Evidence integration summary judgment
	highest dose tested (2.5 mg/kg-d) (NTP, 2018). • Increased relative thyroid weight in males at ≥1.25 mg/kg-d and females at ≥0.312 mg/kg-d (NTP, 2018).	thyroid weight (males and females). • Coherence of increased thyroid weight and decreased fT4.			
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways (or other information)	**Primary evidence evaluated Key findings, interpretation, and limitations**		**Evidence stream summary**		
Hypothalamic-pituitary-thyroid axis	*Interpretation:* The results suggest that PFDA may impair the ability of the HPT axis to respond to physiological stimulation. *Key findings:* • Decreased T3 and T4 levels after TRH stimulation in vivo. *Limitations:* one-time i.p. exposure; single study.		The mechanistic and supplementary data provide limited, inconsistent information on how PFDA may be affecting thyroid hormone homeostasis, and the results may be confounded by overt systemic toxicity due to the high doses used in the i.p. studies.		
Plasma protein binding	*Interpretation:* The results suggest that PFDA may impair the binding of thyroid hormones to plasma transport proteins. *Key findings:* • PFDA decreased the plasma protein uptake of T3 and T4. *Limitations:* one-time i.p. exposure; few studies.				
Activity of thyroid-sensitive hormones	*Interpretation:* The data indicate activation of thyroid-sensitive enzymes in a manner that suggests PFDA increases thyroid activity in rats. *Key findings:*				

Evidence stream summary and interpretation			Evidence integration summary judgment
	• PFDA increased the activities of L-glycerol-3-phosphate dehydrogenase, cytosolic lactate dehydrogenase and cytosolic malic enzyme, which are thyroid-sensitive hormones. *Limitations:* one-time i.p. exposure; few studies.		
Binding to thyroid receptor	*Interpretation:* PFDA is capable of binding to the thyroid hormone receptor. *Key findings:* • Under in vitro conditions, PFDA was shown to bind to the human thyroid hormone receptor. PFDA did not exhibit antagonistic or agonistic effects on the thyroid receptor pathway. Limitations: Single study available.		
Other evidence	*Interpretation:* Effects after i.p. injection is consistent with results in orally exposed rats. *Key findings:* • Altered T3 and T4 levels. *Limitations:* Effects on T3 levels were inconsistent among the i.p. studies, which could be due to differences in experimental design and the time at which thyroid hormones were measured		

3.2.9. Urinary Effects

Human Studies

Nine epidemiological studies (14 publications) investigated the relationship between PFDA exposure and urinary effects, including GFR and uric acid (see Figure 3-86). Two studies were considered uninformative due to lack of consideration of potential confounding (Zhang et al., 2019; Seo et al., 2018). The remaining studies were classified as low confidence primarily due to concerns for reverse causality (with potential for bias away from the null). In essence, as described in Watkins et al. (2013), decreased renal function could plausibly lead to higher levels of PFAS (including PFDA) in the blood due to reduced excretion. This hypothesis is supported by data presented by Watkins et al. (2013), although there is some uncertainty in their conclusions due to the use of modeled exposure data as a negative control and the potential for the causal effect to occur in addition to reverse causality. The results least likely to be affected by reverse causality were analyses in two studies stratified by glomerular filtration stage, (Jain, 2019; Zeng et al., 2019c) and one study with a prospective design (Blake et al., 2018).

Three studies (Lin et al., 2020b; Blake et al., 2018; Qin et al., 2016) reported associations between PFDA exposure and impaired renal function (i.e., lower GFR, higher serum uric acid), although only Blake et al. (2018) was statistically significant and the associations in Qin et al. (2016) and Lin et al. (2020b) were limited to one sex (girls in Qin et al. (2016) and men in Lin et al. (2020b)) (see Table 3-43). Conversely, Wang et al. (2019) reported higher GFR and lower odds of chronic kidney disease with higher exposure. The remaining studies reported null associations with renal function, including the studies that stratified by glomerular function stage. Overall, there is unexplained inconsistency in the direction of the association. More importantly, because of the potential for reverse causation for this outcome, there is considerable uncertainty in interpreting the available evidence.

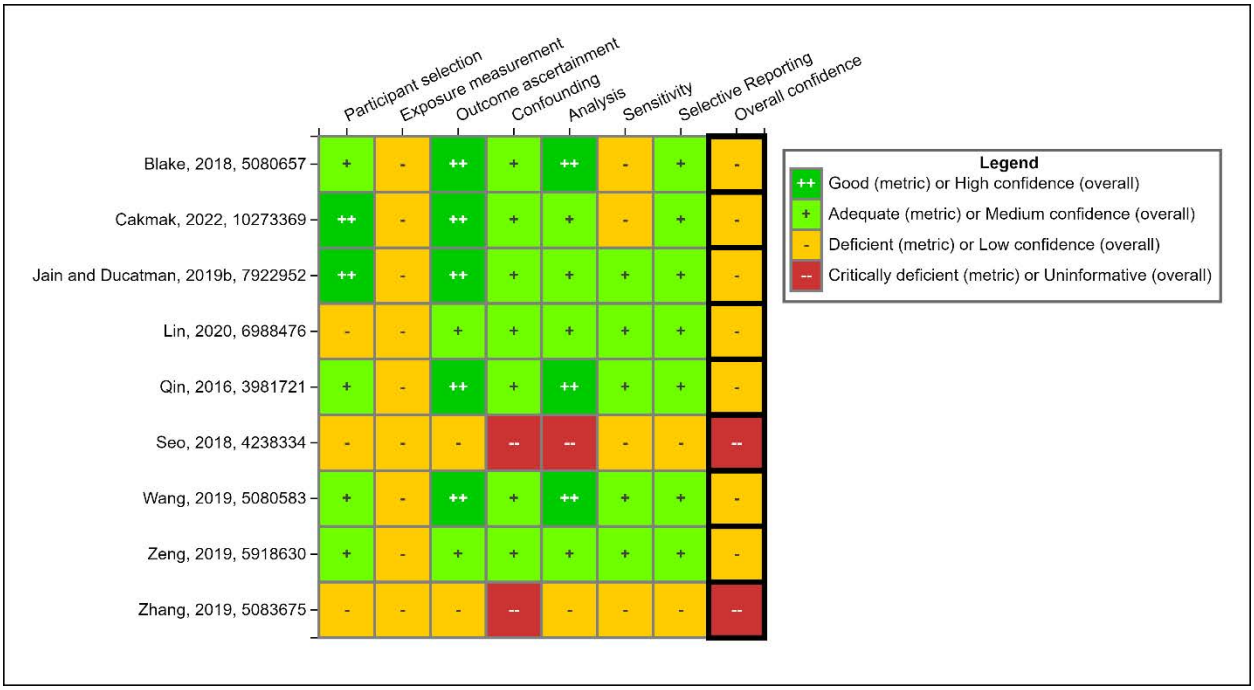


Figure 3-86. Evaluation results for human studies assessing effects of PFDA exposure on urinary effects. Refer to HAWC Human Urinary Effects for details on the study evaluation review.

Table 3-43. Associations between serum PFDA and urinary effects in *low* confidence epidemiological studies

Reference	Population	Median exposure (IQR) (ng/mL)	Result			
Glomerular filtration rate						
Blake et al. (2018)	Prospective cohort of residents near a uranium processing site (1990–2008); U.S.; 210 adults	0.1 (0.1–0.2)	Percent change (95% CI) in eGFR per IQR change in PFDA **−2.2 (−4.3, −0.1) ***			
Jain (2019)	Cross-sectional study (NHANES) (2007–2014); U.S.; 4,057 adults	0.2 in GF-1 group	Adjusted geometric mean (95% CI) by glomerular function stage			
			GF stage	All participants	Men	Women
			GF-1	0.25 (0.24, 0.26)	0.26 (0.25, 0.28)	0.23 (0.22, 0.24)
			GF-2	0.27 (0.25, 0.29)	0.28 (0.26, 0.31)	0.26 (0.24, 0.28)
			GF-3A	0.33 (0.26, 0.43)	0.31 (0.25, 0.38)	0.37 (0.35, 0.39)
			GF-3B/4		0.21 (0.21, 0.22)	0.24 (0.19, 0.31)

Reference	Population	Median exposure (IQR) (ng/mL)	Result			
				0.23 (0.19, 0.28)		
Wang et al. (2019)	Cross-sectional study (2015–2016); China; 1,612 adults	0.9 (0.5, 1.5)	Mean change (95% CI) in eGFR per ln-unit change in PFDA **1.04 (0.27, 1.81)***			
Uric acid						
Scinicariello et al. (2020)	Cross-sectional study (NHANES) (2009–2014); U.S.; 4,917 adults	Mean (SD) 0.2 (0.01)	β (95% CI) in serum uric acid for quartiles vs. Q1 Without chronic kidney disease Q2: 0.00 (−0.09, 0.10) Q3: −0.05 (−0.17, 0.07) Q4: 0.12 (0.00, 0.24) With chronic kidney disease Q2: 0.34 (−0.03, 0.72) Q3: 0.19 (−0.13, 0.52) Q4: 0.26 (−0.09, 0.61)			
			OR (95% CI) in hyperuricemia for quartiles vs. Q1 Without chronic kidney disease Q2: 0.94 (0.66, 1.34) Q3: 0.86 (0.57, 1.25) Q4: 1.30 (0.94, 1.80) With chronic kidney disease Q2: 1.32 (0.66, 2.65) Q3: 0.98 (0.60, 1.61) Q4: 1.26 (0.64, 2.46)			
Zeng et al. (2019c)	Cross-sectional study (2015–2016); China; 384 adults	0.9 (0.5–1.5)	Mean difference per log-unit change in PFDA 0.01 (−0.06, 0.08)			
Qin et al. (2016)	Cross-sectional study (2009–2010); Taiwan; 225 children and adolescents (mean age: 13.6 yr)	0.9 (0.8–1.2)	Mean change (95% CI) in serum uric acid per ln-unit change in PFDA			
			All participants 0.08 (−0.11, 0.28)	Boys 0.05 (−0.23, 0.34)	Girls 0.18 (−0.09, 0.46)	
			OR (95% CI) for high uric acid per quartile change in PFDA			
			1.3 (0.8, 1.9)	1.0 (0.6, 1.7)	1.8 (0.9, 3.7)	
Lin et al. (2020b)	Cross-sectional study (2016-2017); Taiwan; 397 older adults (55–75 yr)	1.6 (1.2–2.4)	β (95% CI) in serum uric acid for quartiles vs. Q1			
			All participants NR	Men Q2: 0.31 (−0.38, 0.99) Q3: 0.68 (−0.02, 1.37)	Women Q2: −0.09 (−0.45, 0.27) Q3: −0.1 (−0.02, 1.37)	

Reference	Population	Median exposure (IQR) (ng/mL)	Result		
				Q4: 0.68 (−0.04, 1.4)	Q4: −0.18 (−0.54, 0.19)
Creatinine					
Cakmak et al. (2022)	Cross-sectional study (2007-2017); Canada; 6,045 adults	Mean 0.2	% change per 1 mean increase in PFDA −1.5 (−3.7, 0.7)		
Chronic kidney disease					
Wang et al. (2019)	Cross-sectional study (2015–2016); China; 1,612 adults	0.9 (0.5, 1.5)	OR (95% CI) for chronic kidney disease per ln-unit change in PFDA **0.7 (0.6, 0.9)***		

*$p < 0.05$.

Animal Studies

Two 28-day studies using B6C3F1/N mice and/or SD rats are available to examine effects relevant to the evaluation of urinary system toxicity after PFDA exposure (Frawley et al., 2018; NTP, 2018). The studies reported on histopathology, serum biomarkers of effect and organ weights. Overall study confidence was high for most endpoints evaluated in these studies with the exception of histopathology in Frawley et al. (2018), which had incomplete reporting of null data (results were only discussed qualitatively) resulting in a medium confidence rating (see Figure 3-87).

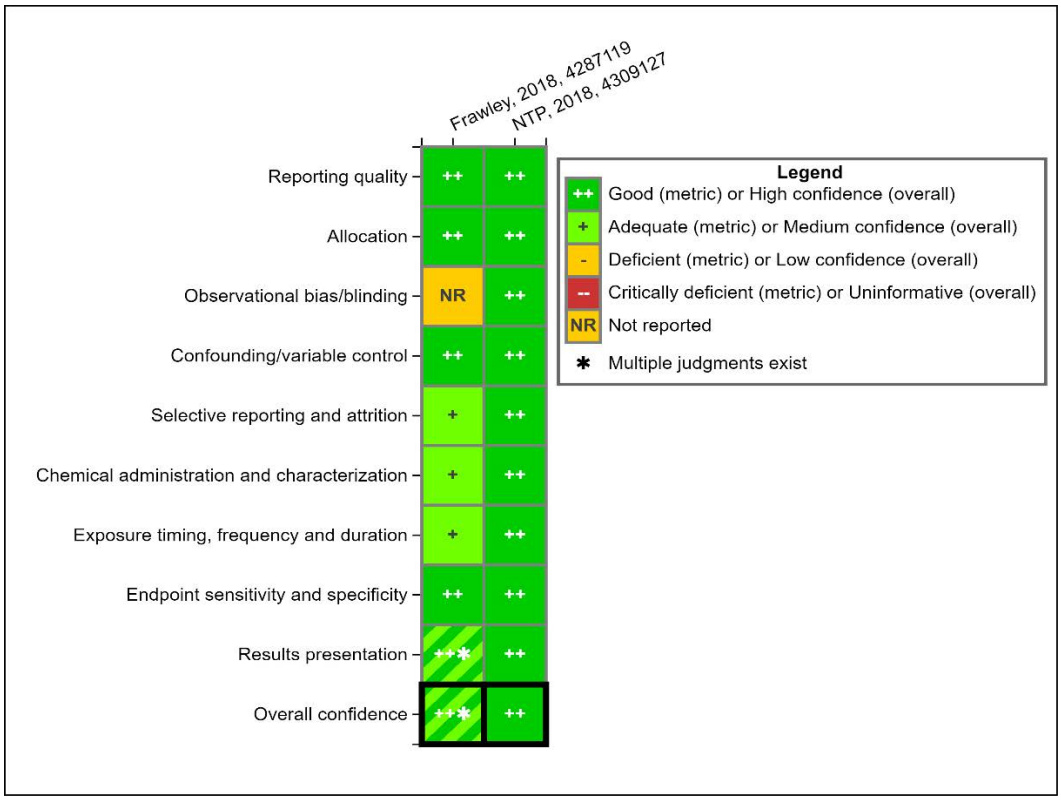


Figure 3-87. Evaluation results for animal studies assessing effects of PFDA exposure on urinary effects. Refer to HAWC for details on the study evaluation review.

Histopathology

The kidney and urinary bladder were evaluated for histopathology across a *high* confidence (NTP, 2018) and a *medium* confidence study (Frawley et al., 2018) in rats exposed for 28 days (see Figure 3-88). NTP (2018) found no evidence of histopathological lesions in the urinary bladder of males and females at the only dose examined (2.5 mg/kg-day). Chronic progressive nephropathy (CPN) graded as minimal occurred in the kidneys of nearly all dose groups, including controls, in this study (NTP, 2018) (see Figure 3-88). A reduction in the incidence of CPN was noted in males and females at the highest dose tested (0% and 30% incidence at 2.5 mg/kg-day in females and males respectively compared with 60% in controls) (NTP, 2018); but there was no clear dose-response effect and incidences were in some instances increased at doses lower than 2.5 mg/kg-day (i.e., 0.156–1.25 mg/kg-day) in both sexes compared with controls. The other 28-day gavage study reported no effects in kidney histopathology in female rats up to doses of 0.5 mg/kg-day (Frawley et al., 2018). Taken together, the high-dose decrease in CPN incidence in rats in one study is not interpreted as biologically significant, and overall, the histopathology data were considered null.

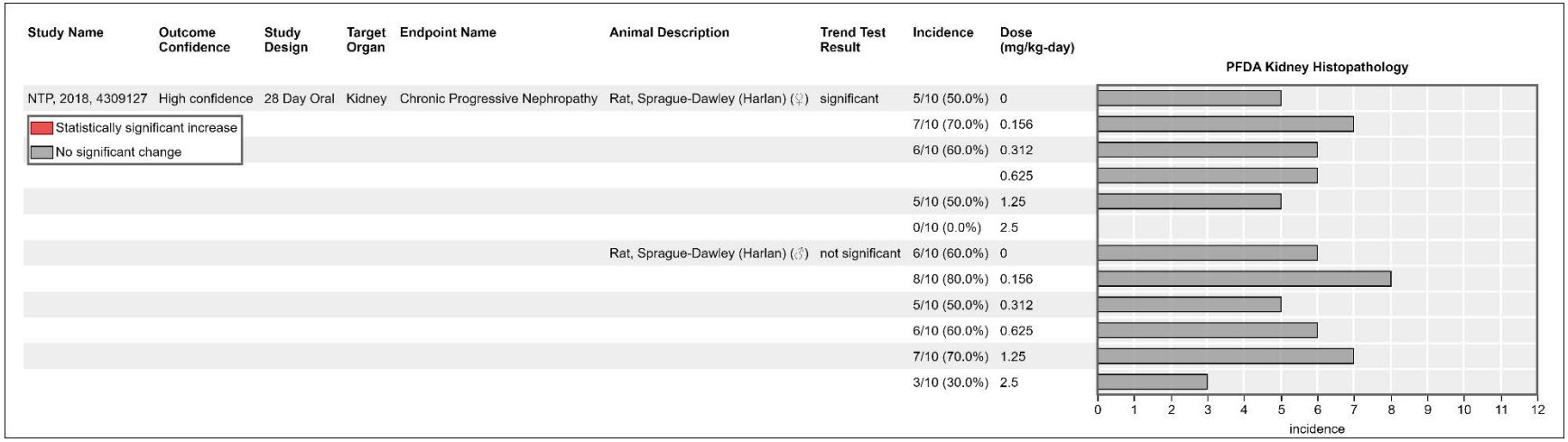


Figure 3-88. Kidney histopathology effects following exposure to PFDA in 28-day rat study. (Results can be viewed by clicking the HAWC link.)

Serum biomarkers

Serum biomarkers of kidney injury and/or function, namely blood urea nitrogen (BUN) and creatinine were measured in rats in one *high* confidence study (NTP, 2018) (see Table 3-44 and Figure 3-89). Creatinine is a waste product of creatine metabolism produced in muscle tissue and BUN is a waste product of protein metabolism in the liver. Both creatinine and BUN are removed from the blood by the kidneys and often used as indicators of kidney function. Dose-related increases in circulating BUN levels occurred in males and females, most notably at 1.25 and 2.5 mg/kg-day (25%–50% compared with controls). In contrast, a significant downward trend was reported for creatinine levels, reaching 4–11% decrease compared with controls at ≥1.25 mg/kg-day. The decreases in creatinine levels were accompanied by significant decreases in glucose levels at similar doses (31%–51% compared with controls; data not shown in Table 3-44 or Figure 3-89) and likely reflect the marked systemic toxicity associated with high-dose PFDA exposure (see Section 3.2.10 for more details) (NTP, 2018).

Table 3-44. Percent change relative to controls in serum biomarkers of kidney function in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Blood urea nitrogen (BUN)					
Male SD rats	−9	−13	5	**25**	**25**
Female SD rats	4	−2	11	**38**	**50**
Creatinine					
Male SD rats	0	4	−8	**−11**	**−11**
Female SD rats	−4	−5	−3	−4	**−10**

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors.

Organ weight

Absolute and relative kidney weights were measured in the two 28-day gavage studies using mice and/or rats (Frawley et al., 2018; NTP, 2018). There are some uncertainties surrounding the most toxicologically relevant organ weight metric so both absolute and relative kidney weights were evaluated herein (Craig et al., 2015; Bailey et al., 2004) (see Table 3-45 and Figure 3-89). Absolute and relative kidney weights of female rats displayed a positive trend, reporting increases of up to 11% and 13%, respectively, at a dose of 0.5 mg/kg-day in 1 of 2 study cohorts exposed to similar experimental conditions (Frawley et al., 2018). Kidney weights (absolute and relative) increased in response to PFDA exposure in the second study cohort, but the changes were relatively small (0%–5%) and a dose-related trend was not established. No appreciable body weight changes were reported in this study up to the highest dose tested (0.5 mg/kg-day) (Frawley et al., 2018). A

separate study observed significant increases in relative kidney weight of 12%–45% compared with controls in male and female rats at doses ≥0.625 mg/kg-day (NTP, 2018). Conversely, absolute kidney weight increased significantly in females by 9% and 15% at 0.312 and 0.625 mg/kg-day, respectively, but decreases were observed in both males and females at 2.5 mg/kg-day (10% and 15% from controls, respectively) (NTP, 2018). The apparent decreases in absolute kidney weight at higher doses may be associated with concurrent reductions in body weight occurring in the exposed animals (up 38% compared with controls at 2.5 mg/kg-day) (see Section 3.2.10 for more details) (NTP, 2018). In mice, kidney weights were mostly unchanged by PFDA treatment (0.045–0.71 mg/kg-day) (Frawley et al., 2018). In addition to the uncertainties due to confounding effects with decreased body weight at the highest PFDA doses (≥1.25 mg/kg-day), the observed kidney weight changes in rats are not supported by significant histopathological findings in these animals (Frawley et al., 2018; NTP, 2018).

Table 3-45. Percent change relative to controls in kidney weights (absolute and relative to body weight) due to PFDA exposure in short-term oral toxicity studies

Animal group	Dose (mg/kg-d)						
	0.045	0.089	0.125–0.179	0.25–0.36	0.5–0.71	1.25	2.5
Absolute kidney weight							
28 d; female SD rats –Histopathology cohort Frawley et al. (2018)			6	6	**11**		
28 d; female SD rats – MPS cohort Frawley et al. (2018)			2	2	5		
28 d; female SD rats NTP (2018)			6	**9**	**15**	6	**−15**
28 d; male SD rats NTP (2018)			5	−1	8	−2	**−10**
28 d; female B6C3F1/N mice Frawley et al. (2018)	1	9	1	−1	−3		
Relative kidney weight							
28 d; female SD rats –Histopathology cohort Frawley et al. (2018)			7	**9**	**13**		
28 d; female SD rats – MPS cohort Frawley et al. (2018)			3	0	4		
28 d; female SD rats NTP (2018)			2	5	**15**	**20**	**34**
28 d; male SD rats NTP (2018)			2	0	**12**	**24**	**45**
28 d; female B6C3F1/N mice Frawley et al. (2018)	−2	1	2	−5	−7		

Bold values indicate instances for which statistical significance ($p < 0.05$) compared with controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Effect	Endpoint Name	Organ	Study Name	Outcome Confidence	Experiment Name	Species, Strain (sex)	Trend Test Result
Clinical Chemistry	Blood Urea Nitrogen (BUN)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Creatinine (CREAT)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Histopathology	Chronic Progressive Nephropathy	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Histopathology	Kidney	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Urinary Bladder Histopathology	Bladder	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not applicable
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
Organ Weight	Kidney Weight, Absolute (Histophatology Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Absolute (MPS Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Right Kidney Weight, Absolute	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Kidney Weight, Absolute (Hematology Study)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
	Kidney Weight, Relative (Histopathology Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Relative (MPS Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Right Kidney Weight, Relative	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Relative (Hematology Study)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant

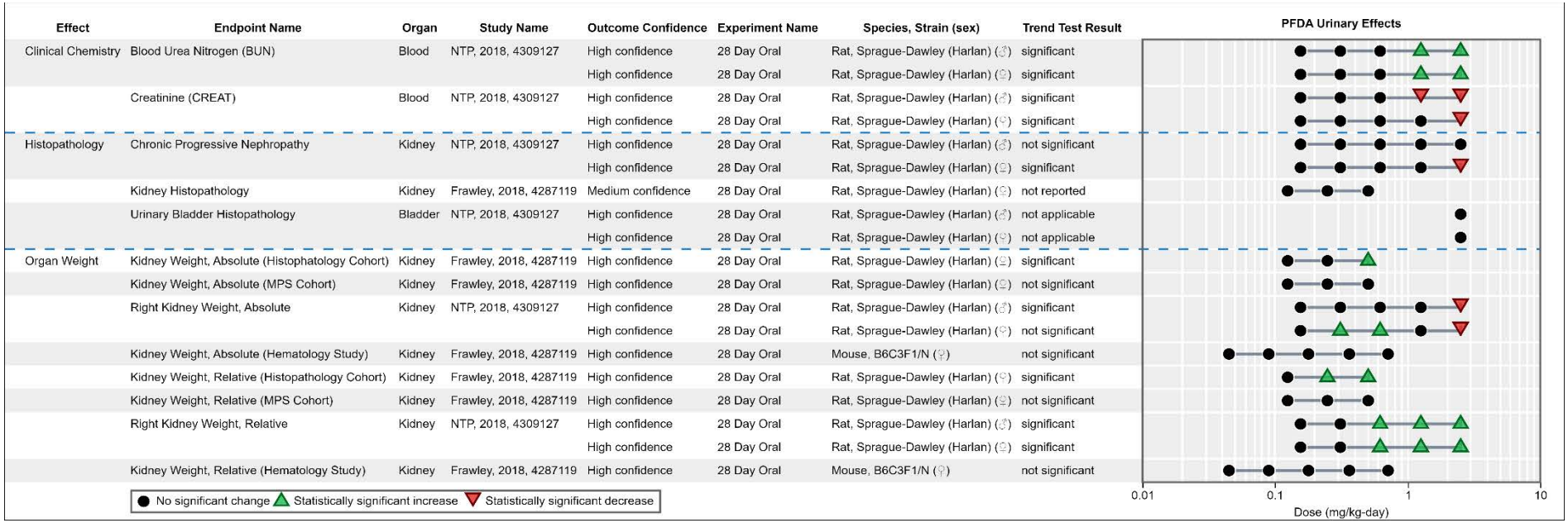


Figure 3-89. Urinary effects following exposure to PFDA in short-term oral studies in animals. (Results can be viewed by clicking the HAWC link.)

Evidence Integration

The evidence for potential urinary system effects in humans is considered *indeterminate.* Associations between PFDA exposure and impaired renal function were reported in two *low* confidence epidemiological studies. However, there is considerable uncertainty in the interpretation of these findings due to the potential for reverse causation and some unexplained inconsistency in the direction of association across studies.

The evidence for potential urinary system effects in experimental animals is limited to two *high/medium* confidence studies using mice and/or rats exposed for 28 days (Frawley et al., 2018; NTP, 2018) {-*}. Although alterations in BUN and creatine levels were observed at ≥1.25 mg/kg-day in rats, there is no coherent pattern of effects (BUN levels increased and creatinine levels decreased) or supportive information (i.e., histopathology) to determine the toxicological relevance of the changes that occurred (NTP, 2018). Histopathological examinations of rat kidney and urinary bladder were mostly unremarkable across two studies (Frawley et al., 2018; NTP, 2018). Finally, the interpretation of the absolute and relative kidney weight changes in rats at doses ≥0.312 mg/kg-day is complicated by the lack of coherent histopathological findings (Frawley et al., 2018; NTP, 2018), inconsistencies in the direction of changes across experiments, and confounding effects from significant body weight reductions at the highest doses tested (≥1.25 mg/kg-day) (NTP, 2018). In summary, the sparse and uncertain evidence from animal studies is considered *indeterminate*. The absence of any long-term studies (subchronic/chronic) via the oral route or other relevant routes of exposure increases uncertainty in the evaluation of potential urinary system toxicity in animals following PFDA exposure.

Altogether, based on the available human and animal studies, there is ***inadequate evidence*** to assess whether PFDA exposure can cause urinary system effects in humans (see Table 3-46).

Table 3-46. Evidence profile table for PFDA exposure and urinary effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.8: Human Studies)					⊙⊙⊙ ***Evidence inadequate*** *Primary basis:* Evidence from epidemiological studies and experimental animals is *indeterminate*. *Human relevance, cross-stream coherence, susceptibility, and other inferences:* No specific factors are noted.
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
Seven *low* confidence studies	• Three studies reported some associations between PFDA exposure and impaired renal function (i.e., lower GFR or higher serum uric acid). • One study reported associations in the opposite direction and three others were null	• No factors noted	• *Low* confidence studies due to potential for reverse causality • Unexplained inconsistency	⊙⊙⊙ *Indeterminate* There is some evidence of urinary effects with PFDA exposure across two *low* confidence studies but considerable concerns for reverse causality and inconsistency.	
Evidence from in vivo animal studies (see Section 3.2.8: Animal Studies)					
<u>Histopathology</u> One *high and 1 medium* confidence studies in rats for 28 d	• Mostly null findings for kidney and urinary bladder histopathology in rats up to 2.5 mg/kg-d across 2 studies. • A high-dose (2.5 mg/kg-d) decrease in the incidence of CPN in rats reported in one study was not interpreted as biologically significant.	• No factors noted	• No factors noted	⊙⊙⊙ *Indeterminate* Lack of coherent effects in *high* and *medium* confidence studies in rats and mice exposed up to 2.5 mg/kg-d for 28 d.	

Evidence stream summary and interpretation					Evidence integration summary judgment
<u>Serum biomarkers</u> One *high* confidence study in rats for 28 d	• Increased BUN levels and decreased creatinine levels in rat serum at ≥1.25 mg/kg-d (alterations in creatinine levels coincide with body weight reductions)	• *High* confidence study	• *Lack of expected coherence* in the directionality of BUN and creatinine changes • Potential confounding by body weight decreases		
<u>Organ weight</u> Two *high* confidence studies (encompassing 4 experiments) in mice and/or rats for 28 d	• Absolute and relative kidney weight changes in rats at doses ≥0.312 mg/kg-d (directionality of effects varied across experiments and organ weight measures); no effects in mice up to 0.71 mg/kg-d	• *High* confidence studies	• *Unexplained inconsistency* across experiments, species, and organ weight measures		

3.2.10. General Toxicity

The potential for PFDA exposure-induced general toxicity is specifically discussed given that PFDA has been shown to cause a "wasting syndrome" in rodents, which is characterized by decreased food intake and reduced body weight (Goecke-Flora et al., 1995). In animals, decreased body weights can be indicative of nonspecific overt toxicity and some effects that occur at doses associated with this and other frank effects should be interpreted cautiously when drawing conclusions about organ/system-specific hazards. Thus, this section informs judgments drawn for other potential health hazards, but a specific evidence integration judgment is not drawn.

Human Studies

No human studies were available to inform the potential for PFDA exposure to cause general toxicity.

Animal Studies

Animal toxicity studies reporting general toxicity with repeated dose exposure to PFDA include two 28-day gavage studies, four dietary exposure studies (7–14 days) in mice and/or rats, and two drinking water studies (12–49 days) in mice. The endpoints measured in these studies include body weight (Li et al., 2022; Wang et al., 2020; Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Takagi et al., 1992, 1991), clinical observations (NTP, 2018), and survival (Wang et al., 2020; NTP, 2018) (see Figure 3-90). Three studies (Li et al., 2022; Frawley et al., 2018; NTP, 2018) were evaluated as *high* confidence for all general toxicity endpoints tested (see Figure 3-90). Four studies (Wang et al., 2020; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992) were evaluated as *medium* confidence for all general toxicity endpoints tested while the Takagi et al. (1991) study was evaluated as *low* confidence for the body weight endpoint (see Figure 3-90). Key issues regarding study quality evaluation in the *medium* and *low* confidence studies were related to exposure sensitivity (no analytical verification methods or quantitative data on food consumption).

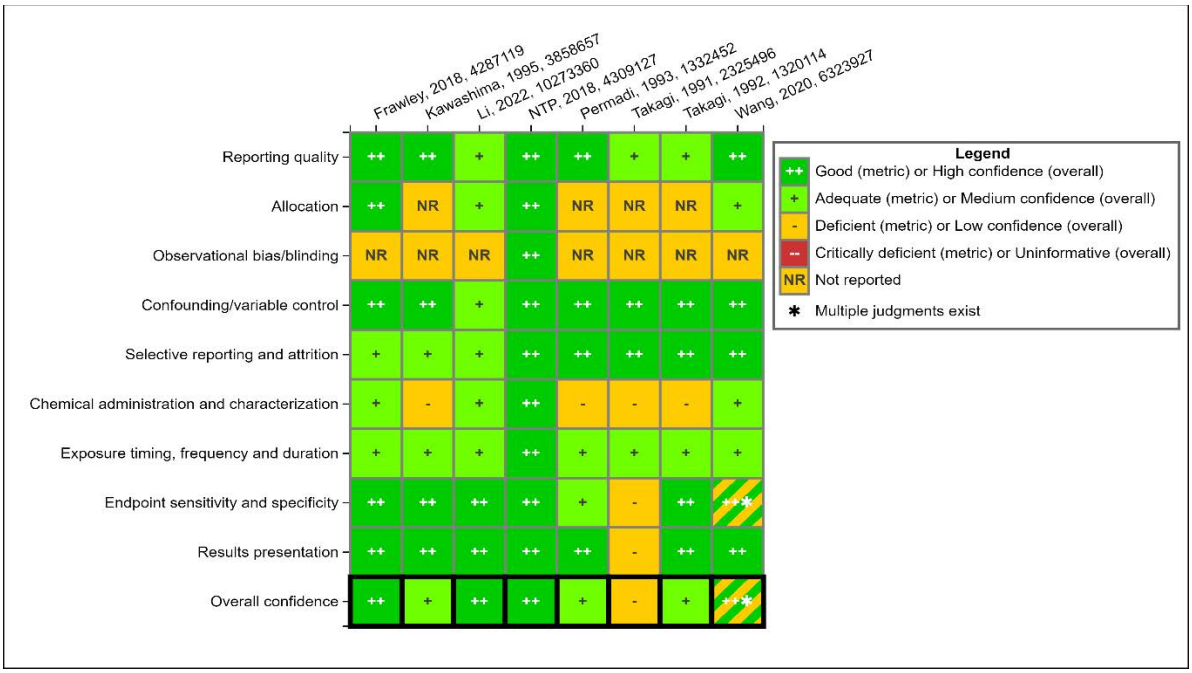


Figure 3-90. Evaluation results for animal studies assessing effects of PFDA exposure on general toxicity. Refer to HAWC for details on the study evaluation review.

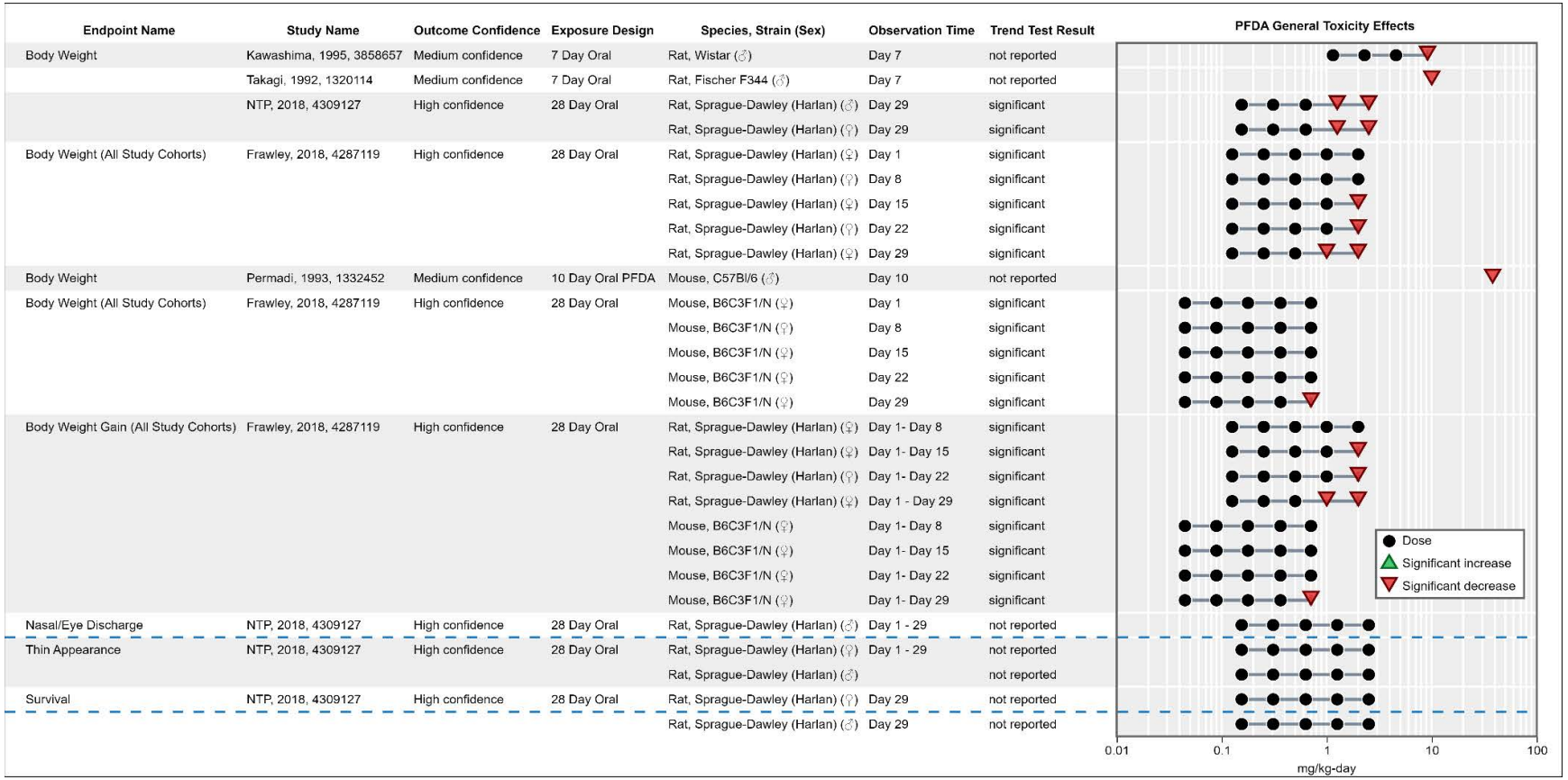
Body weight

PFDA-induced body weight suppression was observed to be dose-dependent in short-term animal studies in rats (Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Takagi et al., 1992, 1991) and mice (Li et al., 2022; Wang et al., 2020; Frawley et al., 2018; Permadi et al., 1993) (see Figure 3-91). In rats treated with doses ranging from 1.0 to 10 mg/kg-day, reductions in mean body weight and body weight gain ranged from 4% to 38% and from 21% to 103%, respectively, compared with controls. In mice, changes in body weight were less than 5% at doses ≤0.71 mg/kg-day but decreases reached 53% at 6.6 mg/kg-day. In the 28-day high confidence study that included multiple study cohorts (Frawley et al., 2018), the study authors reported that 2 of 88 rats in the 2.0 mg/kg-day exposure group were euthanized due to marked reductions in body weight (>20%) occurring within the first 5 days of the study initiation (Frawley et al., 2018). This evidence of PFDA-induced acute toxicity was also observed in several single intraperitoneal (i.p.) injection studies as discussed below. Furthermore, PFDA-induced decreased body weight in female rats was more severe with longer treatment durations (Frawley et al., 2018). For example, body weight was decreased by 4% at day 15, by 13% at day 22, and 22% at day 29 at 2.0 mg/kg-day. Also, in this study, reduced body weight was observed to be more sensitive to dose at day 29 compared with earlier time points (statistically significant at 1.0 mg/kg-day on day 29 compared with 2.0 mg/kg-day for days 15 and 22). The NTP (2018) study also showed similar results for multiple time point data for body weight. For example, in male rats treated with the highest dose (2.5 mg/kg-day), body weight was decreased by 13%, 27%, and 38% on day 15, day 22, and day 29,

respectively. The possible contribution of decreased food consumption to decreased body weight is unknown in some of these studies because food consumption was not measured.

Clinical observations and survival

Clinical observations and survival data are available from a *high* confidence gavage study in SD rats exposed for 28 days (NTP, 2018). Additionally, a *medium* confidence study reported effects on survival in male CD-1 mice exposed to PFDA in the drinking water for 49 days (Wang et al., 2020). PFDA exposure was associated (albeit not statistically significant) with thin appearance in male and female SD rats at the highest exposure dose tested (2.5 mg/kg-day) (see Figure 3-91). The incidence rate was 30% in males and 10% in females compared with 0% for the corresponding controls. Nasal/eye discharge was observed in 1 out 10 male rats in the control, 0.156, 0.0625, 1.25 and 2.5 mg/kg-day exposure groups. No other clinical observations were reported. All exposed animals survived and were euthanized at study termination. In summary, 28-day gavage exposure to PFDA caused mild clinical symptoms in rats (thin appearance) but had no effect on survival in this study. However as discussed above, Frawley et al. (2018) reported that two (of 88) rats were euthanized due to severe weight loss caused by 5 days of exposure to PFDA at 2.0 mg/kg-day. In mice exposed to PFDA for up to 49 days, the mortality rate was reported to be significantly increased at 6.6 mg/kg-day (Wang et al., 2020).



Endpoint Name	Study Name	Outcome Confidence	Exposure Design	Species, Strain (Sex)	Observation Time	Trend Test Result
Body Weight	Kawashima, 1995, 3858657	Medium confidence	7 Day Oral	Rat, Wistar (♂)	Day 7	not reported
	Takagi, 1992, 1320114	Medium confidence	7 Day Oral	Rat, Fischer F344 (♂)	Day 7	not reported
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	Day 29	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 29	significant
Body Weight (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 8	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 15	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 22	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 29	significant
Body Weight	Permadi, 1993, 1332452	Medium confidence	10 Day Oral PFDA	Mouse, C57Bl/6 (♂)	Day 10	not reported
Body Weight (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	Day 1	significant
				Mouse, B6C3F1/N (♀)	Day 8	significant
				Mouse, B6C3F1/N (♀)	Day 15	significant
				Mouse, B6C3F1/N (♀)	Day 22	significant
				Mouse, B6C3F1/N (♀)	Day 29	significant
Body Weight Gain (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 8	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 15	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 22	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1 - Day 29	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 8	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 15	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 22	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 29	significant
Nasal/Eye Discharge	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	Day 1 - 29	not reported
Thin Appearance	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1 - 29	not reported
				Rat, Sprague-Dawley (Harlan) (♂)		not reported
Survival	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 29	not reported
				Rat, Sprague-Dawley (Harlan) (♂)	Day 29	not reported

Figure 3-91. PFDA general toxicity effects. (Results can be viewed by clicking the HAWC link.)

Mechanistic Studies and Supplemental Information

Several intraperitoneal (i.p.) studies using a single injection, have demonstrated that PFDA induces a "wasting syndrome" in rodents, which is characterized by decreased food intake and reduced body weight (Goecke-Flora et al., 1995). In these studies, decreased body weight (5 to 72% compared with controls or pretreatment values) was observed in rats at doses ranging from 20 to 100 mg/kg PFDA (Unkila et al., 1992; Bookstaff et al., 1990; Chen et al., 1990; Ylinen and Auriola, 1990; Gutshall et al., 1988; Van Rafelghem and Andersen, 1988; Van Rafelghem et al., 1988a; Kelling et al., 1987; Langley and Pilcher, 1985; Olson and Andersen, 1983). Generally, across rodent species, i.p. injection of PFDA at doses ≥20 mg/kg-day, even acutely, caused generalized acute toxicity. Whereas significant decreases in food intake were also observed in rats at 40 to 80 mg/kg, body weights were reduced compared with both ad libitum and pair-fed controls suggesting that PFDA decreased body weight is not only related to reduced food intake but also a direct effect of PFDA on body weight. In guinea pigs, body weight gain (32% decrease) and food intake (11% decrease) were significantly reduced at 20 mg/kg PFDA via the i.p. route (Chinje et al., 1994). In a study that tested multiple species, rats lost a maximum of 45% of their pretreatment body weight at 50 mg/kg PFDA, hamsters lost 26% at 50 mg/kg and 41% at 100 mg/kg, and mice lost 25% at 150 mg/kg (Van Rafelghem et al., 1987b). Multiple other i.p. studies reported effects on body weight and food intake, but the data were presented qualitatively or graphically, and percent changes were not calculated. Doses for these studies ranged from 10 to 100 mg/kg (Kudo and Kawashima, 2003; Wilson et al., 1995; Chen et al., 1994; Glauert et al., 1992; Arand et al., 1991; Powers and Aust, 1986). Most of the studies described here used a single injection of PFDA, highlighting the acute toxicity and rapid weight loss caused by PFDA treatment. It is important to note that the doses used in the mechanistic/supplemental studies are much higher than the doses in which body weight was decreased in some of the toxicity studies. For example, decreases in body weight interpreted as biologically significant were observed in rats at ≥1.25 mg/kg-day from the NTP (2018) study.

Summary of Animal and Mechanistic/Supplemental Information

The available studies for PFDA-induced general toxicity were mostly *high* and *medium* confidence (see Figure 3-90) and evaluated endpoints related to general toxicity (body weight, clinical observations, and survival) in multiple strains (SD, Wistar, Fisher F344, C57BL/6N, and B6C3F1/N) of male and female rats and mice via gavage and dietary exposure for up to 28 days (Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991). Reduced body weight was consistently observed in all available animal studies, with biologically significant effects occurring at doses as low as 1.25 mg/kg-day in rats from the NTP (2018) study. The consistent effect of PFDA on body weight that appears to be time- and dose-related coupled with clinical observations (i.e., thin appearance) in rats provide support for PFDA-induced general toxicity. Furthermore, multiple acute i.p. studies across different species reported

decreased body weight indicative of "wasting syndrome" at doses ranging from 20 to 100 mg/kg, but primarily at ≥40 mg/kg-day.

3.2.11. Other Health Effects

Short-term oral exposure studies (*high/medium* confidence) in experimental animals evaluated potential health effects related to the hematological, respiratory, digestive, dermal, musculoskeletal, and adult nervous system (please see Section 3.2.7 for the synthesis of evidence on Neurodevelopmental Effects). The available evidence from these animal studies is briefly summarized below. Given the limitations of the evidence base and the lack of consistent or coherent effects of PFDA exposure, there is ***inadequate evidence*** to determine whether any of the evaluated outcomes below might represent potential human health hazards of PFDA exposure. Additional studies on these health effects could modify these interpretations.

Animal Studies

Other health effects

Hematological parameters (see HAWC data visualization for PFDA hematological effects) were evaluated across two studies using B6C3F1/N mice and/or SD rats with gavage exposure for 28-days (Frawley et al., 2018; NTP, 2018). No significant effects were found in mice up to 0.71 mg/kg-day (Frawley et al., 2018). In rats, mean corpuscular hemoglobin (amount of hemoglobin per red blood cell [RBC]; MCH) and mean corpuscular hemoglobin concentration (amount of hemoglobin per unit of RBC volume; MCHC) decreased at the two highest doses (0.25 and 0.5 mg/kg-day) in one study (Frawley et al., 2018); however, the changes did not show a dose-response gradient and were relatively small (6%–7% compared with controls). In the other rat study, a significant dose-related trend was reported for several hematological parameters (NTP, 2018). Erythrocyte (RBCs) counts increased (9%–23%) in males and females and hematocrit (proportion of RBCs in blood; 6%–16%) and hemoglobin (7%–19%) concentrations increased in females only at doses ≥1.25 mg/kg-day. These changes were accompanied by decreases in reticulocyte counts (immature RBCs) of 54%–91%, and slight decreases in mean corpuscular volume (average volume of RBCs; decreases of 3%–7%) and MCH (4%) and slight increases in MCHC (2%–4%) in males and females at similar doses. In addition, the platelet count in females decreased by up to 30% in females at the highest dose group, 2.5 mg/kg-day. In summary, although there is some potential evidence of hematological effects in rats with PFDA exposure (NTP, 2018), the observed changes occurred mostly in the presence of significant systemic toxicity (i.e., reduced body weights at ≥2.5 mg/kg-day), which limits the interpretation of the findings.

Histopathology of the dermal, musculoskeletal, nervous, and special senses (eye and harderian gland) systems was examined in the control and 2.5 mg/kg-day dose groups in adult SD rats in one 28-day study that reported null findings (NTP, 2018). The digestive and respiratory systems were examined histologically in SD rats across two, 28-days studies (Frawley et al., 2018;

NTP, 2018). No lesions were identified in stomach or lungs of rats at doses of 0.125–0.5 mg/kg in one study (Frawley et al., 2018). The second study found lesions in the esophagus, forestomach, lungs, and nose of exposed rats (NTP, 2018) (see HAWC data visualization for PFDA Digestive and Respiratory Histopathology). Increased incidence of forestomach lesions (epithelium hyperplasia, inflammation, and ulcer) was reported in males and inflammation was reported in the lungs and esophagus of females. The incidence rates for these lesions were low (10%–20%) and restricted to the highest dose group (2.5 mg/kg-day). The nose lesions (epithelium degeneration, hyperplasia, and chronic inflammation) were increased in both males and females (10%–50% incidence) across 0.158–2.5 mg/kg-day, but there was no clear dose-response relationship, and these morphological changes were also observed in the control group (0%–20% incidence). Overall, the limited information available for these organ systems impedes further evaluation of the biological significance of the histopathological results.

3.3. CARCINOGENICITY

3.3.1. Cancer

Human Studies

Eight studies evaluated the risks of cancer associated with exposures to PFDA (Velarde et al., 2022; Liu et al., 2021b; Omoike et al., 2021; Lin et al., 2020a; Tsai et al., 2020; Wielsøe et al., 2017; Christensen et al., 2016; Hardell et al., 2014) (Figure 3-92). Five cancer studies by (Velarde et al., 2022; Omoike et al., 2021; Lin et al., 2020a; Wielsøe et al., 2017; Christensen et al., 2016) were evaluated as *Uninformative* (Figure 3-92).

The study of risks of prostate cancer (Hardell et al., 2014) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant period, potential for confounding, insufficiencies in the analysis, and concerns about sensitivity (see Figure 3-92). Hardell et al. (2014) reported a nonsignificantly increased risk of prostate cancer among men with PFDA concentrations in blood that were above the median value. The study of risks of thyroid cancer (Liu et al., 2021b) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant period, deficiencies regarding the outcome definition, and potential for confounding, (see Figure 3-92). Liu et al. (2021b) reported significantly decreased risk of thyroid cancer associated with increasing quartiles of PFDA. The study of risks of breast cancer (Tsai et al., 2020) was *low* confidence because of concern about the exposure measurement not representing the etiologically relevant period, potential for confounding, and concerns about low sensitivity (see Figure 3-92). Tsai et al. (2020) reported nonsignificantly increased risk of breast cancer per ln-transformed unit increase in PFDA concentration in blood among women ≤50 years of age; and nonsignificantly decreased risk of breast cancer per ln-transformed unit increase in PFDA concentration in blood among women >50 years of age. In

summary, the available epidemiologic evidence on PFDA and the risk of cancer is limited and generally *uninformative*.

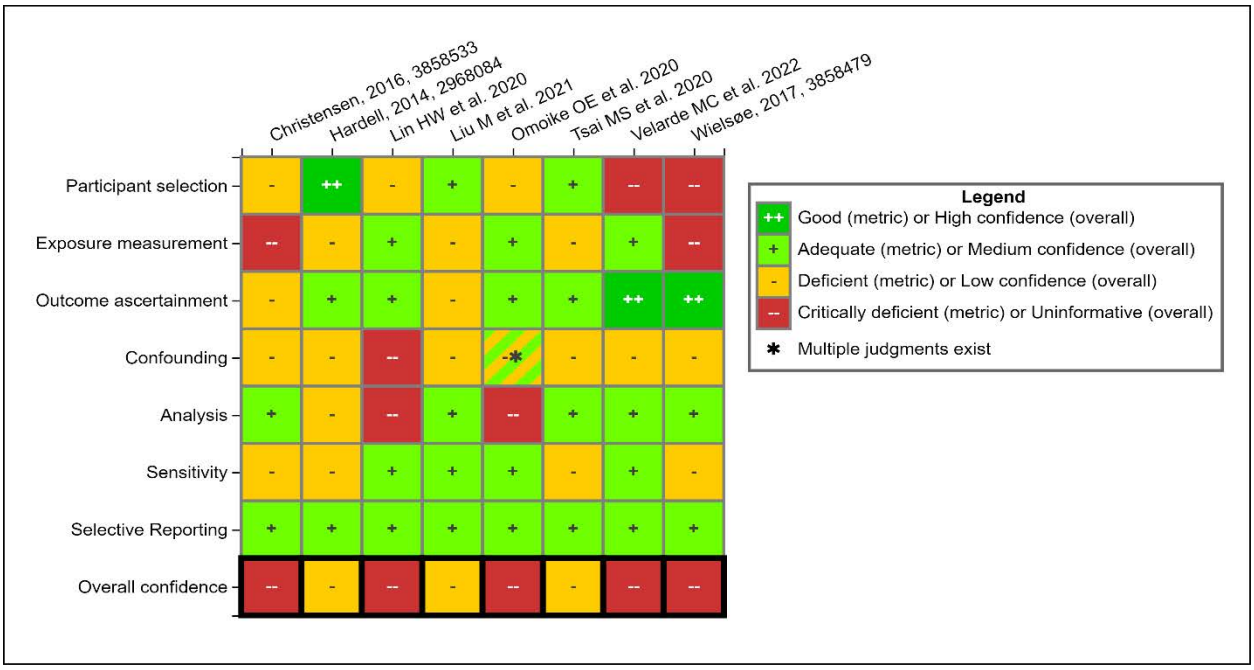


Figure 3-92. Evaluation results for epidemiological studies assessing effects of PFDA exposure on cancer. Refer to HAWC Human Cancer for details on the study evaluation review.

Animal Studies

There are no long-term animal bioassay studies available for PFDA. One short-term study reported null findings for neoplastic histopathology in male and female rats gavaged with doses of 0–2.5 mg/kg-day for 28 days (NTP, 2018). The study performed a complete necropsy of control and PFDA-exposed groups, examining various tissues (i.e., esophagus, intestine, liver, pancreas, salivary glands, stomach, blood vessel, heart, adrenal cortex, adrenal medulla, parathyroid gland, pituitary gland, thyroid gland, epididymis, preputial gland, prostate seminal vesicle, testes, clitoral gland, ovary, uterus, bone marrow, lymph node, spleen, thymus, mammary gland, skin, bone, brain, lung, nose, eye, harderian gland, kidney, and urinary bladder). However, the study was considered *low* confidence for the assessment of carcinogenicity due to the inadequacy of the short-term exposure duration for evaluating the long-term development of potential cancers. Although 28-day studies may be able to provide some information on preneoplastic lesions, the study duration does not cover the entire spectrum of tumor development and promotion for nearly all cancer types and thus they are insensitive.

Mechanistic Studies and Supplemental Information

The scope of the analysis for evaluating putative mechanisms of carcinogenicity for PFDA focused on the synthesis of genotoxicity studies based on data availability. A more comprehensive and rigorous MOA investigation was not attempted because of the sparse and *low* confidence human and animal studies available, as well as insufficient information for the evaluation of alternative carcinogenic mechanisms (e.g., mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression) or considerations for human relevance of tumor responses in animals, susceptible populations and lifestages and anticipated shape of dose-response relationships. This approach is in agreement with the proposed framework for cancer MOA analysis in the EPA *Guidelines for Carcinogen Risk Assessment*, which states that "the framework supports a full analysis of mode-of-action information, but it can also be used as a screen to decide whether sufficient information is available to evaluate or whether the data gaps are too substantial to justify further analysis" (U.S. EPA, 2005).

Studies evaluating the genotoxic, mutagenic and clastogenic potential of PFDA from in vitro assays with prokaryotic organisms and mammalian cells and in vivo assays in rats and mice are summarized in Table 3-47. Mutagenicity test results in *S. typhimurium* (TA98, TAl00, TA1535, TA1537, and TA1538) and *E. coli* strains (WP2 *uvrA* pKM101) across several studies were consistently negative for PFDA in the presence or absence of S9 rat liver metabolism system (NTP, 2005; Kim et al., 1998; Godin et al., 1992; Myhr et al., 1990). Similarly, PFDA had no effect on mutation frequency in L5178Y mouse-lymphoma cells and in the HGPRT forward mutation assay in Chinese hamster ovary (CHO) cells with or without S9 metabolic activation (Godin et al., 1992; Myhr et al., 1990; Rogers et al., 1982).

PFDA was inactive for the in vitro transformation of BALB/C-3T3 mouse cells (Godin et al., 1992) and in the sister chromatic exchange (SCE) assays in CHO cells but induced chromosomal aberrations indicative of clastogenic effects under conditions of S9 metabolic activity (Godin et al., 1992; Myhr et al., 1990). PFDA caused DNA double-strand breaks (DSB) in human gastric adenocarcinoma AGS and SGC cell lines, although the details of the study exposure methodology including information on the test article concentrations were not provided (Liu et al., 2019a). The mechanisms of PFDA-induced DSB were attributed to the downregulation of X-ray repair cross complementing 4 (XRCC4) expression and nonhomologous end-joining (NHEJ) inactivation. These events led to impairment of DNA damage repair and inhibition of p53 expression and apoptosis, contributing to the observed alterations in cell sensitivity to chemotherapy (Liu et al., 2019a). Elevated levels of DSB were also detected in mice with PFDA treatment (dosing regimen was not specified) (Liu et al., 2019a). Xu et al. (2019b) also showed increases in DNA strand breaks, 8-hydroxy-2'-deoxyguanosine (8OHdG) formation, and ROS levels, indicative of oxidative DNA damage in primary mouse hepatocytes exposed to PFDA. In vivo experiments in rats showed increase in oxidative DNA damage (8OHdG levels) in liver tissue after dietary PFDA treatment at 10 mg/kg-day for 2 weeks (Takagi et al., 1991) but no effects were reported with a lower dose

(1.4 mg/kg-day) administered via i.p. for up to 8 weeks (Kim et al., 1998). There were no effects on frequency of micronucleated polychromatic or normochromatic erythrocytes in blood after repeated dose PFDA treatment (0.156–2.5 mg/kg-day) via gavage (NTP, 2012). PFDA was not associated with induction of unscheduled DNA synthesis (UDS) in primary hepatocytes isolated from rats after single-dose exposure (≥11 mg/kg); however, increase in S-phase DNA synthesis was observed in the exposed rats (Godin et al., 1992; Myhr et al., 1990).

In summary, PFDA does not appear to elicit a strong genotoxic response as demonstrated by the lack of activity in most assays described above, including mutagenicity tests in prokaryotic organisms and mammalian cells; SCE and cell transformation assays in vitro; and UDS, oxidative DNA damage, and micronucleus assays in rats. Nevertheless, there is some evidence of potential clastogenic effects in CHO cells, S-phase induction in rat hepatocytes, double-strand DNA breaks in human and mouse gastric cells, and oxidative DNA damage in primary mouse hepatocytes.

Table 3-47. Test evaluating genotoxicity and mutagenicity

Test	Materials and methods	Results	Conclusions	References
Genotoxicity studies in prokaryotic organisms				
Ames assay	*S. typhimurium* strains (TA98, TAl00, TA1535, TA1537, and TA1538) were tested with or without S9 rat liver homogenate and with a preincubation period. PFDA concentrations ranged from 33.3 to 10, 000 µg/plate.	No increase in the number of reverent colonies was observed with PFDA in any of the tester strains in the presence or absence of S9 metabolic activation.	There is no evidence of PFDA mutagenicity in *S. typhimurium* strains.	Godin et al. (1992); Myhr et al. (1990)
Ames assay	*S. typhimurium* strains (TA98 and TA1535) were incubated with PFDA (1 to 100 g/plate) with or without S9.	Test results were negative in the two strains tested irrespective of the presence of S9.	There is no evidence of PFDA mutagenicity in *S. typhimurium* strains.	Kim et al. (1998)
Ames assay	*S. typhimurium* strains (TA98 and TA100) and *E. coli* strain (WP2 *uvrA* pKM101) in the presence or absence of S9. Concentrations of PFDA were 0−10,000 µg/plate.	Test results were negative in all bacterial strains irrespective of the presence of S9.	There is no evidence of PFDA mutagenicity in *S. typhimurium* and *E. coli* strains.	NTP (2005)
Genotoxicity studies in mammalian cells – in vitro				
Mutagenicity assay	L5178Y mouse-lymphoma cells were treated with PFDA (0.01–500 µg/mL) for 24 h and plated in the presence of selective agents to evaluate mutation frequency (ouabain, excess thymidine, methotrexate, cytosine arabinoside and thioguanine) and in nonselective medium to evaluate survival.	Mutagenicity tests showed no significant results in any of the selective systems.	There is no evidence of PFDA mutagenicity in L5178Y cells.	Rogers et al. (1982)
CHO/HGPRT forward mutation assay	CHO cells were treated with PFDA concentrations ranging from 0.005 to 0.5 mg/mL with or without S9.	The results were negative for PFDA-mediated induction of forward mutations in the HGPRT locus in CHO cells under conditions of S9 metabolic activation and nonactivation.	There is no evidence of PFDA mutagenicity in CHO cells in the HGPRT forward mutation assay.	Godin et al. (1992); Myhr et al. (1990)

Test	Materials and methods	Results	Conclusions	References
Cytogenetic assays in CHO cells	CHO cells were treated with PFDA to evaluate induction of SCE and chromosomal aberrations with or without S9. PFDA concentrations of 0.167 to 5,000 µg/mL were tested in the SCE assays and 7.50 to 201 µg/mL were used in the chromosomal aberration assay.	The results of the SCE assay were negative in the presence or absence of S9 metabolic activation. PFDA did induce chromosomal aberrations at 151 and 201 µg/mL but only under conditions of metabolic S9 activation. Cytotoxicity was observed at a concentration of 201 µg/mL in the chromosomal aberration assay.	Induction of chromosomal aberrations provides evidence of clastogenic activity of PFDA in combination with S9. PFDA did not cause DNA damage in the SCE assay.	Godin et al. (1992); Myhr et al. (1990)
In vitro transformation of BALB/C-3T3 cells	BALB/C-3T3 mouse cells were treated with PFDA at doses of 40.0 to 650 µg/mL with or without S9.	PFDA failed to significantly increase morphological transformation in BALB/C-3T3 cells in the presence or absence of S9 metabolism.	There is no evidence of malignant transformation with PFDA in cultured BALB/C-3T3 mouse cells.	Godin et al. (1992)
DNA damage (double-strand breaks)	Human gastric adenocarcinoma AGS and SGC cell lines treated with PFDA (concentration not specified).	PFDA induced double-strand DNA breaks, reduced DNA repair activity, altered expression of DNA repair gene pathways (e.g., NHEJ), inhibited apoptosis via p53 downregulation and affected chemotherapy sensitivity of human gastric cells.	PFDA can cause double-strand DNA damage in vitro by altering DNA repair mechanisms.	Liu et al. (2019a)
DNA damage (strand breaks and oxidative damage [8OHdG])	Primary hepatocytes isolated from male C57BL/6 mice and exposed to PFDA at doses of 0.1, 1, 10, 100 µM.	PFDA increased DNA strand breaks and levels of 8OHdG and ROS in primary mouse hepatocytes (statically significant only at highest dose for ROS but there was a dose-response gradient).	There is evidence of oxidative DNA damage with PFDA in vitro exposure.	Xu et al. (2019b)

Test	Materials and methods	Results	Conclusions	References
Genotoxicity studies in mammalian species – in vivo				
UDS and S-phase induction assays	Adult male F344 rats were treated by oral gavage with a dose of PFDA (5.5 to 44.0 mg/kg) and primary hepatocyte cultures were prepared ~15–48 hr after treatment to examine nuclear labeling.	PFDA was found to be inactive in the UDS assays but induced a significant increase in the number of S-phase cells at doses ≥11.0 mg/kg.	S-phase induction provides some in vivo evidence of genotoxicity with PFDA.	Godin et al. (1992); Myhr et al. (1990)
Oxidative DNA damage (8OHdG)	Male Fischer F344 rats were treated with PFDA (0.01% or 10 mg/kg-d) via the diet for 14 d. DNA was isolated from the liver and kidney of rats after treatment for analysis of 8OHdG formation.	8OHdG levels were significantly increased by PFDA treatment in rat liver but no effects were seen in the kidney.	PFDA (10/mg/kg-d) caused oxidative DNA damage in rat liver after repeated dose exposure via the diet.	(Takagi et al., 1991)
Oxidative DNA damage (8OHdG)	Female Sprague-Dawley rats were treated with a dose of 10 mg/kg PFDA via i.p. once a week for a 2- or 8-wk period. DNA was isolated from rat liver after treatment for analysis of 8OHdG formation.	8OHdG levels were not significantly affected by PFDA treatment in the two time points analyzed.	PFDA (1.4 mg/kg-d) did not cause oxidative DNA damage in rat liver after repeated dose exposure via i.p. administration.	Kim et al. (1998)
Micronucleus assay	Male and female Sprague-Dawley rats (5/group) were exposed daily to PFDA by oral gavage at doses of 0, 0.156, 0.312, 0.625, 1.25 and 2.5 (males only) mg/kg for 28 d.	Test results were negative for the increase in frequency of micronucleated polychromatic or normochromatic erythrocytes in rat blood.	There is no evidence of PFDA (0.156–2.5 mg/kg-d) genotoxicity in the erythrocyte micronucleus assay.	NTP (2012)
DNA damage (double-strand breaks)	Mice were exposed to PFDA via drinking water (dosing regimen was not specified)	PFDA induced double-strand DNA breaks in mouse gastric cells.	PFDA can cause double-strand DNA damage in vivo.	Liu et al. (2019a)

CA = chromosomal aberration; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; LD50 = median lethal dose; ROS = reactive oxygen species; SCE = sister chromatic exchange; UDS = unscheduled DNA synthesis.

Evidence Integration

The available evidence to evaluate the potential for PFDA exposure to lead to the development of any cancer type consists of sparse and minimally informative studies in humans and animals and limited mechanistic information from genotoxicity studies. Specifically, the single *low* confidence study of prostate cancer (reporting an association that was not statistically significant) in exposed humans, as well as the single *low* confidence null study in rats with poor sensitivity that was due to short-term duration, are of limited utility for drawing a conclusion regarding potential carcinogenicity with PFDA exposure. The results from genotoxicity studies were mostly null, although a few studies provided some evidence of potential genotoxic effects in response to PFDA (i.e., clastogenic effects in CHO cells, S-phase induction in rat hepatocytes, double-strand DNA breaks in human and mouse gastric cells, and oxidative DNA damage in primary mouse hepatocytes). Considering evidence for all potential cancer types across the available human, animal, and mechanistic studies and based on the EPA cancer guidelines (U.S. EPA, 2005), the evidence base is judged to be ***inadequate to assess the carcinogenic potential*** of PFDA in humans.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

The available ***evidence indicates*** hazards likely exist with respect to liver, immune, developmental, and male and female reproductive effects in humans, given sufficient perfluorodecanoic acid (PFDA) exposure conditions.[18] Additionally, the available ***evidence suggests*** that PFDA exposure might also have the potential to cause cardiometabolic and neurodevelopmental effects in humans given sufficient exposure conditions[19]. These judgments were derived primarily from epidemiological studies and studies in experimental animals, the latter exposed to PFDA during short-term (7–28 days) and developmental (GD 6–15) oral exposures. On the other hand, there is ***inadequate evidence*** for urinary, endocrine, and other health effects to determine the potential for health hazards in humans with PFDA exposure. A summary of the justifications for the evidence integration judgments for each of the main hazard sections is provided below.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause liver effects in humans, given sufficient exposure condition[20] is based on animal evidence of concordant effects for increased liver weight, alterations in levels of serum biomarkers of liver injury (ALT, AST, ALP, bile salts/acids, bilirubin and blood proteins), and some evidence of hepatocyte degenerative or necrotic changes that provide support for the adversity of PFDA-induced liver toxicity reported in *high* and *medium* confidence studies in rats and mice exposed to PFDA doses ≥0.156 mg/kg-day. Although associations between serum ALT levels and PFDA exposure in epidemiological studies of adult*s* were observed, the epidemiological evidence overall is uncertain due to unexplained inconsistency in the results for other clinical markers and a lack of clear evidence of adversity. Mechanistic studies in rodents and limited evidence from in vitro studies and animal models considered more relevant to humans provide support for the biological plausibility and human relevance of the apical effects observed in animals and suggest a possible PPARα-dependent and independent MOA for PFDA-induced liver toxicity.

[18]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.
[19]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.
[20]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause immunosuppression in humans, given sufficient exposure conditions[21], is based on *moderate* human evidence of immunosuppression primarily from two *medium* confidence studies in children and one *low* confidence study in adults at levels of 0.3 ng/mL (median exposure in studies observing an adverse effect). Although some evidence for coherent immunomodulatory responses consistent with immunosuppression (decreases in phagocytic activity of liver microphages, spleen cell counts and immune organ weights and immune organ histopathology) was identified in short-term, *high*, and *medium* confidence studies in rats and mice at ≥0.089 mg/kg-day, the animal evidence overall is uncertain. Issues with overt organ and general systemic toxicity pose limitations with respect to the interpretation of the animal evidence. Although possible effects of hypersensitivity-related responses were reported in one epidemiological study and one high-exposure study in mice (21.4 mg/kg-day), outstanding uncertainties remain to draw specific conclusions for this outcome.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause developmental toxicity, given sufficient exposure conditions[22], is based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA doses ≥0.5 mg/kg-day, supported by evidence of decreased birth and childhood weight from studies of exposed humans in which PFDA was measured during pregnancy. The conclusion is further supported by coherent epidemiological evidence for biologically related effects (e.g., decreased birth length).

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause potential adverse effects to the male reproductive system in humans, given sufficient exposure conditions[23], is based on a coherent pattern of effects on sperm counts, testosterone levels, and male reproductive histopathology and organ weights at doses ≥0.625 mg/kg-day in adult rats exposed for 28 days (*high* confidence for most endpoints evaluated). Although the MOA for PFDA-induced male reproductive effects is unknown, a few acute i.p. and in vitro rodent studies suggest a possible mechanism via disruption of Leydig cell function and impaired steroidogenesis. Evidence from a *medium* confidence epidemiological study reported nonstatistically significant decreases in testosterone levels and altered sperm parameters but the findings are inconsistent and imprecise.

[21]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.
[22]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.
[23]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause female reproductive toxicity in humans given sufficient exposure conditions[24] is based primarily on the results of a *high* confidence study in rats showing biologically coherent effects on uterus weight and the estrous cycle after oral exposure to PFDA at ≥1.25 mg/kg-day for 28 days. Although human studies are available for examining associations between PFDA and female reproductive toxicity (e.g., fecundity), the results were mostly null, possibly due to their low sensitivity for observing effects.

The hazard identification judgment that the ***evidence suggests*** PFDA exposure has the potential to cause cardiometabolic effects in humans given sufficient exposure conditions [25] is based primarily on associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in a few epidemiological studies. However, evidence is largely inconsistent across studies, which adds considerable uncertainty. Evidence in experimental animals from a *high* confidence rat study was *indeterminate*.

The hazard identification judgment that the ***evidence suggests*** PFDA exposure has the potential to cause neurodevelopmental effects in humans given sufficient exposure conditions[26] is based on associations between PFDA exposure and outcomes related to attention and behavior, although there is high degree of uncertainty due to inconsistencies and imprecision in the results. No relevant animal studies were available.

Finally, there is ***inadequate evidence*** to evaluate the potential for PFDA exposure to cause effects on the endocrine system, urinary system, and other health outcomes in adult humans (i.e., respiratory, digestive, dermal, musculoskeletal, and hematological systems, and nonspecific clinical chemistry). The available data from human and/or animal studies for these health outcomes was largely limited or lacked consistency and coherence. Further, the absence of studies examining the potential for effects of PFDA exposure on the thyroid in developing organisms, or on mammary glands, represent data gaps in light of associations observed for other PFAS, such as PFBS, PFOS, and PFOA (ATSDR, 2021; U.S. EPA, 2018), see Table 4-1 below.

[24]The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

[25]Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

[26]Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

Table 4-1. Hazard conclusions across published EPA PFAS human health assessments

			EPA PFAS assessments[a,b]				
Health outcome	**PFDA**	**PFHxA**	**PFBA**	**PFBS**	**GenX chemicals**	**PFOA[c]**	**PFOS[c]**
Thyroid	–	+	+	+	–[d]	±	±
Liver	+	+	+	–	+	+	+
Developmental	+	+	+	+	±	+	+
Reproductive	+	–	–	–	±	±	±
Immunotoxicity	+	–	–	–	±	+	+
Renal	–	–	–	+	±	±	±
Hematological	–	+	–	–[d]	±	–	–
Ocular	–	–[d]	–	–[d]	–[d]	–	–
Serum Lipids	±	–[d]	–[e]	–	–[d]	+	+
Hyperglycemia	–	–[d]	–[e]	–[d]	–[d]	±	±
Nervous System	±[e]	–	–[e]	–[d]	–[d]	±	±
Cardiovascular	±	–[d]	–[e]	–	–[d]	+	+
Cancer	–	–	–	–	±	+	+

[a]Assessments used multiple approaches in summarizing their noncancer hazard conclusions; for comparison purposes, the conclusions are presented as follows: '+' = *evidence demonstrates* or *evidence indicates* (e.g., PFDA), or *evidence supports* (e.g., PFBS); '±' = *suggestive evidence*; '–' = *inadequate evidence* (e.g., PFDA) or *equivocal evidence* (e.g., PFBS); and '–/–' = *sufficient evidence to conclude no hazard* (no assessment drew this conclusion).

[b]The assessments all followed the EPA carcinogenicity guidelines (2005); a similar presentation to that used to summarize the noncancer judgments is applied for the cancer hazard conclusions, as follows: '+' = *carcinogenic to humans or likely to be carcinogenic to humans*; '±' = *suggestive evidence of carcinogenic potential*; '–' = *inadequate information to assess carcinogenic potential*; and '–/–' = *not likely to be carcinogenic to humans* (no assessment drew this conclusion).

[c]The hazard conclusions were taken from the EPA OW assessments for PFOA (U.S. EPA, 2024b) and PFOS (U.S. EPA, 2024a).

[d]No data available for this outcome for this PFAS, so '– 'entered by default.

[e]Data available for PFDA includes neurodevelopmental outcomes in humans.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

Given the limited scope and utility of the available evidence across human, animals, and genotoxicity studies, the evidence is judged to be insufficient to determine whether PFDA exposure (via any exposure route) might affect the development of any specific cancer types. In accordance with EPA cancer guidelines (U.S. EPA, 2005), a weight-of-evidence descriptor of ***inadequate to assess the carcinogenic potential*** is assigned for PFDA.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES

Understanding of potential areas of susceptibility to the identified human health hazards of PFDA can help to inform expectations of variability in responses across individuals, as well as uncertainties and confidence in candidate toxicity values (see Section 5.2). The available human and animal studies indicate that early life represents a susceptible lifestage for the effects of PFDA exposure. Two medium confidence studies reported immune effects (i.e., decreased antibody response) in children exposed to PFDA during gestation and childhood (Grandjean et al., 2017b) and (Grandjean et al., 2017a; Grandjean et al., 2012). Additionally, developmental effects (i.e., fetal growth restriction, gestational duration, postnatal growth, and spontaneous abortion) were reported in multiple high-quality studies (Buck Louis et al., 2018; Gyllenhammar et al., 2018; Meng et al., 2018; Lind et al., 2017a; Swedish Environmental Protection Agency, 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Wang et al., 2016; Robledo et al., 2015). The strongest and most consistent evidence was observed for fetal growth restriction. Potentially coherent with these epidemiological observations, effects in developing rodents (decreased fetal body weight, skeletal variations, decreased live fetuses per litter) after maternal exposure also support the potential for early-life susceptibility. Young individuals may also be susceptible to PFDA-induced male reproductive effects. Although no animal studies and only a few human studies are available that examined reproductive effects in early lifestages (i.e., pubertal development and anogenital distance), effects on sperm motility and testosterone were consistently reported in exposed human and rodent adults (NTP, 2018; Zhou et al., 2016; Joensen et al., 2013). Given the potential for PFDA to impair androgen function, boys exposed during critical developmental lifestages may be susceptible as exposure during gestation and early postnatal lifestages could result in agenesis of the male reproductive system and/or infertility.

Although inconclusive, some effects on thyroid hormone homeostasis were observed in adult rats (NTP, 2018). Although no studies are available that assessed the effect of PFDA on thyroid hormones in developing organisms, young individuals exposed during gestation, early childhood, and puberty may be a susceptible population given that triiodothyronine (T3) and thyroxine (T4) levels play critical roles in bone growth and brain development (O'Shaughnessy et al., 2019) at these lifestages (i.e., both pregnancy and early life). PFDA was also observed to disrupt estrous cyclicity in female rats with potential implications for impaired fertility (NTP, 2018).

Therefore, although the current evidence does not explicitly address the potential for a linkage between these observations and impaired fertility in women, women of reproductive age may also be susceptible to the effects of PFDA exposure.

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

The available ***evidence indicates*** that oral exposure to perfluorodecanoic acid (PFDA) is likely to cause adverse hepatic, immune, developmental, and male and female reproductive effects in humans given sufficient exposure conditions, on the basis of findings from epidemiological and animal toxicity studies. This section aims to characterize the dose levels associated with these identified hazards and derive toxicity values as presented below. Additionally, the available ***evidence suggests*** PFDA exposure might have the potential to cause cardiometabolic and neurodevelopmental effects in humans given sufficient PFDA exposure conditions, on the basis of findings from a limited number of epidemiological studies; the results are considered too uncertain, however, to support the derivation of toxicity values. For all other health effects (i.e., endocrine, urinary, hematology, special senses [eye and harderian gland], dermal and musculoskeletal systems), the ***evidence is inadequate*** to assess the hazard potential; therefore, these endpoints were not considered for the derivation of toxicity values.

There are no available studies to inform the potential for PFDA to cause adverse health effects via inhalation exposure, therefore, the derivation of reference concentrations (RfCs) is precluded (see Section 5.2.4). Likewise, evidence pertaining to the evaluation of carcinogenicity was considered ***inadequate to assess carcinogenic potential*** of PFDA in humans, precluding the derivation of cancer toxicity values via any exposure route (see Section 5.3).

5.2. NONCANCER TOXICITY VALUES

The noncancer toxicity values (i.e., oral reference doses [RfDs]) derived in this section are estimates of an exposure for a given duration to the human population (including susceptible subgroups and/or lifestages) that are likely to be without an appreciable risk of adverse health effects (see Section 1.2.1). The RfD derived in Section 5.2.1 corresponds to chronic, lifetime exposure. In addition, a less-than-lifetime toxicity value (referred to as a "subchronic RfD") is derived in Section 5.2.3. This subchronic RfD can be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures). Both the lifetime and subchronic RfD include organ/system-specific RfDs (osRfDs) associated with each health effect considered for point-of-departure (POD) derivation, as supported by the available data. These toxicity values might be useful in some contexts (e.g., when assessing the potential cumulative effects of multiple chemical exposures occurring simultaneously). Section 5.2.4 summarizes the conclusion that no information exists to inform the potential toxicity of inhaled PFDA or to derive an inhalation RfC.

5.2.1. Oral Reference Dose (RfD) Derivation

Study/Endpoint Selection

Data sufficient to support dose-response analyses for oral PFDA exposure were available for all identified human health hazards (see Section 4.1): hepatic, immune, developmental, and male and female reproductive effects. Rationales for study selection and the specifics of RfD calculations, as well as the determination of confidence in quantitative estimates are detailed in this section.

The following general considerations were used to prioritize studies for estimating PODs for potential use in toxicity value derivation. Dependent on the evidence for each identified hazard, *high* or *medium* confidence human studies that were deemed influential to the hazard conclusions and suitable for dose-response analysis were prioritized for POD derivation and compared with PODs derived from animal data when possible. Human studies were available for developmental and immunotoxicity effects. For other health effects (i.e., hepatic, and male and female reproductive effects), only evidence from animal studies was considered influential for hazard identification; therefore, these data were prioritized for dose-response assessment. Given the lack of comprehensive subchronic or chronic animal studies, *medium* and *high* confidence short-term studies in animals of longer exposure duration (e.g., 28 days versus 7 or 14 days) and with exposure levels near the lower dose range of doses tested across the evidence base were preferred along with *medium* or *high* confidence animal studies evaluating exposure during development. These types of *medium* and *high* confidence human and animal studies increase the confidence in the resultant RfD because they represent data with lower risk of bias and reduce the need for low-dose and exposure duration extrapolation (see Appendix C).

A summary of endpoints and rationales considered for toxicity value derivation is presented below.

Hepatic Effects

The hazard conclusions for PFDA-induced liver effects are based primarily on *moderate* evidence from short-term animal studies (see Section 3.2.1). In humans, an association between PFDA exposure and ALT levels in the blood was identified, but there was considerable uncertainty due to potential for confounding by other PFAS. As such, only animal studies were considered for dose-response analysis. The database of animal studies examining liver effects includes several short-term studies in rats and mice (Wang et al., 2020; Frawley et al., 2018; NTP, 2018; Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991; Harris and Birnbaum, 1989). In particular, two *high* confidence studies in SD rats gavaged with PFDA for 28-days were prioritized for the derivation of candidate values because they included several hepatic endpoints that together provided coherent evidence of liver toxicity with PFDA exposure across histopathology, organ weights and/or clinical chemistry (Frawley et al., 2018; NTP, 2018) (see Table 5-1). Additionally, these studies had the longest exposure duration (28 days) and

examined the lower range of PFDA doses (dose range of observed effects is 0.156–2.5 mg/kg-day) across the available studies examining hepatic effects.

PFDA induced changes in serum liver biomarkers, hepatocyte lesions and increased liver weights in rats across the two 28-day studies (Frawley et al., 2018; NTP, 2018). Although some of the individual changes have the potential to represent adaptive responses (e.g., increased liver weights and hypertrophy), the constellation of coherent liver effects, most notably consistent effects across multiple serum biomarkers of hepatocyte and biliary injury and histological findings of structural hepatocyte degeneration (necrosis), provide clear evidence of adversity (see "Consideration for potentially adaptive versus adverse responses" under Section 3.2.1 for more details). Alterations in the levels of serum enzymes such as ALT, AST, and ALP and other functional biomarkers (bile salt/acids, bilirubin, and blood proteins [albumin, globulin, and total protein]) were reported in the 28-day study that evaluated clinical chemistry (NTP, 2018). Increases in AST and ALP levels were consistent across sexes and dose groups and generally occurred at lower doses that did not induce significant body weight changes or other general systemic effects (0.156–0.625 mg/kg-day PFDA). Similarly, dose-related increases in relative liver weights were reported in male and female rats at ≥0.125 mg/kg-day across the two 28-day studies (Frawley et al., 2018; NTP, 2018). As discussed in Section 3.2.1, relative liver weight is generally preferred over absolute liver weight; as information on the former was available, changes in absolute liver weight were not considered for dose-response analyses. Given there is no clear indication of sex-specific differences in sensitivity with respect to PFDA-induced liver effects in the available animal toxicity studies, data for both male and female SD rats for these endpoints were advanced for dose-response modeling.

Corroborative hepatocyte lesions such as cytoplastic alterations and vacuolization, hypertrophy and necrosis were reported in rats at higher doses (≥0.625 mg/kg-day) across the two 28-day studies prioritized for dose-response analysis (Frawley et al., 2018; NTP, 2018). The histopathological observations showed a clear progression in severity across lesions and dose groups. These findings provide additional support for the adversity of the progressive effects on the liver with PFDA exposure but were not prioritized for dose-response analysis due to the presence of more sensitive liver endpoints (i.e., serum AST and ALP levels, and relative liver weight; see Table 5-1).

Table 5-1. Endpoints considered for dose-response modeling and derivation of points of departure for liver effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased serum ALT	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male and female	No	Dose-dependent effects were only observed in females and occurred at higher doses compared with other liver findings

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased serum AST	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male and female	Yes	Dose-dependent effects were consistent across sexes and concordant with liver weight and liver histopathology findings
Increased serum ALP	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male and female	Yes	Effects were consistent across sexes and dose groups and concordant with liver weight and liver histopathology findings.
Other serum biomarkers (increased bile salts/acids and bilirubin, and decreased albumin and globulin)	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male and female	No	Effects were mostly consistent across sexes but occurred at higher doses compared with other liver findings
Hepatocyte lesions	NTP (2018); *high* confidence (cytoplasmic alterations and vacuolization, hypertrophy, and necrosis)	Gavage, 28 d	SD rat, male and female	No	Effects were consistent across sexes and studies but occurred at higher doses compared with other liver findings
	Frawley et al. (2018); *high* confidence (necrosis)	Gavage, 28 d	SD rat, male	No	
Increased relative liver weight	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male and female	Yes	Dose-dependent effects were consistent across studies, cohorts, sexes and were concordant with serum biomarker and liver histopathology findings. There was no reason to prioritize one dataset over the other.
	Frawley et al. (2018); *high* confidence	Gavage, 28 d	SD rat, female (included three experimental cohorts)	Yes	

Immune Effects

As described in Section 3.2.2, the strongest evidence for immune effects was from epidemiological studies that provided *moderate* evidence of immunosuppression (Shih et al., 2021; Timmermann et al., 2021; Grandjean et al., 2017b; Grandjean et al., 2017a; Kielsen et al., 2016; Grandjean et al., 2012); thus, this outcome was prioritized for dose-response analysis and studies of hypersensitivity (which collectively provided *slight* human evidence) were not considered. Given

the uncertainties with the animal data described in Section 3.2.2 (e.g., influence of systemic toxicity), only the human data were considered for the derivation of PODs.

The two *medium* confidence epidemiological studies of antibody response following vaccination providing the primary support for the hazard judgment were conducted in different birth cohorts of the Faroe Islands population (see Table 3-11). These studies include measures of PFDA exposure taken perinatally (pregnancy week 32 to 2 weeks postpartum); at 18 months; and at 5, 7, and 13 years and measures of antibody levels at 5, 7, and 13 years for both diphtheria and tetanus. The relevant etiologic window of exposure for this outcome is not known. Although there were some heterogeneous results (see Section 3.2.2), the direction of association across these combinations of different timings of exposure and outcome measurement were generally consistent, indicating immunosuppression (i.e., decreased antibody response with higher exposure). However, selecting the most informative exposure-outcome combination(s) for POD derivation is complicated by the lack of a clear etiologic window. In a follow-up publication without new data, the study authors performed benchmark dose modeling for a subset of the data presented in Grandjean et al. (2012), specifically antibody levels at age 7 and PFDA concentrations at age 5, and antibody levels at age 5 (prebooster) and perinatal PFDA concentrations (Budtz-Jørgensen and Grandjean, 2018b). These were selected by the authors due to the strong inverse associations observed and the results were considered reasonably representative of the study results overall. After review of the BMD methods and additional modeling details (Budtz-Jørgensen and Grandjean, 2018b) for completeness and appropriateness (see Appendix C.1.1), EPA used the authors' analytic regression results for this Toxicological Review (see Table 5-2).

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFDA measured at age 5 years, against log2-transformed antitetanus 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. Three model shapes of PFDA were evaluated by Budtz-Jørgensen and Grandjean (2018a): a linear model, a piecewise-linear model with a knot at the median, and a logarithmic function. Tables 5-3 and Table 5-4 shows the best fitting model results of PFDA and tetanus from this analysis of the combination of the Faroe cohort born in 1997–2000 Grandjean et al. (2012) with the cohort born in 2007–2009 (Grandjean et al., 2017b). These results differ from those shown separately for the two cohorts in Section 3.2 because the PFDA exposure has not been log2-transformed (only the antibody concentrations have). The published results from the "log-log" models in which both exposure and outcome are log-transformed (Grandjean et al., 2017b; Grandjean et al., 2012) are not statistically suited to derive points of departure for toxicity values, whereas the untransformed PFDA exposure models are suited to do so Budtz-Jørgensen and Grandjean (2018a).

The regression model used to estimate the results in Table 5-3 and Table 5-4 were from "single-PFAS models" in which only one PFAS exposure (here, PFDA) was included and "multi-PFAS models," which were parallel models fit with control of PFOA and PFOA. Two-time windows of exposure and outcome were evaluated: PFDA exposure measured at age 5 years with tetanus

antibodies measured at age 7 years; and PFDA measured perinatally, and tetanus antibodies measured at age 5 years. The details of the regression analyses were provided to EPA by the authors and are available in (Budtz-Jørgensen and Grandjean, 2018b). Detailed interpretations of these results with discussion of potential confounding are provided in Appendix C.1.1 in which these results are used to estimate benchmark doses (BMDs) and lower bound benchmark doses (BMDLs), which identify point of departures for reference doses.

Table 5-2. Endpoints considered for dose-response modeling and derivation of points of departure for immune effects in humans

Endpoint	Study reference and confidence	POD derived?	Notes
Antibody concentrations for diphtheria and tetanus	Grandjean et al. (2012) [Birth cohort 1997–2000 with follow-up to age 7] and (Grandjean et al., 2017a) [Birth cohort 1997–2000 with follow-up to age 13]; Grandjean et al. (2017b) [Birth cohorts from 1997–2000 and 2007–2009 with follow-up to age 5]; *medium* confidence	No	Effect was generally coherent with epidemiological evidence for other antibody effects. However, while these results contribute to understanding the hazard for PFDA, the analytic models in these specific publications used log-transformed exposure and log-transformed outcome variables and such log-log models cannot be used for BMD calculations and thus PODs were not derived.
Antibody concentrations for diphtheria and tetanus	Budtz-Jørgensen and Grandjean (2018a); Birth cohorts 1997–2000 and 2007–2009 using different analyses of combined data from Grandjean et al. (2012) and Grandjean et al. (2017a) *medium* confidence	Yes	Effect generally coherent with epidemiological evidence for other antibody effects. Results were based on analytic models using log-transformed outcome and untransformed exposure, which were suitable for BMD calculations and POD derivations (see Appendix C.1.1 for more details on BMD modeling results).

Table 5-3. Results from the analyses of PFDA measured in serum at age 5 years (and measured perinatally) and log2(tetanus antibody concentrations) measured at ages 5 and 7 years in a single-PFAS models and multi-PFAS models from (Budtz-Jørgensen and Grandjean, 2018a, b)

Exposure	Outcome	Model shape (best fit)	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum[a]	95% CI[b]	Slope (β) fit	95% One-sided lower bound slope (β_{LB}) per ng/mL in serum[c]
PFDA at age 5 yr	Tetanus antibodies at age 7 yr	Linear	No	−1.55	(−2.73, −0.370)	p = 0.01	−2.55
PFDA at age 5 yr	Tetanus antibodies at age 7 yr	Linear	Yes	−0.98	(−2.31, 0.355)	p = 0.15	−2.10
Perinatal PFDA	Tetanus antibodies at age 5 yr	Linear	No	−0.343	(−1.25, 0.563)	p = 0.46	−1.10
Perinatal PFDA	Tetanus antibodies at age 5 yr	Linear	Yes	−0.038	(−1.12, 1.05)	p = 0.95	−0.874

[a]This slope is used to estimate the benchmark dose (BMD). See Appendix C.1.1.
[b]EPA computed the 95% CI from the β and SE(β) provided in (Budtz-Jørgensen and Grandjean, 2018b).
[c]This slope is used to estimate the lower bound benchmark dose (BMDL). See Appendix C.1.1.

Table 5-4. Results from the analyses of PFDA measured in serum at age 5 years (and measured perinatally) and log2(diphtheria antibody concentrations) measured at ages 5 and 7 years in a single-PFAS models and multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b)

Exposure	Outcome	Model shape (best fit)	PFOS and PFOA adjusted	Slope (β) per ng/mL in serum[a]	95% CI[b]	Slope (β) fit	95% One-sided lower bound slope (β_{LB}) per ng/mL in serum[c]
PFDA at age 5 yr	Diphtheria antibodies at age 7 yr	Linear	No	−0.894	(−1.99, 0.206)	p = 0.11	−1.82
PFDA at age 5 yr	Diphtheria antibodies at age 7 yr	Linear	Yes	−0.297	(−1.54, 0.948)	p = 0.64	−1.35
Perinatal PFDA	Diphtheria antibodies at age 5 yr	Piecewise Linear	No	−3.70	(−8.11, 0.708)	p = 0.10	−7.40
Perinatal PFDA	Diphtheria antibodies at age 5 yr	Piecewise Linear	Yes	−2.47	(−3.94, −1.00)	p = 0.001	−3.70

[a]This slope is used to estimate the benchmark dose (BMD). See Appendix C.1.1.
[b]EPA computed the 95% CI from the β and SE(β) provided in (Budtz-Jørgensen and Grandjean, 2018b).
[c]This slope is used to estimate the lower bound benchmark dose (BMDL). See Appendix C.1.1.

Developmental Effects

Uncertainties in the human evidence of developmental effects resulted in a judgment of *slight* (see Section 3.2.3); however, the database includes several well-conducted *medium* and *high* confidence epidemiological studies reporting birth weight deficits of varying magnitude in male or female neonates or both. Birth weight deficits (and several other developmental endpoints) were generally larger and more consistent among studies that sampled maternal serum later in pregnancy including postpartum measures. This observation suggests that those samples may be most prone to potential bias from changing pregnancy hemodynamics, but the complex patterns of influence due to pregnancy hemodynamics are not completely understood. Nevertheless, the apparent influence of pregnancy hemodynamics introduces considerable uncertainty in the interpretation of these associations of PFDA-induced developmental effects and was a major contributing factor in the overall evidence integration judgment for this health effect (see Section 3.2.3). Despite these concerns regarding sample timing, decreased birth weight was the focus of dose-response analysis, given the accuracy in measurement of the endpoint, and the abundance of high-quality studies. There is considerably less uncertainty related to pregnancy hemodynamics in studies based on maternal serum samples collected during the first trimester.

Twenty-eight epidemiological studies (8 *high* and 10 *medium* confidence) evaluated associations between PFDA and fetal growth restriction, including 26 studies examining mean birth weight. Given the abundance of *high* confidence studies, *low* and *medium* confidence studies were not considered for POD derivation; thus, four *high* confidence studies were considered as they provided consistent evidence of associations within the overall population and across both sexes. Among the eight *high* confidence studies detailed in Table 5-5, two studies Buck Louis et al. (2018); Bach et al. (2016) were not considered further, as they did not find evidence of an inverse association between PFDA exposures and mean birth weight in the overall population. Two studies were not advanced because they reported vastly different findings across the sexes Lind et al. (2017a); Wang et al. (2016) with no clear biological explanation for this inconsistency (see discussion in Section 3.2.3).

Three of the four remaining studies examined PFDA during the third trimester Luo et al. (2021); Yao et al. (2021); Valvi et al. (2017) and one examined PFDA across the first and second trimesters (Wikström et al., 2020). Two *high* confidence studies, Valvi et al. (2017) and Wikström et al. (2020), were selected for dose-response quantification. In the (Wikström et al., 2020) study, 96% of samples were collected during the first trimester and the remaining during the early weeks of the second trimester; sensitivity analyses showed no differences when second trimester samples were excluded. The Valvi et al. (2017) has a unique design that may increase study sensitivity by sampling all participants during the same gestational week (i.e., 34). These two studies had a low overall risk of bias and reliable exposure measurements with sufficient exposure contrasts (PFDA median/interquartile ranges: 0.26/0.15 and 0.28/0.16 ng/mL, respectively for Wikström et al. (2020); Valvi et al. (2017)) and other characteristics that allowed for adequate study sensitivity to

detect associations (see Table 5-5). As noted above, the Valvi et al. (2017) and Wikström et al. (2020) studies selected for dose-response quantification reported results consistent in magnitude that allowed the consideration of sex-specific and overall population results. A limitation of the Valvi et al. (2017) study advancing to dose response is that it did not have early trimester samples (third trimester only) and may be prone to some potential bias due to pregnancy hemodynamics (see more details in Appendix F). Despite these important concerns regarding sample timing, as noted above, derivation of a POD(s) for developmental outcomes using the Valvi, 2017 study was considered potentially informative to toxicity value derivation for birth weight effects reported by (Wikström et al., 2020).

The one available *high* confidence animal study that examined developmental toxicity in mice treated with PFDA (Harris and Birnbaum, 1989) provided *moderate* evidence of developmental toxicity (see Section 3.2.3). Several endpoints from this study were considered suitable for POD derivation (see Table 5-6) and for comparison to PODs derived from the human studies. Harris and Birnbaum (1989) reported developmental effects in C57BL/6N mice treated either on GD 10–13 (0–32 mg/kg-day) or GD 6–15 (0–12.8 mg/kg-day). Harris and Birnbaum (1989) reported statistically significant changes for increased % resorptions per litter and decreased number of live fetuses GD 6–15 component of the study. However, these effects were not considered for dose-response analysis because their interpretation is confounded by overt maternal toxicity (i.e., mortality) observed at the same dose. Statistically significant and dose-dependent decreases in fetal body weight were also observed in both the GD 10–13 and the GD 6–15 experiments. Data for decreased fetal body weight from the GD 6–15 experiment were prioritized for dose-response analysis over data from the GD 10–13 experiment, since the former experiment encompasses a larger developmental window. Statistically significant and dose-dependent increases in variations (i.e., delayed braincase and phalanges ossification and absence of fifth sternebrae) were also reported, but there were methodological concerns and uncertainty regarding the adversity of these endpoints (see Section 3.2.3) that precluded their consideration for dose-response analysis.

Table 5-5. Mean birth weight deficit studies considered for dose-response modeling and derivation of points of departure for developmental effects in humans

Study reference and confidence	Population-overall population, sex-specific and all births vs. term births only	PFDA biomarker sample timing	POD derived?	Notes
Valvi et al. (2017); *high* confidence	Overall population; sex-specific; all births	Trimester 3	Yes	Effect was large in magnitude and coherent with findings in mice and epidemiological evidence for other

Study reference and confidence	Population-overall population, sex-specific and all births vs. term births only	PFDA biomarker sample timing	POD derived?	Notes
				biologically related effects (e.g., decreased postnatal growth and birth length).
Wikström et al. (2020), *high* confidence	Overall population; sex-specific; all births	Trimesters 1–2 (94% in T1)	Yes	Effect was statistically significant, large in magnitude, and coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., decreased postnatal growth and birth length).
Luo et al. (2021), *high* confidence	Overall population; term births	Trimester 3	No	Effect size was statistically significant and moderate in magnitude. Results are coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., preterm birth, postnatal growth, and other fetal growth measures such as birth length).
Yao et al. (2021), *high* confidence	Overall population; sex-specific; all births	Trimester 3	No	Effect size was moderate in magnitude. Results are coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., preterm birth, postnatal growth, and other fetal growth measures such as birth length).
Wang et al. (2016); *high* confidence	Sex-specific; term births	Trimester 3	No	Study reported sex-specific findings that were not consistent across male and female neonates.
Bach et al. (2016); *high* confidence	Sex-specific; term births	Trimester 1	No	Study reported sex-specific findings that were not consistent across male and female neonates.
Buck Louis et al. (2018), *high* confidence	Overall population; term births	Trimester 2	No	Study did not detect inverse associations between mean birth weight and PFDA.
Lind et al. (2017a), *high* confidence	Sex-specific; all births	Trimester 1	No	Study reported sex-specific findings that were not consistent across male and female neonates.

Table 5-6. Endpoints considered for dose-response modeling and derivation of points of departure for developmental effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased % resorptions per litter	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	Effect was observed at the same dose as significant maternal mortality.
Decreased live fetuses per litter	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	Effect was observed at the same dose as significant maternal mortality.
Decreased fetal body weight	Harris and Birnbaum (1989); *medium* confidence	Gavage, GD 10–13	C57BL/6N mouse, male and female	No	Fetal body weight data from GD 10–13 was not advanced in lieu of the more sensitive data available from GD 6–15.
Decreased fetal body weight	Harris and Birnbaum (1989); *medium* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	Yes	Effect displayed a dose-response trend and was coherent with other developmental changes in mice and humans.
Skeletal variations (i.e., delayed braincase ossification; absence of fifth sternebrae; delayed phalanges ossification)	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	The adversity and interpretation of these effects is unclear (see Section 3.2.3).

Male Reproductive Effects

The hazard conclusions for PFDA-induced male reproductive effects are driven by *moderate* evidence from a single, *high* confidence study in rats gavaged for 28 days (NTP, 2018). The available evidence from human studies was *indeterminate* (see Section 3.2.4); thus, these human studies were not considered further for POD derivation.

The single, 28-days study in adult male rats examining reproductive effects was considered *low* confidence for sperm evaluations based on potential reduced sensitivity due to inadequate exposure duration. Otherwise, the study would have been considered *high* confidence for sperm measures and was considered *high* confidence for other, related male reproductive endpoints. Thus, the coherent results across multiple measures, including sperm evaluations, in this well-conducted study provide support for advancing the study for dose-response modeling. Effects in male rats included significant decreases in testicular and epididymal sperm counts at doses

≥1.25 mg/kg-day (NTP, 2018). Although there are concerns about exposure sensitivity for sperm evaluations, the alterations in sperm counts are supported by concordant effects for histopathology and organ weight measures in the testis and epididymis evaluated. The decreases in absolute epididymal sperm counts (although not testicular sperm counts) displayed a dose-response gradient and thus were prioritized for POD derivation (see Table 5-7).

A consistent pattern of mild degenerative changes was detected in the testes and epididymis of exposed rats at the two highest doses (NTP, 2018). These doses were associated with significant body weight decreases (21%–38%) but concerns over potential confounding with overt systemic toxicity were mitigated by mechanistic evidence suggesting that male reproductive effects are only affected by severe changes in body weight (72%; see "Mechanistic Studies and Supplemental Information" in Section 3.2.4). Increased incidence of Leydig cell atrophy was observed at doses ≥1.25 mg/kg-day, which is consistent with reductions in spermatogenesis and serum testosterone levels reported in this same 28-day rat study and with mechanistic evidence that suggests PFDA targets Leydig cells and disrupts steroidogenesis (see "Mechanistic Studies and Supplemental Information" in Section 3.2.4). As such, this endpoint was selected for dose-response modeling (see Table 5-7). Other corroborative histopathological lesions (germinal epithelium degeneration, seminiferous tubule spermatid retention, epididymal duct germ cell exfoliation and hypospermia in the epididymis) were not advanced, as these lesions occurred mostly in the high-dose group (2.5 mg/kg-day) and had low to medium incidence rates (10%–40% compared with 0%–10% for controls). Finally, decreases in absolute testicular and epididymal weights and serum testosterone levels identified in rats were also advanced for POD derivation. Absolute weights are the preferred measure for testis and epididymis as these organs appeared to be conserved even with body weight changes (Creasy and Chapin, 2018; U.S. EPA, 1996b). The changes in organ weights and testosterone levels demonstrated a dose-response effect and were concordant with other male reproductive findings occurring at similar doses (≥1.25 mg/kg-day) (NTP, 2018).

Table 5-7. Endpoints considered for dose-response modeling and derivation of points of departure for male reproductive effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Decreased testicular sperm counts	NTP (2018); *low* confidence	Gavage, 28 d	SD rat, male	No	Effects provide corroborative evidence of male reproductive toxicity but were not dose dependent.

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Decreased absolute epididymis sperm counts (cauda)	NTP (2018); *low* confidence due to concern for potential insensitivity	Gavage, 28 d	SD rat, male	Yes	Effects displayed a dose-response pattern and were coherent with other male reproductive findings
Leydig cell atrophy	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male	Yes	Effects were coherent with other male reproductive findings and mechanistic evidence supporting biological plausibility
Other histopathological lesions in the testes and epididymis	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male	No	Effects provide corroborative evidence of male reproductive toxicity but were less sensitive compared with other findings
Decreased serum testosterone levels	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male	Yes	Effects displayed a dose-response pattern and were coherent with other male reproductive system findings
Decreased absolute testis weight	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male	Yes	
Decreased absolute epididymis weight (cauda and whole)	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, male	Yes	

Female Reproductive Effects

The available human evidence was judged to be *indeterminate* and thus these data were not considered for dose-response analysis (see Section 3.2.5). Only one animal study (NTP, 2018) evaluated female reproductive effects that were due to PFDA exposure; the study was evaluated as *high* confidence for all endpoints examined and provided *moderate* evidence for female reproductive toxicity. The NTP (2018) study reported reproductive effects in female rats exposed to PFDA (doses of 0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) via gavage for 28 days (see Table 5-8). Statistically significant dose-dependent changes were observed for the number of days spent in estrus and diestrus and for absolute and relative uterus weights; these endpoints were advanced for POD derivation. Although Bailey et al. (2004) provided guidance on the preferred measure (relative or absolute) for many organs (e.g., liver), both relative and absolute uterus weight were carried forward for POD derivation because it is unclear which is the preferred measure for this organ. Endpoints related to estrous cyclicity were also advanced for POD

derivation. Under normal conditions, the estrus stage is highlighted by sexual receptivity (Goldman et al., 2007). PFDA was shown to decrease the number of days spent in estrus in female rats, which could result in decreased opportunities for mating and ultimately in reductions or delays in fertility. PFDA was also reported to cause a continuous state of diestrus (NTP, 2018). Per EPA's *Guidelines for Reproductive Toxicity Risk Assessment*, "Persistent diestrus indicates temporary or permanent cessation of follicular development and ovulation, and thus at least temporary infertility." Refer to Section 3.2.5 for a more detailed discussion. Whereas the study authors also reported increased testosterone in female rats, this effect was not considered further because its biological relevance to the development of PFDA-induced female reproductive toxicity is unclear.

Table 5-8. Endpoints considered for dose-response modeling and derivation of points of departure for female reproductive effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Decreased estrus time	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Increased diestrus time	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Decreased absolute and relative uterus weight	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Increased testosterone	NTP (2018); *high* confidence	Gavage, 28 d	SD rat, female	No	The toxicological significance of this effect in females for the purposes of this assessment is unclear.

Estimation or Selection of Points of Departure (PODs) for RfD Derivation

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR selected to represent a minimal, biologically significant level of change. The BMD technical guidance (U.S. EPA, 2012a) sets up a hierarchy by which BMRs are selected, with the first and preferred approach using a biological or toxicological basis to define what minimal level of response or change is biologically significant. If that biological or toxicological information is lacking, the BMD technical guidance recommends alternative BMRs, specifically a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data (see Appendix D for more details). In cases when a biological or toxicological basis to define what minimal level of

response or change is biologically significant is lacking, a BMR of less than 1 SD is also considered when there are concerns about the severity of the effect, or effects occur in a sensitive lifestage. The BMRs selected for dose-response modeling of PFDA-induced health effects are listed in Table 5-9 along with the rationale for their selection.

Table 5-9. Benchmark response levels selected for BMD modeling of PFDA health outcomes

Endpoint	BMR	Rationale
Liver effects		
Increased serum enzymes in adult rats (ALT and ALP)	1 standard deviation	No information is readily available that allows for determining a minimally biologically significant response. The *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on 1 standard deviation (SD) for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.
Increased relative liver weight in adult rats	10% relative deviation	A 10% increase in liver weight is considered a minimally biologically significant response level in adult animals and has been used as the BMR for benchmark dose modeling in prior IRIS assessments.
Immune effects		
Decreased antibody concentrations for diphtheria and tetanus in children	½ standard deviation	Diphtheria and tetanus are serious and sometimes fatal infections. Immunomodulatory effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards. In addition, childhood represents a sensitive lifestage. Given the potential severity of this outcome, a BMR of both 1 SD and ½ SD were considered (see additional discussion in Appendix C.1.1). Ultimately, it was concluded that a BMR of ½ SD is best supported based on the severity of the outcome and the sensitive lifestage.
Developmental effects		
Decreased birth weight in humans	5% extra risk of exceeding adversity cutoff (hybrid approach)	A 5% extra risk is commonly used for dichotomous developmental endpoints as recommended by the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a). For birth weight, a public health definition of low birth weight (2,500 g) exists, and the hybrid approach was used to estimate the dose at which the extra risk of falling below that cutoff equaled 5% (see additional discussion in Appendix C.1.2).
Decreased fetal weight in mice	5% relative deviation	A 5% change was used because the developmental effects were observed during a sensitive lifestage. A

Endpoint	BMR	Rationale
		5% change in markers of growth/development in gestational studies (e.g., fetal weight) is considered a minimally biologically significant response level and has been used as the BMR for benchmark dose modeling in prior IRIS assessments (U.S. EPA, 2012b, 2004, 2003).
Male reproductive effects		
Increased Leydig cell atrophy in adult rats	10% extra risk	No information is readily available that allows for determining a minimally biological significant response. A 10% ER is recommended as the standard BMR for dichotomous endpoints in the absence of information for a biologically based BMR (U.S. EPA, 2012a).
Decreased epididymal sperm counts in adult rats	1 standard deviation	No information is readily available that allows for determining a minimally biological significant response. The *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.
Decreased serum testosterone in adult rats		
Decreased testicular weight in adult rats		
Decreased epididymal weight in adult rats		
Female reproductive effects		
Decreased estrus time in adult rats	5% relative deviation	Given that the PFDA-induced alterations in estrous cyclicity are possible indicators of infertility, which is an outcome of serious concern to the human population, a BMR of 5% RD is selected for these effects. Further support for the BMR of 5% RD is provided by the large magnitude of these effects. Specifically, PFDA induced a continuous state of diestrus in 100% of rats at the highest dose tested.
Increased diestrus time in adult rats		
Decreased absolute and relative uterus weight in adult rats	1 standard deviation	No information is readily available that allows for determining a minimally biologically significant response. The *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.

When modeling was feasible, the estimated BMDLs were used as PODs (see Table 5-10). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix C. When dose-response modeling was not feasible, or adequate modeling results were not obtained, no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) values were identified based on biological rationales when possible and used as the POD. NOAELs and LOAELs were determined based on the dose at

which biologically significant changes were identified, which takes precedence over statistical significance. For example, for relative liver weight, a 10% change is generally viewed as a biologically significant level of change, taking into consideration the study-specific variability. If no biological rationale for selecting the NOAEL/LOAEL is available, statistical significance was used as the basis for selection. The PODs (based on BMD modeling or NOAEL/LOAEL selection) for the endpoints advanced for dose-response analysis are presented in Table 5-10.

Application of Animal-Human Pharmacokinetic Extrapolation of PFDA Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Table 5-10 displays the POD and estimated HED PODs for liver, immune, developmental, and male and female reproductive endpoints from animal and/or human studies selected for the derivation of candidate values. Given that the available studies tested the free acid form of PFDA, normalization from a salt to the free acid using a molecular weight conversion was not performed, but formulas for providing such conversions are included in later tables.

Table 5-10. PODs considered for the derivation of PFDA candidate values

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD (mg/kg-d)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Liver effects						
Increased AST	28-d study (NTP, 2018); *high* confidence	SD rat, male	$BMDL_{1SD}$, Hill CV	0.123	3.36	4.93×10^{-4}
		SD rat, female	NOAEL[c] (1% increase)	0.625	25.15	3.70×10^{-3}
Increased ALP		SD rat, male	NOAEL[d] (9% increase)	0.156	4.25	6.25×10^{-4}
		SD rat, female	NOAEL[c] (14% increase)	0.156	5.60	8.24×10^{-4}
Increased relative liver weight		SD rat, male	$BMDL_{10RD}$, Hill CV	0.170	4.90	7.21×10^{-4}
		SD rat, female	$BMDL_{10RD}$, Hill CV[(e)]	0.112	4.03	5.92×10^{-4}
	28-d study (Frawley et al., 2018); *high* confidence	SD rat, female (histopathology study cohort)	$BMDL_{10RD}$, Exp2 CV	0.222	8.67	1.27×10^{-3}
		SD rat, female (MPS study cohort)	$BMDL_{10RD}$, Linear CV	0.187	7.04	1.04×10^{-3}
		SD rat, female (TDAR study cohort)	NOAEL[c] (2% increase)	0.125	4.49	6.61×10^{-4}
Immune effects (developmental)						
Decreased serum antitetanus	Budtz-Jørgensen and Grandjean	Human, male and female	$BMDL_{1/2SD}$ Linear	–	4.11×10^{-4}	6.04×10^{-8}

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD (mg/kg-d)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
antibody concentrations in children at age 7 yr and PFDA measured at age 5 yr	(2018a); Grandjean et al. (2012); *medium* confidence					
Decreased serum antidiphtheria antibody concentrations at age 7 yr and PFDA concentrations at age 5 yr	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	–	4.07×10^{-4}	5.98×10^{-8}
Decreased serum antitetanus antibody concentrations at age 5 yr and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	–	7.02×10^{-4}	1.03×10^{-7}
Decreased serum antidiphtheria antibody concentrations at age 5 yr and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	–	2.57×10^{-4}	3.78×10^{-8}
Developmental effects						
Decreased birth weight	Valvi et al. (2017); *high* confidence[f]	Human, male and female	$BMDL_{5RD}$, Hybrid	–	2.8×10^{-4}	4.12×10^{-8}
	Valvi et al. (2017); *high* confidence[f]	Human, male	$BMDL_{5RD}$, Hybrid	–	2.2×10^{-4}	3.23×10^{-8}
	Valvi et al. (2017); *high* confidence[f]	Human, female	$BMDL_{5RD}$, Hybrid	–	2.4×10^{-4}	3.53×10^{-8}

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD (mg/kg-d)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
	(Wikström et al., 2020); *high* confidence[g]	Human, male and female[h]	$BMDL_{5RD}$, Hybrid	–	3.7×10^{-4}	5.44×10^{-8}
	(Wikström et al., 2020); *high* confidence[g]	Human, male	$BMDL_{5RD}$, Hybrid	–	3.3×10^{-4}	4.85×10^{-8}
	(Wikström et al., 2020); *high* confidence[g]	Human, female	$BMDL_{5RD}$, Hybrid	–	3.1×10^{-4}	4.56×10^{-8}
Decreased fetal body weight	Developmental study (GD 6-15) (Harris and Birnbaum, 1989); *medium* confidence	C57BL/6N mouse, male and female	NOAEL[(c)] (4% decrease)	1	–	6.68×10^{-2}
Male reproductive effects						
Decreased cauda epididymis sperm count	28-d study (NTP, 2018); *low* confidence	SD rat, male	$BMDL_{1SD}$, Exp3 CV	0.963	37.28	5.48×10^{-3}
Increased Leydig cell atrophy	28-d study (NTP, 2018); *high* confidence		NOAEL[d] (0% change)	0.625	21.36	3.14×10^{-3}
Decreased serum testosterone			NOAEL[d] (25% decrease)	0.625	21.36	3.14×10^{-3}
Decreased absolute testis weight			$BMDL_{1SD}$, Linear CV	1.074	42.50	6.25×10^{-3}
Decreased absolute cauda epididymis weight			$BMDL_{1SD}$, Linear CV	0.582	20.01	2.94×10^{-3}
Decreased absolute whole epididymis weight			$BMDL_{1SD}$, Linear NCV	0.546	18.88	2.77×10^{-3}
Female reproductive effects						
Decreased number of days spent in estrus	28-d study (NTP, 2018); *high* confidence	SD rat, female	$BMDL_{5RD}$, Linear CV	0.128	4.60	6.76×10^{-4}
Increased number of days spent in diestrus			$BMDL_{5RD}$, Exp2 CV	0.200	7.65	1.12×10^{-3}
Decreased relative uterus weight			NOAEL[c] (12% increase)	0.625	25.15	3.70×10^{-3}

Endpoint	Study/ confidence	Strain/ species/sex	POD type/model	POD (mg/kg-d)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-d)
Decreased absolute uterus weight			NOAEL[c] (12% increase)	0.625	25.15	3.70×10^{-3}

[a]For PODs based on rat toxicity studies, POD internal concentration (POD_{int}) values were estimated by linear interpolation of the observed end-of-study serum concentrations in (NTP, 2018), as described in Section 3.1.7 and Appendix G.2.2. POD_{int} values from human epidemiological studies were determined directly from the analyses of response vs. blood concentration data.

[b]$POD_{HED} = POD_{int} \times CL_H$ for extrapolation from rat toxicity and human epidemiological studies, with $CL_H = 0.147$ mL/kg-d $= 1.47 \times 10^{-4}$ L/kg-d (see Table 3-3). For the developmental mouse endpoint, $POD_{HED} = POD \times DDEF$, where DDEF = 0.067. For DDEF derivation details, see Section 3.1.7.

[c]No models provided adequate fit; therefore, a NOAEL approach was selected.

[d]After visual inspection, data were not considered amenable for BMD modeling due to obvious nonmonotonicity in the dose response; therefore, a NOAEL approach was used instead.

[e]Highest dose group was dropped to allow for adequate model fit.

[f]Trimester 3 maternal biomarker samples.

[g]Ninety-six percent of samples during the first trimester and the remaining during the early weeks of the second trimester; sensitivity analyses showed no differences when trimester 2 samples excluded.

[h]Sex-specific results were available for both males and females separately; these were consistent in magnitude with the overall result.

Derivation of Candidate Lifetime Toxicity Values for the RfD

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFDA. The identified potential areas of susceptibility to PFDA exposure-induced health effects, including in children and possibly in women of reproductive age (see Section 4.3), can help inform uncertainty factor (UF) value selection and, subsequently, confidence in toxicity values. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate POD_{HED} values are listed in Table 5-11 below. For liver and male and female reproductive effects, quantitative information is limited to studies in which animals were exposed for ≤28 days. Serum levels of PFDA are not predicted to reach steady state in these studies because the half-life of PFDA in rodents is longer than 28 days. Hence, continued exposure to the same dose will likely result in higher serum and tissue levels, leading to greater effects. Furthermore, for each of these identified hazards, little information is available to assess the extent to which the specific changes caused by PFDA exposure for 28 days might be expected to worsen with PFDA exposure for a lifetime, even if serum and tissue levels remained constant at the levels reached after 28 days. Separately, human equivalent PODs for these endpoints were much less sensitive (several orders of magnitude) than the PODs for developmental and immune effects from the epidemiological studies (see Table 5-10). As such, for liver, male reproductive, and female reproductive effects, derivation of candidate lifetime values was not attempted given the high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting. However, these endpoints were considered for the derivation of the subchronic RfD (see Section 5.2.3).

Developmental effects observed in mice from the Harris and Birnbaum (1989) study, albeit observed after exposure during a sensitive lifestage, were not considered for derivation of a candidate lifetime value. Specifically, given the availability of PODs for developmental effects from *high* confidence human studies that were observed to be more sensitive than the POD from the rodent study (by 6–7 orders of magnitude; see Table 5-10), the available human data were given preference. It is important to note that the (Valvi et al., 2017) study was not considered for the derivation of candidate toxicity values for developmental effects given the limitations described above. However, the PODs determined from the (Valvi et al., 2017) study are informative for the PODs and resulting RfDs for developmental effects based on birth weight data from the (Wikström et al., 2020) study.

Table 5-11. Uncertainty factors for the development of the candidate lifetime toxicity values for PFDA

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to developmental and immunological effects observed in humans.
UF_H	10	A UF_H of 10 is applied for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFDA exposure in humans.
UF_S	1	A UF_S of 1 is applied to developmental delays (i.e., decreased birth body weight) [Wikström et al. (2020); and reduced antibody responses in children Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a). The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure in adulthood (U.S. EPA, 1991). Additional considerations for the UFS for immune effects are discussed below.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. BMDLs were available for both the developmental and immune effects in the epidemiological studies advanced for candidate value derivation.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of *high/medium* confidence short-term studies in rodents and a high confidence developmental study in mice. The database for PFDA also includes several *high/medium* confidence epidemiological studies most informative for immune and developmental effects, which are sensitive effects of PFDA exposure. However, uncertainties remain regarding the lack of studies examining effects with long-term exposure in adults—including in women of reproductive age (which may have increased susceptibility), studies of potential multigenerational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity after PFDA exposure during development. In all, the data are too sparse to conclude with certainty that the quantified developmental effects are likely to be the most sensitive; thus, a UF_D of 1 was not selected. However, a UF_D of 10 was also not selected give the availability of data from well-conducted studies on a range of health outcomes in multiple species, including sensitive evaluations of developmental and immune endpoints in humans. See discussion below for additional details.
UF_C	See Table 5-12	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans, and accounts for uncertainty regarding the pharmacokinetic and pharmacodynamic differences across species. The datasets considered for derivation of candidate lifetime values were from human studies, so a $UF_A = 1$ was applied to all PODs after the application of dosimetric approaches for estimation of HEDs as described above.

For immune effects, both a duration extrapolation uncertainty factor (UF_S) = 3 and a value of $UF_S = 1$ were considered to account for extrapolation from less than chronic data, ultimately

selecting a $UF_S = 1$. A $UF_S = 10$ was not considered as the developmental period is recognized as a susceptible lifestage for these types of effects and therefore exposure during this time window can be considered more relevant to the induction of developmental effects than exposure in adulthood (U.S. EPA, 1991). The reduced antibody responses were measured in children 5–7 years of age. The HED calculations used for these immune effects assume chronic exposure, so an RfD based on them will assure that serum PFDA levels remain below the POD irrespective of exposure duration. Also, development is recognized as a sensitive period for effects on immune system responses. According to the WHO/IPCS Immunotoxicity Guidance for Risk Assessment, developmental immunotoxicity encompasses the prenatal, neonatal, juvenile, and adolescent lifestages and should be viewed differently from the immune system of adults from a risk assessment perspective (IPCS, 2012). Special considerations for developmental immunotoxicity include increased dose sensitivity, potential for effects to become permanent even after cessation of exposure, broader spectrum of adverse effects and "rewiring of the immune system" (IPCS, 2012), which indicates a greater health risk for early-life exposures to immunotoxicants compared with adults. Given PFDA's long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFDA for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure to the subjects from conception until the age when the response was evaluated. Further, the consequences of perturbed immune system function (in this case, suppressed antibody responses leading potentially to increased disease) during development are expected to be generally more severe and longer lasting than those that manifest in healthy adults. Taken together, the observed immune effects in children considered to be the result of prolonged exposure to PFDA and the enhanced susceptibility of the developmental immune system to chemical pollutants, attenuate concerns of potentially increased sensitivity with longer-term exposures. As such, a $UF_S = 1$ rather than a $UF_S = 3$ was applied for immune effects in children. Uncertainties regarding possible more sensitive latent effects of these impacts on the immune system during early-life exposures leading to unpredictable outcomes later in life, for example in other susceptible lifestages of reduced immunocompetence such as pregnancy and most notably old age, are addressed as part of the justification for selecting a database uncertainty factor (UFD) > 1, as discussed below.

For PFDA, both a $UF_D = 10$ and a $UF_D = 3$ were considered due to the limited database (e.g., the lack of a two-generation developmental/reproductive toxicity study) and a $UF_D = 3$ ultimately was applied. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. The PFDA database does include a *medium* confidence (Harris and Birnbaum, 1989) developmental toxicity study in mice. Despite its quality, however, that study fails to cover potential transgenerational impacts of longer-term exposures evaluated in a two-generation study. The 1994 *Reference Concentration Guidance* (U.S. EPA, 1994) and 2002 *Reference Dose Report* (U.S. EPA, 2002); (U.S. EPA, 2002) support applying a UF_D in

situations when such a study is missing. The 2002 *Reference Dose Report* (U.S. EPA, 2002); (U.S. EPA, 2002) states that "[i]f the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproductive study is missing." Further, (U.S. EPA, 2002) states "[i]f the RfD/RfC is based on human data, a similar assessment regarding the completeness of the database is necessary. Information on life stages and organ systems may come from either animal or human studies. If data on specific life stages or organ systems are unavailable or limited data suggest that availability of more extensive data might decrease the POD, this should be taken into account in assigning a database UF." Consideration of the PFDA, PFBA (a short-chain perfluoroalkyl carboxylic acid),[27, 28] PFBS (a short-chain perfluoroalkane sulfonic acid with a 4-carbon backbone), [29] PFHxA (a short-chain perfluoroalkyl carboxylic acid), and PFHxS (a long-chain perfluoroalkane sulfonic acid)[30] databases together, however, diminish the concern that the availability of a multigenerational reproductive study would result in reference values far below those currently derived for PFDA. Although limited in their ability to assess reproductive health or function, measures of possible reproductive toxicity occurred at doses equal to or higher than those that resulted in effects in other organ systems (e.g., thyroid, liver) when measured after exposure to PFDA for 28 days (NTP, 2019). Similar results were observed for the animal databases for PFOA and PFOS indicating reproductive effects were not uniquely sensitive markers of toxicity for these long-chain PFAS (ATSDR, 2021). Further, no notable male or female reproductive effects were observed in epidemiological or toxicological studies investigating exposure to PFHxS (MDH, 2019). Therefore, considering the limited chemical-specific information alongside information gleaned from structurally related compounds, the lack of a multigenerational reproductive study is not considered a major concern relative to UF_D selection for PFDA.

The lone animal developmental study (Harris and Birnbaum, 1989) for PFDA also did not evaluate postnatal developmental effects. Effects on postnatal development (e.g., delayed eye opening; reduced postnatal growth) have been observed in rodents exposed to other long-chain PFAS such as PFOA (ATSDR, 2021). Overall, the available information on potential PFDA-induced postnatal developmental effects is sparse, introducing uncertainty as to whether more sensitive developmental effects of PFDA might occur and may be of concern relative to UF_D selection.

Another gap in the PFDA database is the lack of measures of thyroid toxicity in gestationally exposed offspring or after longer-than-28-day PFDA exposures, and the lack of a developmental

[27]The systematic review protocol for PFDA (see Appendix A) defines perfluoroalkyl carboxylic acids with seven or more perfluorinated carbon groups and perfluoralkane sulfonic acids with six or more perfluorinated carbon groups as "long-chain" PFAS. Thus, PFHxA and PFBA are considered short-chain PFAS, whereas PFHxS is considered a long-chain PFAS.
[28]IRIS Toxicological Review of Perfluorobutanoic Acid (PFBA, CASRN 375-22-4) and Related Salts {U.S. EPA, 2022, 10692791}.
[29]Human health toxicity values for perfluorobutane sulfonic acid (CASRN 375-73-5) and related compound potassium perfluorobutane sulfonate (CASRN 29420-49-3), (U.S. EPA, 2021b).
[30]Health Based Guidance for Water: Toxicological Summary for: Perfluorohexane sulfonate (PFHxS), MDH (2019).

neurotoxicity study. Thyroid hormones are critical in myriad physiological processes and must be maintained at sufficient levels during times of brain development in utero and after birth. Although no PFDA-specific data on thyroid hormone levels following gestational exposure are available, effects on thyroid hormone homeostasis were observed in a study in adult rats exposed to PFDA for 28 days (NTP, 2018), and disrupted thyroid signaling has been shown to be a consequence of exposure to other PFAS (U.S. EPA, 2021b). Therefore, anticipating that potentially sensitive effects that were due to PFDA exposure also could have been observed had thyroid hormone levels been measured in the Harris and Birnbaum (1989) developmental study, or in longer-term studies, is reasonable. Thus, the lack of data for PFDA-induced effects on thyroid levels in developing animals or with prolonged exposure or data on potential thyroid dependent neurodevelopmental effects is a source of uncertainty.

Lastly, the potential for sensitive effects following long-term exposure durations represents an area of uncertainty for the PFDA database. While the potential for more sensitive effects is mitigated mostly by the availability of sensitive PODs (compared with other PODs) for developmental effects from human studies, there are no comprehensive subchronic and chronic animal studies available for PFDA. The longest exposure study treated mice for 30–49 days via drinking water but tested only one high-PFDA dose (6.6 mg/kg-day) and evaluated limited endpoints (body weight and survival) (Wang et al., 2020). No chemical-specific information is available to judge the degree to which the existing endpoints in the PFDA Toxicological Review would be more sensitive with extended durations. Given that the PODs used to derive candidate values were from studies of developmental exposure, this uncertainty cannot be fully addressed through the application of a UF_S. Specifically, for immune effects, there is a lack of epidemiological studies or studies in animals examining the effects of PFDA exposures that encompass later developmental periods (e.g., late childhood and adolescence) or other potentially susceptible lifestages such as pregnancy and old age. In addition, the available studies include limited or no evaluation of immunotoxicity categories other than immunosuppression, namely sensitization and allergic response, and autoimmunity and autoimmune disease.

Given the residual concerns for potentially more sensitive effects outlined above, a database uncertainty factor is considered necessary. Specifically, a value of 3 was selected for the UF_D to account for the uncertainty surrounding the lack of an evaluation of postnatal or multigenerational effects in animals, specific investigations of potential effects on thyroid function after developmental exposure or neurodevelopmental effects, and comprehensive long-term studies in multiple species.

The uncertainty factors described in Table 5-11 and the text above were applied and the resulting candidate values are shown in Table 5-12. The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor as shown below.

$$Candidate\ values\ for\ PFDA = POD_{HED} \div UFc \quad (5\text{-}1)$$

Table 5-12. Candidate RfD values for PFDA

Endpoint	Study/ confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Immune effects (developmental)										
Decreased serum antitetanus antibody concentration in children at age 7 yr and PFDA measured at age 5 yr	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	6.04×10^{-8}	1	10	1	1	3	30	2×10^{-9}
Decreased serum antidiphtheria antibody levels at age 7 yr and PFDA concentrations at age 5 yr	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	5.98×10^{-8}	1	10	1	1	3	30	2×10^{-9}
Decreased serum antitetanus antibody levels at age 5 y and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.03×10^{-7}	1	10	1	1	3	30	3×10^{-9}
Decreased serum antidiphtheria antibody levels at age 5 yr and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	3.78×10^{-8}	1	10	1	1	3	30	1×10^{-9}
Developmental effects										
Decreased birth weight	(Wikström et al., 2020) *high* confidence	Human, male and female	5.44×10^{-8}	1	10	1	1	3	30	2×10^{-9}
	(Wikström et al., 2020) *high* confidence	Human, male	4.85×10^{-8}	1	10	1	1	3	30	2×10^{-9}

Endpoint	Study/ confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
	(Wikström et al., 2020) *high* confidence	Human, female	4.56×10^{-8}	1	10	1	1	3	30	2×10^{-9}

[a]The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

5.2.2. Selection of Lifetime Toxicity Value(s)

Selection of Organ/System-Specific Oral Reference Doses (osRfDs)

From among the candidate values presented in Table 5-12, organ/system-specific RfDs (osRfDs) are selected for the individual organ systems identified as hazards in Section 3. The osRfD values selected were associated with decreased serum antibody concentrations in children for immune effects and decreased birth weight for developmental effects. The confidence decisions about the studies, evidence base, quantification of the POD, and overall osRfD are fully described in Table 5-13, along with the rationales for selecting those confidence levels. In deciding overall confidence, confidence in the evidence base is prioritized over the other confidence decisions. The overall confidence in the osRfD for immune effects is medium, and the confidence in the osRfD for developmental effects is medium-low. Selection of the overall RfD is described in the following section.

Table 5-13. Confidence in the organ/system-specific (osRfDs) for PFDA

Confidence categories	Designation	Discussion
Immune (developmental) osRfD = 2×10^{-9} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) was rated as *medium* primarily due to relatively limited PFDA exposure contrasts, which can decrease study sensitivity in general (HAWC link). Given that the results in this study were statistically *significant* and that PFOS, PFOA, and PFNA were not considered meaningful confounders (see Section 3.2.2 and Appendix C.1), EPA concluded that while there were potential study sensitivity concerns at the evaluation stage, the results clearly showed that those concerns were not borne out, and confidence in this study to derive an osRfD was judged to be *high*.

Confidence categories	Designation	Discussion
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for immune effects is medium based on consistent findings of reduced antibody responses from two *medium* confidence birth cohort studies (Grandjean et al., 2012); (Grandjean et al., 2017a); (Grandjean et al., 2017b) and a low confidence study in adults (Kielsen et al., 2016). Short-term studies in animals of *high/medium* confidence provide supportive evidence of immunosuppression after PFDA exposure (Frawley et al., 2018); (NTP, 2018). Some residual uncertainties regarding unexplained inconsistency and potential confounding by other co-occurring PFAS from epidemiological studies and issues with concomitant overt target organ and systemic toxicity in animal studies lower confidence in the available evidence for this hazard. Other limitations include the lack of epidemiological studies or long-term/chronic studies in animals examining effects on the immune system across different developmental lifestages and immunotoxicity categories, including sensitization and allergic response and autoimmunity and autoimmune disease.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium.* The POD is based on BMD modeling at the lower end of the range of the observed data and a $BMDL_{1/2SD}$ estimate that is associated with a small degree of uncertainty due to potential confounding by PFOA (see Appendix D.1.1 for more details). The POD for decreased tetanus antibodies at age 7 yr was judged to be *medium* confidence based on a good model fit and was supported by the nearly identical POD for decreased diphtheria antibodies at age 7 yr. Both PODs support the osRfD. An estimate for human clearance was applied to estimate the POD_{HED} using PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on uncertainty in the pharmacokinetic modeling of PFDA above). There is also uncertainty as to the most sensitive window of vulnerability with respect to the exposure/outcome measurement timing (BMDs/BMDLs were estimated from PFDA levels measured at age 5 or perinatally and antitetanus antibody concentrations measured at age 7 or 5); (Grandjean et al., 2017b) reported that *estimated* PFDA "concentrations at 3 mo and 6 mo showed the strongest inverse associations with antibody concentrations at age 5 yr, particularly for tetanus." Thus, it is possible that adverse effects during infancy could be more sensitive than between ages 5 and 7 yr.
Overall confidence in osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is driven by *medium* confidence in the evidence base for immune effects and the quantification of the POD.

Confidence categories	Designation	Discussion
Developmental osRfD = 2×10^{-9} mg/kg-d		
Confidence in study[a] used to derive osRfD	*Medium*	Confidence in the Wikström et al. (2020) study for hazard identification was rated as *high* (HAWC link) for developmental effects. The study was selected for dose-response analysis due to low overall risk of bias and reliable exposure measurements, which had sufficient exposure contrasts and other characteristics that allowed for adequate study sensitivity to detect associations. The Wikström et al. (2020) study demonstrated associations consistent in magnitude for boys, girls, and the overall population. Overall, mean birth weight was considered the most precise and accurate endpoint and not anticipated to be subject to much error. This study was advanced for dose-response analysis, given minimal presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). Wikström et al. (2020) also adjusted for sample timing in their multivariate models and show no differences in models also restricted to trimester 1 samples only. Some uncertainty remains on the potential for confounding by other PFAS (concern primarily for PFNA), which were not examined in this study. Given the potential quantitative impact of this uncertainty, confidence in the use of this study for dose-response analysis was judged as *medium* rather than *high*.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for developmental effects is *medium*. There was consistent evidence for reduced birth weight among multiple human studies, including high-quality studies. However, unlike the Wikström et al. (2020) study used here and noted above, some uncertainty remains in many studies given the predominance of associations that were detected for studies with later pregnancy sampling. The human database also showed some coherence across different measures of fetal growth restriction. In animals, the lone developmental study reported effects on fetal growth that are coherent with effects observed in humans. Some residual uncertainty regarding potential confounding by other co-occurring PFAS from epidemiological studies lowers confidence in the available evidence for this hazard.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on a BMD hybrid approach within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is driven by *medium-low* confidence in the evidence base for developmental effects (i.e., fetal growth restriction).

[a]All study evaluation details can be found on HAWC.

Selection of Overall Oral Reference Dose (RfD) and Confidence Statement

Organ/system-specific and overall RfD values for PFDA selected in the previous section are summarized in Table 5-14.

Table 5-14. Organ/System-specific and overall lifetime RfDs for PFDA

System	Toxicity value	Basis	POD_{HED} (mg/kg-d)[a]	UF_C	osRfD or RfD (mg/kg-d)	Confidence
Immune (developmental)	osRfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr	6.04×10^{-8} based on BMDL½ SD from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a)	30	2×10^{-9}	*Medium*
Developmental	osRfD	Decreased birth weight in males and females	5.44×10^{-8} based on BMDL5%RD from (Wikström et al., 2020)	30	2×10^{-9}	*Medium-low*
Immune /developmental	Overall lifetime RfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr Decreased birth weight in males and females	6.04×10^{-8} based on BMDL½ SD from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) 5.44×10^{-8} based on BMDL5%RD from (Wikström et al., 2020)	30	2×10^{-9}	*Medium*

[a]The details of the BMD modeling approach and results can be found in Appendix C.

From the identified human health effects of PFDA and derived osRfDs for immune and developmental effects (see Table 5-14), an overall ***RfD of 2 × 10⁻⁹ mg/kg-day based on decreased serum antibody concentrations and decreased birth weight in humans*** was selected. As described in Table 5-14, confidence in the RfD is *medium*, based on *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental (Wikström et al., 2020) endpoints using BMD modeling. This RfD is the same for both developmental and immune critical effects given that the PODs for these two osRfDs were similar (i.e., 6.04×10^{-8} and 5.44×10^{-8}, respectively) and

that identical UFs were applied. Selection of the overall RfD is presumed to be protective of all other potential health effects in humans, based on the currently available evidence. Finally, the immune osRfD and developmental osRfD are based on effects observed in males and females indicating that the overall RfD would be protective for both sexes.

Overall, the immune and developmental endpoints from epidemiological studies of PFDA were preferentially advanced for the derivation of candidate lifetime values. For immune effects, osRfDs were derived for decreased serum antibody levels (for both diphtheria and tetanus) in children (male and female) at different timing of exposure and outcome measurement combinations, specifically antibody levels at age 7 and PFDA concentrations at age 5, and antibody levels at age 5 and perinatal PFDA concentrations (Budtz-Jørgensen and Grandjean, 2018a) (see Table 5-12). The toxicity value (osRfD) for immune effects of 2×10^{-9} mg/kg-day was based on deleterious effects observed in children showing decreased antibody concentrations for both tetanus and diphtheria at age 7 years related to serum PFDA concentrations measured at age 5 years. The PODs for decreased tetanus and diphtheria antibody concentrations were nearly identical ($BMDL_{1/2SD[HED]}$ of 6.04×10^{-8} mg/kg-day for tetanus and 5.98×10^{-8} mg/kg-day for diphtheria) and were close to the PODs for other outcome-exposure combinations (see Table 5-10), which further supports the selected osRfD. Although both tetanus and diphtheria are rare in the U.S., the findings that PFDA exposure reduced antibody responses may be broadly indicative of developmental immunosuppression impacting overall immune function in these children. The lowest serum PFDA concentration measured at age 5 years was 0.05 ng/mL and the 10th percentile was 0.2 ng/mL (Grandjean and Bateson, 2021) so the estimated $BMD_{½SD}$ (0.411 ng/mL) for this endpoint 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 (see Appendix C.1.1 for more details).

For developmental effects, given that the candidate toxicity values are identical (see Table 5-10), the osRfD of 2×10^{-9} mg/kg-day ($BMDL_{5RD[HED]}$ of 5.44×10^{-8} mg/kg-day) based on reduced birth weight in males and females from the Wikström et al. (2020) study was selected. Although this osRfD is not based on the lowest POD for reduced birth weight from the (Wikström et al., 2020) study, it is more representative of the general human population (males and females combined) than the comparisons in males or females only. There is some uncertainty with PODs considered from the Valvi et al. (2017) study because it is not based on early sampling and may be prone to bias from pregnancy hemodynamics to some unknown degree. As discussed in Appendix F, there is only one developmental study (Gyllenhammar et al., 2018) for PFDA that collected and was able to analyze maternal hemodynamics data such as GFR and/or albumin. This study did not report any evidence of confounding following statistical adjustment of different GFR measures for any of the PFAS examined, which is consistent with no demonstrated confounding by either GRR (Manzano-Salgado et al., 2017); (Whitworth et al., 2012) or albumin (Sagiv et al., 2018) for other PFAS examined in other studies. However, existing meta-analyses for both PFOA (Steenland et al., 2018) and PFOS (Dzierlenga et al., 2020) only detected birth weight deficits for later trimester

sampling (e.g., beyond the first trimester). A similar detailed analysis was precluded for PFDA given that there are only two studies that examined any first trimester measures. Overall, there was limited evidence of any patterns of larger birth weight associations with sample timing for PFDA, but possible associations could not be evaluated further given limited available data as well as disparate exposure measures, distributions, and contrasts being examined. In contrast, the Wikström et al. (2020) study was prioritized for RfD derivation as it was a high confidence study that predominately sampled maternal plasma during the first trimester thereby reducing uncertainty relating to pregnancy hemodynamics. Further confidence in the osRfD derived from the (Wikström et al., 2020) study is provided by the fact that the PODs from the (Wikström et al., 2020) and (Valvi et al., 2017) studies are relatively close (see Table 5-14 above). While not presented in this Toxicological Review, additional birth weight studies were BMD modeled to provide a sensitivity analysis for the comparison of birth weight effects; please see Table C-8 of the Supplemental Appendices. These studies are either medium confidence and/or have later trimester sampling and thus not considered in the dose-response analysis. The PODs from these birth weight studies are relatively close (varying by ~threefold), providing further confidence in using the POD from the (Wikström et al., 2020) study for RfD derivation. In addition to the quantitative implications, the close proximity of the BMDLs from a multitude of birth weight studies increases the confidence in deriving osRfDs despite *slight* evidence of developmental effects in humans.

5.2.3. Subchronic toxicity values for oral exposure (subchronic oral reference dose [RfD]) derivation

In addition to providing an RfD for lifetime exposure in health systems, this document also provides an RfD for less-than-lifetime ("subchronic") exposures. Datasets considered for the subchronic RfD were based on endpoints advanced for RfD derivation in Table 5-10. Given that the developmental and immune effects were observed in humans exposed to PFDA during susceptible lifestages (postnatal growth/development and immune system effects in children at ages 5–7), these endpoints were also considered for the derivation of candidate subchronic values, applying identical uncertainty factors to those used for the lifetime candidate values (see Table 5-15 below).

Similar to the derivation of the lifetime RfD, the developmental effects observed in mice from the Harris and Birnbaum (1989) study were not advanced for the derivation of candidate subchronic values. The developmental PODs from human studies are 6–7 orders of magnitude more sensitive than the POD from the rodent study (see Table 5-10), and were, therefore, prioritized. In addition, endpoints for hepatic, male reproductive toxicity, and female reproductive toxicity observed in the 28-day rodent study (NTP, 2018) were considered for the derivation of subchronic toxicity values. As compared with the large uncertainty in extrapolating the available 28-day studies to lifetime PFDA exposure in the context of the RfD, it was considered reasonable to try to extrapolate the 28-day study data for the purposes of deriving subchronic candidate values.

The use of animal data for hepatic, male reproductive, and female reproductive endpoints required the application of different uncertainty factors than those used for developmental and immune effects in humans and can be found in Table 5-15.

Table 5-15. Uncertainty factors for the development of the candidate subchronic values for PFDA

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to developmental and immunological effects observed in epidemiological studies.
	3	A UF_A of 3 is applied to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral PFDA exposure. Aspects of the cross-species extrapolation of pharmacokinetic processes have been accounted for by using a PK approach that interpolated measured PFDA serum concentrations in rats from the NTP 28-d bioassay, EPA's custom PK model for mice (incorporating mouse-specific PFDA PK parameters) and a PFDA clearance estimated from human data; however, some residual pharmacokinetic uncertainty remains as does the potential for pharmacodynamic differences. Availability of chemical-specific data justifies the selection of a UF of 3 for PFDA. See discussion below for more details.
UF_H	10	A UF_H of 10 is applied for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFDA exposure in humans.
UF_S	1	A UF_S of 1 is applied to developmental delays (i.e., decreased birth body weight) Wikström et al. (2020); and reduced antibody responses in children (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012).The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than subchronic exposure (U.S. EPA, 1991).
	10	A UF_S of 10 is applied to liver, male reproductive, and female reproductive effects in adult animals (increased AST levels, decreased epididymis weight and decreased number of days in estrus, respectively) because of the short exposure duration (28 d) and the presumption that effects would worsen with longer exposures. See discussion below for more details.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. All PODs considered for candidate subchronic values were BMDLs.

UF	Value	Justification
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of *high/medium* confidence short-term studies in rodents and a *high* confidence developmental study in mice. The database for PFDA also includes several *high/medium* confidence epidemiological studies most informative for immune and developmental effects. However, uncertainties remain regarding the lack of studies of potential multigenerational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity during developmental lifestages. In all, the data are too sparse to conclude with certainty that the quantified developmental effects are likely to be the most sensitive; thus, a UF_D of 1 was not selected. However, a UF_D of 10 was also not selected give the availability of data from well-conducted studies in multiple species, including developmental and short-term rodent studies examining a range of potentially sensitive health outcomes and sensitive evaluations of developmental and immune endpoints in humans.
UF_C	See Table 5-16	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

As described above under "Derivation of candidate lifetime toxicity values for the RfD," and in (U.S. EPA, 2002), five possible areas of uncertainty and variability were considered in deriving the candidate subchronic values for PFDA. In general, the explanations for these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate POD_{HED} values are listed above and in Table 5-15, including the UF_D, which remained at 3 because of data gaps discussed previously in the derivation of the lifetime RfD. One UF that differs between subchronic and chronic RfDs is that for effects (i.e., decreased fetal body weight, increase AST levels, decreased whole epididymis weight, and decreased estrus time) observed in rodents. A UF_A of 3 was applied to account for pharmacokinetic and pharmacodynamic differences between rodents and humans following oral PFDA exposure. As is usual in the application of this uncertainty factor, the pharmacokinetic uncertainty is partly addressed through the application of an adjustment factor, in this case, chemical-specific dosimetric data for estimating human equivalent doses (see "Approach for Pharmacokinetic Extrapolation of PFDA among Rats, Mice, and Humans" in Section 3.1.7 and "Application of Animal-Human Pharmacokinetic Extrapolation of PFDA Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints" in Section 5.2.1). This application leaves some residual uncertainty around the pharmacokinetics and the uncertainty surrounding differences in pharmacodynamic differences between animals and humans. Typically, a UF_A of 3 is applied for this uncertainty when either $BW^{3/4}$ scaling or chemical-specific information is used for dose extrapolation, which is the case for mouse developmental and the rat male and female reproductive endpoints. For the liver endpoint, available mechanistic and supplemental information is considered further in determining the most appropriate value for the UF_A to account for the uncertainty.

Evidence from in vitro studies suggest that PFDA interacts with several human receptor pathways relevant to its mechanism of hepatotoxicity, including PPARα. PFDA can bind and activate

PPARα in vitro, but reduced or no sensitivity toward the human PPARα versus other mammalian isoforms (i.e., mouse, Baikal seal and polar bear PPARα isoforms) is apparent (Ishibashi et al., 2019; Routti et al., 2019; Wolf et al., 2012; Wolf et al., 2008) and similar findings have been demonstrated for some other perfluorinated compounds. If PPARα were the only operant MOA for noncancer effects in the liver, this observation might support reducing the remaining portion of the UF_A to 1, as it could be argued that humans are not as sensitive as wild-type rats are to the hepatic effects of PFDA exposure (note: without evidence to the contrary, as mentioned in the previous paragraph, the toxicodynamic portion of this UF is typically assigned a value of 3 assuming responses manifest in humans could be more sensitive than those observed in animals). Although PPARα appears to be an important mechanism of PFDA-induced liver toxicity in animals and reduced sensitivity in PPAR activation in humans compared with rodents has been suggested, available evidence for PFDA in PPARα null mice, human in vitro assays and in vivo animal models more relevant to humans with respect to PPARα sensitivity (i.e., guinea pigs and Syrian hamsters) suggest that liver effects occur, at least in part, independent of PPARα (see "Summary of Mechanistic Studies" for PFDA in Section 3.2.1). A plausible PPARα-dependent and independent MOA for liver effects is also supported by studies in null and humanized animal models of structurally related long-chain PFAS [C7–C9] (see "Evidence from Related PFAS" in Section 3.2.1), which are mostly lacking for PFDA (a few studies in null mice but no humanized models). Considering the remaining uncertainty in additional MOAs that appear active in PFDA-induced liver effects, and the relative contribution of these MOAs to toxicity in humans compared with rodents, uncertainties surrounding a potential multifaceted MOA for PFDA-induced liver effects, a value of 3 was selected for the UF_A for the purposes of deriving candidate subchronic toxicity values for hepatic effects.

EPA states that for "short-term and longer-term reference values, the application of a UF analogous to the subchronic-to-chronic duration UF also needs to be explored, as there may be situations in which data are available and applicable, but they are from studies in which the dosing period is considerably shorter than that for the reference value being derived" (U.S. EPA, 2002). This is the case for hepatic, male reproductive and female reproductive endpoints derived from the 28-day NTP (2018) study. Although there is no chemical-specific information to evaluate the potential for increased sensitivity with exposures longer than 28-days (e.g., a 90-day subchronic study), the following considerations are outlined to inform the application of the UF_S for duration extrapolation. (U.S. EPA, 2002)

Regarding female reproductive toxicity, PFDA-induced effects on estrous cyclicity were observed to be of large magnitude in the 28-day study. Specifically, PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested (2.5 mg/kg-day) by day 21 (by day 9 of the sixteen days in which vaginal cytology was assessed) (NTP, 2018). Given these data, it is possible that PFDA-induced effects on estrous cyclicity could become more sensitive or lead to more severe downstream effects like infertility with longer exposure durations. For male reproductive effects, the study duration (28 days) was insufficient to cover the entire period of

spermatogenesis in rats (~8 weeks), raising concerns about reduced sensitivity for some of the endpoints evaluated and selected for POD derivation (i.e., sperm evaluations). For liver effects, increases in relative liver weights demonstrated a time dependency across short-term exposures. Relative liver weight increased by 17%–56% at 1.15–10 mg/kg-day in rats exposed for 7–14 days and by 12%–127% at 1–16 mg/kg-day in mice exposed during gestion (GD 10–13 and 6–15). Similar magnitudes of liver weight increases were achieved in rodents after 28-day exposure but at lower PFDA doses (10%–102% at 0.125–2.5 mg/kg-day in rats and 16%–81% at 0.089–0.71 mg/kg-day in mice). The limited data for liver weight suggest potential increase in sensitivity with increasing duration, although there is no information on how liver weight or other sensitive liver endpoints (increased AST and ALP levels) are impacted by longer-term exposures (>28 days). Considering the potential for some health effects (prolonged diestrus, sperm measures, and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of any chemical-specific data on whether the effects observed in the short-term study worsen after subchronic exposure, a UFs of 10 is selected for the purposes of deriving candidate subchronic toxicity values from the 28-day toxicity data.

The uncertainty factors described in Table 5-15 and the text above were applied and the resulting candidate subchronic values are shown in Table 5-16. The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor as shown below.

$$Candidate\ values\ for\ PFDA = POD_{HED} \div UFc \quad (5\text{-}2)$$

Table 5-16. Candidate values for deriving the subchronic RfD for PFDA

Endpoint	Study/ Confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Immune effects (developmental)										
Decreased serum antitetanus antibody concentrations in children at age 7 yr and PFDA measured at 5 yr	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	6.04×10^{-8}	1	10	1	1	3	30	2×10^{-9}
Decreased serum antidiphtheria antibody concentrations at age 7 yr and PFDA concentrations at age 5 yr	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	5.98×10^{-8}	1	10	1	1	3	30	2×10^{-9}

Endpoint	Study/ Confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Decreased serum antitetanus antibody concentrations at age 5 yr and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.03×10^{-7}	1	10	1	1	3	30	3×10^{-9}
Decreased serum antidiphtheria antibody concentrations at age 5 yr and perinatal (pregnancy week 32–2 wk postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	3.78×10^{-8}	1	10	1	1	3	30	1×10^{-10}
Developmental effects										
Decreased birth weight	Wikström et al. (2020); *high* confidence	Human, male and female	5.44×10^{-8}	1	10	1	1	3	30	2×10^{-9}
	Wikström et al. (2020); *high* confidence	Human, male	4.85×10^{-8}	1	10	1	1	3	30	2×10^{-9}
	Wikström et al. (2020); *high* confidence	Human, female	4.56×10^{-8}	1	10	1	1	3	30	2×10^{-9}
Liver effects										
Increased AST	28-d study NTP (2018); *high* confidence	SD rat, male	4.93×10^{-4}	3	10	10	1	3	1,000	5×10^{-7}
		SD rat, female	3.70×10^{-3}	3	10	10	1	3	1,000	4×10^{-6}
Increased ALP		SD rat, male	6.25×10^{-4}	3	10	10	1	3	1,000	6×10^{-7}
		SD rat, female	8.24×10^{-4}	3	10	10	1	3	1,000	8×10^{-7}
Increased relative liver weight		SD rat, male	7.21×10^{-4}	3	10	10	1	3	1,000	7×10^{-7}

Endpoint	Study/ Confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
		SD rat, female	5.92×10^{-4}	3	10	10	1	3	1,000	6×10^{-7}
	28-d study Frawley et al. (2018); *high* confidence	SD rat, female (histo-pathology study cohort)	1.27×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
		SD rat, female (MPS study cohort)	1.04×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
		SD rat, female (TDAR study cohort)	6.61×10^{-4}	3	10	10	1	3	1,000	7×10^{-7}
Male reproductive effects										
Decreased cauda epididymis sperm count	28-d study NTP (2018); *low* confidence	SD rat, male	5.48×10^{-3}	3	10	10	1	3	1,000	5×10^{-6}
Increased Leydig cell atrophy	28-d study NTP (2018); *high* confidence		3.14×10^{-3}	3	10	10	1	3	1,000	3×10^{-6}
Decreased serum testosterone			3.14×10^{-3}	3	10	10	1	3	1,000	3×10^{-6}
Decreased absolute testis weight			6.25×10^{-3}	3	10	10	1	3	1,000	6×10^{-6}
Decreased absolute cauda epididymis weight			2.94×10^{-3}	3	10	10	1	3	1,000	3×10^{-6}
Decreased absolute whole epididymis weight			2.77×10^{-3}	3	10	10	1	3	1,000	3×10^{-6}
Female reproductive effects										
Decreased number of days spent in estrus	28-d study NTP (2018); *high* confidence	SD rat, female	6.76×10^{-4}	3	10	10	1	3	1,000	7×10^{-7}
Increased number of days spent in diestrus			1.12×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}

Endpoint	Study/ Confidence	Strain/ species/ sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Decreased relative uterus weight			3.70×10^{-3}	3	10	10	1	3	1,000	4×10^{-6}
Decreased absolute uterus weight			3.70×10^{-3}	3	10	10	1	3	1,000	4×10^{-6}

[a]The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

Selection of Subchronic Toxicity Value(s)

As described above, candidate subchronic values for several health effects associated with PFDA exposure were derived. The subchronic osRfD values selected were associated with decreased serum antibody concentrations for developmental immune effects, decreased birth weight for developmental effects, increased relative liver weight for liver effects, decreased whole epididymis weight for male reproductive effects, and increased number of days spent in diestrus for female reproductive effects. As discussed earlier, these subchronic osRfDs may be useful for certain decision purposes (i.e., site-specific risk assessments with less-than-lifetime exposures). Confidence in each subchronic osRfD is described in Table 5-17 and includes confidence in the study used to derive the quantitative estimate, the overall health effect, specific evidence base, and quantitative estimate for each subchronic osRfD.

Table 5-17. Confidence in the subchronic organ/system-specific RfDs (subchronic osRfDs) for PFDA

Confidence categories	Designation[a]	Discussion
Immune (developmental) subchronic osRfD = 2×10^{-9} mg/kg-d		
Confidence in study used to derive the subchronic osRfD	*High*	Confidence in Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) was rated as *medium* primarily due to relatively limited PFDA exposure contrasts, which can decrease study sensitivity in general (HAWC link). Given that the results in this study were statistically significant, EPA concluded that while there were potential study sensitivity concerns at the evaluation stage, the results clearly showed that those concerns were not borne out, and confidence in this study to derive an osRfD was judged to be high.
Confidence in the evidence base supporting this hazard	*Medium*	Confidence in the evidence base for immune effects is *medium* based on consistent findings of reduced antibody responses from two *medium* confidence birth cohort studies (Grandjean et al., 2012); (Grandjean et al., 2017a); (Grandjean et al., 2017b) and a *low* confidence study in adults (Kielsen et al., 2016). Short-term studies in animals of *high/medium* confidence provide supportive evidence of immunosuppression after PFDA exposure (Frawley et al., 2018); (NTP,

Confidence categories	Designation[a]	Discussion
		2018). Some residual uncertainties regarding unexplained inconsistency and potential confounding by other co-occurring PFAS from epidemiological studies and issues with concomitant overt target organ and systemic toxicity in animal studies lower confidence in the available evidence for this hazard. Other limitations include the lack of epidemiological studies or long-term/chronic studies in animals examining effects on the immune system across different developmental lifestages and immunotoxicity categories, including sensitization and allergic response and autoimmunity and autoimmune disease.
Confidence in the quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium*. The POD is based on BMD modeling at the lower end of the range of the observed data and a BMDL1/2SD estimate that is associated with a small degree of uncertainty due to potential confounding by PFOA (see Appendix D.1.1 for more details). The POD for decreased tetanus antibodies at age 7 yr was judged to be *medium* confidence based on a good model fit and was supported by the nearly identical POD for decreased diphtheria antibodies at age 7 yr. Both PODs support the osRfD. A health-protective estimate for human clearance was applied to estimate the POD_{HED} using PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above). There is also uncertainty as to the most sensitive window of vulnerability with respect to the exposure/outcome measurement timing (BMDs/BMDLs were estimated from PFDA levels measured at age 5 or perinatally and antitetanus antibody concentrations measured at age 7 or 5); (Grandjean et al., 2017b) reported that estimated PFDA "concentrations at 3 mo and 6 mo showed the strongest inverse associations with antibody concentrations at age 5 yr, particularly for tetanus." Thus, it is possible that adverse effects during infancy could be more sensitive than between ages 5 and 7 yr.
Overall confidence in subchronic osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is driven by *medium* confidence in the evidence base for immune effects and the quantification of the POD.
Developmental subchronic osRfD = 2×10^{-9} mg/kg-d		
Confidence in study[a] used to derive osRfD	*Medium*	Confidence in the Wikström et al. (2020) study for hazard identification was rated as *high* (HAWC link) for developmental effects. The study was selected for dose-response analysis due to low overall risk of bias and reliable exposure measurements, which had sufficient exposure contrasts and other characteristics that allowed for adequate study sensitivity to detect associations. The Wikström et al. (2020) study demonstrated associations consistent in magnitude for boys, girls, and the overall population. Overall, mean birth weight was considered the most precise and accurate endpoint and not anticipated to be subject to much error. This study was advanced for dose-response analysis, given minimal presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). Wikström et al. (2020) also adjusted for sample timing in their

Confidence categories	Designation[a]	Discussion
		multivariate models and show no differences in models also restricted to trimester 1 samples only. Some uncertainty remains on the potential for confounding by other PFAS (concern primarily for PFNA), which were not examined in this study. Given the potential quantitative impact of this uncertainty, confidence in the use of this study for dose-response analysis was judged as *medium* rather than *high*.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for developmental effects is *medium*. There was consistent evidence for reduced birth weight among multiple human studies, including high-quality studies. However, unlike the Wikström et al. (2020) study used here and noted above, some uncertainty remains in many studies given the predominance of associations that were detected for studies with later pregnancy sampling. The human database also showed some coherence across different measures of fetal growth restriction. In animals, the lone developmental study reported effects on fetal growth that are coherent with effects observed in humans. Some residual uncertainty regarding potential confounding by other co-occurring PFAS from epidemiological studies lowers confidence in the available evidence for this hazard.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on a BMD hybrid approach within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in osRfD	*Medium-low*	The overall confidence in the osRfD is *medium* and is driven by *medium-low* confidence in the evidence base for developmental effects (i.e., fetal growth restriction).
Liver subchronic osRfD = 6×10^{-7} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in the NTP (2018) study was rated *high* based on good or adequate ratings for most study quality domains (HAWC link) and characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for liver effects is *medium*. Coherent liver effects for histopathology, serum biomarkers and organ weights were observed across short-term rodent studies (primarily two *high* confidence 28-d studies) that are supported by mechanistic studies of biological plausibility and possible human relevance. Uncertainties remain due to the absence of longer-term toxicity studies (28 d) and limited information from available epidemiological studies and in vivo models to characterize the role of PPARα and other signaling pathways in the mechanisms of hepatotoxicity of PFDA in both humans and animals.

Confidence categories	Designation[a]	Discussion
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within (at the lower end) the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is primarily driven by *medium* confidence in both the evidence base supporting this hazard and the quantification of the POD using BMD modeling of data from a *high* confidence study.
Male reproductive subchronic osRfD = 3×10^{-6} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High-medium*	Confidence in the NTP (2018) study was rated *high-medium* (HAWC link) since most of male reproductive measures were rated as high, including the basis for the subchronic osRfD (decreased whole epididymis weight), with the exception of sperm measures, which suffered from insensitivity due to short-term exposure. This is supported by the study evaluation results (good or adequate ratings for most study quality domains) and characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for male reproductive effects is *medium* to *low*. Coherent effects across several relevant measures, including sperm parameters, histopathology, serum testosterone levels and organ weights were observed in a *high* confidence 28-d rat study. The findings are supported by evidence of biological plausibility from limited number of mechanistic studies. In spite of the available evidence, some outstanding uncertainties in the database remain, including the absence of longer-term exposure studies (>28 d), developmental or multigenerational studies that evaluate effects in both adults and developing humans and animals. Given these evidence base uncertainties, it is likely that this osRfD is under-protective of all male reproductive effects.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is primarily driven by the *medium-low* confidence in the evidence base. The *high* confidence in the study and *medium* confidence in the quantification of the POD does not fully mitigate the uncertainties associated with *medium-low* confidence in the evidence base.
Female reproductive subchronic osRfD = 1×10^{-6} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in the NTP (2018) study is *high* (HAWC link) given the study evaluation results (i.e., rating of good in all evaluation categories) and

Confidence categories	Designation[a]	Discussion
		characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for female reproductive effects is *medium-low*. There were consistent and coherent effects on uterus weight and the estrous cycle in a single *high* confidence study. Despite the available evidence, limitations of the evidence base for female reproductive effects include the lack of informative human studies and the lack of a subchronic study in animals as well as lack of studies that examined the effect of PFDA on female fertility and pregnancy outcomes in exposed animals. There are also no developmental or multigenerational studies that evaluated effects in both adults and developing humans and animals. Given these evidence base uncertainties, it is likely that this osRfD is under-protective of all female reproductive effects.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is primarily driven by the *medium-low* confidence in the evidence base. The *high* confidence in the study and *medium* confidence in the quantification of the POD does not fully mitigate the uncertainties associated with *medium-low* confidence in the evidence base.

[a]All study evaluation details can be found on HAWC.

Table 5-18. Organ/system-specific and overall subchronic RfDs for PFDA

System	Toxicity value	Basis	POD_{HED} (mg/kg-d)[a]	UF_C	osRfD (mg/kg-d)	Confidence
Immune (developmental)	Subchronic osRfD	Decreased serum antibody concentrations for both tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr	6.04×10^{-8} based on BMDL½SD from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a)	30	2×10^{-9}	*Medium*
Developmental	Subchronic osRfD	Decreased birth weight in males and females	5.44×10^{-8} based on BMDL5%RD from Wikström et al. (2020)	30	2×10^{-9}	*Medium-low*
Liver	Subchonic osRfD	Increased liver weight in SD female rats	5.92×10^{-4} based on BMDL10%RD from NTP (2018)	1,000	6×10^{-7}	*Medium*
Male reproductive	Subchronic osRfD	Decreased absolute whole epididymis weight in SD rats	2.77×10^{-3} based on BMDL1SD from NTP (2018)	1,000	3×10^{-6}	*Medium-low*
Female reproductive	Subchronic osRfD	Increased number of days spent in diestrus in SD rats	1.12×10^{-3} based on BMDL5%RD from NTP (2018)	1,000	1×10^{-6}	*Medium-low*
Immune/developmental	Overall subchronic RfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yr and PFDA measured at age 5 yr Decreased birth weight in males and females	6.04×10^{-8} based on BMDL½ SD from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) 5.44×10^{-8} based on BMDL5%RD from (Wikström et al., 2020)	30	2×10^{-9}	*Medium*

[a]The details of the BMD modeling approach and results can be found in Appendix C.

Selection of Subchronic RfD and Confidence Statement

Organ/system-specific and overall subchronic RfD values for PFDA selected in the previous section are summarized in Table 5-18.

From the identified subchronic osRfDs (see Table 5-18), an overall subchronic RfD of 2×10^{-9} mg/kg-day based on decreased serum antibody concentrations and decreased birth weight in humans was selected. As described in Table 5-17, confidence in the RfD is *medium*, based on *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental ((Wikström et al., 2020)) endpoints using BMD modeling. This RfD is the same for both developmental and immune critical effects given that the PODs for these two osRfDs were nearly identical (i.e., 6.04×10^{-8} and 5.44×10^{-8}, respectively) and that identical UFs were applied.

As described above, the toxicity value of 2×10^{-9} mg/kg-day for decreased serum antibody concentrations for both diphtheria and tetanus at age 7 and PFDA measured at age 5 was selected for immune effects Budtz-Jørgensen and Grandjean (2018a); Grandjean et al. (2012); and the toxicity value of 2×10^{-9} mg/kg-day based on reduced birth weight from the Wikström et al. (2020) study was selected for developmental effects.

The PODs calculated in Table 5-10 from 28-day studies in rodents were selected for each health effect for the derivation of the candidate subchronic toxicity values based on several considerations, including whether there is an endpoint with less uncertainty and/or greater sensitivity, and whether the endpoint is protective of both sexes and all lifestages.

For liver effects, the toxicity value of 6×10^{-7} mg/kg-day (BMDL10RD[HED] of 5.92×10^{-4} mg/kg-day) for increased liver weight in female rats in the NTP (2018) study was selected as the liver osRfD because it is a reliable marker of hepatotoxicity and represents a more sensitive reference value than other liver endpoints considered for dose-response modeling (see Table 5-16). For male reproductive effects, endpoints with a *high* confidence rating (i.e., increased Leydig cell atrophy, decreased serum testosterone, decreased testis weight, and decreased epididymis weight [whole and cauda]) were prioritized over endpoints, which suffered from potential sensitivity issues due to short-term study exposure (i.e., decreased epididymal sperm counts). Because the PODs for the prioritized endpoints were similar (HEDs ranging from 2.77×10^{-3} to 6.25×10^{-3}) and consistent with mechanistic evidence that suggest PFDA targets Leydig cells and causes decreased steroidogenesis and androgen deficiency (see Section 3.2.4), the most sensitive POD based on a BMDL1SD(HED) of 2.77×10^{-3} mg/kg-day for decreases in whole epididymis weights was selected for derivation, resulting in a subchronic toxicity value of 3×10^{-6} mg/kg-day for male reproductive effects. Lastly, the osRfD of 1×10^{-6} mg/kg-day (BMDL5RD[HED] of 1.12×10^{-3} mg/kg-day) based on increased number of days spent in diestrus was selected for female reproductive effects given its association with infertility as provided by

EPA's *Guidelines for Reproductive Toxicity Risk Assessment*. This endpoint is also supported by concomitant decreases in estrus time (BMDL5RD[HED] of 6.76×10^{-4} mg/kg-day), for which the association with infertility is less clear.

The subchronic osRfDs for liver, male reproductive, and female reproductive effects derived from short-term animal data were several orders of magnitude higher than the subchronic osRfDs for immune and developmental effects in humans; therefore, they were not considered sufficiently protective for consideration in the selection of the overall subchronic RfD. Also, in the case of male and female reproductive effects, confidence in the respective osRfDs was lower compared with the immune osRfD (*medium-low* vs. *medium*) due to deficiencies in the evidence base for these health effects.

5.2.4. Inhalation Reference Concentration (RfC) Derivation

No studies examining inhalation effects of short-term, subchronic, chronic or gestational exposure for PFDA in humans or animals have been identified, precluding the derivation of an RfC. Existing PBPK models for PFDA were judged insufficiently reliable for estimating human dosimetry for any route of exposure, including possible route-to-route extrapolation. Additionally, no classical PK models were identified that included inhalation dosimetry to support the derivation of an RfC

5.3. CANCER TOXICITY VALUES

Considering the limitations in the evidence base across human, animal, and mechanistic studies of PFDA (see Section 3.3) and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded that the ***evidence is inadequate to assess carcinogenic potential*** of PFDA in humans. The lack of adequate carcinogenicity data for PFDA precludes the derivation of quantitative estimates of either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

REFERENCES

Abdel-Hafiz, HA; Horwitz, KB. (2014). Post-translational modifications of the progesterone receptors [Review]. J Steroid Biochem Mol Biol 140: 80-89. http://dx.doi.org/10.1016/j.jsbmb.2013.12.008.

Adinehzadeh, M; Reo, NV. (1998). Effects of peroxisome proliferators on rat liver phospholipids: sphingomyelin degradation may be involved in hepatotoxic mechanism of perfluorodecanoic acid. Chem Res Toxicol 11: 428-440. http://dx.doi.org/10.1021/tx970155t.

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. Environ Int 130: 104929. http://dx.doi.org/10.1016/j.envint.2019.104929.

Aimuzi, R; Luo, K; Huang, R; Huo, XN; Nian, M; Ouyang, FX; Du, YT; Feng, LP; Wang, WY; Zhang, J. (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environ Pollut 264: 114557. http://dx.doi.org/10.1016/j.envpol.2020.114557.

Al-Eryani, L; Wahlang, B; Falkner, KC; Guardiola, JJ; Clair, HB; Prough, RA; Cave, M. (2015). Identification of Environmental Chemicals Associated with the Development of Toxicant-associated Fatty Liver Disease in Rodents. Toxicol Pathol 43: 482-497. http://dx.doi.org/10.1177/0192623314549960.

Alberti, KG; Eckel, RH; Grundy, SM; Zimmet, PZ; Cleeman, JI; Donato, KA; Fruchart, JC; James, WP; Loria, CM; Smith, SC. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120: 1640-1645. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.192644.

Alexander, EK; Pearce, EN; Brent, GA; Brown, RS; Chen, H; Dosiou, C; Grobman, W; Laurberg, P; Lazarus, JH; Mandel, SJ; Peeters, R; Sullivan, S. (2017). 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid 27: 315-389. http://dx.doi.org/10.1089/thy.2016.0457.

Amin, K. (2012). The role of mast cells in allergic inflammation. Respir Med 106: 9-14. http://dx.doi.org/10.1016/j.rmed.2011.09.007.

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. J Neuroimmunol 330: 90-95. http://dx.doi.org/10.1016/j.jneuroim.2019.03.002.

Anderson, RH; Long, GC; Porter, RC; Anderson, JK. (2016). Occurrence of select perfluoroalkyl substances at U.S. Air Force aqueous film-forming foam release sites other than fire-training areas: Field-validation of critical fate and transport properties. Chemosphere 150: 678-685. http://dx.doi.org/10.1016/j.chemosphere.2016.01.014.

Angrish, MM; Kaiser, JP; Mcqueen, CA; Chorley, BN. (2016). Tipping the balance: Hepatotoxicity and the 4 apical key events of hepatic steatosis [Review]. Toxicol Sci 150: 261-268. http://dx.doi.org/10.1093/toxsci/kfw018.

Arand, M; Coughtrie, MW; Burchell, B; Oesch, F; Robertson, LW. (1991). Selective induction of bilirubin UDP-glucuronosyl-transferase by perfluorodecanoic acid. Chem Biol Interact 77: 97-105. http://dx.doi.org/10.1016/0009-2797(91)90008-U.

ATSDR (Agency for Toxic Substances and Disease Registry). (2018a). An overview of perfluoroalkyl and polyfluoroalkyl substances and interim guidance for clinicians responding to patient exposure concerns. Interim guidance revised 5/7/2018. https://stacks.cdc.gov/view/cdc/77114.

ATSDR (Agency for Toxic Substances and Disease Registry). (2018b). Toxicological profile for perfluoroalkyls. Draft for public comment [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

ATSDR (Agency for Toxic Substances and Disease Registry). (2021). Toxicological profile for perfluoroalkyls [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://dx.doi.org/10.15620/cdc:59198.

ATSDR (Agency for Toxic Substances and Disease Registry). (2024). PFAS: Information for clinicians [Fact Sheet]. https://www.atsdr.cdc.gov/pfas/docs/PFAS-info-for-clinicians-508.pdf.

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. Environ Res 195: 110740. http://dx.doi.org/10.1016/j.envres.2021.110740.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bonefeld-Jørgensen, EC; Bossi, R; Henriksen, TB. (2016). Perfluoroalkyl acids in maternal serum and indices of fetal growth: The Aarhus Birth Cohort. Environ Health Perspect 124: 848-854. http://dx.doi.org/10.1289/ehp.1510046.

Bach, CC; Bech, BH; Nohr, EA; Olsen, J; Matthiesen, NB; Bossi, R; Uldbjerg, N; Bonefeld-Jørgensen, EC; Henriksen, TB. (2015). Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. Environ Res 142: 535-541. http://dx.doi.org/10.1016/j.envres.2015.08.007.

Bach, CC; Matthiesen, NB; Olsen, J; Henriksen, TB. (2018). Conditioning on parity in studies of perfluoroalkyl acids and time to pregnancy: An example from the Danish national birth cohort. Environ Health Perspect 126: 117003. http://dx.doi.org/10.1289/EHP1493.

Baduel, C; Paxman, CJ; Mueller, JF. (2015). Perfluoroalkyl substances in a firefighting training ground (FTG), distribution and potential future release. J Hazard Mater 296: 46-53. http://dx.doi.org/10.1016/j.jhazmat.2015.03.007.

Bailey, SA; Zidell, RH; Perry, RW. (2004). Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? Toxicol Pathol 32: 448-466. http://dx.doi.org/10.1080/01926230490465874.

Bao, J; Shao, LX; Liu, Y; Cui, SW; Wang, X; Lu, GL; Wang, X; Jin, YH. (2022). Target analysis and suspect screening of per- and polyfluoroalkyl substances in paired samples of maternal serum, umbilical cord serum, and placenta near fluorochemical plants in Fuxin, China. Chemosphere 307: 135731. http://dx.doi.org/10.1016/j.chemosphere.2022.135731.

Bao, WW; Qian, ZM; Geiger, SD; Liu, E; Liu, Y; Wang, SQ; Lawrence, WR; Yang, BY; Hu, LW; Zeng, XW; Dong, GH. (2017). Gender-specific associations between serum isomers of perfluoroalkyl substances and blood pressure among Chinese: Isomers of C8 Health Project in China. Sci Total Environ 607-608: 1304-1312. http://dx.doi.org/10.1016/j.scitotenv.2017.07.124.

Barber, JL; Berger, U; Chaemfa, C; Huber, S; Jahnke, A; Temme, C; Jones, KC. (2007). Analysis of per- and polyfluorinated alkyl substances in air samples from Northwest Europe. J Environ Monit 9: 530-541. http://dx.doi.org/10.1039/b701417a.

Barrett, ES; Chen, C; Thurston, SW; Haug, LS; Sabaredzovic, A; Fjeldheim, FN; Frydenberg, H; Lipson, SF; Ellison, PT; Thune, I. (2015). Perfluoroalkyl substances and ovarian hormone

concentrations in naturally cycling women. Fertil Steril 103: 1261-1270.e1263. http://dx.doi.org/10.1016/j.fertnstert.2015.02.001.

Batt, AM; Ferrari, L. (1995). Manifestations of chemically-induced liver-damage. Clin Chem 41: 1882-1887.

Beck, IH; Timmermann, CAG; Nielsen, F; Schoeters, G; Jøhnk, C; Kyhl, HB; Høst, A; Jensen, TK. (2019). Association between prenatal exposure to perfluoroalkyl substances and asthma in 5-year-old children in the Odense Child Cohort. Environ Health 18: 97. http://dx.doi.org/10.1186/s12940-019-0541-z.

Berg, V; Nøst, TH; Hansen, S; Elverland, A; Veyhe, AS; Jorde, R; Odland, JØ; Sandanger, TM. (2015). Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ Int 77: 63-69. http://dx.doi.org/10.1016/j.envint.2015.01.007.

Berg, V; Nøst, TH; Pettersen, RD; Hansen, S; Veyhe, AS; Jorde, R; Odland, JØ; Sandanger, TM. (2017). Persistent organic pollutants and the association with maternal and infant thyroid homeostasis: a multipollutant assessment. Environ Health Perspect 125: 127-133. http://dx.doi.org/10.1289/EHP152.

Bernstein, AS; Kapraun, DF; Schlosser, PM. (2021). A Model Template Approach for Rapid Evaluation and Application of Physiologically Based Pharmacokinetic Models for Use in Human Health Risk Assessments: A Case Study on Per- and Polyfluoroalkyl Substances. Toxicol Sci 182: 215–228. http://dx.doi.org/10.1093/toxsci/kfab063.

Bijland, S; Rensen, PCN; Pieterman, EJ; Maas, ACE; van Der Hoorn, JW; van Erk, MJ; Havekes, LM; Willems van Dijk, K; Chang, SC; Ehresman, DJ; Butenhoff, JL; Princen, HMG. (2011). Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. Toxicol Sci 123: 290-303. http://dx.doi.org/10.1093/toxsci/kfr142.

Birukov, A; Andersen, LB; Andersen, MS; Nielsen, JH; Nielsen, F; Kyhl, HB; Jørgensen, JS; Grandjean, P; Dechend, R; Jensen, TK. (2021). Exposure to perfluoroalkyl substances and blood pressure in pregnancy among 1436 women from the Odense Child Cohort. Environ Int 151: 106442. http://dx.doi.org/10.1016/j.envint.2021.106442.

Biton, Y; Goldenberg, I; Kutyifa, V; Baman, J. R.; Solomon, S; Moss, AJ; Szepietowska, B; Mcnitt, S; Polonsky, B; Zareba, W; Barsheshet, A. (2016). Relative Wall Thickness and the Risk for Ventricular Tachyarrhythmias in Patients With Left Ventricular Dysfunction. J Am Coll Cardiol 67: 303-312. http://dx.doi.org/10.1016/j.jacc.2015.10.076.

Bjerregaard-Olesen, C; Bach, CC; Long, M; Wielsøe, M; Bech, BH; Henriksen, TB; Olsen, J; Bonefeld-Jørgensen, EC. (2019). Associations of Fetal Growth Outcomes with Measures of the Combined Xenoestrogenic Activity of Maternal Serum Perfluorinated Alkyl Acids in Danish Pregnant Women. Environ Health Perspect 127: 17006. http://dx.doi.org/10.1289/EHP1884.

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. Environ Pollut 242: 894-904. http://dx.doi.org/10.1016/j.envpol.2018.07.042.

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. Environ Res 200: 111400. http://dx.doi.org/10.1016/j.envres.2021.111400.

Bloom, MS; Commodore, S; Ferguson, PL; Neelon, B; Pearce, JL; Baumer, A; Newman, RB; Grobman, W; Tita, A; Roberts, J; Skupski, D; Palomares, K; Nageotte, M; Kannan, K; Zhang, C; Wapner, R; Vena, JE; Hunt, KJ. (2022). Association between gestational PFAS exposure and Children's adiposity in a diverse population. Environ Res 203: 111820. http://dx.doi.org/10.1016/j.envres.2021.111820.

Bloom, MS; Kannan, K; Spliethoff, HM; Tao, L; Aldous, KM; Vena, JE. (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol Behav 99: 240-245. http://dx.doi.org/10.1016/j.physbeh.2009.02.005.

Boettiger, EG. (1946). Changes in the glycogen and water content of the rat uterus. Journal of Cellular and Comparative Physiology 27: 9-14. http://dx.doi.org/10.1002/jcp.1030270103.

Bookstaff, RC; Moore, RW; Ingall, GB; Peterson, RE. (1990). Androgenic deficiency in male rats treated with perfluorodecanoic acid. Toxicol Appl Pharmacol 104: 322-333. http://dx.doi.org/10.1016/0041-008X(90)90306-F.

Boone, L; Meyer, D; Cusick, P; Ennulat, D; Bolliger, AP; Everds, N; Meador, V; Elliott, G; Honor, D; Bounous, D; Jordan, H. (2005). Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies [Review]. Vet Clin Pathol 34: 182-188. http://dx.doi.org/10.1111/j.1939-165X.2005.tb00041.x.

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. Environ Health 21: 85. http://dx.doi.org/10.1186/s12940-022-00892-6.

Boros, DL; Revankar, SG. (2017). Granulomatous diseases. 2017.

Boujrad, N; Vidic, B; Gazouli, M; Culty, M; Papadopoulos, V. (2000). The peroxisome proliferator perfluorodecanoic acid inhibits the peripheral-type benzodiazepine receptor (PBR) expression and hormone-stimulated mitochondrial cholesterol transport and steroid formation in Leydig cells. Endocrinology 141: 3137-3148. http://dx.doi.org/10.1210/endo.141.9.7678.

Bräunig, J; Baduel, C; Heffernan, A; Rotander, A; Donaldson, E; Mueller, JF. (2017). Fate and redistribution of perfluoroalkyl acids through AFFF-impacted groundwater. Sci Total Environ 596-597: 360-368. http://dx.doi.org/10.1016/j.scitotenv.2017.04.095.

Bretveld, RW; Thomas, CMG; Scheepers, PTJ; Zielhuis, GA; Roeleveld, N. (2006). Pesticide exposure: the hormonal function of the female reproductive system disrupted? [Review]. Reprod Biol Endocrinol 4: 30. http://dx.doi.org/10.1186/1477-7827-4-30.

Brewster, DW; Birnbaum, LS. (1989). The biochemical toxicity of perfluorodecanoic acid in the mouse is different from that of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 99: 544-554. http://dx.doi.org/10.1016/0041-008X(89)90161-0.

Buck Louis, GM; Chen, Z; Schisterman, EF; Kim, S; Sweeney, AM; Sundaram, R; Lynch, CD; Gore-Langton, RE; Barr, DB. (2015). Perfluorochemicals and human semen quality: The LIFE Study. Environ Health Perspect 123: 57-63. http://dx.doi.org/10.1289/ehp.1307621.

Buck Louis, GM; Zhai, S; Smarr, MM; Grewal, J; Zhang, C; Grantz, KL; Hinkle, SN; Sundaram, R; Lee, S; Honda, M; Oh, J; Kannan, K. (2018). Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environ Int 119: 515-526. http://dx.doi.org/10.1016/j.envint.2018.07.024.

Buck, RC; Franklin, J; Berger, U; Conder, JM; Cousins, IT; de Voogt, P; Jensen, AA; Kannan, K; Mabury, SA; van Leeuwen, SP. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins [Review]. Integr Environ Assess Manag 7: 513-541. http://dx.doi.org/10.1002/ieam.258.

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.

Budtz-Jørgensen, E; Grandjean, P. (2018b). Computational details for the paper "Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity".

Buhrke, T; Kibellus, A; Lampen, A. (2013). In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. Toxicol Lett 218: 97-104. http://dx.doi.org/10.1016/j.toxlet.2013.01.025.

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). Sci Total Environ 827: 153900. http://dx.doi.org/10.1016/j.scitotenv.2022.153900.

Caldwell, ASL; Middleton, LJ; Jimenez, M; Desai, R; Mcmahon, AC; Allan, CM; Handelsman, DJ; Walters, KA. (2014). Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. Endocrinology 155: 3146-3159. http://dx.doi.org/10.1210/en.2014-1196.

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. Sci Total Environ 569-570: 1107-1113. http://dx.doi.org/10.1016/j.scitotenv.2016.06.177.

Cao, L; Quan, XB; Zeng, WJ; Yang, XO; Wang, MJ. (2016). Mechanism of hepatocyte apoptosis [Review]. Journal of Cell Death 9: 19-29. http://dx.doi.org/10.4137/JCD.S39824.

Cao, W; Liu, X; Liu, X; Zhou, Y; Zhang, X; Tian, H; Wang, J; Feng, S; Wu, Y; Bhatti, P; Wen, S; Sun, X. (2018). Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a Chinese birth cohort. Environ Int 116: 197-205. http://dx.doi.org/10.1016/j.envint.2018.04.015.

Carlson, LA; Angrish, M; Shirke, AV; Radke, EG; Schulz, B; Kraft, A; Judson, R; Patlewicz, G; Blain, R; Lin, C; Vetter, N; Lemeris, C; Hartman, P; Hubbard, H; Arzuaga, X; Davis, A; Dishaw, LV; Druwe, IL; Hollinger, H; ... Thayer, KA. (2022). Systematic evidence map for over one hundred and fifty per- and polyfluoroalkyl substances (PFAS) [Review]. Environ Health Perspect 130: 56001. http://dx.doi.org/10.1289/EHP10343.

Carney, EW; Kimmel, CA. (2007). Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs. Birth Defects Res B Dev Reprod Toxicol 80: 473-496.

Carwile, JL; Seshasayee, SM; Aris, IM; Rifas-Shiman, SL; Claus Henn, B; Calafat, AM; Sagiv, SK; Oken, E; Fleisch, AF. (2021). Prospective associations of mid-childhood plasma per- and polyfluoroalkyl substances and pubertal timing. Environ Int 156: 106729. http://dx.doi.org/10.1016/j.envint.2021.106729.

CDC (Centers for Disease Control and Prevention). (2022a). Biomonitoring data tables for environmental chemicals: analysis of whole blood, serum, and urine samples, NHANES 1999-2018. https://www.cdc.gov/exposurereport/data_tables.html.

CDC (Centers for Disease Control and Prevention). (2022b). National report on human exposure to environmental chemicals. Atlanta, GA: U.S. Department of Health and Human Services. https://www.cdc.gov/exposurereport/.

Chang, CJ; Barr, DB; Ryan, PB; Panuwet, P; Smarr, MM; Liu, K; Kannan, K; Yakimavets, V; Tan, Y; Ly, V; Marsit, CJ; Jones, DP; Corwin, EJ; Dunlop, AL; Liang, D. (2022). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. Environ Int 158: 106964. http://dx.doi.org/10.1016/j.envint.2021.106964.

Charles, D; Berg, V; Nøst, TH; Huber, S; Sandanger, TM; Rylander, C. (2020). Pre- and post-diagnostic blood profiles of perfluoroalkyl acids in type 2 diabetes mellitus cases and controls. Environ Int 145: 106095. http://dx.doi.org/10.1016/j.envint.2020.106095.

Chen, H; Huang, CY; Wilson, MW; Lay, LT; Robertson, LW; Chow, CK; Glauert, HP. (1994). Effect of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cell proliferation and toxicity in Sprague-Dawley rats. Carcinogenesis 15: 2847-2850. http://dx.doi.org/10.1093/carcin/15.12.2847.

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, LC; Borges, T; Glauert, HP; Knight, SAB; Sunde, RA; Schramm, H; Oesch, F; Chow, CK; Robertson, LW. (1990). Modulation of selenium-dependent glutathione peroxidase by perfluorodecanoic acid in rats: Effect of dietary selenium. J Nutr 120: 298-304. http://dx.doi.org/10.1093/jn/120.3.298.

Chen, Q; Huang, R; Hua, L; Guo, Y; Huang, L; Zhao, Y; Wang, X; Zhang, J. (2018a). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: A prospective birth cohort study. Environ Health 17: 1-12. http://dx.doi.org/10.1186/s12940-018-0352-7.

Chen, Q; Zhang, X; Zhao, Y; Lu, W; Wu, J; Zhao, S; Zhang, J; Huang, L. (2019). Prenatal exposure to perfluorobutanesulfonic acid and childhood adiposity: A prospective birth cohort study in Shanghai, China. Chemosphere 226: 17-23. http://dx.doi.org/10.1016/j.chemosphere.2019.03.095.

Chen, WL; Bai, FY; Chang, YC; Chen, PC; Chen, CY. (2018b). Concentrations of perfluoroalkyl substances in foods and the dietary exposure among Taiwan general population and pregnant women. J Food Drug Anal 26: 994-1004. http://dx.doi.org/10.1016/j.jfda.2017.12.011.

Chen, X; Feng, X; Sun, X; Li, Y; Yang, Y; Shan, G; Zhu, L. (2022). Quantifying indirect contribution from precursors to human body burden of legacy PFASs based on paired blood and one-week duplicate diet. Environ Sci Technol 56: 5632-5640. http://dx.doi.org/10.1021/acs.est.1c07465.

Chinje, E; Kentish, P; Jarnot, B; George, M; Gibson, G. (1994). Induction of the CYP4A subfamily by perfluorodecanoic acid: The rat and the guinea pig as susceptible and non-susceptible species. Toxicol Lett 71: 69-75. http://dx.doi.org/10.1016/0378-4274(94)90200-3.

Chiu, WA; Lynch, MT; Lay, CR; Antezana, A; Malek, P; Sokolinski, S; Rogers, RD. (2022). Bayesian estimation of human population toxicokinetics of PFOA, PFOS, PFHxS, and PFNA from studies of contaminated drinking water. Environ Health Perspect 130: 127001. http://dx.doi.org/10.1289/EHP10103.

Christensen, JVR; Bangash, KK; Weihe, P; Grandjean, P; Nielsen, F; Jensen, TK; Petersen, MS. (2021). Maternal exposure to perfluoroalkyl chemicals and anogenital distance in the offspring: A Faroese cohort study. Reprod Toxicol 104: 52-57. http://dx.doi.org/10.1016/j.reprotox.2021.06.016.

Christensen, KY; Raymond, M; Meiman, J. (2019). Perfluoroalkyl substances and metabolic syndrome. Int J Hyg Environ Health 222: 147-153. http://dx.doi.org/10.1016/j.ijheh.2018.08.014.

Christensen, KY; Raymond, M; Thompson, BA; Anderson, HA. (2016). Perfluoroalkyl substances in older male anglers in Wisconsin. Environ Int 91: 312-318. http://dx.doi.org/10.1016/j.envint.2016.03.012.

Corsini, E; Sangiovanni, E; Avogadro, A; Galbiati, V; Viviani, B; Marinovich, M; Galli, CL; Dell'Agli, M; Germolec, DR. (2012). In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicol Appl Pharmacol 258: 248-255. http://dx.doi.org/10.1016/j.taap.2011.11.004.

Corton, JC; Peters, JM; Klaunig, JE. (2018). The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions [Review]. Arch Toxicol 92: 83-119. http://dx.doi.org/10.1007/s00204-017-2094-7.

Craig, EA; Yan, Z; Zhao, QJ. (2015). The relationship between chemical-induced kidney weight increases and kidney histopathology in rats. J Appl Toxicol 35: 729-736. http://dx.doi.org/10.1002/jat.3036.

Creasy, D; Bube, A; de Rijk, E; Kandori, H; Kuwahara, M; Masson, R; Nolte, T; Reams, R; Regan, K; Rehm, S; Rogerson, P; Whitney, K. (2012). Proliferative and nonproliferative lesions of the rat and mouse male reproductive system [Review]. Toxicol Pathol 40: 40S-121S. http://dx.doi.org/10.1177/0192623312454337.

Creasy, DM; Chapin, RE. (2018). Chapter 17 - Male reproductive system. In Fundamentals of Toxicologic Pathology (Third Edition). London, UK: Academic Press of Elsevier. http://dx.doi.org/10.1016/B978-0-12-809841-7.00017-4.

Dalsager, L; Christensen, N; Halekoh, U; Timmermann, CAG; Nielsen, F; Kyhl, HB; Husby, S; Grandjean, P; Jensen, TK; Andersen, HR. (2021a). 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. Environ Int 149: 106395. http://dx.doi.org/10.1016/j.envint.2021.106395.

Dalsager, L; Christensen, N; Husby, S; Kyhl, H; Nielsen, F; Høst, A; Grandjean, P; Jensen, TK. (2016). Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environ Int 96: 58-64. http://dx.doi.org/10.1016/j.envint.2016.08.026.

Dalsager, L; Jensen, TK; Nielsen, F; Grandjean, P; Bilenberg, N; Andersen, HR. (2021b). No association between maternal and child PFAS concentrations and repeated measures of ADHD symptoms at age 2½ and 5 years in children from the Odense Child Cohort. Neurotoxicol Teratol 88: 107031. http://dx.doi.org/10.1016/j.ntt.2021.107031.

Darrow, DC; Soule, HC; Buckman, TE. (1928). Blood volume in normal infants and children. J Clin Invest 5: 243-258. http://dx.doi.org/10.1172/JCI100156.

Das, KP; Wood, CR; Lin, MT; Starkov, AA; Lau, C; Wallace, KB; Corton, JC; Abbott, BD. (2017). Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378: 37-52. http://dx.doi.org/10.1016/j.tox.2016.12.007.

Davidson, MH. (2010). Update on CETP inhibition. 4: 394-398. http://dx.doi.org/10.1016/j.jacl.2010.08.003.

Davies, B; Morris, T. (1993). Physiological parameters in laboratory animals and humans [Review]. Pharm Res 10: 1093-1095. http://dx.doi.org/10.1023/A:1018943613122.

De Boo, HA; Harding, JE. (2006). The developmental origins of adult disease (Barker) hypothesis [Review]. Aust N Z J Obstet Gynaecol 46: 4-14. http://dx.doi.org/10.1111/j.1479-828X.2006.00506.x.

de Simone, G; Daniels, S. R.; Kimball, TR; Roman, MJ; Romano, C; Chinali, M; Galderisi, M; Devereux, RB. (2005). Evaluation of concentric left ventricular geometry in humans - Evidence for age-related systematic underestimation. Hypertension 45: 64-68. http://dx.doi.org/10.1161/01.HYP.0000150108.37527.57.

Dent, MP; Carmichael, PL; Jones, KC; Martin, FL. (2015). Towards a non-animal risk assessment for anti-androgenic effects in humans [Review]. Environ Int 83: 94-106. http://dx.doi.org/10.1016/j.envint.2015.06.009.

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. Environ Res 191: 110110. http://dx.doi.org/10.1016/j.envres.2020.110110.

Dong, GH; Tung, KY; Tsai, CH; Liu, MM; Wang, D; Liu, W; Jin, YH; Hsieh, WS; Lee, YL; Chen, PC. (2013). Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect 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. Ecotoxicol Environ Saf 173: 461-468. http://dx.doi.org/10.1016/j.ecoenv.2019.02.061.

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. Environ Int 134: 105295. http://dx.doi.org/10.1016/j.envint.2019.105295.

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. Environ Res 211: 112903. http://dx.doi.org/10.1016/j.envres.2022.112903.

Dzierlenga, AL; Robinson, VG; Waidyanatha, S; Devito, MJ; Eifrid, MA; Gibbs, ST; Granville, CA; Blystone, CR. (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. http://dx.doi.org/10.1080/00498254.2019.1683776.

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.

Eick, SM; Demicco, E; Valeri, L; Woodruff, TJ; Morello-Frosch, R; Hom Thepaksorn, EK; Izano, MA; Cushing, LJ; Wang, Y; Smith, SC; Gao, S; Park, JS; Padula, AM. (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. Environ Health 19: 100-100. http://dx.doi.org/10.1186/s12940-020-00654-2.

EMEA (European Medicines Agency). (2008). Non-clinical guideline on drug induced hepatotoxicity. (EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003355.pdf.

Ernst, A; Brix, N; Lauridsen, LLB; Olsen, J; Parner, ET; Liew, Z; Olsen, LH; Ramlau-Hansen, CH. (2019). Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish National Birth Cohort. Environ Health Perspect 127: 17004. http://dx.doi.org/10.1289/EHP3567.

Evans, H; Killoran, KE; Mitre, E. (2014). Measuring Local Anaphylaxis in Mice. J Vis Exp 14: e52005. http://dx.doi.org/10.3791/52005.

Evans, TJ; Ganjam, VK. (2011). Chapter 2 - Reproductive anatomy and physiology. In RC Gupta (Ed.), Reproductive and Developmental Toxicology (1st ed., pp. 7-32). San Diego, CA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-382032-7.10002-5.

Fàbrega, F; Kumar, V; Benfenati, E; Schuhmacher, M; Domingo, JL; Nadal, M. (2015). Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. Toxicol Environ Chem 97: 814-827. http://dx.doi.org/10.1080/02772248.2015.1060976.

Feghali, M; Venkataramanan, R; Caritis, S. (2015). Pharmacokinetics of drugs in pregnancy. Semin Perinatol 39: 512-519. http://dx.doi.org/10.1053/j.semperi.2015.08.003.

Fleisch, AF; Rifas-Shiman, SL; Mora, AM; Calafat, AM; Ye, X; Luttmann-Gibson, H; Gillman, MW; Oken, E; Sagiv, SK. (2017). Early-life exposure to perfluoroalkyl substances and childhood metabolic function. Environ Health Perspect 125: 481-487. http://dx.doi.org/10.1289/EHP303.

Foster, P; Gray, LE, Jr. (2013). Toxic responses of the reproductive system. In CD Klaassen (Ed.), Casarett & Doull's toxicology: The basic science of poisons (8th ed., pp. 861-906). New York,

NY: McGraw-Hill Education. https://www.mheducation.com/highered/product/casarett-doull-s-toxicology-basic-science-poisons-9th-edition-klaassen/9781259863745.html.

Fraser, AJ; Webster, TF; Watkins, DJ; Strynar, MJ; Kato, K; Calafat, AM; Vieira, VM; Mcclean, MD. (2013). Polyfluorinated compounds in dust from homes, offices, and vehicles as predictors of concentrations in office workers' serum. Environ Int 60: 128-136. http://dx.doi.org/10.1016/j.envint.2013.08.012.

Frawley, RP; Smith, M; Cesta, MF; Hayes-Bouknight, S; Blystone, C; Kissling, GE; Harris, S; Germolec, D. (2018). Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague-Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days. J Immunotoxicol 15: 41-52. http://dx.doi.org/10.1080/1547691X.2018.1445145.

Friis-Hansen, B. (1961). Body water compartments in children: Changes during growth and related changes in body composition. Pediatrics 28: 169-181.

Fu, Y; Wang, T; Fu, Q; Wang, P; Lu, Y. (2014). Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf 106: 246-252. http://dx.doi.org/10.1016/j.ecoenv.2014.04.039.

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. J Occup Health 57: 1-12. http://dx.doi.org/10.1539/joh.14-0136-OA.

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.

Gao, K, e; Zhuang, T; Liu, X; Fu, J; Zhang, J; Fu, J, ie; Wang, L; Zhang, A; Liang, Y; Song, M; Jiang, G. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. Environ Sci Technol 53: 6529-6538. http://dx.doi.org/10.1021/acs.est.9b00715.

Gao, Y; Luo, J; Zhang, Y; Pan, C; Ren, Y; Zhang, J; Tian, Y; Cohort, SB. (2022). Prenatal Exposure to Per- and Polyfluoroalkyl Substances and Child Growth Trajectories in the First Two Years. Environ Health Perspect 130: 37006. http://dx.doi.org/10.1289/EHP9875.

Gardener, H; Sun, Q; Grandjean, P. (2021). PFAS concentration during pregnancy in relation to cardiometabolic health and birth outcomes. Environ Res 192: 110287. http://dx.doi.org/10.1016/j.envres.2020.110287.

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. Environ Int 74: 160-169. http://dx.doi.org/10.1016/j.envint.2014.10.013.

Getz, GS; Reardon, CA. (2012). Animal models of atherosclerosis [Review]. Arterioscler Thromb Vasc Biol 32: 1104-1115. http://dx.doi.org/10.1161/ATVBAHA.111.237693.

Glauert, HP; Srinivasan, S; Tatum, VL; Chen, LC; Saxon, DM; Lay, LT; Borges, T; Baker, M; Chen, LH; Robertson, LW; Chow, CK. (1992). Effects of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cellular antioxidants and lipid peroxidation in rats. Biochem Pharmacol 43: 1353-1359. http://dx.doi.org/10.1016/0006-2952(92)90513-I.

Glynn, A; Kotova, N; Dahlgren, E; Lindh, C; Jakobsson, K; Gyllenhammar, I; Lignell, S; Nälsén, C. (2020). Determinants of serum concentrations of perfluoroalkyl acids (PFAAs) in school children and the contribution of low-level PFAA-contaminated drinking water. Environ Sci Process Impacts 22: 930-944. http://dx.doi.org/10.1039/c9em00497a.

Godin, CS; Myhr, BC; Lawlor, TE; Young, RR; Murli, H; Cifone, MA. (1992). Assessment of the potential genotoxicity of perfluorodecanoic acid and chlorotrifluoroethylene trimer and

tetramer acids. Fundam Appl Toxicol 18: 557-569. http://dx.doi.org/10.1016/0272-0590(92)90115-X.

Goecke-Flora, CM; Wyman, JF; Jarnot, BM; Reo, NV. (1995). Effect of the peroxisome proliferator perfluoro-n-decanoic acid on glucose transport in the isolated perfused rat liver. Chem Res Toxicol 8: 77-81. http://dx.doi.org/10.1021/tx00043a010.

Goeden, HM; Greene, CW; Jacobus, JA. (2019). A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. J Expo Sci Environ Epidemiol 29: 183-195. http://dx.doi.org/10.1038/s41370-018-0110-5.

Goldman, JM; Murr, AS; Cooper, RL. (2007). The rodent estrous cycle: Characterization of vaginal cytology and its utility in toxicological studies [Review]. Birth Defects Res B Dev Reprod Toxicol 80: 84-97. http://dx.doi.org/10.1002/bdrb.20106.

Goodrich, JA; Alderete, TL; Baumert, BO; Berhane, K; Chen, Z; Gilliland, FD; Goran, MI; Hu, X; Jones, DP; Margetaki, K; Rock, S; Stratakis, N; Valvi, D; Walker, DI; Conti, DV; Chatzi, L. (2021). Exposure to Perfluoroalkyl Substances and Glucose Homeostasis in Youth. Environ Health Perspect 129: 97002. http://dx.doi.org/10.1289/EHP9200.

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Chen, CJ; Ito, S; Araki, A; Kobayashi, S; Matsuura, H; Kishi, R. (2017). Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environ Int 104: 132-138. http://dx.doi.org/10.1016/j.envint.2017.01.024.

Goudarzi, H; Miyashita, C; Okada, E; Kashino, I; Kobayashi, S; Chen, CJ; Ito, S; Araki, A; Matsuura, H; Ito, YM; Kishi, R. (2016). Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environ Int 94: 124-132. http://dx.doi.org/10.1016/j.envint.2016.05.020.

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 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 at (accessed

Grandjean, P; Heilmann, C; Weihe, P; Nielsen, F; Mogensen, UB; Budtz-Jørgensen, E. (2017a). Serum vaccine antibody concentrations in adolescents exposed to perfluorinated compounds. Environ Health Perspect 125: 077018. http://dx.doi.org/10.1289/EHP275.

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. J Immunotoxicol 14: 188-195. http://dx.doi.org/10.1080/1547691X.2017.1360968.

Grandjean, P; Timmermann, CAG; Kruse, M; Nielsen, F; Vinholt, PJ; Boding, L; Heilmann, C; Mølbak, K. (2020). Severity of COVID-19 at elevated exposure to perfluorinated alkylates. PLoS ONE 15: e0244815. http://dx.doi.org/10.1371/journal.pone.0244815.

Gump, BB; Wu, Q; Dumas, AK; Kannan, K. (2011). Perfluorochemical (PFC) exposure in children: Associations with impaired response inhibition. Environ Sci Technol 45: 8151-8159. http://dx.doi.org/10.1021/es103712g.

Guo, J; Zhang, J; Wang, Z; Zhang, L; Qi, X; Zhang, Y; Chang, X; Wu, C; Zhou, Z. (2021). Umbilical cord serum perfluoroalkyl substance mixtures in relation to thyroid function of newborns: Findings from Sheyang Mini Birth Cohort Study. Chemosphere 273: 129664. http://dx.doi.org/10.1016/j.chemosphere.2021.129664.

Gutshall, DM; Pilcher, GD; Langley, AE. (1988). Effect of thyroxine supplementation on the response to perfluoro-n-decanoic acid (PFDA) in rats. J Toxicol Environ Health 24: 491-498. http://dx.doi.org/10.1080/15287398809531179.

Gutshall, DM; Pilcher, GD; Langley, AE. (1989). Mechanism of the serum thyroid hormone lowering effect of perfluoro-n-decanoic acid (PFDA) in rats. J Toxicol Environ Health 28: 53-65. http://dx.doi.org/10.1080/15287398909531328.

Guyatt, G; Oxman, AD; Akl, EA; Kunz, R; Vist, G; Brozek, J; Norris, S; Falck-Ytter, Y; Glasziou, P; DeBeer, H; Jaeschke, R; Rind, D; Meerpohl, J; Dahm, P; Schünemann, HJ. (2011). GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 64: 383-394. http://dx.doi.org/10.1016/j.jclinepi.2010.04.026.

Gyllenhammar, I; Diderholm, B; Gustafsson, J; Berger, U; Ridefelt, P; Benskin, JP; Lignell, S; Lampa, E; Glynn, A. (2018). Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int 111: 191-199. http://dx.doi.org/10.1016/j.envint.2017.12.002.

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A; Küttler, K; Malarkey, DE; Maronpot, RR; Nishikawa, A; Nolte, T; Schulte, A; Strauss, V; York, MJ. (2012). Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes—Conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicol Pathol 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.

Hallberg, L; Högdahl, AM; Nilsson, L; Rybo, G. (1966). Menstrual blood loss--a population study. Variation at different ages and attempts to define normality. Acta Obstet Gynecol Scand 45: 320-351. http://dx.doi.org/10.3109/00016346609158455.

Han, W; Gao, Y; Yao, Q; Yuan, T; Wang, Y; Zhao, S; Shi, R; Bonefeld-Jorgensen, EC; Shen, X; Tian, Y. (2018). Perfluoroalkyl and polyfluoroalkyl substances in matched parental and cord serum in Shandong, China. Environ Int 116: 206-213. http://dx.doi.org/10.1016/j.envint.2018.04.025.

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. Environ Res 99: 253-261. http://dx.doi.org/10.1016/j.envres.2004.12.003.

Harbison, RD; Bourgeois, MM; Johnson, GT. (2015). Hamilton & Hardy's industrial toxicology (6th ed.). Hoboken, NJ: John Wiley & Sons, Inc. http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470929731,subjectCd-CH50.html.

Hardell, E; Kärrman, A; van Bavel, B; Bao, J; Carlberg, M; Hardell, L. (2014). Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environ Int 63: 35-39. http://dx.doi.org/10.1016/j.envint.2013.10.005.

Harris, MH; Oken, E; Rifas-Shiman, SL; Calafat, AM; Bellinger, DC; Webster, TF; White, RF; Sagiv, SK. (2021). Prenatal and childhood exposure to per- and polyfluoroalkyl substances (PFAS) and child executive function and behavioral problems. Environ Res 202: 111621. http://dx.doi.org/10.1016/j.envres.2021.111621.

Harris, MH; Oken, E; Rifas-Shiman, SL; Calafat, AM; Ye, X; Bellinger, DC; Webster, TF; White, RF; Sagiv, SK. (2018). Prenatal and childhood exposure to per- and polyfluoroalkyl substances (PFASs) and child cognition. Environ Int 115: 358-369. http://dx.doi.org/10.1016/j.envint.2018.03.025.

Harris, MH; Rifas-Shiman, SL; Calafat, AM; Ye, X; Mora, AM; Webster, TF; Oken, E; Sagiv, SK. (2017). Predictors of per- and polyfluoroalkyl substance (PFAS) plasma concentrations in 6-10 year old American children. Environ Sci Technol 51: 5193-5204. http://dx.doi.org/10.1021/acs.est.6b05811.

Harris, MW; Birnbaum, LS. (1989). Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam Appl Toxicol 12: 442-448. http://dx.doi.org/10.1016/0272-0590(89)90018-3.

Harris, MW; Uraih, LC; Birnbaum, LS. (1989). Acute toxicity of perfluorodecanoic acid in C57BL/6 mice differs from 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol 13: 723-736. http://dx.doi.org/10.1016/0272-0590(89)90330-8.

Harrison, EH; Lane, JS; Luking, S; Van Rafelghem, MJ; Andersen, ME. (1988). Perfluoro-n-decanoic acid: Induction of peroxisomal beta-oxidation by a fatty acid with dioxin-like toxicity. Lipids 23: 115-119. http://dx.doi.org/10.1007/BF02535290.

Hashem, MS; Kalashyan, H; Choy, J; Chiew, SK; Shawki, AH; Dawood, AH; Becher, H. (2015). Left ventricular relative wall thickness versus left ventricular mass index in non-cardioembolic stroke patients. Medicine (Baltimore) 94: e872. http://dx.doi.org/10.1097/MD.0000000000000872.

Heo, JJ; Lee, JW; Kim, SK; Oh, JE. (2014). Foodstuff analyses show that seafood and water are major perfluoroalkyl acids (PFAAs) sources to humans in Korea. J Hazard Mater 279: 402-409. http://dx.doi.org/10.1016/j.jhazmat.2014.07.004.

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300. http://dx.doi.org/10.1177/003591576505800503.

Hjermitslev, MH; Long, M; Wielsøe, M; Bonefeld-Jørgensen, EC. (2020). Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. Sci Total Environ 698: 134118. http://dx.doi.org/10.1016/j.scitotenv.2019.134118.

Holder, C; Deluca, N; Luh, J; Alexander, P; Minucci, JM; Vallero, DA; Thomas, K; Cohen Hubal, EA. (2023). Systematic evidence mapping of potential exposure pathways for per- and polyfluoroalkyl substances based on measured occurrence in multiple media [Review]. Environ Sci Technol 57: 5107-5116. http://dx.doi.org/10.1021/acs.est.2c07185.

Hotchkiss, AK; Rider, CV; Blystone, CR; Wilson, VS; Hartig, PC; Ankley, GT; Foster, PM; Gray, CL; Gray, LE. (2008). Fifteen years after "Wingspread"--environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go [Review]. Toxicol Sci 105: 235-259. http://dx.doi.org/10.1093/toxsci/kfn030.

Høyer, BB; Bonde, JP; Tøttenborg, SS; Ramlau-Hansen, CH; Lindh, C; Pedersen, HS; Toft, G. (2017). Exposure to perfluoroalkyl substances during pregnancy and child behaviour at 5 to 9 years of age. Horm Behav 101: 105-112. http://dx.doi.org/10.1016/j.yhbeh.2017.11.007.

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. Environ Int 119: 37-46. http://dx.doi.org/10.1016/j.envint.2018.05.051.

Huang, Q; Liu, L; Wu, Y; Wang, X; Luo, L; Nan, B; Zhang, J; Tian, M; Shen, H. (2019a). Seminal plasma metabolites mediate the associations of multiple environmental pollutants with semen quality in Chinese men. Environ Int 132: 105066. http://dx.doi.org/10.1016/j.envint.2019.105066.

Huang, R; Chen, Q; Zhang, L; Luo, K; Chen, L; Zhao, S; Feng, L; Zhang, J. (2019b). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy. Environ Health 18: 5. http://dx.doi.org/10.1186/s12940-018-0445-3.

Huo, X; Zhang, L; Huang, R; Feng, L; Wang, W; Zhang, J; Cohort, SB. (2020). Perfluoroalkyl substances exposure in early pregnancy and preterm birth in singleton pregnancies: a prospective cohort study. Environ Health 19: 60. http://dx.doi.org/10.1186/s12940-020-00616-8.

ICH Expert Working Group. (2005). Immunotoxicity studies for human pharmaceuticals S8 11. In ICH Harmonised Tripartite Guideline.

Inoue, K; Ritz, B; Andersen, SL; Ramlau-Hansen, CH; Høyer, BB; Bech, BH; Henriksen, TB; Bonefeld-Jørgensen, EC; Olsen, J; Liew, Z. (2019). Perfluoroalkyl substances and maternal thyroid

hormones in early pregnancy; findings in the Danish National Birth Cohort. Environ Health Perspect 127: 117002. http://dx.doi.org/10.1289/EHP5482.

IPCS (International Programme on Chemical Safety). (1996). Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Geneva, Switzerland: World Health Organization. http://www.inchem.org/documents/ehc/ehc/ehc180.htm.

IPCS (International Programme on Chemical Safety). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland: World Health Organization. https://apps.who.int/iris/handle/10665/330098.

IQEHC (Institute for Quality and Efficiency in Health Care). (2014). Deciding whether or not to treat subclinical hypothyroidism. https://www.informedhealth.org/underactive-thyroid-hypothyroidism.2713.en.pdf?all_backgrounds=0&all_details=0&all_lexicons=0&all_reports=0&overview=1&print=1&theme=0.

Ishibashi, H; Hirano, M; Kim, EY; Iwata, H. (2019). In vitro and in silico evaluations of binding affinities of perfluoroalkyl substances to baikal seal and human peroxisome proliferator-activated receptor α. Environ Sci Technol 53: 2181-2188. http://dx.doi.org/10.1021/acs.est.8b07273.

Itoh, S; Araki, A; Miyashita, C; Yamazaki, K; Goudarzi, H; Minatoya, M; Ait Bamai, Y; Kobayashi, S; Okada, E; Kashino, I; Yuasa, M; Baba, T; Kishi, R. (2019). Association between perfluoroalkyl substance exposure and thyroid hormone/thyroid antibody levels in maternal and cord blood: The Hokkaido Study. Environ Int 133: 105139. http://dx.doi.org/10.1016/j.envint.2019.105139.

Jaeschke, H; Lemasters, JJ. (2003). Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology 125: 1246-1257. http://dx.doi.org/10.1016/S0016-5085(03)01209-5.

Jain, RB. (2019). Synergistic impact of co-exposures to toxic metals cadmium, lead, and mercury along with perfluoroalkyl substances on the healthy kidney function. Environ Res 169: 342-347. http://dx.doi.org/10.1016/j.envres.2018.11.037.

Jain, RB. (2020a). Impact of the co-occurrence of obesity with diabetes, anemia, hypertension, and albuminuria on concentrations of selected perfluoroalkyl acids. Environ Pollut 266 Pt. 2: 115207. http://dx.doi.org/10.1016/j.envpol.2020.115207.

Jain, RB. (2020b). Variabilities in concentrations of selected perfluoroalkyl acids among normotensives and hypertensives across various stages of glomerular function. Arch Environ Occup Health 76: 1-11. http://dx.doi.org/10.1080/19338244.2020.1732856.

Jain, RB. (2021). Perfluoroalkyl acids and their isomers, diabetes, anemia, and albuminuria: Variabilities with deteriorating kidney function. Ecotoxicol Environ Saf 208: 111625. http://dx.doi.org/10.1016/j.ecoenv.2020.111625.

Jain, RB; Ducatman, A. (2019a). Roles of gender and obesity in defining correlations between perfluoroalkyl substances and lipid/lipoproteins. Sci Total Environ 653: 74-81. http://dx.doi.org/10.1016/j.scitotenv.2018.10.362.

Jain, RB; Ducatman, A. (2019b). Selective associations of recent low concentrations of perfluoroalkyl substances with liver function biomarkers: nhanes 2011 to 2014 data on us adults aged ≥20 years. J Occup Environ Med 61: 293-302. http://dx.doi.org/10.1097/JOM.0000000000001532.

Janis, JA; Rifas-Shiman, SL; Seshasayee, SM; Sagiv, S; Calafat, AM; Gold, DR; Coull, BA; Rosen, CJ; Oken, E; Fleisch, AF. (2021). Plasma concentrations of per- and polyfluoroalkyl substances and body composition from mid-childhood to early adolescence. J Clin Endocrinol Metab 106: e3760–e3770. http://dx.doi.org/10.1210/clinem/dgab187.

Je, I, -G; Kim, D, uk-Sil; Kim, S, -W; Lee, S; Lee, HS; Park, E, -K; Khang, D; Kim, SH. (2015). Tyrosol suppresses allergic inflammation by inhibiting the activation of phosphoinositide 3-kinase in mast cells. PLoS ONE 10: e0129829. http://dx.doi.org/10.1371/journal.pone.0129829.

Jensen, RC; Andersen, MS; Larsen, PV; Glintborg, D; Dalgård, C; Timmermann, CAG; Nielsen, F; Sandberg, MB; Andersen, HR; Christesen, HT; Grandjean, P; Jensen, TK. (2020a). Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study. Environ Health Perspect 128: 77001. http://dx.doi.org/10.1289/EHP5184.

Jensen, RC; Glintborg, D; Gade Timmermann, CA; Nielsen, F; Kyhl, HB; Frederiksen, H; Andersson, AM; Juul, A; Sidelmann, JJ; Andersen, HR; Grandjean, P; Andersen, MS; Jensen, TK. (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. Environ Res 182: 109101. http://dx.doi.org/10.1016/j.envres.2019.109101.

Jensen, RC; Glintborg, D; Timmermann, CAG; Nielsen, F; Kyhl, HB; Andersen, HR; Grandjean, P; Jensen, TK; Andersen, M. (2018). Perfluoroalkyl substances and glycemic status in pregnant Danish women: The Odense Child Cohort. Environ Int 116: 101-107. http://dx.doi.org/10.1016/j.envint.2018.04.010.

Jensen, TK; Andersen, LB; Kyhl, HB; Nielsen, F; Christesen, HT; Grandjean, P. (2015). Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PLoS ONE 10: e0123496. http://dx.doi.org/10.1371/journal.pone.0123496.

Ji, K; Kim, S; Kho, Y; Paek, D; Sakong, J; Ha, J; Kim, S; Choi, K. (2012). Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environ Int 45: 78-85. http://dx.doi.org/10.1016/j.envint.2012.03.007.

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. Environ Int 64: 40-47. http://dx.doi.org/10.1016/j.envint.2013.12.001.

Jin, H; Zhang, Y; Jiang, W; Zhu, L; Martin, JW. (2016). Isomer-Specific Distribution of Perfluoroalkyl Substances in Blood. Environ Sci Technol 50: 7808-7815. http://dx.doi.org/10.1021/acs.est.6b01698.

Joensen, UN; Veyrand, B; Antignac, JP; Blomberg Jensen, M; Petersen, JH; Marchand, P; Skakkebæk, NE; Andersson, AM; Le Bizec, B; Jørgensen, N. (2013). PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum Reprod 28: 599-608. http://dx.doi.org/10.1093/humrep/des425.

Jones, MEE; Boon, WC; Proietto, J; Simpson, ER. (2006). Of mice and men: The evolving phenotype of aromatase deficiency [Review]. Trends Endocrinol Metab 17: 55-64. http://dx.doi.org/10.1016/j.tem.2006.01.004.

Joshi-Barve, S; Kirpich, I; Cave, MC; Marsano, LS; McClain, CJ. (2015). Alcoholic, nonalcoholic, and toxicant-associated steatohepatitis: Mechanistic similarities and differences [Review]. CMGH 1: 356-367. http://dx.doi.org/10.1016/j.jcmgh.2015.05.006.

Kadel, S; Kovats, S. (2018). Sex Hormones Regulate Innate Immune Cells and Promote Sex Differences in Respiratory Virus Infection [Review]. Front Immunol 9: 1653. http://dx.doi.org/10.3389/fimmu.2018.01653.

Kang, H; Lee, HK; Moon, HB; Kim, S; Lee, J; Ha, M; Hong, S; Kim, S; Choi, K. (2018). Perfluoroalkyl acids in serum of Korean children: Occurrences, related sources, and associated health outcomes. Sci Total Environ 645: 958-965. http://dx.doi.org/10.1016/j.scitotenv.2018.07.177.

Karásková, P; Venier, M; Melymuk, L; Bečanová, J; Vojta, Š; Prokeš, R; Diamond, ML; Klánová, J. (2016). Perfluorinated alkyl substances (PFASs) in household dust in Central Europe and North America. Environ Int 94: 315-324. http://dx.doi.org/10.1016/j.envint.2016.05.031.

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. Reprod Toxicol 68: 145-153. http://dx.doi.org/10.1016/j.reprotox.2016.08.002.

Kashino, I; Sasaki, S; Okada, E; Matsuura, H; Goudarzi, H; Miyashita, C; Okada, E; Ito, YM; Araki, A; Kishi, R. (2020). Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environ Int 136: 105355. http://dx.doi.org/10.1016/j.envint.2019.105355.

Kass, GEN. (2006). Mitochondrial involvement in drug-induced hepatic injury. Chem Biol Interact 163: 145-159. http://dx.doi.org/10.1016/j.cbi.2006.06.007.

Kawabata, K; Matsuzaki, H; Nukui, S; Okazaki, M; Sakai, A; Kawashima, Y; Kudo, N. (2017). Perfluorododecanoic acid induces cognitive deficit in adult rats. Toxicol Sci 157: 421-428. http://dx.doi.org/10.1093/toxsci/kfx058.

Kawashima, Y; Kobayashi, H; Miura, H; Kozuka, H. (1995). Characterization of hepatic responses of rat to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. Toxicology 99: 169-178. http://dx.doi.org/10.1016/0300-483X(95)03027-D.

Kelling, CK; Van Rafelghem, MJ; Menahan, LA; Peterson, RE. (1987). Effects of perfluorodecanoic acid on hepatic indices of thyroid status in the rat. Biochem Pharmacol 36: 1337-1344. http://dx.doi.org/10.1016/0006-2952(87)90091-8.

Khalil, N; Ducatman, AM; Sinari, S; Billheimer, D; Hu, C; Littau, S; Burgess, JL. (2020). Per- and polyfluoroalkyl substance and cardio metabolic markers in firefighters. J Occup Environ Med 62: 1076-1081. http://dx.doi.org/10.1097/JOM.0000000000002062.

Kielsen, K; Shamim, Z; Ryder, LP; Nielsen, F; Grandjean, P; Budtz-Jørgensen, E; Heilmann, C. (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol 13: 270-273. http://dx.doi.org/10.3109/1547691X.2015.1067259.

Kim, HY; Kim, KN; Shin, CH; Lim, YH; Kim, JI; Kim, BN; Hong, YC; Lee, YA. (2020a). The relationship between perfluoroalkyl substances concentrations and thyroid function in early childhood: A prospective cohort study. Thyroid 30: 1556-1565. http://dx.doi.org/10.1089/thy.2019.0436.

Kim, K; Bennett, DH; Calafat, AM; Hertz-Picciotto, I; Shin, HM. (2020b). Temporal trends and determinants of serum concentrations of per- and polyfluoroalkyl substances among Northern California mothers with a young child, 2009-2016. Environ Res 186: 109491. http://dx.doi.org/10.1016/j.envres.2020.109491.

Kim, OJ; Kim, S; Park, EY; Oh, JK; Jung, SK; Park, S; Hong, S; Jeon, HL; Kim, HJ; Park, B; Park, B; Kim, S; Kim, B. (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, S; Choi, K; Ji, K; Seo, J; Kho, Y; Park, J; Kim, S; Park, S; Hwang, I; Jeon, J; Yang, H; Giesy, JP. (2011). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol 45: 7465-7472. http://dx.doi.org/10.1021/es202408a.

Kim, SC; Hong, JT; Jang, SJ; Kang, WS; Yoo, HS; Yun, YP. (1998). Formation of 8-oxodeoxyguanosine in liver DNA and hepatic injury by peroxisome proliferator clofibrate and perfluorodecanoic acid in rats. J Toxicol Sci 23: 113-119. http://dx.doi.org/10.2131/jts.23.2_113.

Kim, SJ; Choi, EJ; Choi, GW; Lee, YB; Cho, HY. (2019). Exploring sex differences in human health risk assessment for PFNA and PFDA using a PBPK model. Arch Toxicol 93: 311-330. http://dx.doi.org/10.1007/s00204-018-2365-y.

Kim, SK; Kannan, K. (2007). Perfluorinated acids in air, rain, snow, surface runoff, and lakes: Relative importance of pathways to contamination of urban lakes. Environ Sci Technol 41: 8328-8334. http://dx.doi.org/10.1021/es072107t.

Kim, YR; White, N; Bräunig, J; Vijayasarathy, S; Mueller, JF; Knox, CL; Harden, FA; Pacella, R; Toms, LL. (2020c). Per- and poly-fluoroalkyl substances (PFASs) in follicular fluid from women experiencing infertility in Australia. Environ Res 190: 109963. http://dx.doi.org/10.1016/j.envres.2020.109963.

Kjeldsen, LS; Bonefeld-Jørgensen, EC. (2013). Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int 20: 8031-8044. http://dx.doi.org/10.1007/s11356-013-1753-3.

Klein, SL; Flanagan, KL. (2016). Sex differences in immune responses [Review]. Nat Rev Immunol 16: 626-638. http://dx.doi.org/10.1038/nri.2016.90.

Kleiner, DE; Weerasinghe, P; Buja, LM. (2012). Oncosis: An important non-apoptotic mode of cell death. Exp Mol Pathol 93: 302-308. http://dx.doi.org/10.1016/j.yexmp.2012.09.018.

Knobeloch, L; Imm, P; Anderson, H. (2012). Perfluoroalkyl chemicals in vacuum cleaner dust from 39 Wisconsin homes. Chemosphere 88: 779-783. http://dx.doi.org/10.1016/j.chemosphere.2012.03.082.

Konwick, BJ; Tomy, GT; Ismail, N; Peterson, JT; Fauver, RJ; Higginbotham, D; Fisk, AT. (2008). Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. Environ Toxicol Chem 27: 2011-2018. http://dx.doi.org/10.1897/07-659.1.

Koponen, J; Winkens, K; Airaksinen, R; Berger, U; Vestergren, R; Cousins, IT; Karvonen, AM; Pekkanen, J; Kiviranta, H. (2018). Longitudinal trends of per- and polyfluoroalkyl substances in children's serum. Environ Int 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; Honda, M; Chu, DB; Han, X; Shao, Y; Kannan, K; Urbina, EM; Trasande, L. (2017). Serum perfluoroalkyl substances and cardiometabolic consequences in adolescents exposed to the World Trade Center disaster and a matched comparison group. Environ Int 109: 128-135. http://dx.doi.org/10.1016/j.envint.2017.08.003.

Kotthoff, M; Müller, J; Jürling, H; Schlummer, M; Fiedler, D. (2015). Perfluoroalkyl and polyfluoroalkyl substances in consumer products. Environ Sci Pollut Res Int 22: 14546-14559. http://dx.doi.org/10.1007/s11356-015-4202-7.

Kudo, N. (2015). Metabolism and pharmacokinetics. In JC DeWitt (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances (pp. 151-175). New York, NY: Springer International Publishing. http://dx.doi.org/10.1007/978-3-319-15518-0_6.

Kudo, N; Kawashima, Y. (2003). Induction of triglyceride accumulation in the liver of rats by perfluorinated fatty acids with different carbon chain lengths: Comparison with induction of peroxisomal beta-oxidation. Biol Pharm Bull 26: 47-51. http://dx.doi.org/10.1248/bpb.26.47.

Kudo, N; Suzuki, E; Katakura, M; Ohmori, K; Noshiro, R; Kawashima, Y. (2001). Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem Biol Interact 134: 203-216. http://dx.doi.org/10.1016/S0009-2797(01)00155-7.

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. Environ Int 134: 105259. http://dx.doi.org/10.1016/j.envint.2019.105259.

Kwon, EJ; Shin, JS; Kim, BM; Shah-Kulkarni, S; Park, H; Kho, YL; Park, EA; Kim, YJ; Ha, EH. (2016). Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med 58: e198-e205. http://dx.doi.org/10.1097/JOM.0000000000000739.

Langley, AE; Pilcher, GD. (1985). Thyroid, bradycardic and hypothermic effects of perfluoro-n-decanoic acid in rats. J Toxicol Environ Health 15: 485-491. http://dx.doi.org/10.1080/15287398509530675.

Lankova, D; Lacina, O; Pulkrabova, J; Hajslova, J. (2013). The determination of perfluoroalkyl substances, brominated flame retardants and their metabolites in human breast milk and infant formula. Talanta 117: 318-325. http://dx.doi.org/10.1016/j.talanta.2013.08.040.

Lau, C. (2015). Perfluorinated compounds: An overview. In JC DeWitt (Ed.), Toxicological effects of perfluoroalkyl and polyfluoroalkyl substances (pp. 1-21). New York: Springer. http://dx.doi.org/10.1007/978-3-319-15518-0_1.

Lee, ES; Han, S; Oh, JE. (2016). Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reprod Toxicol 59: 53-59. http://dx.doi.org/10.1016/j.reprotox.2015.10.020.

Lee, JK; Kim, SH. (2018). Correlation between mast cell-mediated allergic inflammation and length of perfluorinated compounds. J Toxicol Environ Health A 81: 1-12. http://dx.doi.org/10.1080/15287394.2018.1440188.

Lee, YA; Kim, JH; Jung, HW; Lim, YH; Bae, S; Kho, Y; Hong, YC; Shin, CH; Yang, SW. (2018). The serum concentrations of perfluoroalkyl compounds were inversely associated with growth parameters in 2-year old children. Sci Total Environ 628-629: 226-232. http://dx.doi.org/10.1016/j.scitotenv.2018.02.050.

Leehy, KA; Regan Anderson, TM; Daniel, AR; Lange, CA; Ostrander, JH. (2016). Modifications to glucocorticoid and progesterone receptors alter cell fate in breast cancer [Review]. J Mol Endocrinol 56: R99-R114. http://dx.doi.org/10.1530/JME-15-0322.

Lefevre, J; Mcclintock, MK. (1988). Reproductive senescence in female rats - A longitudinal-study of individual-differences in estrous cycles and behavior. Biol Reprod 38: 780-789. http://dx.doi.org/10.1095/biolreprod38.4.780.

Lenters, V; Portengen, L; Rignell-Hydbom, A; Jönsson, BA; Lindh, CH; Piersma, AH; Toft, G; Bonde, JP; Heederik, D; Rylander, L; Vermeulen, R. (2016). Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect 124: 365-372. http://dx.doi.org/10.1289/ehp.1408933.

Levin, S; Selmer, D; Z, R. (1993). Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. Toxicol Pathol 21: 1-14. http://dx.doi.org/10.1177/019262339302100101.

Levitt, D; Liss, A. (1986). Toxicity of perfluorinated fatty acids for human and murine B cell lines. Toxicol Appl Pharmacol 86: 1-11. http://dx.doi.org/10.1016/0041-008x(86)90394-7.

Lewiński, A; Stasiak, M. (2017). Physiopathology, diagnosis, and treatment of secondary hypothyroidism. In Hypothalamic-Pituitary Diseases. Basel, Switzerland: Springer International Publishing. http://dx.doi.org/10.1007/978-3-319-38681-2_7-1.

Li, J; Cai, D; Chu, C; Li, QQ; Zhou, Y; Hu, LW; Yang, BY; Dong, GH; Zeng, XW; Chen, D. (2020a). Transplacental Transfer of Per- and Polyfluoroalkyl Substances (PFASs): Differences between Preterm and Full-Term Deliveries and Associations with Placental Transporter mRNA Expression. Environ Sci Technol 54: 5062-5070. http://dx.doi.org/10.1021/acs.est.0c00829.

Li, J; Cao, H; Feng, H; Xue, Q; Zhang, A; Fu, J. (2020b). Evaluation of the estrogenic/antiestrogenic activities of perfluoroalkyl substances and their interactions with the human estrogen receptor by combining in vitro assays and in silico modeling. Environ Sci Technol 54: 14514-14524. http://dx.doi.org/10.1021/acs.est.0c03468.

Li, KM; Zhao, Q; Fan, ZY; Jia, SY; Liu, Q; Liu, FY; Liu, SL. (2022). The toxicity of perfluorodecanoic acid is mainly manifested as a deflected immune function. Mol Biol Rep 49: 4365-4376. http://dx.doi.org/10.1007/s11033-022-07272-w.

Li, LQ; Shigematsu, Y; Hamada, M; Hiwada, K. (2001). Relative wall thickness is an independent predictor of left ventricular systolic and diastolic dysfunctions in essential hypertension. Hypertens Res 24: 493-499. http://dx.doi.org/10.1291/hypres.24.493.

Li, M; Zeng, XW; Qian, ZM; Vaughn, MG; Sauvé, S; Paul, G; Lin, S; Lu, L; Hu, LW; Yang, BY; Zhou, Y; Qin, XD; Xu, SL; Bao, WW; Zhang, YZ; Yuan, P; Wang, J; Zhang, C; Tian, YP; Nian, M; Xiao, X; Fu, C; Dong, GH. (2017). Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environ Int 102: 1-8. http://dx.doi.org/10.1016/j.envint.2017.03.006.

Liang, H; Wang, Z; Miao, M; Tian, Y; Zhou, Y; Wen, S; Chen, Y; Sun, X; Yuan, W. (2020). Prenatal exposure to perfluoroalkyl substances and thyroid hormone concentrations in cord plasma in a Chinese birth cohort. Environ Health 19: 127. http://dx.doi.org/10.1186/s12940-020-00679-7.

Liao, Q; Tang, P; Fan, H; Song, Y; Liang, J; Huang, H; Pan, D; Mo, M; Leilei; Lin, M; Chen, J; Wei, H; Long, J; Shao, Y; Zeng, X; Liu, S; Huang, D; Qiu, X. (2023). Association between maternal exposure to per- and polyfluoroalkyl substances and serum markers of liver function during pregnancy in China: A mixture-based approach. Environ Pollut 323: 121348. http://dx.doi.org/10.1016/j.envpol.2023.121348.

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. Environ Health Perspect 128: 47007. http://dx.doi.org/10.1289/EHP6202.

Liew, Z; Ritz, B; Bach, CC; Asarnow, RF; Bech, BH; Nohr, EA; Bossi, R; Henriksen, TB; Bonefeld-Jørgensen, EC; Olsen, J. (2018). Prenatal exposure to perfluoroalkyl substances and iq scores at age 5; a study in the danish national birth cohort. Environ Health Perspect 126: 067004. http://dx.doi.org/10.1289/EHP2754.

Liew, Z; Ritz, B; Bonefeld-Jørgensen, EC; Henriksen, TB; Nohr, EA; Bech, BH; Fei, C; Bossi, R; von Ehrenstein, OS; Streja, E; Uldall, P; Olsen, J. (2014). Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. Am J Epidemiol 180: 574-581. http://dx.doi.org/10.1093/aje/kwu179.

Liew, Z; Ritz, B; von Ehrenstein, OS; Bech, BH; Nohr, EA; Fei, C; Bossi, R; Henriksen, TB; Bonefeld-Jørgensen, EC; Olsen, J. (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. Environ Health Perspect 123: 367-373. http://dx.doi.org/10.1289/ehp.1408412.

Lim, JJ; Suh, Y; Faustman, EM; Cui, JY. (2021). Regulation of transporters by perfluorinated carboxylic acids in HepaRG cells. Drug Metab Dispos 50: 1396-1413. http://dx.doi.org/10.1124/dmd.121.000477.

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 J Med Sci 82: 323-333. http://dx.doi.org/10.18999/nagjms.82.2.323.

Lin, TW; Chen, MK; Lin, CC; Chen, MH; Tsai, MS; Chan, DC; Hung, KY; Chen, PC. (2020b). Association between exposure to perfluoroalkyl substances and metabolic syndrome and related outcomes among older residents living near a Science Park in Taiwan. Int J Hyg Environ Health 230: 113607. http://dx.doi.org/10.1016/j.ijheh.2020.113607.

Lind, DV; Priskorn, L; Lassen, TH; Nielsen, F; Kyhl, HB; Kristensen, DM; Christesen, HT; Jørgensen, JS; Grandjean, P; Jensen, TK. (2017a). Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 68: 200-206. http://dx.doi.org/10.1016/j.reprotox.2016.08.019.

Lind, PM; Lind, L; Salihovic, S; Ahlström, H; Michaelsson, K; Kullberg, J; Strand, R. (2022). Serum levels of perfluoroalkyl substances (PFAS) and body composition - A cross-sectional study

in a middle-aged population. Environ Res 209: 112677. http://dx.doi.org/10.1016/j.envres.2022.112677.

Lind, PM; Salihovic, S; van Bavel, B; Lind, L. (2017b). Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ Res 152: 157-164. http://dx.doi.org/10.1016/j.envres.2016.10.002.

Liu, B; Wei, B; Mo, M; Song, Y; Tang, C; Tang, P; Guo, X; Tan, C; Liu, S; Huang, D; Qiu, X. (2021a). Exposure to perfluoroalkyl substances in early pregnancy and the risk of hypertensive disorders of pregnancy: A nested case-control study in Guangxi, China. Chemosphere 288: 132468. http://dx.doi.org/10.1016/j.chemosphere.2021.132468.

Liu, C; Xu, X; Huo, X, ia. (2014). Anogenital distance and its application in environmental health research [Review]. Environ Sci Pollut Res Int 21: 5457-5464. http://dx.doi.org/10.1007/s11356-014-2570-z.

Liu, F; Fan, Z; Song, N; Han, M; Yan, M; Guo, LH; Jihui, J; Liu, S. (2019a). XRCC4, which is inhibited by PFDA, regulates DNA damage repair and cell chemosensitivity. J Cell Biochem 120: 12665-12676. http://dx.doi.org/10.1002/jcb.28534.

Liu, G; Dhana, K; Furtado, JD; Rood, J; Zong, G; Liang, L; Qi, L; Bray, GA; Dejonge, L; Coull, B; Grandjean, P; Sun, Q. (2018). Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Med 15: e1002502. http://dx.doi.org/10.1371/journal.pmed.1002502.

Liu, G; Zhang, B; Hu, Y; Rood, J; Liang, L; Qi, L; Bray, GA; Dejonge, L; Coull, B; Grandjean, P; Furtado, JD; Sun, Q. (2020a). Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environ Health 19: 5. http://dx.doi.org/10.1186/s12940-020-0561-8.

Liu, H; Pan, Y; Jin, S; Li, Y; Zhao, L; Sun, X; Cui, Q; Zhang, B; Zheng, T; Xia, W; Zhou, A; Campana, AM; Dai, J; Xu, S. (2020b). Associations of per-/polyfluoroalkyl substances with glucocorticoids and progestogens in newborns. Environ Int 140: 105636. http://dx.doi.org/10.1016/j.envint.2020.105636.

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. Environ Int 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; Chu, C; Wu, LY; Liang, LX; Qin, SJ; Zeeshan, M; Zhou, Y; Hu, LW; Liu, RQ; Zeng, XW; Dong, GH; Zhao, XM. (2022). Per- and perfluoroalkyl substances alternatives, mixtures and liver function in adults: A community-based population study in China. Environ Int 163: 107179. http://dx.doi.org/10.1016/j.envint.2022.107179.

Liu, M; Zhang, G; Meng, L; Han, X; Li, Y; Shi, Y; Li, A; Turyk, ME; Zhang, Q; Jiang, G. (2021b). 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. Environ Sci Technol 56: 6144-6151. http://dx.doi.org/10.1021/acs.est.1c02850.

Liu, X; Zhang, L; Chen, L; Li, J; Wang, Y; Wang, J; Meng, G; Chi, M; Zhao, Y; Chen, H; Wu, Y. (2019b). Structure-based investigation on the association between perfluoroalkyl acids exposure and both gestational diabetes mellitus and glucose homeostasis in pregnant women. Environ Int 127: 85-93. http://dx.doi.org/10.1016/j.envint.2019.03.035.

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.

Louis, GM; Sapra, KJ; Barr, DB; Lu, Z; Sundaram, R. (2016). Preconception perfluoroalkyl and polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. Reprod Toxicol 65: 11-17. http://dx.doi.org/10.1016/j.reprotox.2016.06.011.

Louis, GMB; Peterson, CM; Chen, Z; Hediger, ML; Croughan, MS; Sundaram, R; Stanford, JB; Fujimoto, VY; Varner, MW; Giudice, LC; Kennedy, A; Sun, L; Wu, Q; Kannan, K. (2012). Perfluorochemicals and endometriosis: The ENDO study. Epidemiology 23: 799-805. http://dx.doi.org/10.1097/EDE.0b013e31826cc0cf.

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. http://dx.doi.org/10.1097/EDE.0000000000000552.

Lumley, T. (2004). Analysis of complex survey samples. J Stat Softw 9. http://dx.doi.org/10.18637/jss.v009.i08.

Lumley, T. (2023). Survey: Analysis of complex survey samples (Version 4.2-1). Retrieved from https://cran.r-project.org/package=survey

Luo, D; Wu, WX; Pan, YA; Du, BB; Shen, MJ; Zeng, LX. (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. Environ Sci Technol 55: 11859-11873. http://dx.doi.org/10.1021/acs.est.1c02670.

Luo, J; Xiao, J; Gao, Y; Ramlau-Hansen, CH; Toft, G; Li, J; Obel, C; Andersen, SL; Deziel, NC; Tseng, WL; Inoue, K; Bonefeld-Jørgensen, EC; Olsen, J; Liew, Z. (2020). Prenatal exposure to perfluoroalkyl substances and behavioral difficulties in childhood at 7 and 11 years. Environ Res 191: 110111. http://dx.doi.org/10.1016/j.envres.2020.110111.

Luo, M; Tan, Z; Dai, M; Song, D; Lin, J; Xie, M; Yang, J; Sun, L; Wei, D; Zhao, J; Gonzalez, FJ; Liu, A. (2017). Dual action of peroxisome proliferator-activated receptor alpha in perfluorodecanoic acid-induced hepatotoxicity. Arch Toxicol 91: 897-907. http://dx.doi.org/10.1007/s00204-016-1779-7.

Lyall, K; Yau, VM; Hansen, R; Kharrazi, M; Yoshida, CK; Calafat, AM; Windham, G; Croen, LA. (2018). Prenatal maternal serum concentrations of per- and polyfluoroalkyl substances in association with autism spectrum disorder and intellectual disability. Environ Health Perspect 126: 017001. http://dx.doi.org/10.1289/EHP1830.

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. Toxicol Sci 106: 319-328. http://dx.doi.org/10.1093/toxsci/kfn177.

Mamsen, LS; Björvang, RD; Mucs, D; Vinnars, MT; Papadogiannakis, N; Lindh, CH; Andersen, CY; Damdimopoulou, P. (2019). Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environ Int 124: 482-492. http://dx.doi.org/10.1016/j.envint.2019.01.010.

Mamsen, LS; Jönsson, BAG; Lindh, CH; Olesen, RH; Larsen, A; Ernst, E; Kelsey, TW; Andersen, CY. (2017). Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Sci Total Environ 596-597: 97-105. http://dx.doi.org/10.1016/j.scitotenv.2017.04.058.

Manzano-Salgado, CB; Casas, M; Lopez-Espinosa, MJ; Ballester, F; Iñiguez, C; Martinez, D; Costa, O; Santa-Marina, L; Pereda-Pereda, E; Schettgen, T; Sunyer, J; Vrijheid, M. (2017). Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environ Int 108: 278-284. http://dx.doi.org/10.1016/j.envint.2017.09.006.

Mariash, CN; Kaiser, FE; Schwartz, HL; Towle, HC; Oppenheimer, JH. (1980). Synergism of thyroid-hormone and high-carbohydrate diet in the induction of lipogenic enzymes in the rat - Mechanisms and implications. J Clin Invest 65: 1126-1134. http://dx.doi.org/10.1172/JCI109766.

Marty, MS; Allen, B; Chapin, RE; Cooper, R; Daston, GP; Flaws, JA; Foster, PMD; Makris, SL; Mylchreest, E; Sandler, D; Tyl, RW. (2009). Inter-laboratory control data for reproductive

endpoints required in the OPPTS 870.3800/OECD 416 reproduction and fertility test. Birth Defects Res B Dev Reprod Toxicol 86: 470-489. http://dx.doi.org/10.1002/bdrb.20208.

Matthews, DR; Hosker, JP; Rudenski, AS; Naylor, BA; Treacher, DF; Turner, RC. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419. http://dx.doi.org/10.1007/BF00280883.

Mattsson, K; Rignell-Hydbom, A; Holmberg, S; Thelin, A; Jönsson, BA; Lindh, CH; Sehlstedt, A; Rylander, L. (2015). Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environ Res 142: 148-154. http://dx.doi.org/10.1016/j.envres.2015.06.033.

Mayer, C; Boehm, U. (2011). Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci704-U297. http://dx.doi.org/10.1038/nn.2818.

McComb, J; Mills, IG; Berntsen, HF; Ropstad, E; Verhaegen, S; Connolly, L. (2019). Human-based exposure levels of perfluoroalkyl acids may induce harmful effects to health by disrupting major components of androgen receptor signalling in vitro. Exposure and Health 12: 527-538. http://dx.doi.org/10.1007/s12403-019-00318-8.

McCoy, JA; Bangma, JT; Reiner, JL; Bowden, JA; Schnorr, J; Slowey, M; O'Leary, T; Guillette, LJ; Parrott, BB. (2017). Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. Sci Total Environ 605-606: 9-17. http://dx.doi.org/10.1016/j.scitotenv.2017.06.137.

MDH (Minnesota Department of Health). (2019). Health Based Guidance for Water: Toxicological Summary for: Perfluorohexane sulfonate (PFHxS). https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfhxs.pdf.

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. Int J Environ Res Public Health 15: 1832. http://dx.doi.org/10.3390/ijerph15091832.

Mi, X; Lin, SQ; Zhang, XF; Li, JJ; Pei, LJ; Jin, F; Liao, Q; Xie, LM; Wei, LC; Hao, CJ; Zhang, YW; Li, W. (2022). Maternal perfluorinated compound exposure and risk of early pregnancy loss: A nested case-control study [Letter]. Biomed Environ Sci 35: 174-179. http://dx.doi.org/10.3967/bes2022.026.

Miller, BH; Takahashi, JS. (2014). Central circadian control of female reproductive function [Review]. Front Endocrinol (Lausanne) 4: 195. http://dx.doi.org/10.3389/fendo.2013.00195.

Mira-Escolano, MP; Mendiola, J; Mínguez-Alarcón, L; Melgarejo, M; Cutillas-Tolín, A; Roca, M; López-Espín, JJ; Noguera-Velasco, JA; Torres-Cantero, AM. (2014). Longer anogenital distance is associated with higher testosterone levels in women: a cross-sectional study. BJOG 121: 1359-1364. http://dx.doi.org/10.1111/1471-0528.12627.

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. Environ Int 115: 295-300. http://dx.doi.org/10.1016/j.envint.2018.03.033.

Mora, AM; Fleisch, AF; Rifas-Shiman, SL; Woo Baidal, JA; Pardo, L; Webster, TF; Calafat, AM; Ye, X; Oken, E; Sagiv, SK. (2018). Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine aminotransferase levels. Environ Int 111: 1-13. http://dx.doi.org/10.1016/j.envint.2017.11.008.

Morgan, RL; Thayer, KA; Bero, L; Bruce, N; Falck-Ytter, Y; Ghersi, D; Guyatt, G; Hooijmans, C; Langendam, M; Mandrioli, D; Mustafa, RA; Rehfuess, EA; Rooney, AA; Shea, B; Silbergeld, EK; Sutton, P; Wolfe, MS; Woodruff, TJ; Verbeek, JH; Holloway, AC; Santesso, N; Schünemann, HJ. (2016). GRADE: Assessing the quality of evidence in environmental and occupational health. Environ Int 92-93: 611-616. http://dx.doi.org/10.1016/j.envint.2016.01.004.

Myhr, BC; Lawlor, TE; Young, RR; Murli, H; Cifone, MA. (1990). Genotoxicity assessment of perfluorodecanoic acid using a battery of in vitro and in vivo/in vitro assays (pp. 80). Myhr, BC; Lawlor, TE; Young, RR; Murli, H; Cifone, MA.

Nakagawa, T; Ramdhan, DH; Tanaka, N; Naito, H; Tamada, H; Ito, Y; Li, Y; Hayashi, Y; Yamagishi, N; Yanagiba, Y; Aoyama, T; Gonzalez, FJ; Nakajima, T. (2012). Modulation of ammonium perfluorooctanoate-induced hepatic damage by genetically different PPARα in mice. Arch Toxicol 86: 63-74. http://dx.doi.org/10.1007/s00204-011-0704-3.

Natarajan, S; Lipsitz, S. R.; Nietert, PJ. (2002). Self-report of high cholesterol - Determinants of validity in US adults. Am J Prev Med 23: 13-21. http://dx.doi.org/10.1016/s0749-3797(02)00446-4.

Needham, LL; Grandjean, P; Heinzow, B; Jørgensen, PJ; Nielsen, F; Patterson, DG; Sjödin, A; Turner, WE; Weihe, P. (2011). Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45: 1121-1126. http://dx.doi.org/10.1021/es1019614.

Nelson, DL; Frazier, DE, Jr; Ericson, JE; Tarr, MJ; Mathes, LE. (1992). The effects of perfluorodecanoic acid (PFDA) on humoral, cellular, and innate immunity in Fischer 344 rats. Immunopharmacol Immunotoxicol 14: 925-938. http://dx.doi.org/10.3109/08923979209009242.

Nian, M; Li, QQ; Bloom, M; Qian, ZM; Syberg, KM; Vaughn, MG; Wang, SQ; Wei, Q; Zeeshan, M; Gurram, N; Chu, C; Wang, J; Tian, YP; Hu, LW; Liu, KK; Yang, BY; Liu, RQ; Feng, D; Zeng, XW; Dong, GH. (2019). Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. Environ Res 172: 81-88. http://dx.doi.org/10.1016/j.envres.2019.02.013.

Nigam, PK. (2011). Serum lipid profile: fasting or non-fasting? Indian J Clin Biochem 26: 96–97. http://dx.doi.org/10.1007/s12291-010-0095-x.

NIH (National Institutes of Health). (2020). The PhenX Toolkit: protocol - lipid profile. Available online at https://www.phenxtoolkit.org/protocols/view/40201 (accessed

Nilsson, H; Kärrman, A; Westberg, H; Rotander, A; van Bavel, B; Lindström, G. (2010). A time trend study of significantly elevated perfluorocarboxylate levels in humans after using fluorinated ski wax. Environ Sci Technol 44: 2150-2155. http://dx.doi.org/10.1021/es9034733.

Niu, J; Liang, H; Tian, Y; Yuan, W; Xiao, H; Hu, H; Sun, X; Song, X; Wen, S; Yang, L; Ren, Y; Miao, M. (2019). Prenatal plasma concentrations of Perfluoroalkyl and polyfluoroalkyl substances and neuropsychological development in children at four years of age. Environ Health 18: 53. http://dx.doi.org/10.1186/s12940-019-0493-3.

NLM (National Library of Medicine). (2013). HSDB: Perfluoro-n-nonanoic acid. Available online at https://pubchem.ncbi.nlm.nih.gov/source/hsdb/8040 (accessed

NLM (National Library of Medicine). (2017). HSDB: Perfluorohexanesulfonic acid. Available online at https://pubchem.ncbi.nlm.nih.gov/source/hsdb/8274 (accessed

NTP (National Toxicology Program). (2005). Study information: CEBS accession number 002-02652-0001-0000-8: Perfluorodecanoic acid. Available online at https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02652-0001-0000-8 (accessed August 25, 2017).

NTP (National Toxicology Program). (2012). Study information: CEBS accession number 002-02652-0003-0000-0: Perfluorodecanoic acid. Available online at https://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-02652-0003-0000-0 (accessed August 25, 2017).

NTP (National Toxicology Program). (2016). NTP Monograph: Immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). Research Triangle Park, NC: U.S. Department of Health and Human Services, Office of Health

Assessment and Translation. https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf.

NTP (National Toxicology Program). (2018). 28-day evaluation of the toxicity (C20615) of perfluorodecanoic acid (PFDA) (335-76-2) on Harlan Sprague-Dawley rats exposed via gavage [NTP]. U.S. Department of Health and Human Services. http://dx.doi.org/10.22427/NTP-DATA-002-02652-0004-0000-1.

NTP (National Toxicology Program). (2019). NTP technical report on the toxicity studies of perfluoroalkyl carboxylates (perfluorohexanoic acid, perfluorooctanoic acid, perfluorononanoic acid, and perfluorodecanoic acid) administered by gavage to Sprague Dawley (Hsd:Sprague Dawley SD) rats (revised). (Toxicity Report 97). Research Triangle Park, NC. http://dx.doi.org/10.22427/NTP-TOX-97.

O'Shaughnessy, KL; Thomas, SE; Spring, S. R.; Ford, JL; Ford, RL; Gilbert, ME. (2019). A transient window of hypothyroidism alters neural progenitor cells and results in abnormal brain development. Sci Rep 9: 4662. http://dx.doi.org/10.1038/s41598-019-40249-7.

OECD (Organisation for Economic Co-operation and Development). (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). Paris, France. http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/.

Ohmori, K; Kudo, N; Katayama, K; Kawashima, Y. (2003). Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184: 135-140. http://dx.doi.org/10.1016/S0300-483X(02)00573-5.

Okada, E; Sasaki, S; Kashino, I; Matsuura, H; Miyashita, C; Kobayashi, S; Itoh, K; Ikeno, T; Tamakoshi, A; Kishi, R. (2014). Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int 65: 127-134. http://dx.doi.org/10.1016/j.envint.2014.01.007.

Olsen, GW; Burris, JM; Ehresman, DJ; Froehlich, JW; Seacat, AM; Butenhoff, JL; Zobel, LR. (2007). Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115: 1298-1305. http://dx.doi.org/10.1289/ehp.10009.

Olsen, GW; Mair, DC; Lange, CC; Harrington, LM; Church, TR; Goldberg, CL; Herron, RM; Hanna, H; Nobiletti, JB; Rios, JA; Reagen, WK; Ley, CA. (2017). Per- and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000-2015. Environ Res 157: 87-95. http://dx.doi.org/10.1016/j.envres.2017.05.013.

Olson, CT; Andersen, ME. (1983). The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. Toxicol Appl Pharmacol 70: 362-372. http://dx.doi.org/10.1016/0041-008x(83)90154-0.

Omoike, OE; Pack, RP; Mamudu, HM; Liu, Y; Strasser, S; Zheng, S; Okoro, J; Wang, L. (2020). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environ Res 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. Environ Res 196: 110329. http://dx.doi.org/10.1016/j.envres.2020.110329.

Osmond, C; Barker, DJ. (2000). Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women [Review]. Environ Health Perspect 108: 545-553. http://dx.doi.org/10.2307/3454545.

Ou, Y; Zeng, X; Lin, S; Bloom, MS; Han, F; Xiao, X; Wang, H; Matala, R; Li, X; Qu, Y; Nie, Z; Dong, G; Liu, X. (2021). Gestational exposure to perfluoroalkyl substances and congenital heart defects: A

nested case-control pilot study. Environ Int 154: 106567. http://dx.doi.org/10.1016/j.envint.2021.106567.

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]. Environ Int 97: 237-245. http://dx.doi.org/10.1016/j.envint.2016.09.015.

Padula, AM; Ning, X; Bakre, S; Barrett, ES; Bastain, T; Bennett, DH; Bloom, MS; Breton, CV; Dunlop, AL; Eick, SM; Ferrara, A; Fleisch, A; Geiger, S; Goin, DE; Kannan, K; Karagas, MR; Korrick, S; Meeker, JD; Morello-Frosch, R. (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]. Environ Health Perspect 131: (037006) 037001-037011. http://dx.doi.org/10.1289/EHP10723.

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. Environ Health Perspect 127: 127005. http://dx.doi.org/10.1289/EHP4431.

Pan, Y; Zhu, Y; Zheng, T; Cui, Q; Buka, SL; Zhang, B; Guo, Y; Xia, W; Yeung, LW; Li, Y; Zhou, A; Qiu, L; Liu, H; Jiang, M; Wu, C; Xu, S; Dai, J. (2017). Novel Chlorinated Polyfluorinated Ether Sulfonates and Legacy Per-/Polyfluoroalkyl Substances: Placental Transfer and Relationship with Serum Albumin and Glomerular Filtration Rate. Environ Sci Technol 51: 634-644. http://dx.doi.org/10.1021/acs.est.6b04590.

Park, JH; Choi, J; Jun, DW; Han, SW; Yeo, YH; Nguyen, MH. (2019). Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study. J Clin Med 8. http://dx.doi.org/10.3390/jcm8091445.

Pelch, KE; Reade, A; Kwiatkowski, CF; Merced-Nieves, FM; Cavalier, H; Schultz, K; Wolffe, T; Varshavsky, J. (2022). The PFAS-Tox Database: A systematic evidence map of health studies on 29 per- and polyfluoroalkyl substances. Environ Int 167: 107408. http://dx.doi.org/10.1016/j.envint.2022.107408.

Pérez, F; Llorca, M; Köck-Schulmeyer, M; Škrbić, B; Oliveira, LS; da Boit Martinello, K; Al-Dhabi, NA; Antić, I; Farré, M; Barceló, D. (2014). Assessment of perfluoroalkyl substances in food items at global scale. Environ Res 135: 181-189. http://dx.doi.org/10.1016/j.envres.2014.08.004.

Pérez, F; Nadal, M; Navarro-Ortega, A; Fàbrega, F; Domingo, JL; Barceló, D; Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. Environ Int 59: 354-362. http://dx.doi.org/10.1016/j.envint.2013.06.004.

Permadi, H; Lundgren, B; Andersson, K; Depierre, JW. (1992). Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes, enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. Biochem Pharmacol 44: 1183-1191. http://dx.doi.org/10.1016/0006-2952(92)90383-T.

Permadi, H; Lundgren, B; Andersson, K; Sundberg, C; Depierre, JW. (1993). Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica 23: 761-770. http://dx.doi.org/10.3109/00498259309166782.

Perng, W, ei; Rifas-Shiman, SL; Kramer, MS; Haugaard, LK; Oken, E; Gillman, MW; Belfort, MB. (2016). Early Weight Gain, Linear Growth, and Mid-Childhood Blood Pressure A Prospective Study in Project Viva. Hypertension 67: 301-308. http://dx.doi.org/10.1161/HYPERTENSIONAHA.115.06635.

Petersen, KU; Hærvig, KK; Flachs, EM; Bonde, JP; Lindh, C; Hougaard, KS; Toft, G; Ramlau-Hansen, CH; Tøttenborg, SS. (2022). Per- and polyfluoroalkyl substances (PFAS) and male reproductive function in young adulthood; a cross-sectional study. Environ Res 212: 113157. http://dx.doi.org/10.1016/j.envres.2022.113157.

Pirard, C; Dufour, P; Charlier, C. (2020). Background contamination of perfluoralkyl substances in a Belgian general population. Toxicol Lett 333: 13-21. http://dx.doi.org/10.1016/j.toxlet.2020.07.015.

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. Environ Sci Technol 51: 13388-13396. http://dx.doi.org/10.1021/acs.est.7b03299.

Powers, RH; Aust, SD. (1986). The effects of nonadecafluoro-n-decanoic acid on serum retinol and hepatic retinyl palmitate hydrolase activity in male Sprague-Dawley rats. J Biochem Toxicol 1: 27-42. http://dx.doi.org/10.1002/jbt.2570010204.

Qin, XD; Qian, Z; Vaughn, MG; Huang, J; Ward, P; Zeng, XW; Zhou, Y; Zhu, Y; Yuan, P; Li, M; Bai, Z; Paul, G; Hao, YT; Chen, W; Chen, PC; Dong, GH; Lee, YL. (2016). Positive associations of serum perfluoroalkyl substances with uric acid and hyperuricemia in children from Taiwan. Environ Pollut 212: 519-524. http://dx.doi.org/10.1016/j.envpol.2016.02.050.

Rahman, ML; Zhang, C; Smarr, MM; Lee, S; Honda, M; Kannan, K; Tekola-Ayele, F; Buck Louis, GM. (2019). Persistent organic pollutants and gestational diabetes: A multi-center prospective cohort study of healthy US women. Environ Int 124: 249-258. http://dx.doi.org/10.1016/j.envint.2019.01.027.

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. Environ Health 14: 79. http://dx.doi.org/10.1186/s12940-015-0066-z.

Reardon, AJF; Khodayari Moez, E; Dinu, I; Goruk, S; Field, CJ; Kinniburgh, DW; Macdonald, AM; Martin, JW; Study, A. (2019). Longitudinal analysis reveals early-pregnancy associations between perfluoroalkyl sulfonates and thyroid hormone status in a Canadian prospective birth cohort. Environ Int 129: 389-399. http://dx.doi.org/10.1016/j.envint.2019.04.023.

Ren, XM; Qin, WP; Cao, LY; Zhang, J; Yang, Y; Wan, B; Guo, LH. (2016). Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications. Toxicology 366-367: 32-42. http://dx.doi.org/10.1016/j.tox.2016.08.011.

Ren, XM; Zhang, YF; Guo, LH; Qin, ZF; Lv, QY; Zhang, LY. (2015). Structure-activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. Arch Toxicol 89: 233-242. http://dx.doi.org/10.1007/s00204-014-1258-y.

Ren, Y; Jin, L; Yang, F; Liang, H; Zhang, Z; Du, J; Song, X; Miao, M; Yuan, W. (2020). Concentrations of perfluoroalkyl and polyfluoroalkyl substances and blood glucose in pregnant women. Environ Health 19: 88. http://dx.doi.org/10.1186/s12940-020-00640-8.

Ribeiro-Filho, J; Piuvezam, MR; Leite, FC; Costa, HF; Calheiros, AS; Torres, RC; de Azevedo, CT; Martins, MA; Dias, C, daS; Bozza, PT. (2014). Curine inhibits mast cell-dependent responses in mice. J Ethnopharmacol1118-1124. http://dx.doi.org/10.1016/j.jep.2014.06.041.

Robledo, CA; Yeung, E; Mendola, P; Sundaram, R; Maisog, J; Sweeney, AM; Barr, DB; Louis, GM. (2015). Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environ Health Perspect 123: 88-94. http://dx.doi.org/10.1289/ehp.1308016.

Rogers, AM; Andersen, ME; Back, KC. (1982). Mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and perfluoro-n-decanoic acid in L5178Y mouse-lymphoma cells. Mutat Res 105: 445-449. http://dx.doi.org/10.1016/0165-7992(82)90192-0.

Rosen, EM; Brantsæter, AL; Carroll, R; Haug, L; Singer, AB; Zhao, S; Ferguson, KK. (2018). Maternal Plasma Concentrations of Per- and polyfluoroalkyl Substances and Breastfeeding Duration in the Norwegian Mother and Child Cohort. Environmental Epidemiology 2: e027. http://dx.doi.org/10.1097/EE9.0000000000000027.

Rosen, MB; Das, KP; Rooney, J; Abbott, B; Lau, C; Corton, JC. (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387: 95-107. http://dx.doi.org/10.1016/j.tox.2017.05.013.

Rosen, MB; Das, KP; Wood, CR; Wolf, CJ; Abbott, BD; Lau, C. (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology 308: 129-137. http://dx.doi.org/10.1016/j.tox.2013.03.011.

Rosen, MB; Lee, JS; Ren, H; Vallanat, B; Liu, J; Waalkes, MP; Abbott, BD; Lau, C; Corton, JC. (2008). Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. Toxicol Sci 103: 46-56. http://dx.doi.org/10.1093/toxsci/kfn025.

Rosen, MB; Schmid, JR; Corton, JC; Zehr, RD; Das, KP; Abbott, BD; Lau, C. (2010). Gene expression profiling in wild-type and PPAR alpha-null mice exposed to perfluorooctane sulfonate reveals PPAR alpha-independent effects. PPAR Research 2010: 794739. http://dx.doi.org/10.1155/2010/794739.

Rosenmai, AK; Ahrens, L; le Godec, T; Lundqvist, J; Oskarsson, A. (2018). Relationship between peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl substances in HepG2 cells. J Appl Toxicol 38: 219-226. http://dx.doi.org/10.1002/jat.3515.

Routti, H; Berg, MK; Lille-Langøy, R; Øygarden, L; Harju, M; Dietz, R; Sonne, C; Goksøyr, A. (2019). Environmental contaminants modulate the transcriptional activity of polar bear (Ursus maritimus) and human peroxisome proliferator-activated receptor alpha (PPARA). Sci Rep 9: 6918. http://dx.doi.org/10.1038/s41598-019-43337-w.

Sagiv, SK; Rifas-Shiman, SL; Fleisch, AF; Webster, TF; Calafat, AM; Ye, X; Gillman, MW; Oken, E. (2018). Early Pregnancy Perfluoroalkyl Substance Plasma Concentrations and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? Am J Epidemiol 187: 793-802. http://dx.doi.org/10.1093/aje/kwx332.

Salazar-Martinez, E; Romano-Riquer, P; Yanez-Marquez, E; Longnecker, MP; Hernandez-Avila, M. (2004). Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. Environ Health 3: 8-13. http://dx.doi.org/10.1186/1476-069x-3-8.

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. Environ Int 117: 196-203. http://dx.doi.org/10.1016/j.envint.2018.04.052.

Sathyanarayana, S; Beard, L; Zhou, C; Grady, R. (2010). Measurement and correlates of ano-genital distance in healthy, newborn infants. Int J Androl 33: 317-323. http://dx.doi.org/10.1111/j.1365-2605.2009.01044.x.

Schecter, A; Malik-Bass, N; Calafat, AM; Kato, K; Colacino, JA; Gent, TL; Hynan, LS; Harris, TR; Malla, S; Birnbaum, L. (2012). Polyfluoroalkyl compounds in Texas children from birth through 12 years of age. Environ Health Perspect 120: 590-594. http://dx.doi.org/10.1289/ehp.1104325.

Schünemann, H; Hill, S; Guyatt, G; Akl, EA; Ahmed, F. (2011). The GRADE approach and Bradford Hill's criteria for causation. J Epidemiol Community Health 65: 392-395. http://dx.doi.org/10.1136/jech.2010.119933.

Scinicariello, F; Buser, MC; Balluz, L; Gehle, K; Murray, HE; Abadin, HG; Attanasio, R. (2020). Perfluoroalkyl acids, hyperuricemia and gout in adults: Analyses of NHANES 2009-2014. Chemosphere 259: 127446. http://dx.doi.org/10.1016/j.chemosphere.2020.127446.

Scott, HM; Mason, JI; Sharpe, RM. (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds [Review]. Endocr Rev 30: 883-925. http://dx.doi.org/10.1210/er.2009-0016.

Sellers, RS; Mortan, D; Michael, B; Roome, N; Johnson, JK; Yano, BL; Perry, R; Schafer, K. (2007). Society of toxicologic pathology position paper: Organ weight recommendations for

toxicology studies [Review]. Toxicol Pathol 35: 751-755. http://dx.doi.org/10.1080/01926230701595300.

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. Environ Int 113: 149-161. http://dx.doi.org/10.1016/j.envint.2018.01.025.

Shah-Kulkarni, S; Kim, BM; Hong, YC; Kim, HS; Kwon, EJ; Park, H; Kim, YJ; Ha, EH. (2016). Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environ Int 94: 607-613. http://dx.doi.org/10.1016/j.envint.2016.06.024.

Sharpe, RM. (2010). Environmental/lifestyle effects on spermatogenesis [Review]. Philos Trans R Soc Lond B Biol Sci 365: 1697-1712. http://dx.doi.org/10.1098/rstb.2009.0206.

Shi, Y; Yang, L; Li, J; Lai, J; Wang, Y; Zhao, Y; Wu, Y. (2017). Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 169: 396-402. http://dx.doi.org/10.1016/j.chemosphere.2016.11.050.

Shih, YH; Blomberg, AJ; Bind, MA; Holm, D; Nielsen, F; Heilmann, C; Weihe, P; Grandjean, P. (2021). Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. J Immunotoxicol 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. Environ Res 186: 109514. http://dx.doi.org/10.1016/j.envres.2020.109514.

Shinwell, ES; Shlomo, M. (2003). Measured length of normal term infants changes over the first two days of life. J Pediatr Endocrinol Metab 16: 537-540. http://dx.doi.org/10.1515/jpem.2003.16.4.537.

Sigman, M; Lipshultz, LI; Howards, SS. (1997). Evaluation of the subfertile male. In LI Lipschultz; SS Howards (Eds.), Infertility in the male (3rd ed., pp. 173). St. Louis, MO: Mosby.

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). Environ Res 166: 78-85. http://dx.doi.org/10.1016/j.envres.2018.05.019.

Skogheim, TS; Villanger, GD; Weyde, KVF; Engel, SM; Surén, P; Øie, MG; Skogan, AH; Biele, G; Zeiner, P; Øvergaard, KR; Haug, LS; Sabaredzovic, A; Aase, H. (2020). Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health 223: 80-92. http://dx.doi.org/10.1016/j.ijheh.2019.10.003.

Skogheim, TS; Weyde, K, jell Vegard F; Aase, H; Engel, SM; Suren, P; Oie, MG; Biele, G; Reichborn-Kjennerud, T; Brantsaeter, AL; Haug, LS; Sabaredzovic, A; Auyeung, B; Villanger, GD. (2021). Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children. Environ Res 202: 111692. http://dx.doi.org/10.1016/j.envres.2021.111692.

Smit, LAM; Lenters, V; Høyer, BB; Lindh, CH; Pedersen, HS; Liermontova, I; Jönsson, BAG; Piersma, AH; Bonde, JP; Toft, G; Vermeulen, R; Heederik, D. (2015). Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 70: 653-660. http://dx.doi.org/10.1111/all.12605.

Soukup, T; Zacharova, G; Smerdu, V; Jirmanova, I. (2001). Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status. Physiol Res 50: 619-626.

Stahl, LL; Snyder, BD; Olsen, AR; Kincaid, TM; Wathen, JB; Mccarty, HB. (2014). Perfluorinated compounds in fish from U.S. urban rivers and the Great Lakes. Sci Total Environ 499: 185-195. http://dx.doi.org/10.1016/j.scitotenv.2014.07.126.

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. Environ Int 131: 104983. http://dx.doi.org/10.1016/j.envint.2019.104983.

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. Environ Health Perspect 125: 067016. http://dx.doi.org/10.1289/EHP641.

Starling, AP; Engel, SM; Richardson, DB; Baird, DD; Haug, LS; Stuebe, AM; Klungsoyr, K; Harmon, Q; Becher, G; Thomsen, C; Sabaredzovic, A; Eggesbo, M; Hoppin, JA; Travlos, GS; Wilson, RE; Trogstad, LI; Magnus, P, er; Longnecker, MP. (2014a). Perfluoroalkyl Substances During Pregnancy and Validated Preeclampsia Among Nulliparous Women in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 179: 824-833. http://dx.doi.org/10.1093/aje/kwt432.

Starling, AP; Engel, SM; Whitworth, KW; Richardson, DB; Stuebe, AM; Daniels, JL; Haug, LS; Eggesbø, M; Becher, G; Sabaredzovic, A; Thomsen, C; Wilson, RE; Travlos, GS; Hoppin, JA; Baird, DD; Longnecker, MP. (2014b). Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. Environ Int 62: 104-112. http://dx.doi.org/10.1016/j.envint.2013.10.004.

Steenland, K; Barry, V; Savitz, D. (2018). Serum perfluorooctanoic acid and birthweight: an updated meta-analysis with bias analysis. Epidemiology 29: 765-776. http://dx.doi.org/10.1097/EDE.0000000000000903.

Sterchele, PF; Vanden Heuvel, JP; Davis, JW; Shrago, E; Knudsen, J; Peterson, RE. (1994). Induction of hepatic acyl-CoA-binding protein and liver fatty acid-binding protein by perfluorodecanoic acid in rats. Lack of correlation with hepatic long-chain acyl-CoA levels. Biochem Pharmacol 48: 955-966. http://dx.doi.org/10.1016/0006-2952(94)90366-2.

Strynar, MJ; Lindstrom, AB. (2008). Perfluorinated compounds in house dust from Ohio and North Carolina, USA. Environ Sci Technol 42: 3751-3756. http://dx.doi.org/10.1021/es7032058.

Stubleski, J; Salihovic, S; Lind, L; Lind, PM; van Bavel, B; Kärrman, A. (2016). Changes in serum levels of perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort. Environ Int 95: 86-92. http://dx.doi.org/10.1016/j.envint.2016.08.002.

Sun, Q; Zong, G; Valvi, D; Nielsen, F; Coull, B; Grandjean, P. (2018). Plasma concentrations of perfluoroalkyl substances and risk of Type 2 diabetes: A prospective investigation among U.S. women. Environ Health Perspect 126: 037001. http://dx.doi.org/10.1289/EHP2619.

Sunderland, EM; Hu, XC; Dassuncao, C; Tokranov, AK; Wagner, CC; Allen, JG. (2019). A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects [Review]. J Expo Sci Environ Epidemiol 29: 131-147. http://dx.doi.org/10.1038/s41370-018-0094-1.

Swedish Environmental Protection Agency. (2017). [Rapport till Naturvårdsverkets hälsorelaterade miljöövervakning: Utvärdering av samband mellan mammors POP-belastning under graviditets- och amningsperioden och deras barns hälsa]. (2215-17-008).

Sweeney, MF; Hasan, N; Soto, AM; Sonnenschein, C. (2015). Environmental endocrine disruptors: Effects on the human male reproductive system [Review]. Rev Endocr Metab Disord 16: 341-357. http://dx.doi.org/10.1007/s11154-016-9337-4.

Takagi, A; Sai, K; Umemura, T; Hasegawa, R; Kurokawa, Y. (1991). Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. Cancer Lett 57: 55-60. http://dx.doi.org/10.1016/0304-3835(91)90063-N.

Takagi, A; Sai, K; Umemura, T; Hasegawa, R; Kurokawa, Y. (1992). Hepatomegaly is an early biomarker for hepatocarcinogenesis induced by peroxisome proliferators. J Environ Pathol Toxicol Oncol 11: 145-149.

Thankamony, A; Pasterski, V; Ong, KK; Acerini, CL; Hughes, IA. (2016). Anogenital distance as a marker of androgen exposure in humans. Andrology 0: 1-10. http://dx.doi.org/10.1111/andr.12156.

Thomsen, ML; Henriksen, LS; Tinggaard, J; Nielsen, F; Jensen, TK; Main, KM. (2021). Associations between exposure to perfluoroalkyl substances and body fat evaluated by DXA and MRI in 109 adolescent boys. Environ Health 20: 73. http://dx.doi.org/10.1186/s12940-021-00758-3.

Tian, Y; Liang, H; Miao, M; Yang, F; Ji, H; Cao, W; Liu, X; Zhang, X; Chen, A; Xiao, H; Hu, H; Yuan, W. (2019). Maternal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances during pregnancy and anogenital distance in male infants. Hum Reprod 34: 1356-1368. http://dx.doi.org/10.1093/humrep/dez058.

Tian, Y; Miao, M; Ji, H; Zhang, X; Chen, A; Wang, Z; Yuan, W; Liang, H. (2021). Prenatal exposure to perfluoroalkyl substances and cord plasma lipid concentrations. Environ Pollut 268: 115426. http://dx.doi.org/10.1016/j.envpol.2020.115426.

Timmermann, CA; Budtz-Jørgensen, E; Jensen, TK; Osuna, CE; Petersen, MS; Steuerwald, U; Nielsen, F; Poulsen, LK; Weihe, P; Grandjean, P. (2017a). Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol 14: 39-49. http://dx.doi.org/10.1080/1547691X.2016.1254306.

Timmermann, CA; Budtz-Jørgensen, E; Petersen, MS; Weihe, P; Steuerwald, U; Nielsen, F; Jensen, TK; Grandjean, P. (2017b). Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reprod Toxicol 68: 164-170. http://dx.doi.org/10.1016/j.reprotox.2016.07.010.

Timmermann, CAG; Andersen, MS; Budtz-Jørgensen, E; Boye, H; Nielsen, F; Jensen, RC; Bruun, S; Husby, S; Grandjean, P; Jensen, TK. (2022). Pregnancy exposure to perfluoroalkyl substances and associations with prolactin concentrations and breastfeeding in the Odense Child Cohort. J Clin Endocrinol Metab 107: e631-e642. http://dx.doi.org/10.1210/clinem/dgab638.

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. Environ Res 203: 111712. http://dx.doi.org/10.1016/j.envres.2021.111712.

Toor, JM; Sikka, SC. (2017). Developmental and reproductive disorders: Role of endocrine disruptors in testicular toxicity. In RC Gupta (Ed.), Reproductive and developmental toxicology (2nd ed., pp. 1111-1121). San Diego, CA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-804239-7.00059-7.

Tropp, J; Markus, EJ. (2001). Effects of mild food deprivation on the estrous cycle of rats. Physiol Behav 73: 553-559. http://dx.doi.org/10.1016/s0031-9384(01)00487-5.

Truong, L; Rericha, Y; Thunga, P; Marvel, S; Wallis, D; Simonich, MT; Field, JA; Cao, D; Reif, DM; Tanguay, RL. (2022). Systematic developmental toxicity assessment of a structurally diverse library of PFAS in zebrafish. J Hazard Mater 431: 128615. http://dx.doi.org/10.1016/j.jhazmat.2022.128615.

Tsai, MS; Chang, SH; Kuo, WH; Kuo, CH; Li, SY; Wang, MY; Chang, DY; Lu, YS; Huang, CS; Cheng, AL; Lin, CH; Chen, PC. (2020). A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environ Int 142: 105850. http://dx.doi.org/10.1016/j.envint.2020.105850.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment. Fed Reg 56: 63798-63826.

U.S. EPA (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report].

(EPA600890066F). Research Triangle Park, NC. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=71993&CFID=51174829&CFTOKEN=25006317.

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Guidelines for reproductive toxicity risk assessment [EPA Report]. (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30004YQB.txt.

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Guidelines for reproductive toxicity risk assessment. Fed Reg 61: 56274-56322.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Health effects test guidelines OPPTS 870.7800 immunotoxicity. (EPA 712-C-98-351). Washington, DC: Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm.

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes [EPA Report]. (EPA630P02002F). Washington, DC. https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2003). Toxicological review of methyl ethyl ketone (CAS No. 78-93-3): In support of summary information on the Integrated Risk Information System (IRIS). (EPA 635/R-03/009). Washington, DC. http://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0071tr.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2004). Toxicological review of boron and compounds. In support of summary information on the Integrated Risk Information System (IRIS) [EPA Report]. (EPA/635/04/052). Washington, DC: U.S. Environmental Protection Agency, IRIS. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P1006CK9.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2005). 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 (U.S. Environmental Protection Agency). (2011). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA100R110001). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2012a). Benchmark dose technical guidance [EPA Report]. (EPA100R12001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance.

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Toxicological review of tetrahydrofuran. In support of summary information on the integrated risk information system (IRIS) (pp. 1-207). (EPA/635/R-11/006F). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (2014). Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation [EPA Report]. (EPA/100/R-14/002F). Washington, DC: Risk Assessment Forum, Office of the Science Advisor. https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2016a). 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 (U.S. Environmental Protection Agency). (2016b). 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 (U.S. Environmental Protection Agency). (2016c). The Third Unregulated Contaminant Monitoring Rule. Available online at https://www.epa.gov/dwucmr/third-unregulated-contaminant-monitoring-rule (accessed

U.S. EPA (U.S. Environmental Protection Agency). (2018). Human health toxicity values for perfluorobutane sulfonic acid (CASRN 375-73-5) and related compound potassium perfluorobutane sulfonate (CASRN 29420-49-3) (public comment draft). (EPA-823-R-18-307). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (2019a). ChemView [Database]. Retrieved from https://chemview.epa.gov/chemview

U.S. EPA (U.S. Environmental Protection Agency). (2019b). CompTox Chemicals Dashboard [Database]. Research Triangle Park, NC. Retrieved from https://comptox.epa.gov/dashboard

U.S. EPA (U.S. Environmental Protection Agency). (2019c). Development of the Proposed Unregulated Contaminant Monitoring Rule for the Fifth Monitoring Cycle (UCMR 5): Public Meeting and Webinar Held July 16, 2019. (815A19001). https://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P100XWUH.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2021a). CompTox chemicals dashboard. Washington, DC. Retrieved from https://comptox.epa.gov/dashboard

U.S. EPA (U.S. Environmental Protection Agency). (2021b). Human health toxicity values for perfluorobutane sulfonic acid (CASRN 375-73-5) and related compound potassium perfluorobutane sulfonate (CASRN 29420-49-3) [EPA Report]. (EPA/600/R-20/345F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=350888.

U.S. EPA (U.S. Environmental Protection Agency). (2022a). CompTox Chemicals Dashboard [Bioactivity] [Database]. Research Triangle Park, NC. Retrieved from https://comptox.epa.gov/dashboard

U.S. EPA (U.S. Environmental Protection Agency). (2022b). Review of EPA's analyses to support EPA's national primary drinking water rulemaking for PFAS. (EPA-SAB-22-008). https://sab.epa.gov/ords/sab/f?p=100:18:16490947993:::18:P18_ID:2601.

U.S. EPA (U.S. Environmental Protection Agency). (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 (U.S. Environmental Protection Agency). (2024a). Final Human health toxicity assessment for perfluorooctane sulfonic acid (PFOS) and related salts [EPA Report]. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division.

U.S. EPA (U.S. Environmental Protection Agency). (2024b). Final human health toxicity assessment for perfluorooctanoic acid (PFOA) and related salts [EPA Report]. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division.

U.S. EPA (U.S. Environmental Protection Agency). (2024c). Framework for estimating noncancer health risks associated with mixtures of per- and polyfluoroalkyl substances (PFAS). (EPA-815-R-24-003). Washington, DC. www.epa.gov/system/files/documents/2024-04/final-pfas-mix-framework-3.25.24_final-508.pdf.

Ulhaq, M; Carlsson, G; Örn, S; Norrgren, L. (2013). Comparison of developmental toxicity of seven perfluoroalkyl acids to zebrafish embryos. Environ Toxicol Pharmacol 36: 423-426. http://dx.doi.org/10.1016/j.etap.2013.05.004.

Unkila, M; Pohjanvirta, R; Tuomisto, J. (1992). Acute toxicity of perfluorodecanoic acid and cobalt protoporphyrin in a TCDD-sensitive and a TCDD-resistant rat strain. Chemosphere 25: 1233-1238. http://dx.doi.org/10.1016/0045-6535(92)90135-E.

Valenti, L, . Pelusi, S.,. Bianco, C.,. Ceriotti, F.,. Berzuini, A.,. Iogna Prat, L.,. Trotti, R.,. Malvestiti, F.,. D'Ambrosio, R.,. Lampertico, P.,. Colli, A.,. Colombo, M.,. Tsochatzis, E.,. A. Fraquelli, M.,. Prati, D.,. (2021). Definition of Healthy Ranges for Alanine Aminotransferase Levels: A 2021 Update. Hematology 5: 1824-1832. http://dx.doi.org/10.1002/hep4.1794.

Valvi, D; Højlund, K; Coull, BA; Nielsen, F; Weihe, P; Grandjean, P. (2021). Life-course Exposure to Perfluoroalkyl Substances in Relation to Markers of Glucose Homeostasis in Early Adulthood. J Clin Endocrinol Metab 106: 2495-2504. http://dx.doi.org/10.1210/clinem/dgab267.

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. Environ Int 107: 205-215. http://dx.doi.org/10.1016/j.envint.2017.07.016.

Van Anders, SM; Watson, NV. (2006). Menstrual cycle irregularities are associated with testosterone levels in healthy premenopausal women. Am J Hum Biol 18: 841-844. http://dx.doi.org/10.1002/ajhb.20555.

van Kerkhof, LWM; Van Dycke, KCG; Jansen, EHJ, M; Beekhof, PK; van Oostrom, CTM; Ruskovska, T; Velickova, N; Kamcev, N; Pennings, JLA; van Steeg, H; Rodenburg, W. (2015). Diurnal variation of hormonal and lipid biomarkers in a molecular epidemiology-like setting. PLoS ONE 10: e0135652. http://dx.doi.org/10.1371/journal.pone.0135652.

Van Rafelghem, MJ; Andersen, ME. (1988). The effects of perfluorodecanoic acid on hepatic stearoyl-coenzyme A desaturase and mixed function oxidase activities in rats. Fundam Appl Toxicol 11: 503-510. http://dx.doi.org/10.1016/0272-0590(88)90114-5.

Van Rafelghem, MJ; Inhorn, SL; Peterson, RE. (1987a). Effects of perfluorodecanoic acid on thyroid status in rats. Toxicol Appl Pharmacol 87: 430-439. http://dx.doi.org/10.1016/0041-008X(87)90248-1.

Van Rafelghem, MJ; Mattie, DR; Bruner, RH; Andersen, ME. (1987b). Pathological and hepatic ultrastructural effects of a single dose of perfluoro-n-decanoic acid in the rat, hamster, mouse, and guinea pig. Fundam Appl Toxicol 9: 522-540. http://dx.doi.org/10.1016/0272-0590(87)90034-0.

Van Rafelghem, MJ; Noren, CW; Menahan, LA; Peterson, RE. (1988a). Interrelationships between energy and fat metabolism and hypophagia in rats treated with perfluorodecanoic acid. Toxicol Lett 40: 57-69. http://dx.doi.org/10.1016/0378-4274(88)90183-X.

Van Rafelghem, MJ; Vanden Heuvel, JP; Menahan, LA; Peterson, RE. (1988b). Perfluorodecanoic acid and lipid metabolism in the rat. Lipids 23: 671-678. http://dx.doi.org/10.1007/BF02535666.

Vanden Heuvel, JP; Kuslikis, BI; Peterson, RE. (1992). Covalent binding of perfluorinated fatty acids to proteins in the plasma, liver and testes of rats. Chem Biol Interact 82: 317-328. http://dx.doi.org/10.1016/0009-2797(92)90003-4.

Vanden Heuvel, JP; Kuslikis, BI; Van Rafelghem, MJ; Peterson, RE. (1991). Disposition of perfluorodecanoic acid in male and female rats. Toxicol Appl Pharmacol 107: 450-459. http://dx.doi.org/10.1016/0041-008X(91)90308-2.

Varshavsky, JR; Robinson, JF; Zhou, Y; Puckett, KA; Kwan, E; Buarpung, S; Aburajab, R; Gaw, SL; Sen, S; Gao, SM; Smith, SC; Park, JS; Zakharevich, I; Gerona, RR; Fisher, SJ; Woodruff, TJ. (2021). Organophosphate flame retardants, highly fluorinated chemicals, and biomarkers of placental development and disease during mid-gestation. Toxicol Sci 181: 215-228. http://dx.doi.org/10.1093/toxsci/kfab028.

Vasilenko, P; Adams, WC; Frieden, EH. (1981). Uterine size and glycogen-content in cycling and pregnant rats - Influence of relaxin. Biol Reprod 25: 162-169. http://dx.doi.org/10.1095/biolreprod25.1.162.

Velarde, MC; Chan, AFO; Sajo, MEJ, V; Zakharevich, I; Melamed, J; Uy, GLB; Teves, JMY; Corachea, AJM; Valparaiso, AP; Macalindong, SS; Cabaluna, ND; Dofitas, RB; Giudice, LC; Gerona, RR. (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.

Verner, MA; Longnecker, MP. (2015). Comment on "enhanced elimination of perfluorooctanesulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling" [Letter]. Environ Sci Technol 49: 5836-5837. http://dx.doi.org/10.1021/acs.est.5b00187.

Vestergaard, S; Nielsen, F; Andersson, AM; Hjøllund, NH; Grandjean, P; Andersen, HR; Jensen, TK. (2012). Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Hum Reprod 27: 873-880. http://dx.doi.org/10.1093/humrep/der450.

Vuong, A; Yolton, K; Webster, GM; Sjodin, A; Calafat, AM; Braun, JM; Dietrich, K; Lanphear, BP; Chen, A. (2016). Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environ Res 147: 556-564. http://dx.doi.org/10.1016/j.envres.2016.01.008.

Vuong, AM; Yolton, K; Wang, Z; Xie, C; Webster, GM; Ye, X; Calafat, AM; Braun, JM; Dietrich, KN; Lanphear, BP; Chen, A. (2018). Childhood perfluoroalkyl substance exposure and executive function in children at 8 years. Environ Int 119: 212-219. http://dx.doi.org/10.1016/j.envint.2018.06.028.

Wahlang, B; Beier, JI; Clair, HB; Bellis-Jones, HJ; Falkner, K; Mcclain, CJ; Cave, MC. (2013). Toxicant-associated steatohepatitis [Review]. Toxicol Pathol 41: 343-360. http://dx.doi.org/10.1177/0192623312468517.

Walaas, O. (1952). Effect of oestrogens on the glycogen content of the rat uterus. European Journal of Endocrinology 10: 175–192. http://dx.doi.org/10.1530/acta.0.0100175.

Wan, HT; Mruk, DD; Wong, CK; Cheng, CY. (2013). Targeting testis-specific proteins to inhibit spermatogenesis: lesson from endocrine disrupting chemicals [Review]. Expert Opin Ther Targets 17: 839-855. http://dx.doi.org/10.1517/14728222.2013.791679.

Wang, B; Fu, J; Gao, K; Liu, Q; Zhuang, L; Zhang, G; Long, M; Na, J; Ren, M; Wang, A; Liang, R; Shen, G; Li, Z; Lu, Q. (2021). Early pregnancy loss: Do Per- and polyfluoroalkyl substances matter? Environ Int 157: 106837. http://dx.doi.org/10.1016/j.envint.2021.106837.

Wang, B; Zhang, R; Jin, F; Lou, H; Mao, Y; Zhu, W; Zhou, W; Zhang, P; Zhang, J. (2017). Perfluoroalkyl substances and endometriosis-related infertility in Chinese women. Environ Int 102: 207-212. http://dx.doi.org/10.1016/j.envint.2017.03.003.

Wang, D; Gao, Q; Wang, T; Kan, Z; Li, X; Hu, L; Peng, CY; Qian, F; Wang, Y; Granato, D. (2020). Green tea polyphenols and epigallocatechin-3-gallate protect against perfluorodecanoic acid induced liver damage and inflammation in mice by inhibiting NLRP3 inflammasome activation. Food Res Int 127: 108628. http://dx.doi.org/10.1016/j.foodres.2019.108628.

Wang, J; Pan, Y; Cui, Q; Yao, B; Wang, J; Dai, J. (2018a). Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. Environ Sci Technol 52: 13553-13561. http://dx.doi.org/10.1021/acs.est.8b04550.

Wang, J; Zeng, XW; Bloom, MS; Qian, Z; Hinyard, LJ; Belue, R; Lin, S; Wang, SQ; Tian, YP; Yang, M; Chu, C; Gurram, N; Hu, LW; Liu, KK; Yang, BY; Feng, D; Liu, RQ; Dong, GH. (2019). Renal function and isomers of perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS): Isomers of C8 Health Project in China. Chemosphere 218: 1042-1049. http://dx.doi.org/10.1016/j.chemosphere.2018.11.191.

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. Environ Health Perspect 124: 1794-1800. http://dx.doi.org/10.1289/ehp.1509998.

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. Int J Hyg Environ Health 218: 639-644. http://dx.doi.org/10.1016/j.ijheh.2015.07.002.

Wang, Y; Rogan, WJ; Chen, PC; Lien, GW; Chen, HY; Tseng, YC; Longnecker, MP; Wang, SL. (2014a). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect 122: 529-534. http://dx.doi.org/10.1289/ehp.1306925.

Wang, Y; Starling, AP; Haug, LS; Eggesbo, M; Becher, G; Thomsen, C; Travlos, G; King, D; Hoppin, JA; Rogan, WJ; Longnecker, MP. (2013). Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environ Health 12: 76. http://dx.doi.org/10.1186/1476-069X-12-76.

Wang, Y; Zhang, L; Teng, Y; Zhang, J; Yang, L; Li, J; Lai, J; Zhao, Y; Wu, Y. (2018b). Association of serum levels of perfluoroalkyl substances with gestational diabetes mellitus and postpartum blood glucose. J Environ Sci 69: 5-11. http://dx.doi.org/10.1016/j.jes.2018.03.016.

Wang, Z; Cousins, IT; Scheringer, M; Buck, RC; Hungerbühler, K. (2014b). Global emission inventories for C4-C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources [Review]. Environ Int 70: 62-75. http://dx.doi.org/10.1016/j.envint.2014.04.013.

Wang, Z; Shi, R; Ding, G; Yao, Q; Pan, C; Gao, Y; Tian, Y. (2022). Association between maternal serum concentration of perfluoroalkyl substances (PFASs) at delivery and acute infectious diseases in infancy. Chemosphere 289: 133235. http://dx.doi.org/10.1016/j.chemosphere.2021.133235.

Washington, JW; Yoo, H; Ellington, JJ; Jenkins, TM; Libelo, EL. (2010). Concentrations, distribution, and persistence of perfluoroalkylates in sludge-applied soils near Decatur, Alabama, USA. Environ Sci Technol 44: 8390-8396. http://dx.doi.org/10.1021/es1003846.

Watkins, DJ; Josson, J; Elston, B; Bartell, SM; Shin, HM; Vieira, VM; Savitz, DA; Fletcher, T; Wellenius, GA. (2013). Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect 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. Toxicol Sci 113: 305-314. http://dx.doi.org/10.1093/toxsci/kfp275.

Weisskopf, MG; Seals, RM; Webster, TF. (2018). Bias amplification in epidemiologic analysis of exposure to mixtures. Environ Health Perspect 126. http://dx.doi.org/10.1289/EHP2450.

Westwood, FR. (2008). The female rat reproductive cycle: A practical histological guide to staging [Review]. Toxicol Pathol 36: 375-384. http://dx.doi.org/10.1177/0192623308315665.

Whalan, JE. (2015). A toxicologist's guide to clinical pathology in animals: Hematology, clinical chemistry, urinalysis. Switzerland: Springer International Publishing. http://dx.doi.org/10.1007/978-3-319-15853-2.

Whitworth, KW; Haug, LS; Baird, DD; Becher, G; Hoppin, JA; Skjaerven, R; Thomsen, C; Eggesbo, M; Travlos, G; Wilson, R; Longnecker, MP. (2012). Perfluorinated compounds and subfecundity in pregnant women. Epidemiology 23: 257-263. http://dx.doi.org/10.1097/EDE.0b013e31823b5031.

WHO (World Health Organization). (2010). WHO laboratory manual for the examination and processing of human semen. In WHO laboratory manual for the examination and processing of human semen (5th ed.). Geneva, Switzerland.

Wielsøe, M; Kern, P; Bonefeld-Jørgensen, EC. (2017). Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health 16: 56. http://dx.doi.org/10.1186/s12940-017-0269-6.

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. Sci Rep 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. Pediatr Res 87: 1093-1099. http://dx.doi.org/10.1038/s41390-019-0720-1.

Wilson, MW; Lay, LT; Chow, CK; Tai, HH; Robertson, LW; Glauert, HP. (1995). Altered hepatic eicosanoid concentrations in rats treated with the peroxisome proliferators ciprofibrate and perfluorodecanoic acid. Arch Toxicol 69: 491-497. http://dx.doi.org/10.1007/s002040050203.

Wilson, VS; Blystone, CR; Hotchkiss, AK; Rider, CV; Gray, LE, Jr. (2008). Diverse mechanisms of anti-androgen action: Impact on male rat reproductive tract development [Review]. Int J Androl 31: 178-187. http://dx.doi.org/10.1111/j.1365-2605.2007.00861.x.

Wise, LA; Wesselink, AK; Schildroth, S; Calafat, AM; Bethea, TN; Geller, RJ; Coleman, CM; Fruh, V; Claus Henn, B; Botelho, JC; Harmon, QE; Thirkill, M; Wegienka, GR; Baird, DD. (2022). Correlates of plasma concentrations of per- and poly-fluoroalkyl substances among reproductive-aged Black women. Environ Res 203: 111860. http://dx.doi.org/10.1016/j.envres.2021.111860.

Wolf, CJ; Schmid, JE; Lau, C; Abbott, BD. (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds. Reprod Toxicol 33: 546-551. http://dx.doi.org/10.1016/j.reprotox.2011.09.009.

Wolf, CJ; Takacs, ML; Schmid, JE; Lau, C; Abbott, BD. (2008). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 106: 162-171. http://dx.doi.org/10.1093/toxsci/kfn166.

Wong, F; Macleod, M; Mueller, JF; Cousins, IT. (2014). Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. Environ Sci Technol 48: 8807-8814. http://dx.doi.org/10.1021/es500796y.

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. Environ Health 16: 115. http://dx.doi.org/10.1186/s12940-017-0332-3.

Workman, CE; Becker, AB; Azad, MB; Moraes, TJ; Mandhane, PJ; Turvey, SE; Subbarao, P; Brook, JR; Sears, MR; Wong, CS. (2019). Associations between concentrations of perfluoroalkyl substances in human plasma and maternal, infant, and home characteristics in Winnipeg, Canada. Environ Pollut 249: 758-766. http://dx.doi.org/10.1016/j.envpol.2019.03.054.

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. J Clin Endocrinol Metab 105: 735-745. http://dx.doi.org/10.1210/clinem/dgz147.

Xie, X; Feng, LP; Weng, XQ; Liu, S; Chen, JM; Guo, XR; Gao, XY; Fei, QY; Hao, G; Jing, CX. (2021). Perfluoroalkyl and polyfluoroalkyl substance exposure and association with sex hormone concentrations: Results from the NHANES 2015-2016. Environ Sci Eur 33: 69. http://dx.doi.org/10.1186/s12302-021-00508-9.

Xu, C; Yin, S; Liu, Y; Chen, F; Zhong, Z; Li, F; Liu, K; Liu, W. (2019a). Prenatal exposure to chlorinated polyfluoroalkyl ether sulfonic acids and perfluoroalkyl acids: Potential role of maternal determinants and associations with birth outcomes. J Hazard Mater 380: 120867. http://dx.doi.org/10.1016/j.jhazmat.2019.120867.

Xu, M; Zhang, T; Lv, C; Niu, Q; Zong, W; Tang, J; Liu, R. (2019b). Perfluorodecanoic acid-induced oxidative stress and DNA damage investigated at the cellular and molecular levels. Ecotoxicol Environ Saf 185: 109699. http://dx.doi.org/10.1016/j.ecoenv.2019.109699.

Yamada, A; Bemrah, N; Veyrand, B; Pollono, C; Merlo, M; Desvignes, V; Sirot, V; Marchand, P; Berrebi, A; Cariou, R; Antignac, JP; Le Bizec, B; Leblanc, JC. (2014). Dietary exposure to perfluoroalkyl acids of specific French adult sub-populations: High seafood consumers, high freshwater fish consumers and pregnant women. Sci Total Environ 491-492: 170-175. http://dx.doi.org/10.1016/j.scitotenv.2014.01.089.

Yamamoto, A; Kawashima, Y. (1997). Perfluorodecanoic acid enhances the formation of oleic acid in rat liver. Biochem J 325 (Pt 2): 429-434. http://dx.doi.org/10.1042/bj3250429.

Yang, BY; Wu, J; Niu, XL; He, CJ; Bloom, MS; Abudoukade, M; Abulizi, M; Xu, AM; Li, BB; Li, L; Zhong, XM; Wu, QZ; Chu, C; Luo, YN; Liu, XX; Zeng, XW; Yu, YJ; Dong, GH; Zou, XG; Liu, T. (2022a). 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 14: 793-805. http://dx.doi.org/10.1007/s12403-021-00454-0.

Yang, J; Wang, H; Du, H; Fang, H; Han, M; Wang, Y; Xu, L; Liu, S; Yi, J; Chen, Y; Jiang, Q; He, G. (2022b). Exposure to perfluoroalkyl substances was associated with estrogen homeostasis in pregnant women. Sci Total Environ 805: 150360. http://dx.doi.org/10.1016/j.scitotenv.2021.150360.

Yang, J; Wang, H; Du, H; Fang, H; Han, M; Xu, L; Liu, S; Yi, J; Chen, Y; Jiang, Q; He, G. (2020). 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; Li, J; Lai, J; Luan, H; Cai, Z; Wang, Y; Zhao, Y; Wu, Y. (2016a). Placental transfer of perfluoroalkyl substances and associations with thyroid hormones: Beijing prenatal exposure study. Sci Rep 6: 21699. http://dx.doi.org/10.1038/srep21699.

Yang, L; Wang, Z; Shi, Y; Li, J; Wang, Y; Zhao, Y; Wu, Y; Cai, Z. (2016b). Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and cord serum. Chemosphere 144: 1631-1638. http://dx.doi.org/10.1016/j.chemosphere.2015.10.063.

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. Sci Total Environ 621: 1542-1549. http://dx.doi.org/10.1016/j.scitotenv.2017.10.074.

Yao, J; Dong, Z; Jiang, L; Pan, Y; Zhao, M; Bai, X; Dai, J. (2023). Emerging and Legacy Perfluoroalkyl Substances in Breastfed Chinese Infants: Renal Clearance, Body Burden, and Implications. Environ Health Perspect 131: 37003-37003. http://dx.doi.org/10.1289/EHP11403.

Yao, J; Pan, Y; Sheng, N; Su, Z; Guo, Y; Wang, J; Dai, J. (2020). Novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs): Occurrence and association with serum biochemical parameters in residents living near a fluorochemical plant in China. Environ Sci Technol 54: 13389-13398. http://dx.doi.org/10.1021/acs.est.0c02888.

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; Zhou, Y; Ding, G; Tian, Y. (2019). Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood

reproductive hormone. Environ Int 129: 573-582. http://dx.doi.org/10.1016/j.envint.2019.03.047.

Yao, Q; Vinturache, A; Lei, X; Wang, Z; Pan, C; Shi, R; Yuan, T; Gao, Y; Tian, Y. (2022). Prenatal exposure to per- and polyfluoroalkyl substances, fetal thyroid hormones, and infant neurodevelopment. Environ Res 206: 112561. http://dx.doi.org/10.1016/j.envres.2021.112561.

Yeung, LW; Guruge, KS; Taniyasu, S; Yamashita, N; Angus, PW; Herath, CB. (2013). Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicol Environ Saf 96: 139-146. http://dx.doi.org/10.1016/j.ecoenv.2013.06.006.

Ylinen, M; Auriola, S. (1990). Tissue distribution and elimination of perfluorodecanoic acid in the rat after single intraperitoneal administration. Pharmacol Toxicol 66: 45-48. http://dx.doi.org/10.1111/j.1600-0773.1990.tb00700.x.

Yoo, H; Washington, JW; Jenkins, TM; Ellington, JJ. (2011). Quantitative determination of perfluorochemicals and fluorotelomer alcohols in plants from biosolid-amended fields using LC/MS/MS and GC/MS. Environ Sci Technol 45: 7985-7990. http://dx.doi.org/10.1021/es102972m.

Yu, G; Jin, M; Huang, Y; Aimuzi, R; Zheng, T; Nian, M; Tian, Y; Wang, W; Luo, Z; Shen, L; Wang, X; Du, Q; Xu, W; Zhang, J. (2021). Environmental exposure to perfluoroalkyl substances in early pregnancy, maternal glucose homeostasis and the risk of gestational diabetes: A prospective cohort study. Environ Int 156: 106621. http://dx.doi.org/10.1016/j.envint.2021.106621.

Zeng, X; Chen, Q; Zhang, X; Li, H; Liu, Q; Li, C; Ma, M; Zhang, J; Zhang, W; Zhang, J; Huang, L. (2019a). Association between prenatal exposure to perfluoroalkyl substances and asthma-related diseases in preschool children. Environ Sci Pollut Res Int 26: 29639-29648. http://dx.doi.org/10.1007/s11356-019-05864-x.

Zeng, XW; Bloom, MS; Dharmage, SC; Lodge, CJ; Chen, D; Li, S; Guo, Y; Roponen, M; Jalava, P; Hirvonen, MR; Ma, H; Hao, YT; Chen, W; Yang, M; Chu, C; Li, QQ; Hu, LW; Liu, KK; Yang, BY; Liu, S; Fu, C; Dong, GH. (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. Sci Total Environ 663: 60-67. http://dx.doi.org/10.1016/j.scitotenv.2019.01.325.

Zeng, XW; Lodge, CJ; Dharmage, SC; Bloom, MS; Yu, Y; Yang, M; Chu, C; Li, QQ; Hu, LW; Liu, KK; Yang, BY; Dong, GH. (2019c). Isomers of per- and polyfluoroalkyl substances and uric acid in adults: Isomers of C8 Health Project in China. Environ Int 133: 105160. http://dx.doi.org/10.1016/j.envint.2019.105160.

Zeng, XW; Qian, Z; Emo, B; Vaughn, M; Bao, J; Qin, XD; Zhu, Y; Li, J; Lee, YL; Dong, GH. (2015). Association of polyfluoroalkyl chemical exposure with serum lipids in children. Sci Total Environ 512-513: 364-370. http://dx.doi.org/10.1016/j.scitotenv.2015.01.042.

Zhang, C; Sundaram, R; Maisog, J; Calafat, AM; Barr, DB; Buck Louis, GM. (2015). A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. Fertil Steril 103: 184-189. http://dx.doi.org/10.1016/j.fertnstert.2014.10.001.

Zhang, R; Ye, J; Wei, Q; Li, M; Xu, K; Li, Z; Lin, W; Liu, P; Chen, R; Ma, A; Zhou, Z. (2018a). Plasma concentration of 14 perfluoroalkyl acids (PFAAs) among children from seven cities in Guangdong, China. Sci Total Environ 616-617: 1469-1476. http://dx.doi.org/10.1016/j.scitotenv.2017.10.167.

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. J Clin Endocrinol Metab 103: 2543-2551. http://dx.doi.org/10.1210/jc.2017-02783.

Zhang, T; Sun, H; Lin, Y; Qin, X; Zhang, Y; Geng, X; Kannan, K. (2013a). Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain

length related maternal transfer. Environ Sci Technol 47: 7974-7981. http://dx.doi.org/10.1021/es400937y.

Zhang, T; Zhang, B; Bai, X; Yao, Y; Wang, L; Shu, Y; Kannan, K; Huang, X; Sun, H. (2019). Health status of elderly people living near e-waste recycling sites: association of e-waste dismantling activities with legacy perfluoroalkyl substances (PFASs). Environ Sci Technol Lett 6: 133-140. http://dx.doi.org/10.1021/acs.estlett.9b00085.

Zhang, Y; Beesoon, S; Zhu, L; Martin, JW. (2013b). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ Sci Technol 47: 10619-10627. http://dx.doi.org/10.1021/es401905e.

Zhang, Y; Pan, C; Ren, Y; Wang, Z; Luo, J; Ding, G; Vinturache, A; Wang, X; Shi, R; Ouyang, F; Zhang, J; Li, J; Gao, Y; Tian, Y. (2022). Association of maternal exposure to perfluoroalkyl and polyfluroalkyl substances with infant growth from birth to 12 months: A prospective cohort study. Sci Total Environ 806: 151303. http://dx.doi.org/10.1016/j.scitotenv.2021.151303.

Zhang, YM; Dong, XY; Fan, LJ; Zhang, ZL; Wang, Q; Jiang, N; Yang, XS. (2017). Poly- and perfluorinated compounds activate human pregnane X receptor. Toxicology 380: 23-29. http://dx.doi.org/10.1016/j.tox.2017.01.012.

Zhao, W; Cui, R; Wang, J; Dai, J. (2017). Inhibition effects of perfluoroalkyl acids on progesterone production in mLTC-1. J Environ Sci 56: 272-280. http://dx.doi.org/10.1016/j.jes.2016.08.026.

Zhao, Y; Jin, H; Qu, J; Zhang, S; Hu, S; Xue, J; Zhao, M. (2022). The influences of perfluoroalkyl substances on the rheumatoid arthritis clinic. BMC Immunol 23: 10. http://dx.doi.org/10.1186/s12865-022-00483-7.

Zhou, W; Zhang, L; Tong, C; Fang, F; Zhao, S; Tian, Y; Tao, Y; Zhang, J. (2017a). Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environ Health Perspect 125: 067012. http://dx.doi.org/10.1289/EHP1203.

Zhou, Y; Hu, LW; Qian, ZM; Chang, JJ; King, C; Paul, G; Lin, S; Chen, PC; Lee, YL; Dong, GH. (2016). Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environ Int 94: 189-195. http://dx.doi.org/10.1016/j.envint.2016.05.018.

Zhou, Y; Hu, LW; Qian, ZM; Geiger, SD; Parrish, KL; Dharmage, SC; Campbell, B; Roponen, M; Jalava, P; Hirvonen, MR; Heinrich, J; Zeng, XW; Yang, BY; Qin, XD; Lee, YL; Dong, GH. (2017b). Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children. Sci Rep 7: 899. http://dx.doi.org/10.1038/s41598-017-01140-5.

Zhu, Y; Qin, XD; Zeng, XW; Paul, G; Morawska, L; Su, MW; Tsai, CH; Wang, SQ; Lee, YL; Dong, GH. (2016). Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. Sci Total Environ 559: 166-173. http://dx.doi.org/10.1016/j.scitotenv.2016.03.187.