Water Quality Control
Commission
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Pamela Jones

STATE OF NEW MEXICO
WATER QUALITY CONTROL COMMISSION

IN THE MATTER OF PROPOSED AMENDMENTS TO 20.6.4 NMAC - ***Standards for Interstate and Intrastate Surface Waters,***

NO. WQCC 25-75 (R)

NEW MEXICO ENVIRONMENT DEPARTMENT,

PETITIONER.

AMIGOS BRAVOS' NOTICE OF INTENT TO PRESENT DIRECT TESTIMONY AND EXHIBITS

Pursuant to 20.6.1.202.A NMAC and the Pre-hearing Order in this matter, Amigos Bravos files its Notice of Intent to Present Direct Testimony and Exhibits. As required by the applicable rule and Pre-hearing Order, Amigos Bravos provides the following information:

1. Identify the person for whom the witness will testify: Jamie DeWitt, Ph.D., DABT, will testify on behalf of Amigos Bravos.

2. Identify each technical witness the person intends to present, and state the qualifications of that witness, including a description of their educational and work background: Amigos Bravos intends to present:

- Jamie DeWitt, Ph.D., DABT, whose educational and work background is set forth in her curriculum vitae, attached as AB Exhibit 2.

3. Include a copy of the direct testimony of each technical witness in narrative form: The full written direct testimony of Dr. DeWitt is submitted as AB Exhibit 3.

4. Include the text of any recommended modifications to the proposed regulatory change: Amigos Bravos' proposed amendments to the amendments to 20.6.4 NMAC proposed by the New Mexico Environment Department in its First Amended Petition are set forth in blue underline in AB Exhibit 1, attached.

5. List and attach all exhibits anticipated to be offered by that person at the hearing, including any proposed statement of reasons for adoption of the rules: Below is a list of all direct exhibits to be offered by Amigos Bravos, which includes testimony setting forth its reasons to adopt its proposed modifications. The list of exhibits includes the Bates stamped page where each exhibit starts.

Exhibit	Description	Bates Stamped Page
1	Proposed amendments to 20.6.4 NMAC	0001
2	Curriculum vitae of Jamie DeWitt, Ph.D., DABT	0004
3	Direct testimony of Jamie DeWitt, Ph.D., DABT	0048
4	EPA DRAFT Human Health Ambient Water Quality Criteria: PFOA and Related Salts	0069
5	EPA DRAFT Human Health Ambient Water Quality Criteria: PFOS and Related Salts	0128
6	EPA DRAFT Human Health Ambient Water Quality Criteria: PFBS and Related Salts	0188
7	EPA Acute Freshwater Aquatic Life Benchmarks for Eight Data-Limited PFAS	0284
8	EPA Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS	0539
9	EPA MCLGs for Three Individual PFAS and a Mixture of Four PFAS	0677

Respectfully submitted,

/s/ Tannis Fox

Tannis Fox
Senior Attorney
Western Environmental Law Center
330 Garfield Street, Suite 304
Santa Fe, New Mexico 87501
505.629.0732
fox@westernlaw.org

Attorney for Amigos Bravos

Certificate of Service

I certify a copy of the foregoing pleading was emailed to the following on July 30, 2026:

Pamela Jones
Commission Administrator
1190 Saint Francis Drive, Suite S2102
Santa Fe, New Mexico 87505
Pamela.jones@env.nm.gov

Brecken Scott
Christal Weatherly
Assistant General Counsels
New Mexico Environment Department
P.O. Box 5469
Santa Fe, New Mexico 87502
brecken.scott@env.nm.gov
Christal.Weatherly@env.nm.gov

Elizabeth Newlin Taylor
Taylor & McCaleb, P.A.
P.O. Box 2540
Corrales, New Mexico 87048-2540
etaylor@taylormccaleb.com

Bruce Wetherbee
60 Thoreau Street, #103
Concord, Massachusetts 01742
editor@thecandlepublishing.com

Jennifer Bradfute
Matthias Sayer
David Kirmse
Bradfute Sayer, P.C.
P.O. Box 90233
Albuquerque, New Mexico 87199
jennifer@bradfutelaw.com
matthias@bradfutelaw.com
david@bradfutelaw.com

Dalva L. Moellenberg
Anthony J. Trujilo
Serafina I. Seluja
Gallagher & Kennedy, P.A.
1239 Paseo De Peralta
Santa Fe, New Mexico 87501
DLM@gknet.com
AJT@gknet.com
Serafina.seluja@gknet.com

Stuart R. Butzier
Benjamin Rossi
Modrall, Sperling, Roehl, Harris & Sisk, P.A.
P.O. Box 2168
Albuquerque, New Mexico 87103
Stuart.butzier@modrall.com
Benjamin.rossi@modrall.com

Felicia Orth
Hearing Officer
Water Quality Control Commission
1190 Saint Francis Drive, Suite S2102
Santa Fe, New Mexico 87505
felicia.l.orth@gmail.com

Eduardo Ugarte, II
Assistant Attorney General
New Mexico Department of Justice
P.O. Box 1508
Santa Fe, New Mexico 87504
eugarte@nmdoj.gov

/s/ Tannis Fox
Tannis Fox

AMIGOS BRAVOS EXHIBIT 1

AMIGOS BRAVOS' PROPOSED AMENDMENTS TO 20.6.4 NMAC[1]

20.6.4.900.I **Mixtures of PFAS.** For mixtures of PFAS with human health-organism only criteria (PFOA, PFOS, and PFBS), a hazard index (HI) will be applied with the following formula: HI = ([PFOA$_{ambient\ water}$]/[0.0036 ng/L] + ([PFOS$_{ambient\ water}$]/[0.07 ng/L]) + ([PFBS$_{ambient\ water}$]/[500 ng/L]). When the HI of the mixture of the three PFAS exceeds a value of one, the water quality standard is exceeded.

20.6.4.900.J Use-specific numeric criteria

(1) Table of numeric criteria: The following table sets forth the numeric criteria in µg/L, unless otherwise indicated, applicable to existing, designated and attainable uses. For metals, criteria represent the total sample fraction unless otherwise specified in the table. Additional criteria that are not compatible with this table are found in Subsections A through I and K through M of 20.6.4.900 NMAC.

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Pollutant	CAS Number	DWS	Irr/Irr storage	LW	WH	Aquatic Life			Type
						Acute	Chronic	HH-OO	
Perfluorooctane sulfonate (PFOS)	Various					71	0.25	7e-5	P
PFOS (mg/kg-ww-iwbt)	Various						0.028		P
PFOS (mg/kg-ww-fwbt)	Various						0.201		P
PFOS (mg/kg-ww-fmt)	Various						0.087		P
Perfluorooctanoic acid (PFOA)	Various					3,100	100	3.6e-6	P
PFOA (mg/kg-ww-iwbt)	Various						1.18		P
PFOA (mg/kg-ww-fwbt)	Various						6.49		P
PFOA (mg/kg-ww-fmt)	Various						0.133		P
Perfluorobutane sulfonate (PFBS)	Various							0.5	P
PFBA (perfluorobutanoic acid)	375-22-4					5,300			P
PFHxA (perfluorohexanoic acid)	307-24-4					4,800			P
PFNA (perfluorononanoic acid)	375-95-1					650			P
PFDA (perfluorodecanoic acid)	335-76-2					500			P
PFBS (perfluorobutane sulfonate)	375-73-5					5000			P

[1] NMED's proposed amendments appear in red underline and Amigos Bravos' proposed amendments appear in blue underline.

PFHxS (perfluorohexanesulfonic acid)	355-46-4					210			P
8:2 FTUCA (8:2 fluorotelomer unsaturated carboxylic acid)	70887-84-2					37			P
7:3 FTCA (7:3 fluorotelomer carboxylic acid)	812-70-4					12			P

AMIGOS BRAVOS EXHIBIT 2

Jamie C. DeWitt

Contact Details:

Department of Environmental and Molecular Toxicology
Oregon State University
Corvallis, OR
jamie.dewitt@oregonstate.edu

EDUCATION & CERTIFICATIONS

Diplomate, American Board of Toxicology. 2017. Recertified through 2027.

Ph.D., Environmental Science and Neural Science.
School of Public and Environmental Affairs and Program in Neural Science. Indiana University, Bloomington, IN. 2004.
Concentrations: Environmental and developmental neurotoxicology and risk assessment
Dissertation title: Developmental intoxication of dioxins and polychlorinated biphenyls in an avian model: Correlations of brain asymmetry, behavior, and related developmental effects

B.S., Environmental Science and Biology.
Lyman Briggs College. 1992. Michigan State University, East Lansing, MI.

PROFESSIONAL EXPERIENCE

Director, Pacific Northwest Center for Translational Environmental Health Research
Oregon State University, Corvallis, OR. September 2023-present.

Professor of Environmental and Molecular Toxicology
Department of Environmental and Molecular Toxicology. Oregon State University, Corvallis, OR. September 2023-present.

Adjunct Professor
Toxicology Program, Biological Sciences, North Carolina State University, Raleigh, NC. March 2023-present.

Adjunct Professor
Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC. January 2024-2025.

Professor of Pharmacology and Toxicology
Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC. July 2021-November 2023.

Adjunct Associate Professor
Toxicology Program, Biological Sciences, North Carolina State University, Raleigh, NC. March 2018-2023.

Associate Professor of Pharmacology and Toxicology
Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC. July 2015-July 2021.

Adjunct Associate Professor of Public Health
Department of Public Health, Brody School of Medicine, East Carolina University, Greenville, NC. July 2015-July 2017.

Adjunct Assistant Professor of Public Health
Department of Public Health, Brody School of Medicine, East Carolina University, Greenville, NC. July 2012-July 2014.

Affiliated Member
The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Research, East Carolina University, Greenville, NC. July 2011-June 2017.

Assistant Professor of Pharmacology and Toxicology
Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC. July 2008-July 2015.

Postdoctoral Trainee in Immunotoxicology
University of North Carolina at Chapel Hill in cooperation with the U.S. Environmental Protection Agency (Training Agreement CT829472), National Health and Environmental Effects Research Laboratory, Experimental Toxicology Division, Immunotoxicology Branch, Research Triangle Park, NC (Advisor: Dr. Robert Luebke). Evaluation of immune function and exploration of immunotoxic mechanisms, including use of knock-out models and molecular techniques, of various xenobiotics (organotins and perfluoroalkyl acids) in rodent models. June 2004-June 2008.

Postdoctoral Research Associate in Environmental and Ecotoxicology
Developmental Neurobiology and Environmental Toxicology Laboratory, School of Public and Environmental Affairs, Indiana University, in cooperation with the U.S. Fish and Wildlife Service Bloomington Ecological Services Field Office, Bloomington, IN (Advisors: Dr. Diane Henshel and Daniel Sparks). Cardiotoxic effects in wild passerine birds developmentally exposed to PCBs. September 2003-May 2004.

Research Assistant in Environmental and Ecotoxicology
Developmental Neurobiology and Environmental Toxicology Laboratory, School of Public and Environmental Affairs, Indiana University, Bloomington, IN (Advisor: Dr. Diane Henshel). Toxicological effects of dioxin and polychlorinated biphenyls after developmental exposure in an avian model, wild birds, and wild fish. August 1995-August 2003.

Field Assistant in Limnology
Lake Lemon Conservancy District, Unionville, IN. Canada goose control, littoral zone revegetation, and monitoring of native and exotic aquatic plant populations. June 2000-May 2003.

Field Assistant in Ecotoxicology
U.S. Fish and Wildlife Service-Bloomington Ecological Services Field Office, Bloomington, IN. Wild bird and macroinvertebrate population monitoring in a metal-contaminated lake, including assessment of fertile eggs for embryonic abnormalities. April 1997-October 1997.

Research Associate in Entomology

Landscape Entomology Division, Department of Entomology, Michigan State University, East Lansing, MI. Research and efficacy tests for forest, ornamental and turf entomological studies for the Michigan Department of Agriculture and Turf Foundation. September 1992-August 1995.

Research Assistant in Entomology

Medical Entomology Division, Department of Entomology, Michigan State University, East Lansing, MI. Assessment of Lyme disease prevalence in deer and dog ticks collected from a Lyme disease endemic area in Michigan. May 1992-May 1993.

PUBLICATIONS

Primary Research Manuscripts

Ahmed A, Wittenborn M, **DeWitt J**, Woodlief T. 2026. Metabolic and circadian bioenergetic alterations following PFOA exposure and high-fat diet in female C57BL/6 mice. *Toxicological Sciences.* 209:kfag025.

Connolly J, Ishihara Y, Sawaya Em, Whitfield V, Garrity N, Sohata R, Tsymbal M, Lundberg A, La Merrill M, **DeWitt J**, Ehrlich A, and Vogel CFA. 2025. Per- and polyfluoroalkyl substances (PFAS) enhance cholesterol accumulation and dysregulate inflammatory response in macrophages. *Cardiovascular Toxicology.*2 5:1455-1470.

Kirkwood-Donelson KI, Chappel J, Tobin E, Dodds JN, Reif DM, **DeWitt JC**, and Baker ES. 2024. Investigating mouse hepatic lipidome dysregulation following exposure to emerging per- and polyfluoroalkyl substances (PFAS). *Chemosphere*. 354:141654.

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Wallis DJ, Kotlarz N, Knappe DRU, Collier DN, Lea SC, Reif D, McCord J, Strynar M, **DeWitt JC**, and Hoppin, JA. 2023. Estimation of the half-lives of recently detected per- and polyfluorinated alkyl ethers in an exposed community. *Environmental Science & Technology*. 54:15348-15355.

Taylor KD, Woodlief TL, Ahmed A, Hu Q, Duncker PC, and **DeWitt JC**. 2023. Quantifying the impact of PFOA exposure on B cell development and antibody production. *Toxicological Sciences*. 194:101-108.

Phelps DW, Palekar AI, Conley HE, Gerrero G, Driggers JH, Linder KE, Kullman SW, Reif DM, Sheats MK, **DeWitt JC**, and Yoder JA. 2023. Legacy and emerging per- and polyfluoroalkyl substances suppress the neutrophil respiratory burst. *Journal of Immunotoxicology*. 20:1:2176953.

Barton KE, Zell-Baran LM, **DeWitt JC**, Brindley S, McDonough CA, Higgins CP, Adgate JL, and Starling AP. 2022. Cross-sectional associations between serum PFASs and inflammatory biomarkers in a population exposed to AFFF-contaminated drinking water. *International Journal of Hygiene and Environmental Health*. 240:113905.

Woodlief T, Vance S, Hu Q, **DeWitt J**. 2021. Immunotoxicity of per- and polyfluoroalkyl substances: Insights into short-chain PFAS exposure. *Toxics*. 9:100.

Zhou J, Baumann K, Mead RN, Skrabal SA, Kieber RJ, Avery GB, Shimizu M, **DeWitt JC**, Sun M, Vance SA, Bodnar W, Zhang Z. 2021. PFOS dominates PFAS composition in ambient fine particulate matter ($PM_{2.5}$) collected across North Carolina nearly 20 years after the end of its US production. *Environmental Science: Processes and Impacts*. 23:580-587.

Jiang Q, Xu X, **DeWitt JC**, Zheng Y. 2021. Using chicken embryo as a powerful tool in assessment of developmental cardiotoxicities. *Journal of Visualized Experiments.* 21:doi: 10.3791/62189.

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vonderEmbse A, Hu Q, **DeWitt J**. 2020. Postnatal toxicant exposure in 3xTgAD mice promotes gene x environment-related early alterations to neuroimmune epigenetic profiles. *Neuroimmunology and Neuroinflammation*, 7:345-359.

Kotlarz N, McCord J, Collier D, Lea CS, Strynar M, Lindstrom AB, Wilkie AA, Islam JY, Matney K, Tarte P, Polera ME, Burdette K, **DeWitt J**, May K, Smart RC, Knappe DRU, Hoppin JA. 2020. Poorly understood PFAS generated as byproducts of fluorochemical manufacturing are in the blood of children and adults living in Wilmington, North Carolina. *Environmental Health Perspectives*, 128: https://doi.org/10.1289/EHP6837.

McDonough C, Choyke S, Ferguson PL, **DeWitt J**, Higgins C. 2020. Bioaccumulation of novel per- and polyfluoroalkyl substances (PFASs) in mice dosed with an aqueous film-forming foam (AFFF). 2020. *Environmental Science & Technology*. 54:5700-5709.

vonderEmbse AN, Hu Q, **DeWitt JC.** 2019. Dysfunctional microglia:neuron interactions with significant female bias in a developmental gene x environment rodent model of Alzheimer's disease. *International Immunopharmacology*. 71:241-250.

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Leetham M, **DeWitt J**, Buck B, Goossens D, Teng Y, Pollard J, McLaurin B, Gerads R, and Keil D. 2016. Oxidative stress and lung pathology following geogenic dust exposure. *Journal of Applied Toxicology*. 36:1276-1283.

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Jiang Q, Ma W, Wu J, Wingard CJ, and **DeWitt JC**. 2016. Perfluorooctanoic acid-induced toxicity in primary cultures of chicken embryo cardiomyocytes. *Environmental Toxicology*. 31:1580-1590.

Khalil N, Chen A, Lee M, Czerwinski SA, Ebert JR, **DeWitt JC**, and Kannan K. 2016. Association of perfluoroalkyl substances, bone mineral density, and osteoporosis in the US population in NHANES 2009-2010. *Environmental Health Perspectives*. 124:81-87.

Wambaugh JF, Setzer W, Pitruzzello AM, Liu J, Reif D, Kleinstreuer N, Ching N, Wang Y, Sipes N, Martin M, Das K, **DeWitt J**, Strynar M, Judson R, Houck K, and Lau C. 2013. Dosimetric anchoring of in vivo and in vitro studies for perfluorooctanoate and perfluorooctanesulfonate. *Toxicological Sciences*. 136:308-327.

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DeWitt JC, Copeland CB, and Luebke RW. 2006. Developmental exposure to 1.0 or 2.5 mg/kg of dibutyltin dichloride does not impair immune function in Sprague-Dawley rats. *Journal of Immunotoxicology*. 3:245-252.

DeWitt JC, Millsap DS, Yeager RL, Heise SS, Sparks DW, and Henshel, DS. 2006. External heart deformities in passerine birds exposed to environmental mixtures of polychlorinated biphenyls during development. *Environmental Toxicology and Chemistry.* 25:541-551.

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Henshel DS, Martin JW and **DeWitt JC**. 1997. Brain asymmetry as a potential biomarker for developmental TCDD intoxication: A dose-response study. *Environmental Health Perspectives*. 105:718-725.

Henshel DS, Martin JW, Norstrom RJ, Elliot J, Cheng KM and **DeWitt JC**. 1997. Morphometric brain abnormalities in double-crested cormorant chicks exposed to polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. *Journal of Great Lakes Research*. 23:11-26.

Walker ED, Smith TW, **DeWitt J**, Beaudo DC, McLean RG. 1994. Prevalence of *Borrelia burgdorferi* in host-seeking ticks (Acari: Ixodidae) from a Lyme disease endemic area in northern Michigan. *Journal of Medical Entomology*. 31(4):524-8.

Reviews/Commentaries

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Brander SM, Swan SH, Mehinto AC, Kidd KA, Weis JS, Belcher SM, **DeWitt JC**, Harper S, and Helbing CC. 2026. Impacts of environmental stressors on fertility and fecundity across taxa, with implications for planetary health. *npj Emerging Contaminants. 2:12.*

DeWitt JC, Goldenman G, Lohmann R, Ng CA, and Wang Z. 2025. Emerging toxicological awareness of per- and polyfluoroalkyl substances: the rising concern over "forever chemicals." *Disease Models and Mechanisms*. 18:dmm052647.

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Edited Books

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DeWitt JC (ed). 2015. *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*. Springer Science + Business Media, LLC (Invited).

Book Chapters

vonderEmbse AN and **DeWitt JC**. 2018. Developmental immunotoxicity (DIT) testing: Current recommendations and the future of DIT testing. In: *Immunotoxicity Testing: Methods and Protocols* (DeWitt JC, Rockwell CE, and Bowman CC, eds), Methods in Molecular Biology Series. Springer Science + Business Media, LLC.

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DeWitt JC and Dietert RR. 2014. Immunotoxicity in autism spectrum disorders. In: *The Comprehensive Guide to Autism* (Patel VB, Martin CR, Preedy V, and Preedy VR, eds). Springer Reference, New York, NY.

Dietert RR, **DeWitt JC**, and Luebke RW. 2012. Reducing the prevalence of immune-based chronic disease. In: *Immunotoxicity, Immune Dysfunction, and Chronic Diseases* (Dietert RR and Luebke RW, eds), Molecular and Integrative Toxicology, Springer Science + Business Media, LLC. pp 419-440.

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Luebke RW, **DeWitt JC**, Germolec DR, Salazar KD, and Kerkvliet NI. 2012. Immunomodulation by persistent organic pollutants. In: *Dioxins and Health, Including Other Persistent Organic Pollutants and Endocrine Disruptors, 3nd Edition* (Schecter A, ed), John Wiley and Sons, Inc., Hoboken, NJ. pp 171-192.

DeWitt JC and Dietert RR. 2012. Postnatal immune dysfunction and its impact on growth parameters. In: *Handbook of Growth and Growth Monitoring in Health and Disease* (Preedy VR, ed), Springer, New York, NY. pp 741-755.

DeWitt JC and Luebke RW. 2010. Immunological Aging. In: *Comprehensive Toxicology*, 2nd Edition, Volume 5 (Lawrence D, ed), Elsevier Limited, Oxford, UK. pp 455-465.

Dietert RR and **DeWitt J**. 2010. Developmental immunotoxicity (DIT): The why, where and how of DIT testing. In: *Immunotoxicity Testing: Methods and Protocols* (Dietert RR, ed), Methods in Molecular Biology. Humana Press, Inc., Totowa, NJ. 598:17-25.

Luebke RW, Beamer CA, Bowman C, **DeWitt JC**, Gowdy K, Johnson VJ, Shepherd DM, and Germolec DR. 2009. Immunotoxicology (developmental immunotoxicology section). In: *General and Applied Toxicology*, 3rd Edition (Marrs T, Ballantyne B, Syversen T, eds.), John Wiley & Sons, Ltd., Chichester, UK, pp 1561-1583.

Other Scholarly Contributions

Portier CJ et al. (90+ co-authors). 2016. Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA). *Journal of Epidemiology and Community Health*. 70:741-745.

DeWitt JC and Luebke RW. 2014. Immunological Aging. *Online Reference Database Biomedical Science*.

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Non-Refereed Articles

DeWitt JC, Brown P, Carignan C, Kasper S, Schaider L, Osimo C, Fitzstevens M. Op-ed: PFAS chemicals – the other immune system threat. *Environmental Health News.* July 6, 2020.

"Toxicant induced brain asymmetry: More than just a bird-brained scheme?" Learned Discourses, *SETAC Globe*, Jan/Feb 2001 (invited).

RESEARCH FUNDING

National Institute of Environmental Health Sciences Environmental Health Sciences Core Centers (P30) — 5 years
Pacific Northwest Center for Translational Environmental Health Research
Role on project: Contact PI/Core Leader

National Institute of Environmental Health Sciences Ruth L. Kirschstein National Research Service Award (T32) — 5 years
Integrated Regional Training Program in Environmental Health Sciences
Role on Project: Lead Principal Investigator/Co-Director

New Jersey Department of Environmental Protection Division of Science and Research — 2 years
T-Dependent Antibody Response (TDAR) for Immunotoxicity
Role on Project: Principal Investigator

North Carolina Collaboratory (subaward from Duke University) — 2 years
The North Carolina Firefighter Cancer Cohort Study (NC-FCCS): Investigating PFAS and Chemical Exposures as Cancer Risks
Role on Project: Principal Investigator

Pacific Northwest Center for Translational Environmental Health Research — 1 year
Pilot Project Award: PFAS in Everyday Consumer Products as a Source of Exposure for Oregonians
Role on project: Project Leader

National Institute of Environmental Health Sciences Environmental Health Sciences Core Centers (P30) — 5 years*
Pacific Northwest Center for Translational Environmental Health Research

Role on project: Contact PI/Project Leader
**Contact PI/Core Leader only for Year 5*

National Institutes of Health (R01) 5 years
Perfluoroalkyl Substances and Risk of Kidney Cancer in US Men and Women
Role on project: Consultant (T. Zheng and L. Lipworth, PIs, Brown University)

North Carolina Biotechnology Center 1 year
Development of a High Throughput Screening Assay for Functional Immunotoxicity
Role on project: Principal Investigator through September 2023. PI role transferred to T. Woodlief at East Carolina University. New role on project: Co-Investigator (no funds).

North Carolina Collaboratory 2 years
Per- and Polyfluoroalkyl Substances (PFAS) in North Carolina: Descriptive Toxicological Needs II
Role on project: Principal Investigator through September 2023. PI role transferred to T. Woodlief at East Carolina University. New role on project: Co-Investigator (no funds).

North Carolina Collaboratory
Instrumentation Award (Seahorse XF Pro)
Role on project: Principal Investigator

North Carolina Collaboratory 1 year
Per- and Polyfluoroalkyl Substances (PFAS) in North Carolina: Descriptive Toxicological Needs I
Role on project: Principal Investigator

Center for Human Health and the Environment at North Carolina State University 1 year
Pilot Project Award: Uncovering PFOA-Induced Metabolic Changes and their Association with B-cell Metabolic Function
Role on project: Principal Investigator of Record (Mentor to Dr. Tracey Woodlief, who wrote and received award)

Brody Brothers Endowment Foundation 1 year
Do Per- and Polyfluoroalkyl Substances found in the Cape Fear River of North Carolina Pose a Risk to the Immune System?
Role on project: Principal Investigator

National Institute of Environmental Health Sciences Superfund Research Program (P42) via subcontract from North Carolina State University 5 years
Center for Environmental and Health Effects of PFAS. BMRP2: Uncovering the Mechanisms of PFAS-Induced Immunotoxicity: An Important Public Health Endpoint
Role on project: Co-Principal Investigator (C. Mattingly and D. Knappe, PIs, NCSU)

Department of Defense 3 years
New Approaches for the Treatment of Neuroinflammatory and Behavioral Consequences of Exposure to Gulf War Illness Chemicals
Role on project: Principal Investigator

United States Environmental Protection Agency via subcontract from Oregon State University 3 years

System Toxicological Approaches to Define and Predict Toxicity of Per- and Polyfluoroalkyl Substances
Role on project: Co-Principal Investigator (R. Tanguay, PI, OSU)

North Carolina Collaboratory 2 years
Per- and Polyfluoroalkyl Substance Testing (PFAST) Network: Effects of novel PFASs on immune function
Role on project: Co-Principal Investigator and Team Co-Lead (with R. Fry, UNC-CH)

North Carolina Collaboratory 6 months
Emerging contaminants in North Carolina, including PFASs, 1,4-dioxiane, and bromide, in air and water
Role on project: Co- Principal Investigator

National Institutes of Health/National Institute of Environmental Health Sciences via subcontract with North Carolina State University 2 years
Assessing Impact of Drinking Water Exposure to GenX in the Cape Fear River Basin
Role on project: Co-Investigator (J. Hoppin, NCSU, PI)

Brody Brothers Endowment Foundation 1 year
Immunomodulatory Effects of Aqueous Film Forming Foam (AFFF): An Effective Fire Suppressant or a Persistent Environmental Contaminant with Unknown Health Consequences?
Role on project: Principal Investigator

Center for Human Health and Environment at NCSU Pilot Project Program 1 year
Pilot Project Award: Discovery of Biomarkers of Effect following Environmentally Relevant Exposure to Pharmaceutical Pollutants
Role on project: Co-Principal Investigator (E. Hvastkovs and K. McCoy, ECU, co-PIs)

Brody School of Medicine Internal Seed/Bridge Grant Program 1 year
Post-translational Modifications to Potassium Channels in Alzheimer's Disease: Triggers of Onset and Progression?
Role on project: Co-Principal Investigator (R. Schwalbe, ECU, co-PI)

The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Research 1 year
Microglia as a Target of Environment x Gene Interactions Part II: Digging into the Biochemistry of Alzheimer's Disease
Role on project: Principal Investigator (R. Schwalbe, ECU, co-PI)

Interdisciplinary Research Collaboration Award (East Carolina University) 6 months
Pharmaceutical and Personal Care Product Contaminants in Fresh Water
Role on Project: Corresponding Faculty PI

Alzheimer's North Carolina 1 year
A Multidisciplinary Approach to Fight Senior Dementia
Role on Project: Co-Investigator (Q. Lu, ECU, PI)

East-West Research Collaboration Award (East Carolina University) 6 months
Pharmaceutical and Personal Care Product Contaminants in Fresh Water
Role on Project: Corresponding Faculty PI

The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Research — 1 year
Microglia as a Target of Environment x Gene Interactions: Exacerbation of Alzheimer's Pathology by Early-life Exposure to Lead
Role on project: Principal Investigator

Bureau of Land Management via subcontract from University of Nevada-LV — 3 years
Nellis Dunes Recreation Area Dust Exposure and Human Health Risk Assessment
Role on project: Co-Principal Investigator (B. Buck, UNLV, PI)

Department of Defense — 1 year
Immunopathogenesis in autism: Regulatory T cells and autoimmunity in neurodevelopment
Role on project: Principal Investigator

School of Public and Environmental Affairs, Indiana University. Ph.D. Student Travel Award and Graduate Student Organization Travel Award. 2001. Funded amount: $500.00
Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry. Student Travel Grant. 1997, 1998, 2001. Funded amounts: $300.00 each year

EDITORIAL BOARDS/AD HOC MANUSCRIPT REVIEWER

Editorial Board Member, *Journal of Immunotoxicology*. 2010-
Editorial Board Member, *Journal of Toxicology and Environmental Health Part A.* 2013-
Associate Editor, *Toxicology and Applied Pharmacology*. 2016-
Series co-Editor (with Sarah Blossom), *Molecular and Integrative Toxicology*. 2016-2022
Editorial Board Member, *Environmental Health Perspectives*. 2017-2025
Editorial Board Member, *NeuroToxicology*. 2018-2026
Editorial Board Member, *Reproductive Toxicology*, 2019-
Editorial Board Member, *Chemosphere*, 2023-
Co-Editor, *Food and Chemical Toxicology*, 2023-2025
Co-Editor-in-Chief, *npj Toxicology*, 2026-

Ad Hoc Reviewer (summary of most common journals):

Advances in Physiology Education
Archives of Toxicology
Chemosphere
Environmental Health
Environmental Health Perspectives
Environmental Research
Environmental Science & Technology
Food and Chemical Toxicology
Human & Experimental Toxicology
International Immunopharmacology
International Journal of Tropical Biology
Journal of Toxicology & Environmental Health
Pharmacological Research
Regulatory Toxicology & Pharmacology
Science of the Total Environment
Toxicology & Applied Pharmacology
Toxicology Reports
Archives of Environmental Contamination & Toxicology
Chemical Research in Toxicology
Drug and Chemical Toxicology
Environment International
Environmental Science & Pollution Research
Epidemiology
GENE
International Aquatic Research
Journal of Environmental Immunology & Toxicology
Journal of Immunotoxicology
NeuroToxicology
PLoS One
Reproductive Toxicology
Southeastern Naturalist
Toxicology Letters
Toxicological Sciences

GRANT REVIEWER & REVIEW PANEL CHAIR

South Carolina Sea Grant, 2024
ZonMw Open Competition, The Netherlands, 2024
American Institute of Biological Sciences, 2023
Department of Defense Strategic Environmental Research and Development Program, 2021-present
National Institute of Environmental Health Sciences, 2020-present
University of North Carolina at Chapel Hill pilot funding programs, 2020
Wisconsin SeaGrant, 2019, 2021
Hudson River Foundation, 2019
Department of Defense Congressionally Directed Medical Research Programs, Review Panel Chair, 2018-2021
CORIS, Consorzio per la Ricerca Sanitaria, 2018
Department of Defense Congressionally Directed Medical Research Programs, 2013-present
Graduate Women in Science Graduate Fellowships, 2011, 2014-2018, 2022
CDC-NIOSH, 2010, 2013, 2016

EXTERNAL REVIEWER

California Environmental Protection Agency (product), 2019-2022, 2026
US Environmental Protection Agency (product), 2019, 2021-2022, 2025
ATSDR, 2017-present (manuscripts, Toxicological Profile, and other documents)
New York State Department of Public Health, 2017 (Cancer incidence investigation: Village of Hoosick Falls, Rensselaer County, New York)

ORAL PRESENTATIONS (Invited)

"Human health risks of PFAS are real." *EnviSMART Webinar, Australian Laboratory for Emerging Contaminants.* Virtual. April 29, 2026.

"PFAS: What they are, where are, why they are a problem." *ClientEarth Summit 2026*. Virtual. March 24, 2026.

"PFAS exposure and health: What we know so far." *Earth Day Celebration, Sierra Club Florida Chapter, Broward Group*. Hollywood, FL. March 8, 2026.

"The Global PFAS Science Panel: Informing decision-makers about hazards and risks of per- and polyfluoroalkyl substances (PFAS)." *Society of Environmental Toxicology and Chemistry Annual Meeting.* Portland, OR. November 19, 2025.

"Immune suppression by exposure to PFAS: Focus on B cell development and metabolism." *International Union of Toxicology*. Beijing, China. October 17, 2025.

"A summary of PFAS health risks." *Webinar on a health sector perspective on forever chemicals, hosted by Health Care Without Harm*. Virtual. September 15, 2025.

"The never-ending cycle of reactive environmental toxicology." *Green Chemistry and Engineering Conference Keynote Panel*. Pittsburgh, PA. June 25, 2025.

"Toxicology in chemical design: A basic toxicology primer." *Green Chemistry and Engineering Conference Student and Postdoc Workshop.* Pittsburgh, PA. June 23, 2025.

"Human health effects of PFAS: Emerging evidence and convincing links." *Canadian Society of Chemistry Annual Meeting*. Ottawa, Ontario. June 18, 2025.

"Common misconceptions about per- and polyfluoroalkyl substances (PFAS) and sustainability: A quick look at the essential use concept." *ECU P2 Webinar Series*. Virtual. March 24, 2025.

"T-cell dependent antibody responses and cell-based alternatives to understand the immunosuppressive effects exogenous agents have on the adaptive immune system." *Society of Toxicology Annual Meeting*. Orlando, FL. March 17, 2025.

"When toxicological research informs policy: Immunotoxicological findings of per- and polyfluoroalkyl substances." *Society of Toxicology Undergraduate Diversity Program, Society of Toxicology Annual Meeting*. Orlando, FL. March 16, 2025.

"A review of current and emerging PFAS health effects and regulatory developments in the US." *Air & Waste Management Association conference on The Science of PFAS*. Raleigh, NC. March 11, 2025.

"PFAS health effects 101. *Academy for Lifelong Learning*. Corvallis, OR. February 25, 2025.

"Overview of PFAS: Need to grow emerging concepts in their hazard identification and exposure assessment." *SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety: Emerging Concepts in Hazard Identification and Exposure Assessment of Per- and Polyfluoroalkyl Substances*. Virtual. December 3, 2024.

"Tools for assessing developmental immunotoxicological impacts" Nonclinical Immunotoxicity Assessment: State of the Science and Future Directions Continuing Education Course. *American College of Toxicology 45th Annual Meeting*. Austin, TX. November 17, 2024.

"Why be concerned about PFAS? A brief overview of adverse health findings" *PFAS Conference on Alternatives*. Cophenhagen, DK. November 8, 2024.

"Developmental and juvenile immunotoxicity" *Education Subcommittee of FDA CDER's Pharmacology and Toxicology Coordinating Committee: Practical Immunology and Immunotoxicology (Fall 2024)*. Virtual. October 18, 2024.

"PFAS health effects 101." *The 17th Annual Santa Fe Advanced Conference on Natural Resource Damages*. Santa Fe, NM. September 20, 2024.

"Effects of PFAS exposure on B cell functions." *EuroTox PFAS Compounds and Immunotoxicity*. Copenhagen, Denmark. September 9, 2024.

"Risks of immunotoxicity from exposure to per- and polyfluoroalkyl substances (PFAS) are real." *Emerging Contaminants Workshop 2024: Pollution and Health*. University of Melbourne, Australia. August 14, 2024.

"Uncovering the role of environmental chemicals in disease: Focus on PFAS and the immune system. *2024 NIEHS Science, Teachers and Research Summer (STaRS) Experience* program. Virtual. July 16, 2024.

"Current state of knowledge on toxicity of per- and polyfluoroalkyl substances (PFAS) and what this means for public health." *Gordon Research Conference on Environmental Sciences: Water*. Holderness, NH. June 23, 2024.

"A complex mixture of PFAS science: A few final thoughts." *National PFAS Conference*. Ann Arbor, MI. June 12, 2024.

"A quick primer on the toxicological hazards and health risks of PFAS." *BofA PFAS Conference*. Virtual. May 31, 2024.

"Experimental evidence and underlying mechanisms of PFAS-induced immunotoxicity: *Focus on Antibody Production*." *Expert workshop on NAMS to address PFAS immunotoxicity: The results of the EFAS tender.* Virtual. May 31, 2024.

"Understanding immunotoxicological risks of exposure to per- and polyfluoroalkyl substances." *UCONN School of Pharmacy Seminar*. Storrs, CT. May 13, 2024.

"Exposure to PFAS and immunosuppression: A potential mechanism of cancer development." *Center for PFAS and Cancer Joint Virtual Symposium*. Virtual. March 8, 2024.

"*In vivo* approaches to assessing developmental immunotoxicity." *Partnership for the Assessment of Risks from Chemicals Workshop on Advancing Developmental Immunotoxicity Testing in PARC*. Virtual. March 1, 2024.

"A quick primer on the toxicological hazards and health risks of PFAS." *Water Quality Research Foundation Webinar on PFAS, Drinking Water, and What's Next*. Virtual. February 8, 2024.

"PFAS toxicity: Data from experimental animal models." *International Association of Fire Fighters ALTS/HRC 2024*. Orlando, FL. January 28, 2024.

"Evaluating immunotoxicological risks of PFAS: A North Carolina perspective." *NM-INSPIRES Center*. Seminar. December 7, 2023.

"The scientific basis for managing PFAS as a chemical class." *2023 APPAM Fall Research Conference*. Atlanta, GA. November 11, 2023.

"A quick primer on the toxicological hazards and health risks of PFAS." *UNM METALS Team*. Webinar. September 21, 2023.

"The risk is real: Immunotoxicological hazards of PFAS matter." *North Carolina Society of Toxicology*. Research Triangle Park, NC. September 14, 2023.

"Will new approach methodologies (NAMS) ever work for developmental immunotoxicological outcomes?" *Eurotox 2023*. Ljubljana, Slovenia. September 11, 2023.

"Immunotoxicity of understudied PFAS found in surface waters of the Cape Fear River of North Carolina, USA." *FLUOROS 2023*. Idstein, Germany. September 1, 2023.

"Absence of evidence for PFAS and cancer in firefighting is NOT evidence of absence." *John P. Redmond Health and Safety Symposium Dominick F. Barbera EMS Conference*. New York, NY. August 23, 2023.

"Trials on the trail of communicating health risks of exposure to per- and polyfluoroalkyl substances (PFAS). *Ohio Valley Society of Toxicology Trainee Workshop*. Virtual. July 28, 2023.

"Uncovering the role of environmental chemicals in disease: Focus on PFAS and the immune system. *2023 NIEHS Science, Teachers and Research Summer (STaRS) Experience* program. Virtual. July 12, 2023.

"Immune system development processes: Special emphasis on relationship to potential mechanisms of MIS-C." *Birth Defects Research and Prevention Annual Meeting*. Charleston, SC. June 28, 2023.

"Origin story of a first generation academic." *IDEA Learners Design Institute*. Chapel Hill, NC. June 21, 2023.

"The role of environmental factors in immunosuppression." *Climate Change and Immune Health Symposium*. Duke University, Durham, NC. June 5, 2023.

"Digging into the health endpoints behind the proposed federal drinking water MCLs for six PFAS." Webinar. *MI EGLE Toxic Steering Group Brown Bag Seminar*. May 25, 2023.

"Human health effects of PFAS exposure." Webinar. *NASEM Board on Agriculture and Natural Resources: Exploring linkages between soil health and human health Meeting 3*. May 16, 2023.

"Impact of early-life exposure to per- and polyfluoroalkyl substances (PFAS) and implications for later life immune-based diseases." *NIEHS Symposium: Latest advances in immunology inform the field of developmental immunotoxicology: A panel discussion*. American Association for Immunology annual meeting. Washington, DC. May 14, 2023.

"Challenges with optimizing DIT tests." *Alternatives to in vivo DIT Workshop 4*. Johns Hopkins University Center for Alternatives to Animal Testing. Baltimore, MD. May 9, 2023.

"Human health effects related to draft rule." Webinar. *First National Primary Drinking Water Standards for Six PFAS*. AAAS EpiCenter. May 2, 2023.

"Toxicological investigations of PFAS and implications for human health." Webinar. *Strategies for Managing and Remediating PFAS and Other Emerging Contaminants.* Environment Analyst UK. April 27, 2023.

"Understanding effects of PFAS exposure on the immune system and what it means for public health." *University of Florida Department of Environmental and Global Health Seminar Series*. University of Florida, Gainesville, FL. March 8, 2023.

"Overview of PFAS research and outreach in NC." *UNC Chapel Hill Workshop on PFAS Characterization, Removal, and Replacement*. UNC Chapel Hill, Chapel Hill, NC. January 6, 2023.

"Health effects of PFAS – Focus on the immune system." *AAAS EPICenter/ASTHO/RESOLVE Webinar on The Science of PFAS Exposure and Effects on Human Health*. Virtual, December 8, 2022.

"The ABCs of PFAS and their immunotoxicity. *Baylor University Department of Environmental Science Seminar Series*. Virtual, December 7, 2022.

"Let's talk immunotoxicity: Approaches for determining adverse immune effects across the lifespan." *Northland Regional Chapter SOT Fall 2022 Meeting.* Virtual, October 28, 2022.

"The expanding world of PFAS toxicity and what it could mean for environmental health." *University of Pennsylvania Center for Excellence in Environmental Toxicology Seminar Series*. University of Pennsylvania, Philadelphia, PA. October 20, 2022.

"PFAS toxicology: The ABCs of PFAS and their toxicology." *New Mexico Water Law CLE*. Santa Fe, NM. September 30, 2022.

"(Eco)toxicology of fluorinated polymers: A few highlights" (with Rainer Lohmann from University of Rhode Island). *Europe's PFAS problem: Situation briefings by independent experts. Session 9 Webinar*. Virtual, September 15, 2022.

"Protecting public health from per- and polyfluoroalkyl substances: Focus on immunotoxicity." *Japanese Society of Immunotoxicology Annual Meeting* and *Special Lecture at Hokkaido University*. Sapporo, Hokkaido, Japan. September 12 and 14, 2022.

"Forever in our water: The toxicity of forever chemicals known as PFAS." *ECU Coastal Studies Institute Lunch and Learn*. Wanchese, NC. August 18, 2022.

"Strategies for grouping PFAS based on toxicological understanding." *NASEM Committee on Toxicology Annual Meeting 2022*. Virtual, July 21, 2022.

"PFAS 101: A basic primer on per- and polyfluoroalkyl substances." *Geoscience Teaching Outdoors*. Greenville, NC. June 23, 2022.

"Longer-term health effects: Immune system and experimental animal data." *3rd Annual PFAS Meeting*. Wilmington, NC. June 16, 2022.

"What do we know about PFAS and human health? A brief overview with a focus on the immune system." *Environmental Health Project Webinar on PFAS, Pregnancy, and Public Health: Experts Weigh in*. Virtual, May 24, 2022.

"Getting to the bottom of PFAS-induced immune dysfunction." *Northeast Waste Management Officials' Association Conference on The Science of PFAS: Public Health and the Environment*. Marlborough, MA. April 6, 2022.

"Effects of perfluoroether acids, understudied PFAS, on the developing immune system." *Society of Toxicology Annual Meeting Symposium Session on "Developmental origins of inflammatory and immune diseases: Identifying the effects of in utero stress on immunological competency."* San Diego, CA. March 29, 2022.

"How toxicological data are used for protecting human health from per- and polyfluoroalkyl substances: Focus on immunotoxicity." *Society of Toxicology Annual Meeting Undergraduate Diversity Program*. San Diego, CA. March 27, 2022.

"What do we know about PFAS and human health? The toxicology of PFAS." *IDEA Learners Design Institute*. Chapel Hill, NC. March 14, 2022.

"Zurich I to Zurich II: In the past five years we haven't done enough to protect public & environmental health." *Zurich II On-Line Workshop*. February 10-11, 2022.

"What are human health concerns of PFAS?" *Orion Expert Network: A Lawyer's Guide to PFAS/AFFF*. Virtual Seminar. February 4, 2022.

"PFAS 101: What are PFAS and why are they health and environmental concerns?" *Ohio FACE Team*. Virtual Seminar. December 22, 2021.

"Properties, applications, and impacts of PFAS on environment, wildlife, and humans." *Lunchlezing, RIVM (The Netherlands)*. Virtual Seminar. November 18, 2021.

"Health effects of PFAS exposure: A summary of major findings." *Firefighter 4th Annual Health & Wellness Conference*. Beavercreek, OH. November 5, 2021.

"The role of a research toxicologist in responding to public health emergencies: An example of PFAS." *Research Triangle Environmental Health Collaborative 2021 Summit on Disaster Research Response and Public Health Emergencies: Creating an Environment for Resilience in North Carolina*. Virtual Seminar. November 3, 2021.

"Per- and polyfluoroalkyl substances – Emerging research for not so emerging contaminants." *Duke Integrated Toxicology and Environmental Health Program's Fall 2021 Series*. Durham, NC. October 1, 2021.

"From the lab bench to the town hall meeting: How studying PFAS has added depth to my research program." *ECU Biology Seminar*. Virtual Seminar. September 16, 2021.

"PFAS 101: A quick primer on why per- and polyfluoroalkyl substances are a problem for occupational and public health." *NFPA 1971 Task Group on Hazardous Substances*. Virtual Talk. July 22, 2021.

"PFAS impacts on the immune system." *Environmental Working Group's Inaugural PFAS Conference: PFAS health harms and solutions session*. Virtual Talk. July 14, 2021.

"Overview of putative health effects of PFAS: Toxicology." *National Academies Committee on Guidance on PFAS Testing and Health Outcomes*. Virtual Talk. July 14, 2021.

"Building capacity through PFAS communication." *SRP Risk Communication Strategies to Reduce Exposures and Improve Health Virtual Workshop.* Virtual Talk (with Katy May). June 21, 2021.

"Human health impacts of PFAS exposure." *The National Governors Association and AAAS EPI Center: Per- and Polyfluoroalkyl Substances (PFAS) and Drinking Water*. Virtual Talk. June 3, 2021.

"Per- and polyfluoroalkyl substances: Emerging research to enhance management." *National Sea Grant Law Center Webinar.* Virtual Talk. May 26, 2021.

"Current efforts and future opportunities." *Center for Human Health and Environment Annual Symposium*. Virtual Talk (with Detlef Knappe). May 15, 2021.

"PFAS toxicity: What we've learned so far." *PFAS Testing Network Virtual Forum*. Virtual Talk. May 4, 2021.

"Current developmental immunotoxicity testing status." *Alternatives to in vivo DIT Workshop, Center for Alternatives to Animal Testing at Johns Hopkins Bloomberg School of Public Health*. Virtual Talk. May 4, 2021.

"The toxicity of PFAS and why it matters." *Cape Fear River Watch First Saturday Seminar*. Virtual Talk. May 1, 2021.

"PFAS Exposure and COVID-19." *The PA Multi-site Health Study Information Session.* Virtual Talk. March 25, 2021.

"From Pollutants to People: How Studying PFAS has given my Laboratory Research Deeper Meaning." *NC Water Resources Research Institute Annual Conference*. Virtual Talk. March 25, 2021.

"An Overview of the Health Effects of PFAS." *Toxic Free NC and Climate Action NC Educational Event.* Virtual Talk. February 16, 2021.

"Unregulated and Emerging Contaminants in North Carolina Waters: Focus on PPCPs and PFAS." *Sierra Club Cypress Group.* Virtual Talk (with Dr. Sid Mitra). January 11, 2021.

"PFAS Immunotoxicity." *Environmental Working Group Press Briefing*. Virtual Talk. December 16, 2020.

"Forever Chemicals." *Tell me about it Tuesdays, Sound Rivers Science Series.* Virtual Talk. December 8, 2020.

"Why Uncovering Immunotoxicological Impacts of Understudied PFAS are Public Health Protective." *PharmTox Seminar, Michigan State University*, Virtual Seminar. November 25, 2020.

"Let's not Forget about the T in the PBMT of PFAS: An Overview of what we know about PFAS Toxicity." *EHSC 8030 Environmental Health Science, College of Public Health, University of Georgia*, Virtual Seminar. November 13, 2020.

"What can Science tell us about Potential Health Effects of PFAS found in NC: Why Understanding Effects of PFAS on the Immune System is Important." *PFAST Network Webinar*. November 6, 2020.

"PFAS 101: A 10-minute Primer on Per- and Polyfluoroalkyl Substances." *NAS Virtual Workshop on Federal Government Human Health PFAS Research.* October 26, 2020.

"Immune Investigations of some of the Understudied PFAS found in the Cape Fear River: What we've learned and were we go from here." *NC Coastal Federation Emerging Contaminants in North Carolina Waters*, Virtual Seminar. October 22, 2020.

"Immunotoxicological Evaluation of Understudied Per- and Polyfluoroalkyl Substances found in North Carolina." *WVU Microbiology & Women in Biomedical Science Seminar,* Virtual Seminar. October 15, 2020.

"PFAS: Why Immune Effects are Relevant Points of Departure for these Multisystem Toxicants." *M-LEEaD Virtual Mini-Symposium on Per- and Polyfluoroalkyl Substances (PFAS): Exposure, Toxicity, and Policy at the University of Michigan*, Virtual Seminar. October 8, 2020.

"Why Uncovering Immunotoxic Outcomes of PFAS can be a Health Protective Strategy." *Department of Environmental Medicine, NIEHS Environmental Health Sciences Center Seminar Series, University of Rochester*, Virtual Seminar. October 1, 2020.

"From Inert to Adverse: What we've learned about PFAS Toxicity in the Past Two Decades." *VME4906 Intro to Water Analysis, University of Florida Gainesville*, Virtual Lecture. September 24, 2020.

"Immunotoxicity of PFAS: Functional Toxicological Outcomes to Support Decision-Making." *Air & Waste Management Association, The Science of PFAS, Chemistry, Health and Multimedia Measurements,* Virtual Conference. September 15, 2020.

"(Eco)toxicology of PFAS: A few Highlights." *Europe's PFAS Problem: Situation Briefings by Independent Experts, European Environmental Bureau,* Virtual Webinar. September 14, 2020.

"Mechanisms of Toxicity for Per- and Polyfluoroalkyl Substances: Are we there yet?" *Chemical Exposures and Impact on Health, ACS Fall 2020 Virtual Meeting & Expo,* Virtual Meeting. August 19, 2020.

"What are PFAS and why should you care about them?" *Climate Action NC*, Virtual Public Meeting. August 13, 2020.

"Biomarkers of Immunotoxicity and Applicability to PFAS and other Environmental Toxicants," *Predicting Human Health Effects from Environmental Exposures: Applying Translatable and Accessible Biomarkers of Effect – A NAS Workshop, National Academies of Science,* Virtual Workshop. 2020.

"Developmental Immunotoxicology." *Society for Birth Defects Research and Prevention Annual Meeting,* Virtual Meeting. 2020.

"Bringing Scientific Evidence to Meet Local Policy Challenges," Session Panelist, *American Association of the Advancement of Science Annual Meeting*, Seattle, WA. February 14, 2020.

"Approaches to Understand Health Risks of Understudied PFAS." *Environmental Health Collaborative 2019 Summit on "PFAS: Integrating Science and Solutions in NC."* Durham, NC. October 23, 2019.

"An Overview of how PFAS are Toxic with the Immune System as a Specific Example." *Michigan Society of Toxicology Fall 2019 Meeting on "PFAS Exposure and Toxicology in Michigan and Beyond."* Ann Arbor, MI. October 18, 2019

"Addressing Public Health Concerns about PFAS: Focus on Immunotoxicology." *Purdue University, Chemical Exposure Research Area, Center for the Environment and School of Health Sciences Seminar Series.* Lafayette, IN. September 10, 2019.

"Immunotoxicological Findings of PFAS: Consistency of Effects between Humans and Rodent Models." *SETAC North America Focused Topic Meting: Environmental Risk Assessment of PFAS.* Durham, NC. August 13, 2019.

"Per- and Polyfluoroalkyl Substances (PFAS): A Lifecycle Perspective." *The Toxicology Forum Summer Meeting*. Alexandria, VA. July 9, 2019.

"What can PFAS do to our Health?" *North Carolina Museum of Natural Sciences Science Café: "Toxic Chemicals and Human Health*." Raleigh, NC. June 20, 2019.

"Immunotoxicological Findings of PFAS: Consistency of Effects between Rodent Models and Humans." *2019 Per- and Polyfluoroalkyl Substances: Second National Conference*. Northeastern University. Boston, MA. June 11, 2019.

"PFAS Testing Network: Team #5: Other Applied R&D." *What's in our Water? A Public Forum on Emerging Contaminants*. NC Coastal Federation. Wilmington, NC. 2019.

"Per- and Polyfluoroalkyl Substances are Immunotoxic: What does this mean for Public Health Protection?" *Environmental and Molecular Toxicology Seminar Series*, Oregon State University. Corvallis, OR. 2019.

"An Overview of what we know about PFAS Toxicology." *PFAS and Other Emerging Contaminants Conference*. American Council of Engineering Companies of North Carolina and Groundwater Professionals of North Carolina. Raleigh, NC. 2019.

"Developmental Immunotoxicology." *American College of Toxicology and Teratology Society Practical Reproductive and Developmental Toxicology*. Gaithersburg, MD. 2019.

"PFAS Toxicity." *North Carolina Waterworks Operators Association Lab Technology Day*. Raleigh, NC. 2019.

"PFAS and Health: What we've learned in the Past Two Years." *Center for Human Health and the Environment Third Annual Symposium*. North Carolina State University, Raleigh, NC. 2019.

"Three Reasons that Per- and Polyfluoroalkyl Substances do not belong in our Bodies." *University of North Carolina Wilmington Department of Chemistry and Biochemistry Seminar*, Wilmington, NC. 2019.

"PFAS Exposure: What are the Health Implications?" *Cape Fear Public Utility Authority Board Workshop*, Wilmington, NC. 2018.

"Impact of Early-Life Environmental Factors on the Developing Immune system." *PPTOX VI*, Faroe Islands. 2018.

"Health Effects of GenX: What do we know and what do we need to know to Protect Public Health?" *45th Annual Meeting of the Cape Fear River Assembly*, Wilmington, NC. 2018.

"How do we know Whether a Chemical is Toxic?" *Osher Lifelong Learning Institute Sea & Coffee Series*, University of North Carolina at Wilmington, Wilmington, NC. 2018.

"PFASs, AFFFs, PFAAs: An Alphabet Soup of Emerging Aquatic Contaminants with Immunotoxic Potential." *NCSU Toxicology Program Seminar Series*, North Carolina State University, Raleigh, NC. 2018.

"How Per- and Polyfluoroalkyl Substances (PFASs), as EDCs, can Fool the Developing Brain's Immune System." *EDC-NC Annual Meeting*. RTP, NC. 2018.

"Research Challenges Associated with PFASs: Ubiquitous Multisystem Toxicants." *The Toxicology Forum Winter Meeting*. Washington DC. 2018.

"Environmental Triggers of Underlying Neuroimmune Susceptibilities: Critical Events in Development." *Webinar*, Autism Research Institute. 2018.

"Are Replacements for the Legacy PFASs Indisputably Safe Alternatives?" *Webinar*, Toxic-Free Future. 2018.

"Per and Polyfluoroalkyl Substances: Complex Chemicals that Challenge Policies for Environmental Health Protection and Risk Communication." *Brown Bag Lunch*, RTI International, RTP, NC. 2018.

"From Legacies to Alternatives: What we know and what we need to know about the Toxicity of Per- and Polyfluoroalkyl Substances." *Weekly Seminar*, NC Department of Health and Human Services, Raleigh, NC. 2017.

"The Never-Ending Story of Per- and Polyfluoroalkyl Substances: Immunotoxicity from Legacies to Alternatives." *Mid Atlantic Society of Toxicology Annual Meeting*, Edison, NJ. 2017.

"A Potential Never-Ending Story of Chemical Water Pollution in LMICs: Proliferation of Legacy and Replacement PFASs." *International Society of Exposure Science*, Research Triangle Park, NC. 2017.

"The Science behind GenX." *Water Wednesday*, Clean Cape Fear, Wilmington, NC. 2017.

"Is it Possible to Untangle Underlying Developmental Susceptibilities from Exogenous Triggers in ASD?" *Autism Think Tank*, Autism Research Institute, Dallas-Fort Worth, TX. 2017.

"Urgent Research Needs for Better Understanding the Toxicity of PFASs." *Northeast Superfund Research Program Meeting*, Northeastern University, Boston, MA. 2017.

"Emerging Toxicological Knowledge and Data Gaps for "Novel" PFASs." *Public Workshop on Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) in Carpets, Rugs, Indoor Upholstered Furniture, and Their Care and Treatment Products*, Safer Consumer Products Program, Department of Toxic Substances Control, California Environmental Protection Agency, Sacramento, CA. 2017.

"Pharmaceuticals and Personal Care Products as Emerging Pollutants in Coastal Waters (with Dr. Siddhartha Mitra). *Science on the Sound Symposium*, Coastal Studies Institute, Wanchese, NC. 2017.

"Emerging Aquatic Contaminants and Health: Finding Solutions with Transdisciplinary Teams." Coastal Health Initiative, East Carolina University, Greenville, NC. 2016.

"Water Pollution: Is seeing believing?" *Love a Sea Turtle Second Annual Environmental Symposium*, River Park North, Greenville, NC. 2016.

"Developmental Immunotoxicology." *Middle Atlantic Reproduction and Teratology Association*, Covance Research Products, Inc., Denver, PA. 2015.

"A Little Bit of this and a Little Bit of that…How do we Understand Risks of Agents in just a Drop of Water?" *Love a Sea Turtle First Annual Environmental Symposium*, River Park North, Greenville, NC. 2015.

"Updates on Alzheimer's Disease Research in the DeWitt Lab at East Carolina University" (with Annalise vonderEmbse). *Senior Services Community Health Program*, Vidant Medical Center, Greenville, NC. 2015.

"Immunomodulatory Effects of Perfluoroalkyl Substances in Rodents and Humans." *Immunotoxicology in Food and Ingredient Safety Assessment: Approaches and Case Studies, SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety*, Washington, DC. 2015.

"Updates on Alzheimer's Disease Research in the DeWitt Lab at East Carolina University" (with Annalise vonderEmbse). *Alzheimer's Professional Partnership-Greenville*, Greenville, NC. 2015.

"From Sink to Sea: Evaluating Health Impacts of Pills and Perfumes after we Wash them away" (with Krista McCoy). *FaculTea Seminar*, East Carolina University, Greenville NC. 2014.

"Better Living through Chemistry: A Tale of Two Toxicants." *Department of Chemistry Seminar*, East Carolina University, Greenville, NC. 2014.

"The Nuts and Bolts of Interdisciplinary Toxicological Research" (with Christie Sayes). *Western Carolina University Department of Biology Spring Seminar Series*. Cullowhee, NC. 2014.

"Alzheimer's Disease and Neurodegenerative Disorders Research at East Carolina University" (with Annalise vonderEmbse). *Alzheimer's Professional Partnership-Goldsboro*, Goldsboro, NC. 2015.

"Endocrine Disruption of the Neuro-immune Interface." *The Collaborative on Health and the Environment Partnership Call* (Teleseminar). 2014
"Contaminated Drinking Water: A Case Study of Perfluorinated Compounds." *Coastal Water Resources Center*, East Carolina University, Greenville, NC. 2013.

"Villains and Heroes in the Battle for Clean Water" (with Siddhartha Mitra and Anthony Cannon). *STEM at Starlight*, Greenville, NC. 2013.

"The Nuts and Bolts of Alzheimer's Disease Research at East Carolina University." *Senior Services Community Health Program*, Vidant Medical Center, Greenville, NC. 2013.

"Alzheimer's Disease and Neurodegenerative Disorders Research at East Carolina University." *Alzheimer's Professional Partnership-Greenville*, Greenville, NC. 2013.

"A Neuroimmune Investigation of an Endocrine-Disrupting Compound". *Department of Biology Seminar*, East Carolina University, Greenville, NC. 2013.

"A Neuroimmune Investigation of an Endocrine-Disrupting Compound: How Bisphenol A may Disrupt Learning and Memory through Immunomodulation." *Endocrine Disrupting Chemicals Forum*, Research Triangle Park, NC. 2013.

"Undecafluoro-2-methyl-3-oxahexanoic Acid Versus Perfluorooctanoic Acid: Is Polyfluorination a Less Immunotoxic Option than Perfluorination?" *Department of Environmental and Molecular Toxicology Seminar*, North Carolina State University, Raleigh, NC. 2013.

"Early Life Triggers of Developmental Immunotoxicity." *Society for Toxicologic Pathology*, Annual Meeting, Denver, CO. 2011.

"Is the Pathway to Autism Paved with Environmental Chemicals?" *Department of Comparative Medicine seminar*. East Carolina University, Greenville, NC. 2011.

"PPAR Involvement in PFAA Immunotoxicity." *U.S. EPA PFAA Days III Workshop*, U.S. Environmental Protection Agency, Research Triangle Park, NC. 2010.

"Are Environmental Contaminants (Developmental) Immunotoxicants? A Case Study of a Fluorinated Compound." *Department of Microbiology and Immunology Seminar*, East Carolina University, Greenville, NC. 2010.

"Developmental Immunotoxicity of PFOA, an Emerging Contaminant." *Department of Biology Seminar*, East Carolina University, Greenville, NC. 2009.

"The Immunotoxicity of Perfluorooctanoic Acid (PFOA)." *Department of Physiology Seminar*, Brody School of Medicine, East Carolina University, Greenville, NC. 2008.

"Immunotoxic Potentials of PFOA." *U.S. EPA PFAA Days II Workshop*, U.S. Environmental Protection Agency, Research Triangle Park, NC. 2008.

"Chasing Down the Mechanism of Perfluorooctanoic Acid-Induced Immunomodulation: Knock-outs and Adrenalectomies." *National Health and Environmental Effects Research Laboratory Work in Progress*, U.S. Environmental Protection Agency, Research Triangle Park, NC. 2007.

"Wildlife Immunotoxicology." Immunotoxicology course. College of Veterinary Medicine, North Carolina State University. 2006.

"Immunotoxicity of Individual Organotin Compounds in Sprague-Dawley Rats." *Society for Risk Analysis* 25th Annual Meeting, Orlando, FL. 2005.

"Immune Function in Rats Exposed to Organotins as Adults or During Development." *National Health and Environmental Effects Research Laboratory Work in Progress*, U.S. Environmental Protection Agency, Research Triangle Park, NC. 2005.

"Brain Asymmetry in Domestic Hatchling Chickens Developmentally Exposed to TCDD: A Histological Examination." *Society of Environmental Toxicology and Chemistry* 24th Annual Meeting, Austin, TX. 2003.

"Service Learning and Scientific Research." Indiana University Community Outreach and Partnerships in Service-Learning Workshop, Indiana University, Bloomington, IN. 2004.

"Toxic Effects of Mercury." Clean Air Indiana Speak out on the Clear Skies Initiative, Indiana University, Bloomington, IN. 2003.

"Environmental Health Concerns for Toxics in Indiana Superfund Sites." Indiana Public Interest Research Group (INPIRG) Teach-In on Indiana Superfund Issues, Indiana University, Bloomington, IN. 2002.

"Introduction to Environmental Toxicology." Techniques in Environmental Science and Environmental and People courses. School of Public and Environmental Affairs, Indiana University-Bloomington. 2000, 2001, 2003.

"Bioaccumulation and Biomagnification of Environmental Chemicals in Colonial Fish-eating Waterbirds." Introduction to Environmental Sciences course. School of Public and Environmental Affairs, Indiana University-Bloomington. 2000 and 2001.

"Women in the Sciences: Abolishing Gender Apartheid." IU Skills for Leadership Conference, Office of Women's Affairs, Indiana University, Bloomington, IN. 1999.

ORAL PRESENTATIONS

"Immunomodulation associated with perfluoroether acids, understudied PFAS." *Society of Environmental Toxicology and Chemistry Annual Meeting.* Virtual Meeting. November 10, 2021.

"Developing an Understanding of the Effects of PFAS on the Immature Immune System." *Symposium on Developmental toxicity of per- and polyfluoroalkyl substances (PFAS): Current in vivo approaches and application to human health risk assessment, Society of Toxicology Annual Meeting*, Virtual Meeting. 2020.

"Immunotoxicity Evaluation as a Tool for Protecting Public and Environmental Health from PFAS." *Society of Environmental Toxicology and Chemistry* 40th Annual Meeting, Toronto, Ontario. 2019.

"Immunotoxicological Findings of an Aqueous Film-Forming Foam Formulation." *Society of Environmental Toxicology and Chemistry annual meeting*, Sacramento, CA. 2018.

"Perspectives from the AAMC Mid-Career Women Faculty Professional Development Seminar "MIDWIMS"). *Brody Women Faculty Committee*. Greenville, NC. 2017.

"Immunopathogenesis in Autism: Regulatory T Cells and Markers of Autoimmunity in Mice Developmentally Exposed to Perflurooctanoic Acid (PFOA). *27th Annual NeuroToxicology Conference*, Annual Meeting, Durham, NC. 2011.

"PFOA-induced Immunomodulation in mice: An Overview." *Society of Toxicology* 48th Annual Meeting, Baltimore, MD. 2009.

"Pathways of PFOA-mediated Immunosuppression." *Society of Toxicology* 48th Annual Meeting, Baltimore, MD. 2009.

"Dose-response of Perfluorooctanoic Acid-Induced Immunomodulation in Adult C57BL/6 Mice." *Society of Toxicology* 46th Annual Meeting, Charlotte, NC. 2007.

"Immune Function in Rats Developmentally Exposed to Dibutyltin Dichloride." *Society of Toxicology* 45th Annual Meeting, San Diego, CA. 2006.
"Neurotoxic Effects in Avian Species: Implications for Human and Ecological Health." School of Public and Environmental Affairs *2nd Annual Young Researchers Conference*, Indiana University, Bloomington, IN. 2002.

"TCDD-Induced Brain Asymmetry and Behavior: What do Individual Chicks Have to Say?" *Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry*, Hueston Woods State Park, College Corner, OH. 2000.

"Behavioral and Morphological Changes in Domestic Chicks Exposed to TCDD or PCB-126 at Embryonic Day 0 or Embryonic Day 4." *Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry*, Indiana University, Bloomington, IN. 1997.

"Behavioral Changes in Domestic Hatchling Chicks Exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) *in ovo*." *Conference on Chlorine in the Environment*, Massachusetts Institute of Technology, Boston, MA. 1996.

"Behavioral Assessment of Hatchling Chicks Exposed to TCDD *in ovo*: Preliminary Results." *Great Lakes Bioeffects Workgroup*. Wright State University, Dayton, OH and *Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry*, Eastern Kentucky University, Richmond, KY. 1996.

CONTINUING EDUCATION COURSES

Mid-Career Women Faculty Leadership Development Seminar
Stress as a Confounding Factor in Toxicology Studies
Rodent Pathology (Immunopathology)
Basic Embryology and Developmental Toxicology
Grants 101: Professional Grant Writing Workshop
Immunology for Toxicologists
Risk Communication for the General Public
Estrogen Mimics in Health and Disease
Methods for Assessment of Neurotoxicity

PROFESSIONAL ORGANIZATIONS

2023-present	Pacific Northwest Association of Toxicologists Regional Chapter of SOT
2019-2025	The Toxicology Forum
2017-2018	International Society of Exposure Science
2009-2023	Carolinas Society of Environmental Toxicology and Chemistry
2005-Present	Society of Toxicology (SOT)
2005-2023	North Carolina Chapter of the Society of Toxicology
1997-Present	Society of Environmental Toxicology and Chemistry (SETAC)
1996-2004	Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry
1996-2004	Great Lakes Bioeffects Workgroup

AWARDS AND HONORS

- *Best Oral Presentation Award*, FLUOROS. 2023.
- *Public Communications Award*, Society of Toxicology. 2023.
- *Scholar-Teacher Award,* East Carolina University, Office of Faculty Excellence. 2021-2022.
- *Engagement and Outreach Scholars Academy Scholar*, East Carolina University, Office of Community Engagement and Research. 2019-2020.
- *The Faculty Mentor Award*, East Carolina University Honors College. 2017.
- *Outstanding Young Investigator Award*, Immunotoxicology Specialty Section, Society of Toxicology. 2013.

- *Outstanding Teaching Award*, School of Public and Environmental Affairs, Indiana University. 1999 and 2002.
- *Future Faculty Teaching Fellowship*, Preparing Future Faculty program, Indiana University. 2002.
- *Marian Vinegar Award*, Outstanding Student Presentation at the annual meeting, Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry. 2000.
- *Outstanding Educational Volunteer*, Monroe County Humane Association, Bloomington, IN. 2000.
- *Outstanding Student Poster Award*, Society of Environmental Toxicology and Chemistry 19th Annual Meeting (3rd place). 1998.
- *Teaching Excellence Recognition Award*, School of Public and Environmental Affairs, Indiana University. 1998.

PROFESSIONAL PRACTICE

Select Committee on PFAS (per and polyfluoroalkyl substances). 2025.

- Provided overview of human health effects of PFAS exposure. July 2025.

PFAS 2025 Rulemaking Advisory Committee. 2024-2025.

- Committee supporting the Oregon Department of Environmental Quality as they consider proposed rulemaking to include to per- and polyfluoroalkyl substances (PFAS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in the definition of hazardous substances in Oregon.

PFAS Applied Science Fellow. 2022.

- Research Fellow at the North Carlina Department of Environmental Quality (DEQ) through support of the North Carolina Collaboratory. Worked with DEQ staff on applied PFAS science.

IAFF Cancer Research Committee. 2022-2024.

- Committee of the International Association of Fire Fighters (IAFF) charged with evaluating cancer research projects and providing insight to the IAFF's plan of action regarding cancer research.

U.S. Environmental Protection Agency PFAS Science Advisory Board. 2021-2022.

- External review board charged with reviewing draft documents developed by the US EPA to support National Primary Drinking Water Rulemaking for PFAS.

Flemish Parliamentary Committee of Inquiry PFAS-PFOS Testimony. 2021.

- Provided overview of human health effects of PFAS exposure with a focus on PFOS. July 2021.

Emerging Science for Environmental Health Decisions Standing Committee Member, National Academies of Science, Engineering, and Medicine (NASEM). 2021-2023.

- Charged with convening several workshops each year to explore how new scientific advances, technologies, and research methodologies could deepen understanding of effects of environment on human health.

Guidance on PFAS Testing and Health Outcomes Community Liaison, National Academies of Science, Engineering, and Medicine (NASEM). 2021-2022.

- Community Liaisons charged with providing information to the Committee on various aspects of PFAS, including health and environmental effects as well as personal stories.

North Carolina Secretaries Science Advisory Board Member, NC Department of Environmental Quality (DEQ) and NC Department of Health and Human Services (DHHS). 2019-present.

- Charged with advising the DEQ and DHHS on toxicological effects of contaminants and levels of control necessary for protection of human health and the environment.

North Carolina Cancer Advisory Research Panel Member, NC Policy Collaboratory. 2019-2020.

- Charged by the NC General Assembly with recommending strategies for assessing cancer incidence and mortality rates with respect to temporal and spatial patterns within NC.

Tennessee PFAS External Advisory Group Member, Tennessee Departments of Environment and Conservation and Health. 2019-2022.

- Charged with informing the state of Tennessee about PFAS, including compound characteristics, identification, sampling and measurement, remediation, etc.

U.S. House of Representatives Congressional Testimony. 2019, 2021.

- Committee on Energy and Commerce, *Subcommittee on Environment and Climate Change*, "Protecting Americans at Risk of PFAS Contamination and Exposure." May 15, 2019.
- Committee on Oversight and Reform, *Subcommittee on Environment*, "The Devil they Knew – PFAS Contamination and the Need for Corporate Accountability." July 24, 2019.
- Committee on Appropriations, *Subcommittee on Military Construction, Veterans Affairs, and Related Agencies,* "Remediation and Impact of PFAS." March 24, 2021.

Michigan Science Advisory Workgroup Member, Michigan PFAS Action Response Team (MPART). 2019.

- Charged with advising the state of Michigan on Maximum Contaminant Level recommendations for PFAS.

PFAS Testing Network Executive Advisory Committee Member, NC Collaboratory. 2018-present.

- Responsible for advising the Network on addressing occurrence and effects of PFAS in drinking water resources in the state of NC.

Workshop Participant, Sustainability consequences of chemical exposures: connecting environment, health, and economic assessments. Organized by the European Environmental Agency, Copenhagen, Denmark. June 2018.

- Panelist representing the toxicological sciences.

Global PFAS Science Panel Member. Established from the co-authors of the Zurich Statement (Ritscher et al., 2018). 2017-present.

- Group of academic and government scientists dedicated to fostering development of high-quality scientific research, stewarding information exchange, and coordinating advancement of science and policy to address the class of per- and polyfluoroalkyl substances (PFASs) as a global concern.

Plaintiff Expert Witness, various legal firms, to provide toxicological expertise regarding per- and polyfluoroalkyl substances (PFAS). 2014-present.

- Involved in several settled and ongoing cases involving personal injury, class action, and public utilities across different sites within the U.S. Provide expert reports. Have been deposed in several cases but have not yet testified in a court of law.

Pro-bono Consultation, various environmental protection and advocacy organizations, 2015-present.

- Provide scientific interpretation and opinion regarding toxicity of per- and polyfluoroalkyl substances to individual community members as well as organizations.
- Organizations include *California Environmental Protection Agency, Cape Fear Public Utility Authority, Center for Environmental Health, Green Science Policy Institute, Massachusetts Toxic Use Reduction Program, Silent Spring Institute, Toxic Free Future, Amigos Bravos, and other community-based and occupationally-based organizations.*

Workshop Participant, Is the European Food Safety Authority (EFSA) standard for bisphenol A sufficiently protective of the immune system and should the Dutch government consider a different standard? Organized by representatives of RIVM (National Institute for Public Health and the Environment), Amsterdam, The Netherlands. September 2015.

- Provided immunotoxicological guidance for regulatory consideration of bisphenol A (BPA).

Consultant, CZR Incorporated, Wilmington, NC. May-July, 2014-2019.

- Provided toxicological interpretation of stream water quality monitoring data for heavy metals.

Technical Advisor, Office of Health Assessment and Translation (OHAT), National Toxicology Program, National Institute of Environmental Health Sciences. March 2013. March 2015. April 2016.

- Evaluated OHAT protocol for evaluation of PFOS-PFOA immunotoxicity.

External Peer Reviewer, U.S. Environmental Protection Agency, External peer review of EPA's Draft Health Effects Documents for Perfluorooctanoic acid (PFOA) and Perfluorooctane Sulfonate (PFOS). 2014.

- Scientific peer reviewer of the health effects documents. Nominated and selected.

Working Group Member, International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 110: Perfluorooctanoic acid, Tetrafluoroethylene, Dichloromethane, 1,2-Dichloropropane, and 1,3-Propane sultone. 2014.

- Member of Mechanistic and Other Relevant Data Working Group for perfluorooctanoic acid and tetrafluoroethylene. Invited.

Consultant, Constella Group, LLC, Durham, NC. October 2004-December 2005.

- Summarized immunotoxicology of atrazine for the National Toxicology Program's Report on Carcinogens.

Consultant, Henshel EnviroComm, Bloomington, IN. June 1999-May 2004.

- Representative of the Restoration Advisory Board for Jefferson Proving Ground (Department of Defense) through the Technical Assistance for Public Participation program.

- Interpreted risk assessment documents associated with base clean-up for the general public.

Consultant, Dinosaur Inc., Bloomington, IN. June 2000-September 2000.
- Summarized the potential health and environmental effects of land-applied paper mill sludge.

Consultant, Integrated Pest Management in Schools and Childcare Centers, School of Public and Environmental Affairs Information Clearinghouse, Bloomington, IN. April 2000-September 2000.
- Summarized the potential health effects of pesticides commonly used in schools and childcare centers.

Co-Director, *Summer Program for Exploration of Complex Issues in Environmental Science for Teachers (SPECIES-Teachers)* and *Environmental Education 99*, School of Public and Environmental Affairs, Indiana University, Bloomington, IN. Summer 1999 and 2001.
- Directed hands-on environmental science summer field workshop for Indiana teachers.

Consultant, Brownstown Elementary Fourth Grade, Brownstown, IN. September 1999-May 2000.
- Served as environmental science expert during weekly videoconferences in a "students as environmental scientists" program.

Associate Director, *Research Experience for High School Students*, College of Arts and Sciences, Indiana University, Bloomington, IN. February 1999-October 1999.
- Mentored high school students participating in research in university laboratories, provided weekly counseling, and oversaw development of final research reports and presentations.

Co-Director, *Environment 98* and *Environment 99*, School of Public and Environmental Affairs, Indiana University, Bloomington, IN. Summer 1998 and 1999.
- Directed hands-on environmental science summer field workshop for Indiana students.

Chemical Safety Assistant, Office of Radiation, Chemical and Biological Safety, Michigan State University, East Lansing, MI. August 1993-June 1995.
- Developed Michigan State University's Chemical Hygiene Plan, performed university-wide laboratory safety inspections, and trained new science employees in chemical and laboratory safety.

TEACHING EXPERIENCE

Instructor, Oregon State University
- Experimental Design and Statistics for EMT Students. Graduate special topics course (course director). 2025.
- Fundamentals of Toxicology. Combined graduate and undergraduate (course director). 2024-present.

Instructor, East Carolina University
- Foundations in Biomedical Science. Graduate (topical: drug safety, endocrine pharmacology, toxicology). 2020-2023.
- Pandemic Crisis Management. Third year medical students (small group facilitator). 2020.

- Practical Problems in Biometry. Graduate (course director). 2010-2023 (co-director 2021).
- General Toxicology. Graduate (course co-director). 2009-2023.
- Advanced Toxicology. Graduate (course co-director). 2016-2023.
- Medical Pharmacology. Graduate physician assistant students (topical: endocrine pharmacology, toxicology). 2010-2018.
- Medical Pharmacology. Second year medical students (topical: endocrine pharmacology, toxicology). 2012-2023.
- Pharmacology Mini-Course. First and second year dental students (topical: toxicology, management of poisoned patients, endocrine pharmacology, pharmacokinetics I & II; variable and shared among other instructors). 2012-2023.
- Foundations of Medicine/Problem Based Learning Mini-Course. First and second year medical students (small group facilitator). 2011-2013.
- Foundations of Medicine/PirateMD. First and second year medical students (small group facilitator). 2013-2019.

Instructor, Indiana University

- Analytical Problem Solving (statistics). Undergraduate, honors, 2 semesters.
- Environment and People. Undergraduate (co-instructor), 1 semester.
- Introduction to Statistical Techniques. Undergraduate, 1 semester.
- Introduction to Environmental Sciences. Undergraduate, 1 semester.
- Environmental Risk Analysis. Graduate, 1 semester.
- Outdoor Environmental Awareness (Public land management). Undergraduate recruitment course, 6 semesters.
- Introduction to Risk Assessment and Risk Communication. Undergraduate, 1 semester.
- Environmental Toxicology. Combined undergraduate/graduate, 1 semester.
- Techniques in Environmental Science (field/lab techniques). Undergraduate, 3 semesters.

Teaching Assistant/Coordinator, Indiana University

- Aquatic Habitat Analysis (field techniques). Combined undergraduate/graduate, 3 semesters.
- Terrestrial Habitat Analysis (field techniques). Combined undergraduate/graduate, 3 semesters.
- Environmental Toxicology. Undergraduate and graduate, 3 semesters.
- Teaching Assistant and Undergraduate Teaching Intern Training Workshop. Combined undergraduate and graduate, 6 sessions.

Laboratory Mentor, East Carolina University and Oregon State University

High school students

- Nava Mazumder (high school laboratory research). Project: Microglial impacts following exposure to PFAS. 2023.
- Megan McKeel, Medical Honors Program (high school laboratory research). Project: Developmental effects of PFAS in a rodent model. 2021-2022.
- Abigail Yoon and Andrew Gallagher, Medical Honors Program (high school laboratory research). Project: Body and liver weight changes in rodents exposed to a binary PFAS mixture (virtual). 2020-2021.
- Javier Limon and John Mallett, Medical Honors Program (high school laboratory research). Project: Liver lipid accumulation in livers from rodents exposed to PFAS. 2019-2020.
- Catherine Wondra and Anushka Nandy, Summer Ventures Program (high school laboratory research). Project: Liver lipid accumulation in livers from rodents exposed to PFAS. 2019.
- Alex Beaver and Margarita Anayiotou, Summer Ventures Program (high school laboratory research). Project: Neuroinflammation in a rodent model of Gulf War Illness. 2018.
- Allison Gallagher and Sophie Villani, Medical Honors Program (high school laboratory research). Project Developmental effects of pharmaceutical pollutants in an avian model. 2017-2018.

- Sunnie Li and Alex Reulbach, Summer Ventures Program (high school laboratory research). Project: Neuroinflammation in a rodent model of Gulf War Illness. 2017.
- Matthew Clayton, high school laboratory research for the NC Science Fair. Project: Developmental effects of pharmaceutical pollutants in an avian model. 2016.
- Virginia Billings, Summer Ventures Program (high school laboratory research). Project: Developmental effects of pharmaceutical pollutants in an avian model. 2016.
- Jaein Yoon, Medical Honors Program (high school laboratory research). Project: Developmental effects of pharmaceutical pollutants in an avian model. 2015-2016.
- Chevonne Parker, Summer Ventures Program (high school laboratory research). Project: Neuronal T-cell infiltration following developmental trichloroethylene exposure. 2015.
- Catherine Taylor and Jessi Zhou, Medical Honors Program (high school laboratory research). Project: Microglial responses in an Alzheimer's mouse model developmentally exposed to lead. 2014-2015.
- Janelle Neal, Summer Ventures Program (high school laboratory research). Project: Microglial responses following inhalation exposure to natural dusts. 2014.
- Brian Alloway, Medical Honors Program (high school laboratory research). Project: Peroxisome proliferation in livers of C57BL/6 mice exposed to undecafluoro-2-methyl-oxahexanoic acid (U2M3-OHxA) gavage. 2013-2014.
- Kortney Wager, Summer Ventures Program (high school laboratory research). Project: Developmental effects of BPA on immune responses. 2013.
- Brian Ennis and Jonathan Reed, Medical Honors Program (high school laboratory research). Project: Teratogenicity of PFOS in early chicken embryos. 2012-2013.
- Willa Chen, Summer Ventures Program (high school laboratory research). Project: Developmental effects of PFOA in primary cardiomyocyte cultures from chickens. 2012.
- Elizabeth Fox and Samantha Rouse, Medical Honors Program (high school laboratory research). Project: Teratogenicity of PFOS in early chicken embryos. 2011-2012.
- Pranavi Sanka, Summer Ventures Program (high school laboratory research). Project: Developmental effects of PFOS: T cell infiltration into mouse brains. 2011.
- Erin Morris and Andrew Wood, Medical Honors Program (high school laboratory research). Project: Developmental effects of PFOA on T cell infiltration and myelin basic protein levels in mouse brains. 2010-2011.
- Jillian Loftis, Summer Ventures Program (high school laboratory research). Project: Developmental effects of PFOA on glycogen deposition in a chicken model. 2010.
- Clarissa Morrissey, Medical Honors Program (high school laboratory research). Project: Teratogenicity of PFOA in early chicken embryos. 2009-2010.
- Taylor Brundage, Summer Ventures Program (high school laboratory research). Project: Developmental teratogenic effects of PFOA in a chicken model. 2009.
- Ian Bryan, Medical Honors Program (high school laboratory research). Project: Developmental effects of PFOA on liver glucocorticoid receptor levels, and pancreatic alpha and beta cells in a mouse model. 2008-2009.

Undergraduate students

- Olivia Booker, Bioresource Research (undergraduate laboratory research): Peroxisome proliferation and PPAR-alpha in tissues from rodents exposed to PFAS. 2026-present.
- Laila Major, Biomedical Engineering (undergraduate honors thesis research): Macrophage infiltration in skin of rodents exposed to PFAS. 2026-present.
- Nicolas Sousou, Biochemistry (undergraduate laboratory research): Peroxisome proliferation in tissues from rodents exposed to PFAS. 2025-present.
- Kolette Miller, Bioresource Research (undergraduate laboratory research): NK cell cytotoxicity methods development. 2025-present.
- Ekansh Gupta, Honors College (undergraduate honors thesis research): B cell bioenergetic profiles. 2025-present.

- Alex Lowe, Environmental Engineering (undergraduate laboratory research): In vitro B cell methods development. 2025.
- Sydney Luong, Biochemistry (undergraduate laboratory research): Peroxisome proliferation following PFAS exposure in a rodent model. 2024-2025.
- Ashanti Moore, Program in Neuroscience (undergraduate laboratory research): Systematic review of PFAS neurotoxicity. 2023-2024.
- Sadie Graham, Biology (undergraduate laboratory research): Peroxisome proliferation following PFAS exposure in a rodent model. 2023.
- Yveonna West, Program in Neuroscience (undergraduate laboratory research): Neurotoxic effects of PFAS in a rodent model. 2022-present.
- Jessica Bartram, Program in Neuroscience (undergraduate laboratory research): Neurotoxic effects of PFAS in a rodent model. 2022-2023.
- Megan Harmon, Biology (undergraduate laboratory research): PFAS neurobehavioral effects. 2021-2023.
- Nia Moore, Summer Biomedical Research Program and Public Health (undergraduate laboratory research). Project: Developmental immunotoxicity of PFAS in rodent model. 2022-2023.
- Matthew Wittenborn, Biology (undergraduate laboratory research): Quality assurance and quality control. 2021-2022. NCBC intern 2022-2023.
- Olivia Glazer, Program in Neuroscience (undergraduate laboratory research): Inflammatory biomarkers in a rodent model of Gulf War Illness. 2021-2023.
- Cierra Robbins, and Mackenzie Morrow, Program in Neuroscience (undergraduate laboratory research): Inflammatory biomarkers in a rodent model of Gulf War Illness. 2021-2022.
- Jasmine Clark, University Studies (undergraduate laboratory research): PFAS as air pollutants. 2019-2021.
- Jeffery Ayala, and Mark Ibrahim, Program in Neuroscience (undergraduate laboratory research): Microglial morphology in a rodent model of Alzheimer's disease. 2018-2021.
- Payton Anders, Jeffery Ayala, and Mark Ibrahim, Program in Neuroscience (undergraduate laboratory research): Microglial morphology in a rodent model of Alzheimer's disease. 2018.
- Alexis Liberatore, Program in Neuroscience (undergraduate laboratory research): Glucocorticoid receptor expression in avian brains following developmental exposure to pharmaceutical pollutants. 2018-2019.
- Kathleen Ferris, Biology (undergraduate laboratory research): Peroxisome proliferation in livers of rodents exposed to aqueous film forming foam. 2018-2019.
- Robert Strickland, General Studies/Program in Neuroscience (undergraduate laboratory research). Project: Neuroinflammation in a rodent model of Gulf War Illness. 2017.
- Chastity Ward, Summer Biomedical Research Program (undergraduate laboratory research). Project: Immunotoxic effects of AFFF in a rodent model. 2017.
- Christopher Hamby, Mutidisciplinary Studies Program in Neuroscience (undergraduate laboratory research/senior thesis advisor). Project: Microglial morphology in a rodent model of Gulf War Illness. 2016-2017.
- Ishmael Gomez, Summer Biomedical Research Program (undergraduate laboratory research). Project: DAP12 microglial signaling in a rodent model of Alzheimer's disease. 2016.
- Brianna Davidson, Multidisciplinary Studies Program in Neuroscience (undergraduate laboratory research). Project: Synaptic degeneration in a rodent model of Alzheimer's disease. 2016.
- Samuel Vance, Mutidisciplinary Studies Program in Neuroscience (undergraduate laboratory research/senior thesis advisor). Project: Project: Post-translational modifications and Alzheimer's pathology. 2015-2017.

- Waeya Lin, Summer Biomedical Research Program (undergraduate laboratory research). Project: Exacerbation of Alzheimer's pathology by prenatal exposure to lead; Dystrophic microglia. 2015.
- Giovana Fernanda Cosi Bento, Brazil Scientific Mobility Program (undergraduate laboratory research). Project: Exacerbation of Alzheimer's pathology by prenatal exposure to lead; Synaptosomes. 2015.
- Zoe Hinton, Mutidisciplinary Studies Program in Neuroscience (undergraduate laboratory research/senior thesis advisor). Project I: Exacerbation of Alzheimer's pathology by prenatal exposure to lead; Synaptosomes. 2015. Project II: Microglial morphology in a rodent model of Gulf War Illness. 2016-2017.
- Andrew Wood, Biology (undergraduate honors thesis advisor). Project: Exacerbation of Alzheimer's pathology by prenatal exposure to lead; Measurement of amyloid beta. 2014-2015.
- Dakota Johnson, Biology (undergraduate honors thesis advisor). Project: Exacerbation of Alzheimer's pathology by early-life exposure to lead; Measurement of amyloid beta. 2013-2014.
- Sydney Henry, Summer Biomedical Research Program (undergraduate laboratory research). Project: Neurotoxic effects of dust collected from the Nellis Dunes Recreation Area. 2014.
- Andrew Wood, Biology undergraduate student. Project: Developmental effects of BPA on serum IL-4 and IgG. 2013.
- Dominique Baldwin, Biology undergraduate student. Project: Neurotoxic effects of dust collected from the Nellis Dunes Recreation Area. 2013.
- Megan Biller, Summer Biomedical Research Program (undergraduate laboratory research). Project: Microglia in Alzheimer's-prone triple transgenic mice. 2013.
- Blake Rushing, Summer Biomedical Research Program (undergraduate laboratory research). Project: Blood distribution, urinary excretion, and T cell-dependent immunotoxicity of undecafluoro-2-methyl-oxahexanoic acid (U2M3-OHxA) in C57BL/6 mice exposed via gavage. 2012.
- Alvin-Ming-Yun Tsang, Psychology (undergraduate honors thesis advisor). Project: Developmental effects of nanoparticles in a rodent model. 2011-2012.
- Nick Creech, Summer Biomedical Research Program (undergraduate laboratory research). Project: Immunological effects of PFOA on a T-cell independent antigen (DNP-Ficcol) in an adult mouse model. 2010-2011.
- Hatel Patel, Biochemistry undergraduate student. Project 1: Developmental effects of PFOA on liver peroxisomes proliferation in a chicken model. Project 2: Effects of PFOA on myelin basic protein levels in the brains of developmentally-exposed mice. 2009.
- Ian Bryan, Biology and Chemistry undergraduate student. Project: Developmental effects of PFOA and PROS in a chicken model, including early embryo teratogenesis and hatchling glycogen levels. 2009-2012.

Master's students

- Ashtyn Chamberland, Environmental and Molecular Toxicology Master's student. Project: Natural killer cell cytotoxicity following freeze-thaw cycles. 2025-2026
- Kelsie Younce, Environmental and Molecular Toxicology Master's student. Project: Comparison of PFOA immunotoxicity and serum concentrations across two different routes of oral exposure in a rodent model. 2025-2026.
- Alex Soo, Environmental and Molecular Toxicology Master's student. Project: Immunotoxicity of PFAS following dermal exposures in a rodent model. 2025-2026.
- Kellyn Trowse, Biomedical Sciences Master's Student. Project: Developmental neurotoxic effects of PFAS. 2023-2024 (J. Eells thesis advisory committee chair as of September 2023).

- Erica Stewart, Biomedical Sciences Master's Student. Project: Developmental immunotoxicological effects of PFAS. 2020-2021.
- Emma Tobin, Biomedical Sciences Master's Student. Project: Immunotoxicological effects of PFAS of emerging concern in the Cape Fear River. 2019-2021.
- Samuel Vance, Biomedical Sciences Master's Student. Project: Immunotoxicological effects of PFMOAA, a contaminant of emerging concern in the Cape Fear River. 2018-2019.
- Carmen Davis, Environmental Health Master's Student. Project: Developmental effects of Triclosan in an avian model. 2015-2016.
- Cory Boles, Biomedical Sciences Master's Student. Project: Exacerbation of Alzheimer's pathology by early-life exposure to lead. 2013-2015.
- Annalise vonderEmbse, Biomedical Sciences Master's Student. Project: Exacerbation of Alzheimer's pathology by early-life exposure to lead; Effects on microglia. 2012-2014.

Doctoral students

- Shyla Streeter, Environmental and Molecular Toxicology Ph.D. student. Project: Descriptive immunotoxicological effects of understudied PFAS in a rodent model. 2024-present.
- Macon Carroll, Environmental and Molecular Toxicology Ph.D. Student. Project: Mechanisms of B-cell directed immunotoxicity of PFAS. 2023-present.
- Aya Ahmed, Interdisciplinary Doctoral Program in Biology, Biomedicine, and Chemistry Ph.D. Student. Project: B-cell bioenergetics and antibody production. 2022-2025 (T. Woodlief dissertation advisory committee chair 2023-2025).
- Krystal Taylor, Pharmacology and Toxicology Ph.D. Student. Project: Mechanisms of B-cell directed immunotoxicity of PFAS. 2020-2023.
- Jacqueline Meadows, Pharmacology and Toxicology Ph.D. Student. Project: Developmental effects of pharmaceutical pollutants in an avian model. 2015-2019.
- Annalise vonderEmbse, Pharmacology and Toxicology Ph.D. Student. Project: Exacerbation of Alzheimer's pathology by early-life exposure to lead; Effects on microglia. 2014-2017.
- Jason Franklin, Pharmacology and Toxicology Ph.D. Student. Project: Developmental neuroimmunotoxicity of bisphenol a in a rodent model. 2010-2014.
- Qixiao Jiang. Pharmacology and Toxicology Ph.D. Student. Project: Developmental cardiotoxicity of perfluorinated compounds in an avian model. 2009-2013.

Medical students

- Amie McPherson and Danesh Ghiassi, Medical students. Project: Isolation and stimulation of regulatory T cells from spleens of PFOA-exposed mice. 2009.

Postdoctoral Scholars

- Dr. Drake Phelps. Project: Immunotoxicological mechanisms of PFAS. 2023.
- Dr. Tracey Woodlief. Project: Immunotoxicological mechanisms of PFAS. 2019-2020.

Research Faculty

- Dr. Tracey Woodlief. 2021-2023.

Graduate Council Representative, Oregon State University

- Jacob Kreitzer, Comparative Health Sciences MS Student (Advisor: Siva Kolluri, Environmental and Molecular Toxicology). 2025-present.
- Brandon Lindsey, Marine Resource Management MS Student (Advisor: Samuel Chan). 2025-2026.
- Luke Stuntz, Department of Fisheries, Wildlife, and Conservation Sciences MS Student (Advisor: Rachael Orben). 2024.

Advisory, Oregon State University

- Leo Ramirez, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Susan Tilton). 2025-present.
- Kalealani Gurfield, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Kim Anderson). 2025-present.
- Eric Luera, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Kim Anderson). 2025-present.
- Sophia Nelson, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Jennifer Field). 2025-present.
- Mackenzie Allison, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Susan Tilton). 2025-present.
- Ashley Torres, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Robyn Tanguay). 2025-present.
- Kiani Stowers-Fann, Environmental and Molecular Toxicology Professional Master's Student (Advisory committee; Advisor: Jennifer Duringer). 2025-present.
- Owen Brown, Environmental and Molecular Toxicology Professional Master's Student (Advisory committee; Advisor: Robyn Tanguay). 2025-2026.
- Elijah Cowan, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Robyn Tanguay). 2024-present.
- Mireia Roig-Paul, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisors: Jennifer Field & Serhan Mermer). 2024-present.
- Francesca Germano, Environmental and Occupational Public Health Ph.D. Student (Dissertation committee; Advisor: Diana Rohlman). 2024-present.
- Alison Clark, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Kim Anderson). 2023-present.
- Spencer Stinson, Environmental and Molecular Toxicology Ph.D. Student (Dissertation committee; Advisor: Robyn Tanguay). 2023-present.

Advisory, Other

- Yoonsub Kim, Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill Ph.D. Student (Dissertation committee; Advisor: Barbara Turpin). 2025-present.
- Emma Hepworth, Toxicology Ph.D. Student (Dissertation committee; Advisor: Jeff Yoder, NC State). 2023-present.
- Ashley Connors, Toxicology Ph.D. Student (Dissertation committee; Advisor: Jeff Yoder, NC State). 2023-present.
- Wesley Czika, Anatomy and Cell Biology Master's Student (Thesis committee; Advisor: Karen Litwa, East Carolina University). 2023.
- Emma Gilbert, Anatomy and Cell Biology Ph.D. Student (Dissertation committee; Advisor: Chris Geyer, East Carolina University). 2023-present.
- Riley Bessetti, Anatomy and Cell Biology Ph.D. Student (Dissertation committee; Advisor: Karen Litwa, East Carolina University). 2022-2025.
- Maddison Salois, Biochemistry and Molecular Biology Ph.D. Student (Dissertation committee; Advisor: Maranke Koster, East Carolina University). 2022-2023.
- Alexander Bastian, Microbiology and Immunology Ph.D. Student (Dissertation committee; Advisor: Mark Mannie, East Carolina University). 2022.
- Hannah Woolard, Physiology/ Biomedical Sciences Master's Student (Thesis committee; Advisor: Stefan Clemens, East Carolina University). 2021.
- Valeria Fuentes, Chemistry Master's Student (Thesis committee, Advisor: Eli Hvastkovs, East Carolina University). 2021-2022.
- Mandee Schaub, Physiology/Biomedical Sciences Master's Student (Thesis committee; Advisor: Stefan Clemens, East Carolina University). 2021-2022.

- Sarah Lowe, Microbiology and Immunology Ph.D. Student (Dissertation committee; Advisor: Michelle Ratliff, East Carolina University). 2021-2023.
- Cody Hatchet, Biochemistry/Biomedical Sciences Master's Student (Thesis committee; Advisor: Ruth Schwalbe, East Carolina University). 2020-2021.
- David Ogden, Internal Medicine/Biomedical Sciences Master's Student (Thesis committee; Advisor: Mary Jane Thomassen, East Carolina University). 2021.
- Rebecca Nickle, Microbiology and Immunology Ph.D. Student (Dissertation committee; Advisor: Mark Mannie, East Carolina University). 2020-2021.
- Rohan Parek, Pharmacology & Toxicology Ph.D. Student (Dissertation committee, Advisor: Srinivas Sriramula, East Carolina University). 2019-2022.
- Christopher Norton, Microbiology and Immunology Master's Student (Thesis committee; Advisor: Isabelle Lemasson, East Carolina University). 2019-2020.
- Danielle Dillane (Carter), Public Health Ph.D. Student (Dissertation committee; Advisor: Jo Anne Balanay, East Carolina University). 2020-2022.
- Heidi Knecht and Danielle Carter, Public Health Ph.D. Students (Dissertation committee; Advisor: Stephanie Richards, East Carolina University). 2018-2020.
- Megan Rhyne, Environmental Health and Health Education & Promotion Master's Student (Thesis committee; Advisor: Stephanie Richards). 2018.
- Dariel Hopersberger, Microbiology and Immunology Ph.D. Student (Dissertation committee; Advisor: Marty Roop, East Carolina University). 2018-2022.
- Alexandra Hayes, Microbiology and Immunology Master's Student (Thesis committee; Advisor: Rachel Roper, East Carolina University). 2018-2019.
- Kayla DeOca, Microbiology and Immunology Ph.D. Student (Dissertation committee; Advisor: Mark Mannie, East Carolina University). 2018-2021.
- Henry Raab, Coastal Resource Management Ph.D. Student (Dissertation committee; Advisor: Joe Luczkovich, East Carolina University). 2017-2020.
- Ariel Myers, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Rukiyah Van Dross, East Carolina University). 2017-2022.
- Khoa Do, Biomedical Sciences Master's Student (Thesis committee; Advisor: Hu Huang, East Carolina University). 2016-2017.
- John Atkinson, Biology Master's Student (Thesis committee; Advisor: David Rudell, East Carolina University). 2015-2016.
- Blake Rushing, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Mustafa Selim, East Carolina University). 2015-2018.
- Ahmed Aldhafiri, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Ken Soderstrom, East Carolina University). 2014-2019.
- Jason Hoggard, Biomedical Sciences Master's Student (Thesis committee; Advisor: Lance Bridges, East Carolina University). 2014-2015.
- Matthew Edwards, Biology Master's Student (Thesis committee; Advisor: Krista McCoy, East Carolina University). 2014-2015.
- Bevin Blake, Biology Master's Student (Thesis committee; Advisor: Krista McCoy, East Carolina University). 2013-2015.
- Anastasia Weeks, Microbiology and Immunology Master's Student (Thesis committee; Advisor: Mark Mannie, East Carolina University). 2013-2017.
- Samar Rezq, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Abdel Abdel-Rahman, East Carolina University). 2013-2016.
- Partha Nagchowdhuri, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Brian McMillen, East Carolina University). 2013-2018.
- Suelen Demor, Biology Ph.D. Student (Dissertation committee; Advisor: David Chalcraft, East Carolina University). 2013-2017.
- Tessa Holland, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Ken Soderstrom, East Carolina University). 2013-2019.

- Samantha Sellers, Anatomy and Cell Biology Ph.D. Student (Dissertation committee, East Carolina University). 2013.
- Alvin Ming-Yun Tsang, Psychology Undergraduate Student (Honors Thesis Advisor, East Carolina University). 2011-2012.
- Abdullah Aldossari, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Jared Brown, East Carolina University). 2011-2018.
- Michael Smith, Biology Master's Student (Thesis committee; Advisor: Xiaoping Pan, East Carolina University). 2010-2011.
- Pranita Katwa, Pharmacology and Toxicology Ph.D. Student (Dissertation committee; Advisor: Jared Brown, East Carolina University). 2009-2012.

PROFESSIONAL SERVICE

Scientific Community

- Society of Toxicology Women in Toxicology Special Interest Group, Vice-President, 2026-present.
- Science Committee, Society of Environmental Toxicology and Chemistry, 2025-present.
- Society of Toxicology Women in Toxicology Special Interest Group, Vice-President, 2025-2026.
- Society of Toxicology Membership Committee, 2022-2025.
- Co-Chair, Timing is Everything: Developmental Exposure Alters the Path of Immune Cell Maturation and Function continuing education course. 2020 Annual Meeting, Society of Toxicology, Virtual.
- Society of Toxicology Faculty United for Toxicology Undergraduate Recruitment and Education (FUTURE) Committee. 2019-2022.
- Past-President, Society of Toxicology Immunotoxicology Specialty Section. 2019-2020.
- Chair, Program Planning Committee, The Toxicology Forum 45th Annual Summer Meeting (2019), Alexandria, VA.
- Co-Chair, Advanced Immunotoxicity Testing continuing education course. 2019 Annual Meeting, Society of Toxicology, Baltimore, MD.
- President, Society of Toxicology Immunotoxicology Specialty Section. 2018-2019.
- Co-Chair, Introduction to Immunotoxicity Testing continuing education course. 2018 Annual Meeting, Society of Toxicology, San Antonio, TX.
- Society of Toxicology Career Resource and Development Committee. 2018-2019.
- Society of Toxicology Specialty Section Collaboration and Communication Group. 2017-2019.
- Vice-President, Society of Toxicology Immunotoxicology Specialty Section. 2017-2018.
- Program Planning Committee, Volunteers Sub-committee, 2017 Annual Meeting, Society of Environmental Toxicology and Chemistry. 2016-2017.
- Vice-President Elect, Society of Toxicology Immunotoxicology Specialty Section. 2016-2017.
- Program Committee, Society of Toxicology Immunotoxicology Specialty Section. 2015-2016.
- Moderator for Toxicology, Epidemiology, and Human Health session, FLUOROS 2015 meeting, Golden, CO. 2015.
- Program Planning Committee, 2015 Annual Meeting, Society of Environmental Toxicology and Chemistry. 2014-2015.
- Senior Councilor, Immunotoxicology Specialty Section Society of Toxicology. 2014-2015.
- Past-President, North Carolina Society of Toxicology. 2014-2015.
- Junior Councilor, Immunotoxicology Specialty Section Society of Toxicology. 2013-2014.
- President, North Carolina Society of Toxicology. 2013-2014.
- Research Funding Committee, Society of Toxicology. 2012-2014.
- Vice President, North Carolina Society of Toxicology. 2012-2013.
- Vice President-Elect, North Carolina Society of Toxicology. 2011-2012.

- Program Committee, Society of Toxicology Immunotoxicology Specialty Section. 2010-2011.
- Councilor, North Carolina Society of Toxicology. 2009-2011.
- Workshop co-chair and organizer, "Is Modulation of the Immune System by Perfluoroalkyl Acids a Human Health Concern?" Society of Toxicology Annual Meeting, Baltimore, MD. 2009.
- Symposium co-chair and organizer, "Immune Biomarkers in Alternative Species: Implications for Risk Assessment," Society of Toxicology Annual Meeting, Charlotte, NC. 2007.
- Platform session co-chair, "Immunotoxicology: Immune Modulation and Cell Specific Responses," Society of Toxicology Annual Meeting, Charlotte, NC. 2007.
- Postdoctoral Representative and Program Committee member, Immunotoxicology Specialty Section of the Society of Toxicology. 2005-2007.
- Mentor, Association of Women in Science Program (WISP) Mentoring Project, Office of Women's Affairs, Indiana University, Bloomington, IN. 2000-2002.
- Student Board Member, Ohio Valley Chapter of Environmental Toxicology and Chemistry. 2000-2001.

Workplace Community, Oregon State University

- Department of Environmental and Molecular Toxicology, Internal Advisory Board. 2025-present.
- Department of Environmental and Molecular Toxicology, Research Day Planning Committee Co-Chair. 2024-2025.
- Department of Environmental and Molecular Toxicology Academic Programs Committee, 2024-present.
- Department of Environmental and Molecular Toxicology Strategic Planning Committee, 2024-2025.

Workplace Community, East Carolina University

- Interim Director of Postdoctoral Affairs, Office of Postdoctoral Affairs, Division of Research, Economic Development and Engagement, 2023.
- BSOM Promotion and Tenure Committee, 2020-2023.
- Department of Comparative Medicine Promotion and Tenure Committee Chair, 2020-2023.
- Department Pharmacology & Toxicology Personnel Committee Chair, 2019-2023.
- Faculty Research Advisory Committee, Division of Research, Economic Development and Engagement, 2019-2021.
- Vice-Chair, Institutional Animal Care and Use Committee, 2019-2023.
- BSOM Research Committee, 2019-2023.
- Diversity and Equity Leadership Program, Division of Research, Economic Development and Engagement, 2019.
- Postdoctoral Program Liaison, Office of Postdoctoral Affairs, Division of Research, Economic Development and Engagement, 2019-2022.
- LCME Steering Committee and Co-Chair of Academic Environment Sub-Committee, Brody School of Medicine, 2018-2019.
- Department of Comparative Medicine Promotion and Tenure Committee, 2017-2023.
- Coastal Strategic Planning Committee, 2016-2017.
- BSOM Promotion and Tenure Committee, 2015-2018.
- Secretary/Treasurer, Brody Women Faculty Committee, 2015-2017.
- Planning committee for the joint PhD program in Integrated Coastal and Marine Sciences, 2015-2017.
- School of Dental Medicine Admissions Committee, 2014-2018.
- BSOM Sustainability Committee, 2014-2016.
- Committee member, BSOM Research Committee, 2014-2015.
- Five-Year Review Committee for Dean Paul Cunningham, Dean of the Brody School of Medicine (appointed by the Vice Chancellor for Health Sciences). 2014.

- Institutional Animal Use and Care Committee. 2013-2023.
- M1 Curriculum Committee, Brody School of Medicine, East Carolina University. 2013-2016.
- Master Educator Committee, Brody School of Medicine, East Carolina University. 2012-2016.
- Chair, Brody Women Faculty Committee. 2012-2013.
- Brody Vision, Innovation, Achievement (VIA) group. 2011-2015.
- Undergraduate Research and Creative Activity Biomedical Sciences Grant Review Committee. 2011-2018.
- Chair-elect, Brody Women Faculty Committee. 2011-2012.
- Coastal Maritime Council, East Carolina University. 2010-2021.
- Five-Year Review Committee for Dr. David Taylor, Chair of the Department of Pharmacology and Toxicology (appointed by the Dean of the School of Medicine). 2010.
- Shared Resources Committee, Brody School of Medicine, East Carolina University. 2008-2012.
- Faculty of the Interdisciplinary Doctoral Program in Biological Sciences, East Carolina University. 2009-2023.
- Brody Women Faculty Committee, East Carolina University. 2008-present.
- Graduate Faculty, Division of Research and Graduate Studies, East Carolina University. 2008-present.

Other

- Science Olympiad (regional) Event C-Coordinator, "Potions and Poisons." 2018, 2019.
- Science Event Co-Coordinator, "ADdMe to Tox Town," Girl Scouts TechnoQuest Event. 2017.
- Science Event Co-Coordinator, "Biometry in Action," Brody Girl's STEM Day. 2016.
- Science Event Coordinator, "The Water Cycle," Youth Ocean Conservation Summit. 2016.
- STEM volunteer, various events, Love a Sea Turtle, NC Estuarium Sound Rivers. 2015-present.
- Science Event Coordinator, "Marshmallow Genetics" and "DNA Necklaces," ECU Girl's STEM Day. 2014-2016.
- Scientific Expert, Alzheimer's North Carolina fundraising walk, Washington, NC. 2014-2016.
- Über Judge, Blue Heron Bowl, Regional Competition for the National Ocean Sciences Bowl. 2012.
- Judge, North Carolina Region 1 Science and Engineering Fair. 2010. 2011.
- North Carolina Estuarium Docent. 2009-2023.

AMIGOS BRAVOS EXHIBIT 3

STATE OF NEW MEXICO
WATER QUALITY CONTROL COMMISSION

IN THE MATTER OF PROPOSED AMENDMENTS TO 20.6.4 NMAC - *Standards for Interstate and Intrastate Surface Waters,* **NO. WQCC 25-75 (R)**

NEW MEXICO ENVIRONMENT DEPARTMENT,

PETITIONER.

DIRECT TESTIMONY OF JAMIE DeWITT, PH.D., DABT

Background and Qualifications

Q: Please state your name.

A. My name is Jamie DeWitt.

Q: What is your current employment?

A: Since 2023 I have been a Professor in the Department of Environmental and Molecular Toxicology at Oregon State University in Corvallis, Oregon. In this position I teach foundational toxicology to graduate and undergraduate students and I manage a research laboratory that conducts toxicological research on the effects of environmental contaminants on the immune system, including the effects of per- and polyfluoroalkyl substances ("PFAS").

I also serve as Director of the Pacific Northwest Center for Translational Environmental Health Research ("Center") at Oregon State University. The Center supports innovative environmental health research to improve human health by increasing knowledge of interactions between environmental exposures and human biology to help reduce risks of exposure on human health.

In addition, I serve as an Adjunct Professor in the Department of Biological Sciences at North Carolina University (2018 to present) and am a Diplomate of the American Board of

Toxicology ("DABT").

Q: What is your educational background?

A: I received Bachelor of Science degrees in Biology and Environmental Science from Michigan State University and Doctor of Philosophy degrees in Environmental Science and Neural Science from Indiana University-Bloomington.

I completed postdoctoral training in Environmental and Ecotoxicology at Indiana University-Bloomington and in Immunotoxicology at the National Health and Environmental Effects Research Laboratory at the United States Environmental Protection Agency ("EPA") through a cooperative training agreement with the University of North Carolina at Chapel Hill. During my postdoctoral training at Indiana University, I evaluated the developmental cardiotoxicity of polychlorinated biphenyls and dioxins in wild passerine birds for a project funded by the United States Fish and Wildlife Service. At EPA, I evaluated the immunotoxicity of organotin compounds used in polyvinylchloride pipes and of perfluorooctanoic acid ("PFOA") as an emerging contaminant, at the time, in drinking water supplies.

Q: Would you please summarize your work experience, including your experience relating to the study and understanding of PFAS?

A: In 2008 I joined the faculty of the Department of Pharmacology and Toxicology of the Brody School of Medicine at East Carolina University in Greenville, North Carolina as a tenure-track Assistant Professor. In 2015 I was promoted to Associate Professor with tenure and in 2021 I was promoted to Professor. As mentioned, I joined the faculty at Oregon State University in 2023.

I am actively engaged in professional, university, and community service activities. Of particular relevance to the topic of PFAS, I helped to write or review documents from EPA, the

International Agency for Research on Cancer ("IARC") of the World Health Organization, the U.S. National Toxicology Program ("U.S. NTP"), the U.S. Agency for Toxic Substances and Disease Registry ("ATSDR"), the National Academies of Sciences, Engineering, and Medicine, and numerous documents from state agencies. I am part of a group known as the Global PFAS Science Panel that works collaboratively on PFAS reviews and commentaries to help to educate other scientists and decision-makers.

Q: Have you authored articles?

A: Yes. Overall, I have co-authored 27 primary research articles related to PFAS toxicity (first or senior author of 12). These manuscripts include assessments of immunotoxicity, developmental immunotoxicity, developmental neurotoxicity, developmental cardiotoxicity, dosimetric anchoring, and epidemiological associations.

I have co-authored 28 peer-reviewed articles or commentaries on PFAS toxicity and use and two book chapters related to PFAS immunotoxicity, and edited one of the first comprehensive texts on the toxicity of PFAS. My other publications concern toxicological effects of environmental contaminants, including their impact on human diseases. Overall, I have co-authored over 100 scientific publications.

Q: Have you served as a toxicological expert?

A: Yes. I have provided testimony to three subcommittees of U.S. House of Representatives (in 2019 and 2021) and to the New Mexico, North Carolina, and Oregon legislatures.

In New Mexico, I provided testimony earlier this year before the New Mexico Environment Improvement Board ("EIB") on the state of the science regarding the toxicity of PFAS on behalf of the New Mexico Environment Department ("NMED") as the EIB considered adoption of *In the Matter of Adoption of 20.13.2 NMAC, Per- and Poly-Fluoroalkyl Substances*

in Consumer Products (EIB No. 25-61(R)). In that testimony, I provided a high-level overview of human health effects from exposure to PFAS and summarized the current state of the science regarding human health risks that may arise from exposure to PFAS.

I appeared before the Water Quality Control Commission ("Commission") in 2021 during the Commission's last Triennial Review (WQCC No. 20-51(R)). In that rulemaking, I testified on behalf of Amigos Bravos regarding a number of PFAS compounds that should be treated as "toxic pollutants" for purposes of 20.6.4.7.T(2) NMAC; supported the NMED-proposed definition of "emerging contaminants" at 20.6.4.7 NMAC; and supported Amigos Bravos' proposal that NMED should have the authority to require dischargers to establish a baseline for and monitor emerging contaminants in federal permits, a proposal the Commission adopted at 20.6.4.14.F NMAC.

I have been retained on a number of occasions by various law firms representing individual plaintiffs, classes of plaintiffs, and states to provide testimony in lawsuits relating to the state of the science regarding human health risks that may arise from exposure to PFAS.

Q: What advisory panels have you served on?

A: I was part of a steering committee to organize a workshop in 2017 entitled "International Workshop Supporting the Dialogue Between Science and Policy on Per- and Polyfluoroalkyl Substances (PFAS);" the Global PFAS Science Panel, mentioned above, grew out of this workshop. I was one of the members of the Science Advisory Workgroup to the Michigan PFAS Action Response Team. I was appointed as a member of the North Carolina Secretaries Science Advisory Board, which is charged with advising the North Carolina Departments of Environmental Quality and Health and Human Services on toxicological effects of contaminants and levels of control necessary for protection of human health and the environment. I was a

member of the North Carolina Cancer Advisory Research Panel, charged by the North Carolina General Assembly to recommend strategies for assessing cancer incidence and mortality rates within the state. I was a member of the Tennessee PFAS External Advisory Group to the Departments of Environment and Conservation and Health, charged with informing the state about the risks of PFAS. I was a member of the EPA PFAS Science Advisory Board that was charged with evaluating the proposed maximum contaminant levels and maximum contaminant level goals for the six individual PFAS that are currently regulated in drinking water under the Safe Drinking Water Act. I was a community liaison to the National Academies of Sciences, Engineering, and Medicine ("NASEM") Guidance on PFAS Testing and Health Outcomes committee, a committee that released clinical guidance on PFAS to guide medical screening, risk evaluation, and health monitoring for people with elevated levels of PFAS in their blood. I was a PFAS Applied Science Fellow at the North Carolina Department of Environmental Quality ("NC DEQ") where I worked with NC DEQ staff on applied PFAS science. I was part of the PFAS Rulemaking Advisory Committee supporting the Oregon Department of Environmental Quality in considering rulemaking to include two PFAS in the definition of hazardous substances in Oregon. I provided testimony to Australia's Senate Select Committee on PFAS regarding the state of the science on PFAS toxicity.

Q: Is Amigos Bravos' Exhibit 2 an accurate copy of your curriculum vitae?

A: Yes.

Q: Have you provided a list of references upon which you relied for your testimony?

A: Yes. I provided a list of references at the end of my testimony, some of which I cite in my testimony.

Summary of Opinions

Q: Could you please summarize the opinions you will provide in this proceeding.

A. Yes. I offer the following opinions:

- First, I support the numeric water quality standards for perfluorooctane sulfonate ("PFOS") and PFOA for acute and chronic aquatic life proposed by NMED in the table of numeric criteria at 20.6.4.900.J(1) NMAC ("Table of Numeric Criteria")[1], and recommend that the Commission adopt NMED's proposals.
- Second, I recommend the Commission adopt additional numeric water quality standards for various PFAS for which there is sufficient toxicological data in support:
    - I recommend that human health-organism only water quality standard for aquatic life established by EPA for PFOS, PFOA, and perfluorobutane sulfonate ("PFBS") be added to the Table of Numeric Criteria. These values are:
        - PFOS, 0.07 ng/L[2] (7×10^{-5} µg/L[3])[4]
        - PFOA, 0.0036 ng/L (3.6×10^{-6} µg/L)[5]
        - PFBS, 500 ng/L (0.5 µg/L) [6]
    - I recommend that additional PFAS be added to the Table of Numeric Criteria for which EPA has derived acute freshwater aquatic life standards for eight additional

[1] NMED Amended Petition, Att. 2, p. 57.

[2] Nanograms per liter.

[3] Micrograms per liter.

[4] U.S. EPA. 2024a. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctanoic Acid (PFOA) and Related Salts, EPA 822P24001 [AB Ex. 4].

[5] U.S. EPA. 2024b. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, EPA 822P24002 [AB Ex. 5].

[6] U.S. EPA. 2024c. DRAFT Human Health Ambient Water Quality Criteria: Perfluorobutane Sulfonic Acid (PFBS) and Related Salts, EPA 822P24003 [AB Ex. 6].

PFAS[7]:

- perfluorobutanoic acid ("PFBA") (5,300 µg/L)
- perfluorohexanoic acid ("PFHxA") (4,800 µg/L)
- perfluorononanoic acid ("PFNA") (650 µg/L)
- perfluorodecanoic acid ("PFDA") (500 µg/L)
- perfluorobutane sulfonate ("PFBS") (5,000 µg/L)
- perfluorohexanesulfonic acid ("PFHxS") (210 µg/L)
- hexadecafluoro-2-decenoic acid ("8:2 FTUCA") (37 µg/L)
- pentadecafluorodecanoic acid ("7:3 FTCA") (12 µg/L)

The amendments I recommend are set forth below on pages 14-15 of my testimony.

- Third, I recommend that PFAS mixtures for human health-organism only water quality standards should be considered because some mixtures of PFAS can result in dose additive health effects. EPA provides guidance on dose-additivity that I recommend be used to develop a PFAS mixture human health only criterion.[8] For example, EPA has done this for a mixture of four PFAS in drinking water (GenX,[9] PFNA, PFHxS, and PFBS).[10] I recommend the following provision be added as 20.6.4.900.I NMAC:

20.6.4.900.I Mixtures of PFAS. For mixtures of PFAS with human health-organism only criteria (PFOA, PFOS, and PFBS), a hazard index (HI) will be applied with the following formula: HI = ([PFOA$_{ambient\ water}$]/[0.0036 ng/L] + ([PFOS$_{ambient\ water}$]/[0.07 ng/L]) + ([PFBS$_{ambient\ water}$]/[500 ng/L]). When the HI of the mixture of the three PFAS exceeds a value of one, the water quality standard is exceeded.

[7] U.S. EPA. 2024d. Acute Freshwater Aquatic Life Benchmarks for Eight Data-Limited PFAS: PFBA, PFHxA, PFNA, PFDA, PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA, EPA-822-R-24-009 [AB Ex. 7].

[8] U.S. EPA. 2024e. Final Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS), EPA-815-R-24-003 [AB Ex. 8].

[9] "GenX" refers to a technology involving hexafluoropropylene oxide dimer acid ("HFPO-DA") and its ammonium salt.

[10] U.S. EPA. 2024g. Final Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS, EPA-815-R-24-004 [AB Ex. 9].

NMED and Amigos Bravos' proposed amendments that I support are set forth in Amigos Bravos' Exhibit 1.

Background Information on PFAS

Q: Dr. DeWitt, what are PFAS?

A: PFAS are a class of synthetic substances that can be organized into various sub-classes, as shown in Figure 1 below, and are, in the vast majority of cases, either non-degradable or degrade to terminal products that are still PFAS (Cousins et al., 2020).

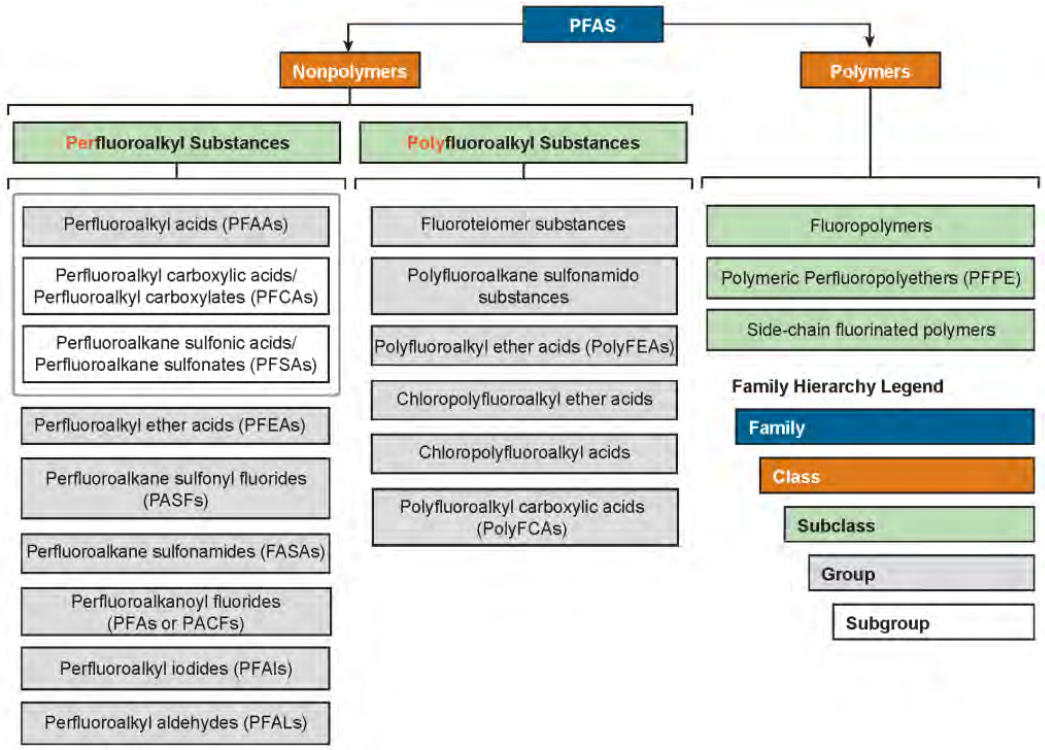


Figure 1. The general classification of PFAS as outlined by the Interstate Technology Regulatory Council (ITRC, 2026)

PFAS are chemically and thermally stable and have properties that make them commercially useful in numerous applications, including processing aids for fluoropolymer manufacture, stain, grease, and water-resistant coatings, and aqueous film forming foams used in

fire suppression activities (Glüge et al., 2020). A consequence of their extensive use and resistance to degradation is their persistence in the environment and when combined with the potential for many PFAS to bioaccumulate, or to move into living organisms from the environment, they are now detectable in tissues and blood of wildlife and humans. The U.S. National Health and Nutrition Examination Survey conducted by the Centers for Disease Control and Prevention has reported detectable concentrations of a subset of PFAS in the serum of 95% of surveyed individuals (ATSDR, 2026), indicating widespread human exposures.

Current estimates by EPA put the total number of chemicals classified as PFAS at over 20,000 individual substances (U.S. EPA, 2026a). PFAS vary by the number of carbons or chain-length, functional groups present (e.g., carboxylic acid, sulfonic acid, hexanoic acid), and other physicochemical characteristics (ITRC, 2026). Not all these substances are produced for industrial or consumer uses; some are breakdown products of "precursor compounds" (McDonough et al., 2022) but nonetheless contribute to environmental concentrations and to human exposures.

EPA defines long-chain PFAS as subsets of perfluoroalkyl acids ("PFAAS"), including perfluoroalkyl carboxylic acids ("PFCAs") with eight or more carbons, which include PFOA and PFNA, as well as perfluoroalkane sulfonates with six or more carbons, which includes PFOS and PFHxS (U.S. EPA, 2009). The bulk of toxicological research efforts concerning PFAS has focused on PFOA and PFOS and other long-chain PFAAs such as PFHxS, presumably because they were widely produced and prevalently used. These substances were produced for decades within the U.S. for the fluoropolymer and telomere industry.

Q: Are PFAS being manufactured within the U.S.?

A: PFOS manufactured within the U.S. was voluntarily discontinued by the 3M Company in 2002 (U.S. EPA, 2009) and PFOA and other long-chain PFCAs were phased out between 2006 and

2015 by eight major U.S. manufacturers through the 2010/15 PFOA Stewardship Program (U.S. EPA, 2026b).

However, these substances may still be present in previously produced and imported items (U.S. EPA, 2026b). Additionally, PFOA and PFOS may result from the breakdown of other PFAS, generally termed as "precursor compounds" (McDonough et al., 2022) and sinks of PFOA, PFOS, and other long chain PFAAs such as PFHxS exist in the environment and contribute to ongoing exposures to living organisms, including humans. One precursor compound that breaks down to PFOA is 8:2 FTCA.

Alternative compounds have been produced to replace PFOA, PFOS, and other long-chain PFAS that have been phased out of production. For example, PFBS is produced as a replacement for PFOS and GenX is produced as a replacement for PFOA. Many of these PFAS fall under the Toxic Substances Control Act ("TSCA") and EPA reviews these under the New Chemicals Program (U.S. EPA, 2019). However, not all PFAS are likely to be subject to TSCA guidelines because some are byproducts of production that may find their way into the environment, are environmental transformation products of precursor compounds, or were being used before TSCA was implemented. These alternative PFAS and PFAS as byproducts are detectable in the environment, including surface water (Hopkins et al., 2018).

Q: Are PFAS found in the environment?

A: Yes. Although alternative and byproduct PFAS also likely are persistent in the environment and are believed by some to be less toxic to wildlife and humans, they appear to be highly mobile in soil and water (U.S. EPA, 2019). This mobility in soil and water means that short-chain PFAS can move to points distant from points of release. Emerging data from studies of these short-chain PFAS indicate that many of these short-chain PFAS elicit toxicities similar to

the long-chain compounds (Fenton et al., 2021).

PFAS that have been studied for their toxicity induce a wide variety of adverse health outcomes in experimental animal models. Epidemiological studies, or studies of people that have been exposed to PFAS through their occupations or from environmental sources such as drinking water, link PFAS exposure to similar adverse health outcomes. These toxicological and epidemiological studies indicate that exposure to PFAS poses a hazard to human health.

PFAS are synthetic substances that do not occur naturally in the environment, and therefore levels in biological tissues and fluids of humans and other organisms indicate exposure from contaminated water, food, and air, from the use of products that contain PFAS, or from occupational settings. A 2020 report by the Environmental Working Group ("EWG") (Andrews and Naidenko, 2020) revealed that the drinking water of 110 million Americans may be contaminated with PFAS. This report highlighted that a major source of PFAS exposure to Americans is via drinking water, which often comes from surface water sources. The EWG recently analyzed data from the EPA's Unregulated Contaminant Monitoring Rule, demonstrating that its 2020 report underestimated PFAS contamination; the new data indicate that over 172 million Americans are drinking water contaminated with PFAS (EWG, 2025).

Drinking water in the U.S. often comes from surface water sources. Community providers deliver drinking water to multiple households and there are multiple types of these facilities across the nation. These facilities draw and treat water before delivering it to households and the source water may come from surface water sources (i.e., lakes and rivers). Surface water can be contaminated by PFAS from a variety of sources, including industrial releases, spills and other accidents, run-off from land-applied biosolids that contain PFAS, release of PFAS-containing wastewater from treatment facilities, and deposition of PFAS in the atmosphere to surface waters.

Living organisms can be exposed to PFAS in surface water through ingestion of the water, contact with the water, or immersion in the water.

Q: What types of studies are being conducted on PFAS?

A: At least six organizations, EPA, the U.S. NTP, the IARC, the ATSDR, NASEM, and the European Food Safety Authority ("EFSA") have completed systematic reviews of toxicity data for PFOA and/or PFOS and additional PFAS (EPA has published toxicity assessments for six PFAS under the Safe Drinking Water Act, ATSDR included 12 PFAS, NASEM included seven, and EFSA included four). These reviews are thorough evaluations of the body of toxicological and/or epidemiological evidence concerning PFOA and/or PFOS, or other PFAS and their likelihood of harm to human health following exposure. Each of these assessments is comprehensive of most of the published studies (up to a specified cut-off date determined by the agency sub-committee charged with writing the document) and accumulated knowledge about these PFAS.

The EPA passed drinking water standards for PFOA, PFOS, PFNA, PFHxS, GenX, and PFBS for protection of public health from ingestion of drinking water contaminated with these PFAS (EPA, 2024f). The health protective values for PFOA and PFOS are based, in part, on their categorization as likely human carcinogens, for PFNA on effects on developing organisms, for PFHxS and PFBS on effects on the thyroid gland, and for GenX on effects on the liver.

The U.S. NTP evaluated data concerning the ability of PFOA and PFOS to affect the immune system and determined that both compounds are presumed to be immune hazards to humans (U.S. NTP, 2016).

The IARC evaluated data concerning links between PFOA or PFOS exposure and cancer and determined that PFOA is carcinogenic to humans (IARC class 1) based on evidence of kidney and testicular cancer from epidemiological studies and supportive evidence from experimental

animal models and that PFOS is possibly carcinogenic to humans (IARC class 2B) based on mechanistic evidence supportive of carcinogenic activity (IARC, 2025).

The ATSDR evaluated data associated with 12 different PFAS and concluded that the following health outcomes were consistently associated with exposure (ATSDR, 2021):

- Pregnancy-induced hypertension/pre-eclampsia (PFOA, PFOS)
- Increases in serum hepatic enzymes, particularly alanine aminotransferase (ALT), and decreases in serum bilirubin levels (PFOA, PFOS, PFHxS)
- Increases in serum lipids, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDA)
- Decreased antibody response to vaccines (PFOA, PFOS, PFHxS, PFDA)
- Small (<20 g or 0.7 ounce decrease in birth weight per 1 ng/mL[11] increase in either PFOA or PFOS blood level) decreases in birth weight (PFOA, PFOS)

NASEM evaluated epidemiological data for seven different PFAS and found sufficient evidence of an association for the following diseases and health outcomes (NASEM, 2022):

- Decreased antibody response (in adults and children)
- Dyslipidemia (in adults and children)
- Decreased infant and fetal growth
- Increased risk of kidney cancer

NASEM also found limited or suggestive evidence of an association for the following diseases and health outcomes (NASEM, 2022):

- Increased risk of breast cancer
- Liver enzyme alterations (in adults and children)
- Increased risk of pregnancy-induced hypertension (gestational hypertension and preeclampsia)
- Increased risk of testicular cancer (in adults)
- Increase risk of thyroid disease and dysfunction (in adults)
- Increased risk of ulcerative colitis (in adults)

EFSA evaluated epidemiological and toxicological data for four different PFAS to calculate a tolerable weekly intake ("TWI") for PFAS in food. This value was 4.4 ng/kg[12] body

[11] Nanograms per milliliter.

[12] Nanograms per kilogram.

weight per week and was based on effects of PFAS exposure on the immune system (EFSA, 2020).

Specific PFAS Proposals

Q: Below are NMED's and Amigos Bravos' proposed additions of PFAS to the Table of Numeric Criteria set forth at 20.6.4.900.J NMAC. NMED's proposals appear in red underline and Amigos Bravos' appear in blue underline:

20.6.4.900.J Use-specific numeric criteria

(1) Table of numeric criteria: The following table sets forth the numeric criteria in µg/L, unless otherwise indicated, applicable to existing, designated and attainable uses. For metals, criteria represent the total sample fraction unless otherwise specified in the table. Additional criteria that are not compatible with this table are found in Subsections A through I and K through M of 20.6.4.900 NMAC.

. . .

Pollutant	CAS Number	DWS	Irr/Irr storage	LW	WH	Aquatic Life			Type
						Acute	Chronic	HH-OO	
Perfluorooctane sulfonate (PFOS)	Various					71	0.25	7e-5	P
PFOS (mg/kg-ww-iwbt)	Various						0.028		P
PFOS (mg/kg-ww-fwbt)	Various						0.201		P
PFOS (mg/kg-ww-fmt)	Various						0.087		P
Perfluorooctanoic acid (PFOA)	Various					3,100	100	3.6e-6	P
PFOA (mg/kg-ww-iwbt)	Various						1.18		P
PFOA (mg/kg-ww-fwbt)	Various						6.49		P
PFOA (mg/kg-ww-fmt)	Various						0.133		P
Perfluorobutane sulfonate (PFBS)	Various							0.5	P
PFBA (perfluorobutanoic acid)	375-22-4					5,300			P
PFHxA (perfluorohexanoic acid)	307-24-4					4,800			P
PFNA (perfluorononanoic acid)	375-95-1					650			P
PFDA (perfluorodecanoic acid)	335-76-2					500			P
PFBS (perfluorobutane sulfonate)	375-73-5					5,000			P
PFHxS (perfluorohexanesulfonic acid)	355-46-4					210			P

8:2 FTUCA (8:2 fluorotelomer unsaturated carboxylic acid)	70887-84-2					37			P
7:3 FTCA (7:3 fluorotelomer carboxylic acid)	812-70-4					12			P

First, what is the basis of your opinion that the Commission should adopt the numeric water quality standards for PFOS and PFOA for acute and chronic aquatic life proposed by NMED in the Table of Numeric Criteria, as shown above?

A: The EPA has published draft human health ambient water quality criteria for these two PFAS.[13] The values for acute and chronic aquatic life were scientifically derived by the EPA for each of these two PFAS—PFOA and PFOS—through a process proscribed by the Clean Water Act. This process is described in a published EPA method[14]. Therefore, these acute and chronic aquatic life values reflect the results of a proscribed and published methodology used by the EPA. Incorporation of these values into the standards for aquatic life will ensure that aquatic species in New Mexico will have clean water in which to live, grow, and reproduce.[15]

Q: What is the basis of your opinion that the Commission should adopt human health-organism only water quality standards for aquatic life for PFOS, PFOA, and PFBS, as shown above?

A: The EPA has published draft human health ambient water quality criteria for these three

[13] U.S. EPA. 2024a. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctanoic Acid (PFOA) and Related Salts, EPA 822P24001 [AB Ex. 4]; U.S. EPA. 2024b. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, EPA 822P24002 [AB Ex. 5].

[14] U.S. EPA. 2000. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. EPA-822-B-00-004.

[15] The EPA posted these draft criteria to the Federal Register in February 2024, with the public comment period ending in April 2025. *See* https://www.epa.gov/wqc/human-health-water-quality-criteria-pfas. The EPA has not yet posted final criteria.

PFAS.[16] The human health-organism only numeric water quality standards were calculated from reference doses ("RfDs") derived by the EPA in its human health toxicity assessments for each of these three PFAS--PFOA, PFOS, and PFBS. The RfDs derived by the EPA were part of its rulemaking process for the current drinking water standards for each of these three PFAS. The RfDs, which form the basis of the human health-organism only numeric water quality standards, underwent a rigorous peer-review process, including inter-EPA peer-review and review by experts external to the EPA. The RfDs also were available to the public for comment during the rulemaking process. Therefore, these human health-organism only numeric water quality standards reflect the results of a process that was transparent and peer-reviewed. Incorporation of these values into the standards for aquatic life will ensure that people are protected from consumption of aquatic organisms that may live in waters contaminated with PFAS.[17]

Q: What is the basis of your opinion that the additional eight PFAS (PFBA, PFHxA, PFNA, PFDA, PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA) be added to the Table of Numeric Criteria for acute freshwater aquatic life standards, as shown above?

A: The EPA has acknowledged that there are likely thousands of PFAS present in the environment beyond PFOA and PFOS, but the majority of these PFAS are data-limited or data-poor. However, based on interest by stakeholders and the availability of aquatic toxicity data, the EPA developed acute benchmarks for eight PFAS, including PFBA, PFHxA, PFNA, PFDA,

[16] U.S. EPA. 2024a. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctanoic Acid (PFOA) and Related Salts, EPA 822P24001 [AB Ex. 4]; U.S. EPA. 2024b. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, EPA 822P24002 [AB Ex. 5]; U.S. EPA. 2024c. DRAFT Human Health Ambient Water Quality Criteria: Perfluorobutane Sulfonic Acid (PFBS) and Related Salts. EPA 822P24003 [AB Ex. 6].

[17] The EPA posted these draft criteria to the Federal Register in February 2024, with the public comment period ending in April 2025. *See* https://www.epa.gov/wqc/human-health-water-quality-criteria-pfas. The EPA has not yet posted final criteria.

PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA.[18] These values therefore reflect the state of the science for these eight PFAS and adding them to the Table of Numeric Criteria will ensure that aquatic species in New Mexico will have clean water in which to live, grow, and reproduce.

Q: Amigos Bravos proposes the following provision be added as 20.6.4.900.I NMAC:

20.6.4.900.I Mixtures of PFAS. For mixtures of PFAS with human health-organism only criteria (PFOA, PFOS, and PFBS), a hazard index (HI) will be applied with the following formula: HI = ([PFOA$_{ambient\ water}$]/[0.0036 ng/L] + ([PFOS$_{ambient\ water}$]/[0.07 ng/L]) + ([PFBS$_{ambient\ water}$]/[500 ng/L]). When the HI of the mixture of the three PFAS exceeds a value of one, the water quality standard is exceeded.

What is the basis of your opinion that PFAS mixtures for human health-organism only water quality standards should be included as a water quality standard?

A: Through the third Unregulated Contaminant Monitory Rule, the EPA established that PFAS co-occur in multiple geographic locations.[19] The EPA finalized a mixtures-based approach for four PFAS, PFHxS, PFNA, HFPO-DA (i.e., "GenX"), and PFBS in drinking water, using the concept of "dose additivity" for the mixture. Dose additivity means that the risk of adverse health effects is equal to the sum of the individual exposures.[20] It is my opinion that because PFOA, PFOS, and PFBS also often co-occur in surface water, NMED can apply the EPA mixtures-based approach for the human health-organism only water quality standards. The approach uses the ambient water quality criteria for human health-organism only for each of the compounds and measured water concentrations to calculate a hazard index ("HI").

[18] U.S. EPA. 2024d. Acute Freshwater Aquatic Life Benchmarks for Eight Data-Limited PFAS: PFBA, PFHxA, PFNA, PFDA, PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA, EPA-822-R-24-009 [AB Ex. 7].

[19] U.S. EPA. 2024g. Final Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS, EPA-815-R-24-004 [AB Ex. 9].

[20] *Id.*

A hazard index for a single chemical is the ratio between measured concentrations and the ambient water quality criteria. For example, if the measured concentration is lower than the ambient water quality criteria, the HI is less than a value of one and no increased health risks are anticipated. If the measured concentration is higher than the ambient water quality criteria, the HI is greater than a value of one and increased health risks are probable.

This approach ensures that humans are adequately protected from mixtures of PFAS that may exist in water in which aquatic organisms consumed by humans may reside. Within the human health ambient water quality criteria documents for PFOA, PFOS, and PFBS,[21] the EPA also references the mixtures-based approach it used setting standards for four PFAS (PFHxS, PFNA, HFPO-DA, and PFBS). Thus, a mixtures-based approach is consistent with EPA recommendations for human health ambient water quality standards. This approach also is consistent with the TWI derived by EFSA for four PFAS (PFOA, PFOS, PFHxS, and PFNA).

This ends my direct testimony, which is accurate to the best of my knowledge.

/s/ Jamie DeWitt, Ph.D., DABT — July 29, 2026
Jamie DeWitt, Ph.D., DABT — Date

[21] U.S. EPA. 2024a. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctanoic Acid (PFOA) and Related Salts, EPA 822P24001 [AB Ex. 4]; U.S. EPA. 2024b. DRAFT Human Health Ambient Water Quality Criteria: Perfluorooctane Sulfonic Acid (PFOS) and Related Salts, EPA 822P24002 [AB Ex. 5]; U.S. EPA. 2024c. DRAFT Human Health Ambient Water Quality Criteria: Perfluorobutane Sulfonic Acid (PFBS) and Related Salts. EPA 822P24003 [AB Ex. 6].

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U.S. EPA. 2024c. DRAFT Human Health Ambient Water Quality Criteria: Perfluorobutane Sulfonic Acid (PFBS) and Related Salts. EPA 822P24003.

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AMIGOS BRAVOS EXHIBIT 4

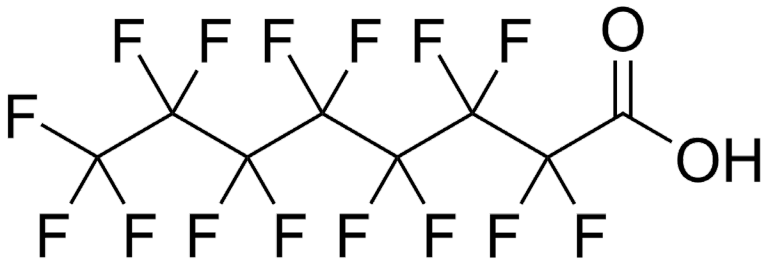
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DRAFT Human Health Ambient Water Quality Criteria:

Perfluorooctanoic Acid (PFOA) and Related Salts



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1 Introduction: Background and Scope

The U.S. Environmental Protection Agency's national recommended ambient water quality criteria (AWQC) for human health are scientifically derived numeric values that define ambient water concentrations that are expected to protect human health from the adverse effects of individual pollutants in ambient water.

Section 304(a)(1) of the Clean Water Act (CWA) requires the EPA to develop and publish, and from time-to-time revise, recommended criteria for the protection of water quality that accurately reflect the latest scientific knowledge. Water quality criteria for human health developed under section 304(a) are based solely on data and scientific judgments about the relationship between pollutant concentrations and human health effects. Section 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting pollutant concentrations in ambient water.

The EPA's recommended section 304(a) criteria provide technical information for states and authorized Tribes[a] to consider and use in adopting water quality standards that ultimately provide the basis for assessing water body health and controlling discharges of pollutants into waters of the United States. Under the CWA and its implementing regulations, states and authorized Tribes are required to adopt water quality criteria to protect the designated uses of waters (e.g., public water supply, aquatic life, recreational use, industrial use). The EPA's recommended water quality criteria do not substitute for the CWA or regulations, nor are they regulations themselves. Thus, the EPA's recommended criteria do not establish legal rights or obligations or impose legally binding requirements and are not final agency actions. States and authorized Tribes may adopt, where appropriate, other scientifically defensible water quality criteria that differ from these recommendations. EPA's water quality standards regulation at 40 CFR 131.20(a) requires states and authorized Tribes to consider any new or updated national section 304(a) recommended criteria as part of their triennial review process, and, if the state or authorized Tribe does not adopt new or revised criteria for parameters that correspond to those new or revised 304(a) criteria, to provide an explanation when it submits its triennial review to EPA. This requirement is to ensure that state or Tribal water quality standards reflect the current science and protect applicable designated uses.

The water quality criteria that are the subject of this document are draft national AWQC recommendations for human health issued under CWA section 304(a). Unless expressly indicated otherwise, all references to "human health criteria," "criteria," "water quality criteria," "ambient water quality criteria recommendations," or similar variants thereof are references to draft national AWQC recommendations for human health.

[a] Throughout this document, the term *states* means the 50 states, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term *authorized Tribe* or *Tribe* means an Indian Tribe authorized for treatment in a manner similar to a state under CWA section 518 for the purposes of section 303(c) water quality standards.

Perfluorooctanoic acid (PFOA) is a member of the per- and polyfluoroalkyl substances (PFAS) class. PFAS are a large class of thousands of synthetic chemicals that have been in use in the United States and around the world since the 1940s (EPA, 2018). The ability for PFAS to withstand heat and repel water and stains makes them useful in a wide variety of consumer, commercial, and industrial products, and in the manufacturing of other products and chemicals. The current scientific evidence has shown the potential for harmful health effects after human exposure to certain PFAS. The persistence and resistance to hydrolysis, photolysis, metabolism, and microbial degradation of PFAS raise additional concerns about long-term exposure and human health effects.

The EPA developed the draft human health criteria (HHC) PFOA to reflect the latest scientific information for input values, including exposure factors (i.e., body weight [BW], drinking water intake [DWI] rate, and fish consumption rate [FCR]), bioaccumulation factors (BAFs), human health toxicity values (i.e., reference dose [RfD] or cancer slope factor [CSF]), and relative source contribution (RSC). The draft criteria are based on the EPA's current *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (EPA, 2000a), which is referred to as the "2000 Methodology" in this document (EPA, 2000a).

2 Problem Formulation

Problem formulation provides a strategic framework for ambient water quality criteria development to systematically identify the major factors and chemical-specific scientific issues to be considered in the assessment (EPA, 2014a). The structure of this draft criteria document is intended to be consistent with general concepts of health assessments as described in the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a).

In developing AWQC, the EPA follows the assessment method outlined in the 2000 Methodology (EPA, 2000a). The 2000 Methodology describes different approaches for addressing water and nonwater exposure pathways to derive human health AWQC depending on the toxicological endpoint of concern, the toxicological effect (noncarcinogenic or carcinogenic), and whether toxicity is considered a linear or threshold effect. Water sources of human exposure include both consuming drinking water and eating fish or shellfish from inland and nearshore water bodies that have been contaminated with pollutants. For pollutants that exhibit a threshold of exposure before deleterious human health effects occur, as is the case for noncarcinogens and nonlinear carcinogens, the EPA applies an RSC. The RSC is the percentage of the total exposure to a contaminant that is attributed to the combination of drinking water and eating freshwater and estuarine fish and shellfish, where the remainder of exposure is allocated to other sources of oral exposure and other routes of exposure. The RSC is calculated by examining the data for other sources of exposure (e.g., air, food, soil) and pathways of exposure following the exposure decision tree for calculation of an RSC described in the 2000 Methodology (EPA, 2000a).

For carcinogenic substances for which the cancer slope factor was quantified using linear low-dose extrapolation, only the exposures from drinking water and fish ingestion are reflected in human health AWQC; that is, nonwater sources are not explicitly included, and no RSC is

applied (EPA, 2000a). This is because in these situations, AWQC are derived with respect to the *incremental* lifetime cancer risk posed by the presence of a substance in ambient water, rather than an individual's total risk from all exposure sources. Therefore, the resulting AWQC represents the ambient water concentration that is expected to increase an individual's lifetime risk of cancer from exposure to the pollutant by no more than one chance in one million (10^{-6}) for the general population (male and female adults, 21 years and older; referred to as "general population" herein), regardless of the additional lifetime cancer risk due to exposure, if any, to that substance from other sources. The EPA calculates AWQC at a 10^{-6} cancer risk level for the general population (EPA, 2000a). The 2000 Methodology recommends that states set human health criteria cancer risk levels for the target general population at either 10^{-5} or 10^{-6} and also notes that states and authorized Tribes can choose a more stringent risk level, such as 10^{-7}.

For substances that are carcinogenic, the EPA takes an integrated approach by considering both cancer and noncancer effects when deriving AWQC (EPA, 2000a,b). Where sufficient data are available, the EPA first derives separate AWQC for both carcinogenic and noncarcinogenic toxicity endpoints and then selects the lower (more health protective) of the two values for the recommended AWQC.

PFOA may exist in multiple forms, such as isomers or associated salts and each form may have a separate Chemical Abstracts Service registry number [CASRN] or no CASRN at all. Additionally, these compounds have various names under different classification systems. PFOA is a strong acid that is generally present as the perfluorooctanoate anion at typical environmental pH values. Therefore, this assessment applies to all isomers of PFOA, as well as nonmetal salts of PFOA that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9. For the purpose of this assessment, "PFOA" will signify the ion, acid or any nonmetal salt of PFOA.

2.1 Uses and Sources of PFOA

PFAS are manufactured chemicals that have been widely used in industrial and consumer processes and products over the past several decades in the United States due to their repellant and surfactant properties. PFAS are persistent chemicals based on their physicochemical properties. Concerns about persistence of PFAS stem from the resistance of these compounds to hydrolysis, photolysis, metabolism, and microbial degradation.

PFOA is a synthetic, fully fluorinated, organic acid that is used in many types of consumer products and in the production of fluoropolymers (EPA, 2016a,b). PFOA is also formed by microbial, metabolic and abiotic degradation of many precursor chemicals. PFOA and its precursors have been used in flame repellents, cosmetics, paints, polishes, and processing aids used in the manufacture of nonstick coatings on cookware. It is one of a large group of perfluoroalkyl substances that are used in consumer and industrial products, etc. to improve their resistance to stains, grease, and water (Gaines, 2023). In 2006, EPA initiated the 2010/2015 PFOA Stewardship Program, resulting in major PFOA producers committing to a 95% reduction in PFOA facility emissions and product contents across the globe by 2010. The 2010/2015 PFOA Stewardship Program further aimed to eliminate PFOA emissions and product content by 2015 (EPA, 2006, 2023a). The EPA has found widespread PFOA contamination in

water, sediments, and soils. Exposure to PFOA can occur through food including fish and shellfish, house dust, air, and contact with consumer products.

2.2 Environmental Fate and Transport in the Environment

Under most environmental conditions PFOA in water rapidly dissociates into ionic components. In aquatic environments, the sorption of PFOA to sediments varies based on the amount of organic carbon present and other site-specific conditions; the range of log(Kd)[b] values reported in the literature for PFOA in sediments is −0.7 to 4.9 (EPA, 2024a). Because of its water solubility and preferential binding to proteins, once PFOA enters a waterbody it can remain dissolved in the water column, sorb to organic particulate matter, or be assimilated by organisms. In the water column, and other environmental compartments, PFOA is stable and resistant to hydrolysis, photolysis, volatilization, and biodegradation (NCBI, 2024; Lange et al. 2006). The persistence of PFOA has been attributed to the strong carbon-fluorine (C-F) bond.

2.3 Occurrence and Detection in Sources Relevant to Ambient Water Quality Criteria

PFOA has been detected in a variety of environmental matrices. The occurrence and detection of PFOA in sources relevant to ambient water quality criteria, including ambient water, fish and shellfish, is described below. Additional occurrence information for sources other than ambient water (e.g., air, food, soil) is summarized in Section 6.2 as part of the determination of the RSC.

2.3.1 Occurrence in Surface Water

Among the PFAS with established analytical methods for detection, PFOA (along with perfluorooctane sulfonic acid [PFOS]) is one of the dominant PFAS compounds detected in ambient water in the United States and worldwide (Ahrens, 2011a; Benskin et al., 2012; Dinglasan-Panlilio et al., 2014; Nakayama et al., 2007; Remucal, 2019; Zareitalabad et al., 2013). Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions in the United States, often targeting sites with known manufacturing or industrial uses of PFAS such as the Great Lakes, the Cape Fear River, and waterbodies near Decatur, Alabama (Boulanger et al., 2004; Hansen et al., 2002; Konwick et al., 2008; Nakayama et al., 2007; 3M, 2000). PFOA concentrations in global surface waters range over seven orders of magnitude, generally in picogram per liter (pg/L) to nanogram per liter (ng/L) concentrations, but sometimes reaching microgram per liter (µg/L) levels (Jarvis et al., 2021; Zareitalabad et al., 2013).

PFOA concentrations in surface water tend to increase with increasing levels of urbanization. Across the Great Lakes region, PFOA was higher in the downstream lakes (Lake Erie and Lake Ontario), which are more heavily impacted by urbanization, and lower in the upstream lakes (Lakes Superior, Michigan, and Huron), which are located in relatively rural and forested areas (Remucal, 2019). Similarly, Zhang et al. (2016) found measured surface water PFOA concentrations in urban areas (urban average PFOA concentration = 10.17 ng/L; n = 20) to be more than three times greater than concentrations in rural areas (rural average PFOA concentration = 2.95 ng/L; n = 17) within New Jersey, New York, and Rhode Island. Seasonal variations in PFOA levels in U.S. surface waters remain largely unknown due to a lack of data.

[b] Log(Kd) is the logarithm of the equilibrium dissociation constant.

2.3.2 Occurrence in Freshwater and Estuarine Fish and Shellfish

PFOA has been detected in freshwater fish fillet samples collected during several national studies in rivers and the Great Lakes; however, PFOA is reported at a lower frequency and at lower levels compared to other PFAS, including PFOS (Table 1). The EPA collaborates with federal agencies, states, Tribes, and other partners to conduct freshwater fish contamination studies as part of a series of statistically based surveys to produce information on the condition of U.S. lakes, streams, rivers, and coastal waters. The National Oceanic and Atmospheric Administration (NOAA) recorded 159 data points available for aquatic organisms in the National Status and Trends Data Portal for PFOA focusing on dreissenid mussel and other mussels, oyster, fish fillet, and fish liver samples. There were six detections reported, ranging from 0.33 ng/g wet weight (ww) to 75.1 ng/g ww; 153 were below the method detection limit (MDL) or not detected (NOAA, 2024).

Table 1. Summary of the EPA national freshwater fish tissue monitoring results for PFOA.

Reference	Most Commonly Sampled Species	Site Description	Results
2008–2009 National Rivers and Streams Assessment (NRSA) (Stahl et al., 2014)	Smallmouth bass Largemouth bass Channel catfish	162 urban river sites across the United States	No PFOA detections reported.
2013–2014 NRSA (EPA, 2020, 2023b)	Channel catfish Largemouth bass Smallmouth bass	349 urban and nonurban river sites across the United States	PFOA detected in 4% of fillet samples. Maximum detected concentration 0.27 ng/g.
2018–2019 NRSA (EPA, 2023c,d)	Channel catfish Smallmouth bass Largemouth bass	290 urban and nonurban river sites across the United States	PFOA detected in 2% of fillet samples. Maximum detected concentration 0.354 ng/g.
2010 National Coastal Condition Assessment (NCCA) Great Lakes Human Health Fish Tissue Study (Stahl et al., 2014)	Lake trout Smallmouth bass Walleye	157 nearshore sites along the U.S. shoreline of the Great Lakes	PFOA detected in 12% of fillet samples. Maximum detected concentration 0.97 ng/g.
2015 NCCA Great Lakes Human Health Fish Tissue Study (EPA, 2021, 2024c)	Lake whitefish Yellow perch Lake trout Walleye	152 nearshore sites along the U.S. shoreline of the Great Lakes	PFOA detected in 14% of fillet samples. Maximum detected concentration 1.93 ng/g.
2022 National Lakes Assessment (EPA, 2024d)	Largemouth Bass Rainbow Trout Bluegill Yellow Perch Black Crappie	413 sampled lakes within the contiguous U.S. (excluding The Great Lakes, Great Salt Lake and lakes which are tidally influenced).	PFOA was detected in < 1% of samples (0.98%). Maximum detected concentration: 1.55 ng/g; median < MDL (0.152 ng/g).

AB Ex. 4

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In addition, Penland et al. (2020) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin-Pee Dee River in North Carolina and South Carolina. PFOA was detected in whole body tissues of unionid mussels (7.41 ng/g ww) and aquatic insects (10.68 ng/g ww), but was not detected in Asian clam, snails, or crayfish. PFOA was measured in muscle tissue of 2 out of 11 sampled fish species: the channel catfish (21.19 ng/g ww) and notchlip redhorse (45.66 ng/g ww).

3 Criteria Formulas: Analysis Plan

Human health AWQC for toxic pollutants may be necessary to protect designated uses of water bodies related to ingestion of water (i.e., public water supply or source water protection) and ingestion of freshwater/estuarine fish and shellfish. See CWA 303(c)(2)(A)–(B). Although the AWQC are based on chronic health effects data (both cancer and noncancer effects), the criteria are intended to also be protective against adverse effects that may reasonably be expected to occur as a result of elevated acute or short-term exposures (EPA, 2000a). Human health AWQC are expected to provide adequate protection not only for the general population over a lifetime of exposure, but also for sensitive life stages and subpopulations who, because of high water- or fish intake rates, or because of biological sensitivities, have an increased risk of receiving a dose that would elicit adverse effect (EPA, 2000a).

The derivation of human health AWQC requires information about both the toxicological endpoints of concern from exposure to water pollutants and human exposure pathways for those pollutants. The EPA only considers the following two primary pathways of human exposure to pollutants present in a particular water body when deriving human health 304(a) AWQC: (1) direct ingestion of drinking water obtained from the water body and (2) consumption of fish and shellfish obtained from the water body.

The equations for deriving human health AWQC are presented as Equations (Eqs.) 1 and 2 for noncancer and nonlinear carcinogenic effects, and Eqs. 3 and 4 for linear carcinogenic effects. The EPA derives two separate recommended human health AWQC based on 1) the consumption of both water and aquatic organisms (Eq. 1), called “water + organism”; and 2) the consumption of freshwater/estuarine fish and shellfish (Eq. 2), called “organism only.” The use of one criterion over the other depends on the designated use of a particular water body or water bodies (i.e., drinking water source and/or fishable waters). The EPA recommends applying organism-only AWQC (Eq. 2) to a water body where the designated use includes supporting fishable uses under section 101(a) of the CWA but the water body is not a drinking water supply source (e.g., nonpotable estuarine waters that support fish or shellfish for human consumption) (EPA, 2000a).

The EPA recommends including the drinking water exposure pathway for ambient surface waters where drinking water is a designated use for the following reasons: (1) drinking water is a designated use for surface waters under the CWA, and therefore, criteria are needed to ensure that this designated use can be protected and maintained; (2) although they are rare, some public water supplies provide drinking water from surface water sources without treatment; (3) even among the majority of water supplies that do treat surface waters, existing

treatments might not be effective for reducing levels of particular contaminants; and (4) in consideration of the agency's goals of pollution prevention, ambient waters should not be contaminated to a level where the burden of achieving health objectives is shifted away from those responsible for pollutant discharges and placed on downstream users that must bear the costs of upgraded or supplemental water treatment (EPA, 2000a).

The equations for deriving the criteria values are as follows (EPA, 2000a):

Equations for Noncancer and Nonlinear Carcinogen HHC:
Consumption of water and organisms:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{DWI + \sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 1)}$$

For consumption of organisms only:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{\sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 2)}$$

Where:
AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)
RfD = reference dose, expressed in milligrams per kilogram-day (mg/kg-d)
RSC = relative source contribution, unitless
BW = body weight, expressed in kg
DWI = drinking water intake, expressed in L/d
$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4
FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d
BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

Equations for Linear Carcinogens HHC:
Consumption of water and organisms:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{c}}{DWI + \sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 3)}$$

For consumption of organisms only:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{c}}{\sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 4)}$$

[c] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

Where:

AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)

RSD = RSD = risk specific dose; the cancer risk level (i.e., a target risk for the population; 1 in 1 million or 10^{-6}) divided by the cancer slope factor (i.e., incidence of cancer relative to dose in units of $[mg/kg/day]^{-1}$), expressed in milligrams per kilogram-day (mg/kg-d)

BW = body weight, expressed in kg

DWI = drinking water intake, expressed in L/d

$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4

FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d

BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

The EPA rounds AWQC to the number of significant figures in the least precise parameter as described in the 2000 Methodology (EPA, 2000a, Section 2.7.3). The EPA used a rounding procedure that is consistent with the 2000 Methodology (EPA, 2000a) and the 2015 HHC update (https://www.epa.gov/wqc/human-health-water-quality-criteria-and-methods-toxics).

4 AWQC Input Parameters

4.1 Exposure Factor Inputs

National recommended HHC establish ambient concentrations of pollutants in waters of the United States which, if not exceeded, will protect the general population from adverse health impacts from those pollutants due to consumption of aquatic organisms (i.e., freshwater and estuarine fish and shellfish) and water (EPA, 2000a). It is the EPA's longstanding practice to set national recommended HHC at a level intended to be adequately protective of a human exposure over a lifetime (EPA, 2000a). To accomplish this, the EPA uses a combination of median values, mean values, and percentile estimates for the HHC inputs consistent with the EPA's 2000 Methodology. The EPA's assumptions afford an overall level of protection targeted at the high end of the general adult population (i.e., the target population or the criteria-basis population) (EPA, 2000a). This approach is reasonably conservative and appropriate to meet the goals of the CWA and the 304(a) criteria program (EPA, 2000a). If the EPA determines that another population of life stage (e.g., pregnant women and their fetuses, young children) is the target then exposure parameters for that target population or life stage could be considered in the derivation of the criteria (EPA, 2000a). Potentially sensitive life stages for PFOA are explored further in a comparative analysis in Appendix B.

4.1.1 Body Weight

The BW for the general adult population including males and females, ages 21 years and older, was selected for the PFOA HHC, consistent with the population selected in the agency's most recent major update to existing 304(a) HHC (EPA, 2015) and the EPA's 2000 Methodology (EPA, 2000a). The EPA used the mean weight for adults ages 21 and older of 80.0 kg, based on National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2006 as reported in Table 8.1 of the EPA's *Exposure Factors Handbook* (EPA, 2011), the EPA's most recent publication of body weight exposure factors.

4.1.2 Drinking Water Intake Rate

For adults ages 21 years and older, the EPA used an updated DWI of 2.3 L/d, rounded from 2.345 L/d. This DWI was estimated using the Food Commodity Intake Database consumption calculator (http://fcid.foodrisk.org) which is based on NHANES 2005–2010 data used to develop the EPA's *Exposure Factors Handbook Update to Chapter 3, Ingestion of Water and Other Select Liquids* (EPA, 2019, Section 3.3.1.1). This rate represents the per capita estimate of combined direct and indirect community water[d] ingestion at the 90th percentile for adults, males and females, ages 21 and older. The EPA selected the per capita rate for the updated DWI because it represents the average daily dose estimates; that is, it includes both people who drank water during the survey period and those who did not, which is appropriate for a national-scale assessment such as the development of CWA section 304(a) national human health criteria development (EPA, 2019, Section 3.2.1). The updated DWI of 2.3 L/d reflects the latest scientific knowledge in accordance with CWA 304(a)(1).

The EPA's selection of the DWI of 2.3 L/d is consistent with the 2000 Methodology's selection of a default rate based on per capita community water ingestion at the 86th percentile for adults surveyed in the U.S. Department of Agriculture's *1994–1996 Continuing Survey of Food Intake by Individuals (CSFII)* analysis (EPA, 2000a, Section 4.3.2.1).

4.1.3 Fish Consumption Rate

The FCR used for the general adult population is 22.0 g/d, or 0.0220 kg/d (EPA, 2014b, Table 9a). This FCR represents the 90th percentile per capita consumption rate of fish from inland and nearshore waters for U.S. adults ages 21 years and older based on NHANES data from 2003–2010. The 95% confidence interval (CI) of the 90th percentile per capita FCR is 19.1 g/d and 25.4 g/d.

As recommended in the 2000 Methodology, the EPA used TL-specific FCRs to better represent human dietary consumption of fish. An organism's trophic position in the aquatic food web can have an important effect on the magnitude of bioaccumulation of certain chemicals. The TL-specific FCRs are numbered 2, 3, and 4, and they account for different categories of fish and shellfish species based on their position in the aquatic food web: TL 2 accounts for benthic filter feeders; TL 3 accounts for forage fish; and TL 4 accounts for predatory fish (EPA, 2000a).

The EPA used the following TL-specific FCRs to derive the AWQC: TL 2 = 7.6 g/d (0.0076 kg/d) (95% CI [6.4, 9.1] g/d); TL 3 = 8.6 g/d (0.0086 kg/d) (95% CI [7.2, 10.2] g/d); and TL 4 = 5.1 g/d (0.0051 kg/d) (95% CI [4.0, 6.4] g/d). Each TL-specific FCR represents the 90th percentile per capita consumption rate of fish and shellfish from inland and nearshore waters from that particular TL for U.S. adults ages 21 years and older (EPA, 2014b, Tables 16a, 17a, and 18a). The sum of these three TL-specific FCRs is 21.3 g/d, which is within the 95% CI of the overall FCR of

[d] *Community water* includes direct and indirect use of tap water for household uses and excludes bottled water and other sources (EPA, 2019, Section 3.3.1.1). *Direct ingestion* is defined as direct consumption of water as a beverage, while *indirect ingestion* includes water added during food preparation (e.g., cooking, rehydration of beverages) but not water intrinsic to purchased foods (EPA, 2019, Section 3.1).

22.0 g/d. The EPA recommends using the TL-specific FCRs when deriving AWQC; however, the overall FCR (22.0 g/d) may be used if a simplified approach is preferred.

4.2 Bioaccumulation Factor (BAF)

4.2.1 Approach

Several attributes of the bioaccumulation process are important to understand when deriving national BAFs for use in developing national recommended section 304(a) AWQC. First, the term *bioaccumulation* refers to the uptake and retention of a chemical by an aquatic organism from all surrounding media, such as water, food, and sediment. The term *bioconcentration* refers to the uptake and retention of a chemical by an aquatic organism from water only. In some cases, experiments conducted in a lab that measure *bioconcentration* can be used to estimate the degree of *bioaccumulation* expected in natural conditions. However, for many chemicals, particularly those that are highly persistent and hydrophobic, the magnitude of bioaccumulation by aquatic organisms can be substantially greater than the magnitude of bioconcentration. In these cases, an assessment of bioconcentration alone underestimates the extent of accumulation in aquatic biota. Accordingly, the EPA guidelines presented in the 2000 Methodology (EPA, 2000a) emphasize using, when possible, measures of *bioaccumulation* as opposed to measures of *bioconcentration* (EPA, 2000a).

The EPA estimated BAFs for the draft PFOA AWQC using the 2000 Methodology (EPA, 2000a) and the associated *Technical Support Document Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003). Specifically, these documents provide a framework for identifying alternative procedures to derive national TL-specific BAFs for a chemical based on the chemical's properties (e.g., ionization and hydrophobicity), metabolism, and biomagnification potential (EPA, 2000a, 2003). As described in the 2000 Methodology, the purpose of the EPA's national BAF is to represent the long-term, average bioaccumulation potential of a chemical in aquatic organisms that are commonly consumed by humans throughout the United States (EPA, 2000a). The EPA evaluated results from field BAF and laboratory bioconcentration factor (BCF) studies on aquatic organisms commonly consumed by humans in the United States for use in developing national trophic-level BAFs. National BAFs are not intended to reflect fluctuations in bioaccumulation over short periods (e.g., a few days) because human health AWQC are generally designed to protect humans from long-term (lifetime) exposures to waterborne chemicals (EPA, 2003).

The EPA followed the approach described in Figure 3-1 of the Technical Support Document, Volume 2 (EPA, 2003). The EPA used the best available data to classify each chemical according to this framework, and to derive the most appropriate BAFs following the 2000 Methodology (EPA, 2000a) and Technical Support Document, Volume 2 (EPA, 2003). Best available data consisted of peer-reviewed literature sources, government reports, and professional society proceedings, when sufficient information was provided to indicate the quality and usability of the data.

The framework provides six procedures to calculate a national BAF based on the pollutant's physical and chemical properties. Each procedure contains a hierarchy of the BAF derivation methods (listed below); however, this hierarchy should not be considered inflexible (EPA, 2000). The four methods are:

1. **BAF Method.** This method calculates national TL-specific BAFs using water and fish and shellfish tissue concentration data obtained from field studies. Field-measured BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of that contaminant in the surrounding water.

   For nonionic organic chemicals, BAFs are normalized to allow a common basis for averaging BAFs from several studies by adjusting for the water-dissolved portions of the chemical.

   In order to calculate representative TL-specific national BAFs used to calculate national recommended 304(a) criteria, the EPA averaged multiple field BAFs using a geometric mean of the normalized BAFs, first by species and then by TL, to calculate the TL baseline BAFs.

2. **BSAF Method.** This method uses biota-sediment accumulation factors (BSAFs) to estimate bioaccumulation. While BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of the contaminant in water, BSAFs divide the concentration in the organism by the concentration in surrounding sediments. BSAFs are useful when calculating site-specific criteria for compounds that are highly hydrophobic—these compounds have potential to cause bioaccumulation in aquatic organisms even when concentrations in the water column are below detection limits.

3. **BCF Method.** This method estimates BAFs from laboratory-measured BCFs. Experiments designed to calculate BCFs aim to measure bioconcentration resulting from an organism's exposure to contaminated water. Unlike BAFs measured in the field, BCF experiments do not capture bioaccumulation from other routes of exposure or biomagnification (the increase in bioaccumulation at higher levels of the food chain). However, BCFs may be used to estimate bioaccumulation if a contaminant's chemical and physical properties indicate that the compound is likely to primarily accumulate in the organism via the water exposure route, and there is no evidence that the contaminant biomagnifies in the food chain. If insufficient field-collected data are available to calculate a national BAF, the EPA may also estimate bioaccumulation using laboratory measured BCFs and a food chain multiplier term, which accounts for biomagnification.

   A similar process to the one described in the BAF method description (above) for normalizing of water-dissolved portions of the chemical and particulate organic carbon content is used for calculating national BAFs from laboratory-measured BCF data. Ionic organic chemicals are normalized, then multiplied by the food chain multiplier if biomagnification is expected to occur. All available BCFs are averaged using a geometric mean across species and then across TL to compute baseline BAFs.

4. **K_{ow} Method.** This method predicts BAFs based on a chemical's octanol-water partition coefficient (K_{ow}), with or without adjustment using a food chain multiplier, as described in Section 5.4 of the Technical Support Document, Volume 2 (EPA, 2003).

4.2.2 Data Selection and Evaluation

The EPA conducted a systematic literature search in October 2022 of publicly available literature sources to determine whether they contained information relevant to calculating national BAFs for human health AWQC, using the 2000 Methodology and Technical Support Document, Volume 2 (EPA, 2000a, 2003). The literature search for reporting the bioaccumulation of PFOA was implemented by developing a series of chemical-based search terms, consistent with the process for derivation of BAFs used in the development of the EPA's Final Aquatic Life Criteria for PFOA (EPA, 2024e) and PFOS (EPA, 2024f) and published in Burkhard (2021). These terms included chemical names and Chemical Abstracts Service Registry Number (CASRN or CAS), synonyms, tradenames, and other relevant chemical forms (i.e., related compounds). Databases searched were Current Contents, ProQuest CSA, Dissertation Abstracts, Science Direct, Agricola, TOXNET, and UNIFY (database internal to the EPA's ECOTOX database). The literature search (including literature published through the first two quarters of 2020) yielded > 37,000 citations that were further refined by excluding citations on analytical methods, human health, terrestrial organisms, bacteria, and where PFOA was not a chemical of study (Burkhard, 2021). The citations meeting the search criteria were reviewed for reported BAFs and/or reported concentrations in which BAFs could be calculated. Data from papers that met the inclusion and data quality screening criteria described below were extracted into the chemical dataset for PFOA.

Specifically, studies were evaluated for inclusion in the dataset used for calculating national BAFs for PFOA using the following evaluation criteria:

- Only BAF studies that included units for tissue, water, and/or BAFs were included.
- Mesocosm, microcosm, and model ecosystem studies were not selected for use in calculating BAFs.
- BAF studies in which concentrations in tissue and/or water were below the minimum level of detection were excluded.
- Only studies performed using freshwater or brackish water were included; high salinity values were excluded.
- Studies of organisms (e.g., damselfly, goby) and tissues (e.g., fish bladder) not commonly consumed by humans or not used as surrogate species for those commonly consumed by humans were excluded.
- Studies in which the BAFs were not found to be at steady state were excluded.
- Initially, for pooled samples, averaging BAF data from multiple locations was only considered acceptable if corresponding tissue and water concentrations were available from matching locations (e.g., a BAF would not have been calculated using water and tissue samples collected from eight separate locations with tissue concentrations collected from only six of these corresponding locations). After further review, for pooled samples, averaging data from multiple locations was considered acceptable if corresponding tissue and water concentrations were available from the overall spatial area of the study.

In addition to the evaluation criteria listed above, PFOA bioaccumulation data were also subsequently evaluated using the following study evaluation criteria outlined in Burkhard (2021) (Table 2).

As noted in Burkhard (2021), study quality determinations based on temporal and spatial coordination were subjective and based on best professional judgement. In the absence of adequate quantifiable information regarding sample location (site coordinates for both water and tissue collection locations) or temporal coordination (specific dates of sample collection), BAF data were given a score of 2 or 3 for these categories.

Table 2. Bioaccumulation factor (BAF) study quality criteria based on suggested criteria in Burkhard (2021).

Criteria	1	2	3
Number of water samples collected	> 3 samples	2–3 samples	1 sample
Number of organism samples collected	> 3 samples	2–3 samples	1 sample
Temporal coordination of water and biota samples	Concurrent collection of samples	Collected within a 1-year time frame	Collected > 1 year time frame
Spatial coordination of water and biota samples	Collected from same locations	Collected from reasonably close locations (1 kilometer (km)–2 km)	Significantly different sampling locations
General experimental design	Assigned a default value of zero for studies in which tissues from individual species were identified and analyzed		Assigned a value of 3 for studies in which tissues were from mixed species or reported as a taxonomic group.

Notes: The scores for each BAF were totaled and used to determine the overall confidence ranking for each individual BAF. The sum of quality values for the five criteria listed in Table 2 were classified as high quality (total score of 4 or 5), medium quality (total score of 5 or 6) or low quality (total score ≥ 7). Only high and medium quality data were included in final national BAFs calculations.

4.2.3 BAFs for PFOA

Following the decision framework presented in Figure 1, the EPA selected one of the four methods to develop a national-level BAF for this chemical. Because PFOA is an organic chemical that predominantly exists in an anionic form in water (EPA, 2024g,h; NCBI, 2024), the BSAF and K_{ow} methods would not be applicable. The EPA selected the BAF estimate using the BAF method (i.e., based on a field-measured BAF) because sufficient field-measured BAF data were available for PFOA.

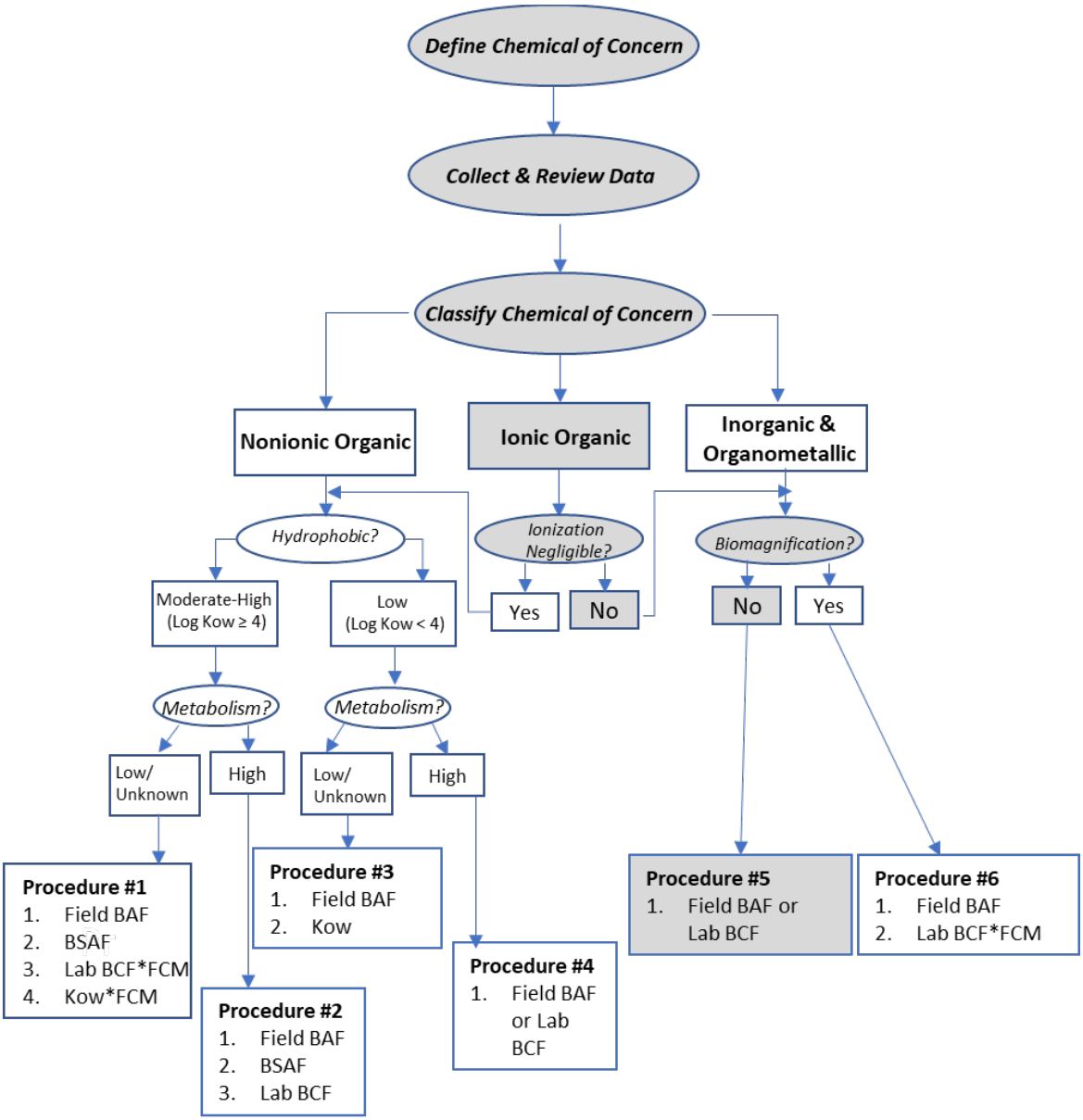


Figure 1. Application of the BAF framework for PFOA; gray boxes indicate steps followed based on available information for PFOA (EPA, 2000a).

The national-level BAF equation adjusts the TL baseline BAFs for nonionic organic chemicals by national default values for lipid content, as well as dissolved and particulate organic carbon content. The partitioning of PFOA is related to protein binding properties (ATSDR, 2021); therefore, the EPA did not normalize measured BAF values for PFOA using lipid content when calculating baseline and national BAFs. The EPA selected the recommended 50th percentile dissolved and particulate organic carbon content for the national-level default values which is consistent with the goal of national BAFs (i.e., as central tendency estimates), as described in Section 6.3 of the Technical Support Document, Volume 2 (EPA, 2003). Adjustments for water-dissolved portions of PFOA is applied to TL baseline BAFs (EPA, 2000a) (see Appendix A).

The EPA followed the framework described in the Technical Support Document, Volume 2 (EPA, 2003), also presented in Figure 1, to select a procedure for estimating national BAFs for PFOA. Based on the characteristics of this chemical, the EPA selected Procedure 5 for deriving a national BAF value. PFOA has the following characteristics:

- Ionic organic chemicals, with ionization not negligible (NCBI, 2024).
- Biomagnification unlikely (Houde et al., 2011; Du et al., 2021; Munoz et al., 2022).

The EPA was able to locate peer-reviewed, field-measured BAFS for TLs 2, 3, and 4 from the sources evaluated for which sufficient information was provided to indicate the quality and usability of the data; therefore, the EPA included only field BAF studies. The EPA used the BAF method to derive the national BAF values for PFOA:

- TL 2 = 22 L/kg
- TL 3 = 49 L/kg
- TL 4 = 31 L/kg

5 Selection of Toxicity Value

5.1 Approach

The EPA considered all available final toxicity values for both noncarcinogenic and carcinogenic toxicological effects after oral exposure to develop AWQC for PFOA. As described in the 2000 Methodology (EPA, 2000a), where data are available, the EPA derives AWQC for both noncarcinogenic and carcinogenic effects and selects the more protective value for the recommended AWQC. (See Section 7, Criteria Derivation: Analysis.)

For noncarcinogenic toxicological effects, the EPA uses a chronic-duration oral reference values (RfVs; RfDs or equivalent) to derive human health AWQC. An RfV is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA, 2002). An RfV may be derived from a toxicological study or a human epidemiological study, from which a point of departure (POD; i.e., a no-observed-adverse-effect level [NOAEL], lowest-observed-adverse-effect level [LOAEL], or benchmark dose [BMD]) can be derived. To derive the RfV, uncertainty factors are applied to the POD to reflect the limitations of the data in accordance with the EPA human health risk assessment methodology (EPA, 2002, 2014a, 2022a).

For carcinogenic toxicological effects, the EPA uses an oral CSF to derive human health AWQC. The oral CSF is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime oral exposure to a stressor. This value may also be derived from animal toxicological studies or human epidemiological studies.

In developing AWQC, the EPA conducts a systematic search of peer-reviewed, publicly available final toxicity assessments to obtain the toxicity value(s) (RfV and/or CSF) for use in developing AWQC. The EPA identified toxicological assessments by systematically searching websites of the following EPA program offices, other national and international programs, and state programs in April 2024:

- EPA, Office of Research and Development
    - Integrated Risk Information System (IRIS) program (EPA, 2024i)
    - Provisional Peer-Reviewed Toxicity Values (PPRTV) (EPA, 2024j)
    - ORD Human Health Toxicity Values (EPA, 2024k)
- EPA, Office of Pesticide Programs (EPA, 2024l)
- EPA, Office of Pollution Prevention and Toxics (EPA, 2024m)
- EPA, Office of Water Drinking Water Health Effects Support Documents (EPA, 2024n)
- U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR, 2024)
- Health Canada (HC, 2023)
- California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (CalEPA, 2024)

After identifying and documenting all available final toxicity values, the EPA followed a systematic process to consider the identified toxicity values and select the toxicity value(s)to derive the AWQC for noncarcinogenic and carcinogenic effects. The EPA selected IRIS toxicity values to derive the draft AWQC if *any* of the following conditions were met:

1. The EPA's IRIS toxicological assessment was the only available source of a toxicity value.
2. The EPA's IRIS toxicological assessment was the most current source of a toxicity value.
3. The toxicity value from a more current toxicological assessment from a source other than the EPA's IRIS program was based on the same principal study and was numerically the same as an older toxicity value from the EPA IRIS program.
4. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not include the relevant toxicity value (chronic-duration oral RfD or CSF).
5. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not introduce new science (e.g., the toxicity value was not based on a newer principal study) or use a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

The EPA selected the toxicity value from a peer-reviewed, publicly available source other than the EPA IRIS program to derive the draft AWQC if *any* of the following conditions were met:

1. The chemical is currently used as a pesticide, and the EPA Office of Pesticide Programs had a toxicity value that was used in pesticide registration decision-making.
2. A toxicological assessment from a source other than the EPA's IRIS program was the only available source of a toxicity value.
3. A more current toxicological assessment from a source other than the EPA's IRIS program introduced new science (e.g., the toxicity value was based on a newer principal study) or used a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

5.2 Toxicity Value for PFOA

5.2.1 Reference Dose

After following the approach outlined in Section 5.1, the EPA identified the final *Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts* (EPA, 2024g). This document is the most recent toxicity assessment identified for PFOA and used the best available science in the evaluation of noncancer risk. The EPA did not identify any other assessments that presented newer scientific information (i.e., unique RfVs) for PFOA.

The EPA's final human health toxicity assessment for PFOA (EPA, 2024g) considered all publicly available human epidemiological, animal toxicological, mechanistic and toxicokinetic evidence relevant to studies that evaluated health effects after oral PFOA exposure. Overall, the available *evidence indicates* that PFOA exposure is likely to cause hepatic, immunological, cardiovascular, and developmental effects in humans, given sufficient exposure conditions (e.g., at levels in humans as low as 1.1 to 5.2 ng/mL and doses in animals as low as 0.3 to 1.0 mg/kg/day). These judgments are based on data from epidemiological studies of infants, children, adolescents, pregnant individuals, and non-pregnant adults, as well as short-term (28-day), subchronic (90-day), developmental (gestational), and chronic (2-year) oral-exposure studies in rodents.

PODs were developed following EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012) and converted to external POD human equivalent doses (POD_{HED}s) using pharmacokinetic modeling. Consistent with the recommendations presented in *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002), the EPA applied uncertainty factors (UFs) to POD_{HED}s to address intraspecies variability, interspecies variability, extrapolation from a lowest observed adverse effect level (LOAEL) to no observed adverse effect level (NOAEL), extrapolation from a subchronic to a chronic exposure duration, and database deficiencies. The EPA derived and considered multiple candidate RfDs from both epidemiological and animal toxicological studies across the four noncancer health outcomes that the EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental).

Decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children (Budtz-Jorgensen and Grandjean, 2018), decreased infant birth weight (Wikström et al., 2020), and increased total cholesterol in adults (Dong et al., 2019) were selected as the co-critical effects

for the overall oral **RfD of 3 × 10^{-8} mg/kg/day** (EPA, 2024g). This RfD was derived by applying a total UF of 10 to account for intraspecies variability (UF_H). Critical effects observed during developmental periods (decreased antibody concentrations in children, decreased birth weight) represent effects in susceptible subpopulations. The RfD based on these effects is considered protective of effects resulting from lifetime exposures to PFOA, as well as short-term risk assessment scenarios, as the observed developmental endpoints can potentially result from a short-term exposure during critical periods of development.

5.2.2 Cancer Slope Factor

Consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a), the EPA's *Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts* (EPA, 2024g) reviewed the weight of the evidence across epidemiological, animal toxicological, and mechanistic studies and concluded that PFOA is *Likely to Be Carcinogenic to Humans* via the oral route of exposure. Epidemiological studies provided evidence of kidney and testicular cancer in humans and some evidence of breast cancer in susceptible subpopulations. Chronic oral animal toxicological studies in Sprague-Dawley rats reported Leydig cell tumors (LCT), pancreatic acinar cell tumors (PACT), and hepatocellular tumors. PFOA exposure is associated with multiple key characteristics of carcinogenicity (Smith, 2016). Available mechanistic data suggest that multiple MOAs could be involved in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in humans and animal models.

To derive a CSF for PFOA, the EPA followed agency risk assessment guidelines and methodologies (EPA, 2005a, 2012, 2022c). EPA conducted benchmark dose modeling and used a similar pharmacokinetic modeling approach as described for the derivation of noncancer RfDs above (see Section 5.2.1). EPA derived and considered multiple candidate CSFs from both epidemiological and animal toxicological studies across multiple tissue types and organ systems (i.e., kidney, liver, pancreas, testes). CSFs were derived for epidemiological data on renal cell carcinoma (RCC) and kidney cancer using weighted linear regressions to calculate quartile-specific relative kidney cancer risks. Relative risks were then converted to the absolute risk scale, yielding an internal CSF, which represents the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was then divided by the selected clearance value and converted to an external dose CSF. For animal toxicological studies, multistage cancer models were used to predict the doses at which the selected BMR for tumor incidence would occur. BMDLs for each tumor type served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type (EPA, 2024g).

The **oral slope factor of 0.0293 (ng/kg/day)$^{-1}$** (29,300 (mg/kg/day)$^{-1}$) for RCC in human males from Shearer et al. (2021) was selected as the basis of the overall CSF for PFOA (EPA, 2024g). Per EPA's *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005a,b) age-dependent adjustment factors were not applied during CSF derivation, as a mutagenic mode of action (MOA) was not determined for PFOA from a review of the available studies, and evidence did not support increased susceptibility to cancer following PFOA exposure during early life.

6 Relative Source Contribution (RSC) Derivation

6.1 Approach

The EPA applies an RSC to the RfD when calculating an AWQC based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to AWQC-related sources (EPA, 2000a). The purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the AWQC), when combined with other identified sources of exposure (e.g., diet, excluding freshwater and estuarine fish and shellfish, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to consumption of ambient water (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with ambient water) and fish and shellfish from inland and nearshore waters relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. The EPA considers any potentially significant exposure source and route when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's 2000 Methodology (EPA, 2000a). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population, depending on the available data.

An RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (EPA, 2000a). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (EPA, 2000a). The EPA's approach recommends a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies.

The EPA's Exposure Decision Tree approach states that when there are insufficient environmental monitoring and/or exposure intake data to permit quantitative derivation of the RSC, the recommended RSC is 20%. In the case of AWQC development, this means that 20% of the exposure equal to the RfD is allocated to the consumption of ambient water and fish and shellfish from inland and nearshore waters and the remaining 80% is reserved for other potential sources, such as diet (excluding fish and shellfish from inland and nearshore waters), air, consumer products, etc. This 20% RSC can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant,

the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (EPA, 2000a). Applying a lower RSC (e.g., 20%) is a more health protective approach to public health and results in a lower AWQC.

To derive an RSC for PFOA, the EPA evaluated the exposure information identified through conducting prior systematic literature searches performed as part of the EPA's final human health toxicity assessment for PFOA (EPA, 2024g). To identify information on PFOA exposure routes and sources to inform RSC determination, the EPA considered primary literature published between 2003–2020 that was collected by the EPA's Office of Research and Development as part of an effort to evaluate evidence for pathways of human exposure to eight PFAS, including PFOA. This search was not date-limited and spanned information collected across the Web of Science, PubMed, and ToxNet/ToxLine (now ProQuest) databases. An updated literature search was conducted and captured relevant literature published through March 2021. Literature captured by this search is housed in the EPA's HERO database (https://hero.epa.gov/). To supplement the primary literature database, the EPA also searched the following gray literature sources in February 2022 for information related to relative exposure of PFOA for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans. The full description of methods used to identify and screen relevant literature is available in the EPA's Final Appendix: Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts (EPA, 2024h). The following description in Section 6.2 is a summary of the information provided in the Appendix of the final PFOA toxicity assessment.

6.2 Summary of Potential Exposure Sources of PFOA Other Than Water and Freshwater and Estuarine Fish/Shellfish

6.2.1 Dietary Sources

A number of studies support food ingestion as a major source of exposure to PFOA based on early studies that modeled the relative contributions of various sources among the general populations of North America and Europe (Fromme et al., 2009; Trudel et al., 2008; Vestergren and Cousins, 2009). The exposure to adults in the U.S. population is typically estimated to be about 2 ng/kg-d to 3 ng/kg-d (Gleason et al., 2017). The dominance of the food ingestion pathway is attributed to bioaccumulation in food from environmental emissions, relatively large amounts of foods being consumed, and high gastrointestinal uptake (Trudel et al., 2008). However, the estimates are highly uncertain due to analytical methods with poor sensitivity, relatively few food items with detectable levels, and levels that can vary greatly depending on sources or location (Gleason et al., 2017).

There is currently no comprehensive, nationwide Total Diet Study (TDS) for PFOA that can be used to draw conclusions about the occurrence and potential risk of PFOA in the U.S. food supply for the general population. In 2021, the FDA released PFAS testing results from their first survey of nationally distributed processed foods, including several baby foods, collected for the TDS (FDA, 2021a). Results of the survey showed that 164 of the 167 foods tested had no detectable levels of PFAS measured. Three food samples (fish sticks, canned tuna, and protein powder) had detectable levels of PFAS but did not include PFOA (FDA, 2021b). PFOA was not

detected in any of the food samples analyzed in the FDA TDS samples of produce, meats, dairy and grain products in 2019 or 2021 (FDA, 2020a,b, 2021c). In a 2018 focused study near a PFAS production plant in the Fayetteville, North Carolina area, PFOA was detected in several produce samples (cabbage, collard greens, kale, mustard greens, swiss chard, and lettuce) (FDA, 2018). In bottled water, PFOA was below the lower limit of quantification (LOQ; 4 ng/L) in all (30) analyzed samples of domestic and imported carbonated water and noncarbonated bottled water (FDA, 2016). The sample size in these studies is limited, and thus, the results cannot be used to draw definitive conclusions about the general levels of PFAS in the U.S. food supply (FDA, 2023). In a 2010 study, PFOA was detected in food samples collected from five grocery stores in Texas (Schecter et al., 2010); based on the results from this study and on dietary intakes from the 2007 USDA food availability data set, the estimated daily exposure to PFOA per capita was 60 ng/day (EPA, 2016a).

As a component of a scientific evaluation on the risks to human health related to PFAS in food, the European Food Safety Authority (EFSA) conducted an exposure assessment using consumption data from the EFSA Comprehensive Food Consumption Database and 69,433 analytical results for 26 PFAS in 1,528 samples of food and beverages obtained from 16 European countries (EFSA, 2020). Samples were collected between the years 2000 and 2016 (74% after 2008), mainly from Norway, Germany, and France. With 92% of the analytical results below the LOD or LOQ, lower bound dietary exposure estimates were obtained by assigning zero to values below LOD/LOQ. Median chronic dietary exposures of PFOA for children and adults were estimated as 0.30 and 0.18 ng/kg body weight per day, respectively. The most important contributor was "fish and other seafood[e]," followed by "eggs and egg products," "meat and meat products," and "fruit and fruit products." "Vegetables and vegetable products" and "drinking water" were also found to be important contributors to dietary PFOA exposure. It is unclear whether or not the contribution from food contact material is reflected in the data.

The 2020 EFSA report highlighted a recent study of aggregate exposure to PFAS from diet, house dust, indoor air, and dermal contact among Norwegian adults (Poothong et al., 2020). Dietary exposures were estimated for 61 study participants using food diaries and data on concentrations from an extensive Norwegian database of concentrations in 68 different food and drinks (including drinking water). For PFOA, dietary intake was by far the greatest contributor to aggregate exposure (contributing 92% of total estimated PFOA intake), but intake from ingestion of house dust represented the dominant pathway for some of the top 20% most highly exposed individuals. On average, measured serum concentrations of PFOA were similar to modeled concentrations based on intakes. It is notable that while the authors reported significant positive correlations between PFOA concentrations in serum and estimated intakes based on surface dust and vacuum cleaner bag dust samples, correlations with estimated dietary intakes were not significant, which the authors attributed to temporal variations in dietary intakes over several years. While the authors did not separately quantify intake from food and drinking water, an earlier article from the same research group

[e] Some dietary studies use the term "seafood" to indicate fish and shellfish from ocean, freshwater, or estuarine water bodies. Information about the water bodies assessed in individual studies is reported in the articles.

(Papadopoulou et al., 2017) reported measured concentrations in duplicate diets with median estimated intake of PFOA approximately three times higher from solid food than from liquids.

Zafeiraki et al. (2019) analyzed about 250 samples of marine fish, farmed fish, crustaceans, bivalves and European eel, caught in Dutch waters or purchased at Dutch markets between 2012 and 2018. Samples were analyzed for 16 PFAS, including PFOA. Brown crab and shrimp had the highest average concentrations of PFOA (0.78 ng/g ww and 0.43 ng/g ww, respectively). PFOA was also detected in farmed fish including eel and trout, and marine fish species including cod, haddock, and sole.

In seafood samples collected for the FDA 2021–2022 seafood survey (FDA, 2022), Young et al. (2022), analyzed concentrations of 20 PFAS, including PFOA, in eight of the most highly consumed marine seafood products in the United States. PFOA was detected most frequently (100% of samples; n = 10) and at the highest average concentrations (8,334 parts per trillion [ppt]) in clams and was also detected in 100% of crab samples (n = 11; 300.9 ppt average concentration). The study reported detections in cod (20% of samples; n = 10; 103.5 ppt average concentration in samples with detections). PFOA was not detected above the MDL (68 ppt or 90 ppt) in tuna, salmon, shrimp, or pollock.

6.2.2 Food Contact Materials

The FDA has authorized the use of PFAS in food contact substances due to their nonstick and grease, oil, and water-resistant properties since the 1960s. There are four categories of products that may contain PFAS (FDA, 2020a,b):

- Nonstick cookware: PFAS may be used as a coating to make cookware nonstick.
- Gaskets, O-Rings, and other parts used in food processing equipment: PFAS may be used as a resin in forming certain parts used in food processing equipment that require chemical and physical durability.
- Processing aids: PFAS may be used as processing aids for manufacturing other food contact polymers to reduce build-up on manufacturing equipment.
- Paper/paperboard food packaging: PFAS may be used as grease-proofing agents in fast-food wrappers, microwave popcorn bags, takeout paperboard containers, and pet food bags to prevent oil and grease from foods from leaking through the packaging.

Paper products used for food packaging are often treated with PFAS for water and grease resistance. In previous testing, sandwich wrappers, french fry boxes, and bakery bags were all been found to contain PFAS (Schreder and Dickman, 2018). Older generation PFAS (e.g., PFOA, PFOS) were manufactured and used in products for decades, and the bulk of the information available on PFAS toxicity relates to the older compounds. However, because newer generation PFAS are more mobile than their predecessors, they migrate more readily into food. In 2016, the FDA deauthorized the remaining uses of long-chain “C8” PFAS in food packaging, which are therefore, no longer used in food contact applications sold in the United States (FDA, 2020a,b).

Schaider et al. (2017) collected 407 samples of food contact papers, beverage containers, and paperboard boxes from locations throughout the United States. Twenty fast food packaging samples of the 407 total samples were selected for more extensive PFAS specific analysis. PFOA, was among the PFAS with the highest detection rates and was detected in six out of 20 samples.

An analysis of popcorn bags, snack bags, and sandwich bags purchased in 2018 from international vendors and grocery stores in the United States found little evidence of PFOA, with only two popcorn bags with content above the limit of quantitation of 5.11 ng per gram (ng/g) of paper (Monge Brenes et al., 2019). The authors presented these results as evidence of a reduction in PFOA concentrations in microwave packaging between 2005 and 2018. In an analysis of microwave popcorn bags from around the world, Zabaleta et al. (2017) reported no measurable concentrations of PFOA in the two bags from the United States, levels typically at about 4 ng/g in those from several European countries, and levels around 50 ng/g in bags from China.

Yuan et al. (2016) analyzed 25 food contact materials purchased in Columbus, Ohio for PFAS as compared to 69 products purchased in China. In food packaging materials from China, of the 15 detected perfluorinated carboxylic acids, PFOA was the most frequently detected (90%) and was detected with the highest median concentration (1.72 ng/g). The authors also report a migration efficiency of PFOA from paper bowl packaging into food stimulants of 1.58%. This is a relatively low efficiency compared to several of the fluorotelomer alcohols (FTOHs) which the authors reported to migrate with greater than 90% efficiency.

Zabaleta et al. (2020) also monitored migration of the PFAS carboxylates (C6 to C10) from packaging materials into cereal, rice, or milk. For each PFAS studied the percent migration to milk exceeded that to rice with the lowest percent migration being that to cereal. The migration percentage of PFOA into cereal, rice, and milk powder products over six months ranged from 1.4%–5.6%.

6.2.3 Consumer Product Uses

A targeted analysis of 29 U.S. and Canadian cosmetic products with high fluorine content (Whitehead et al., 2021) found high concentrations of FTOH, including 8:2 FTOH, commonly present in the formulations. A fraction of 8:2 FTOH is believed to undergo metabolic transformation into PFOA. In addition to direct contact with personal care products, products and articles (and the use of these) may be sources in the indoor environment that manifest as measured occurrence in house dust and indoor air. An earlier investigation of consumer exposure to PFOA by Trudel et al. (2008) used mechanistic modeling together with information on product use habits to estimate oral and dermal exposures from clothes, carpet, upholstery, and food contact materials. Noting that PFOA may be contained as a contaminant in older and in new products, the authors estimated exposure via both mill-treated and home-treated carpets. The authors concluded that contact with consumer products is not a significant contributor to total exposure, but that since PFOA may be a contaminant in even new products, consumer exposure may continue to occur, particularly via both mill-treated and home-treated carpets. The authors also point out that carpet and other textiles are likely to be continuing

sources of PFOA in house dust. In contrast, in an analysis of 116 articles of commerce from the United States, the EPA (2009) identified carpets and related products as potentially the most significant source of perfluorinated carboxylic acids (PFCAs) out of 13 total product categories analyzed. PFOA was detected in all 13 product types. Other important indoor sources of PFCAs include floor wax/sealant and home textiles, upholstery, and apparel. In a similar analysis of 52 European products collected between 2014–2016, Borg and Ivarrson (2017) reported that PFOA was the most commonly detected PFAS and was detected in all samples except those that did not contain any detectable levels of PFAS. Notably, the authors specifically targeted products that were known or suspected to contain PFAS in their analyses.

Liu et al. (2014) investigated trends in PFAS content of household goods between 2007 and 2011. They reported that while PFOA concentrations displayed an overall downward temporal trend with significant reductions observed in nearly all product categories, PFOA was still detected in many products. Kotthoff et al. (2015) similarly reported broad detection of PFOA in a 2010 sampling effort that collected 115 European consumer products, including carpets, leather, outdoor textiles, cooking materials, and others. PFOA was detected in all but one sample type, often at the highest median concentration compared to other PFCAs. The product samples with the highest concentrations of PFOA included ski wax (median concentration of 15.5 µg/kg), leather products (median concentration of 12.4 µg/m^2), and outdoor materials (median concentration of 6 µg/m^2). PFOA has also been detected in textile samples of outdoor apparel from Europe and Asia (Gremmel et al., 2016; van der Veen et al., 2020). PFOA was detected in jackets ranging from concentrations of 0.02–4.59 µg/m^2 (Gremmel et al., 2016). Interestingly, the level of almost all individual PFAS, including PFOA, and total PFAS increased when the textiles were subjected to weathering (i.e., increased ultraviolet [UV] radiation, temperature, and humidity for 300 hours to mimic the average lifespan of outdoor apparel) (van der Veen et al., 2020).

6.2.4 Indoor Dust

Several studies suggest that PFOA and its precursors in indoor air and/or house dust may be an important exposure source for some individuals (Shoeib et al., 2011; Schlummer et al., 2013; Gebbink et al., 2015; Poothong et al., 2020). PFOA is generally a dominant ionic PFAS constituent in indoor air and dust, frequently occurring above detection limits and at relatively high concentrations in all or most samples (Shoeib et al., 2011; Kim et al., 2019; Wu et al., 2015; Poothong et al., 2020; Makey et al., 2017; Byrne et al., 2017; Fraser et al., 2013).

PFOA was measured at the highest concentrations (geometric mean concentrations ranging from 41.4–45.0 ng/g) and frequencies (ranging from 89%–91% detected) in dust sampled from Californian households (Wu et al., 2015). Similarly, PFOA was found at the second highest levels (mean concentration of 1.98 ng/g) of 15 PFAS measured in dust samples taken from households in Seoul, South Korea (Kim et al., 2019). PFOA was detected in all dust samples from that study. Makey et al. (2017) measured PFOA and PFOA precursors in dust and found weak correlations between concentrations in dust and serum PFOA concentrations in pregnant Canadian participants. One study in Alaska Natives found no correlation between dust and serum PFOA concentrations (Byrne et al., 2017).

6.2.5 Ambient Air

Perfluoroalkyl chemicals have been found in ambient air globally, with the highest concentrations observed or expected in urban areas and nearest to industrial facilities, areas where AFFF firefighting foams are used, wastewater treatment plants, waste incinerators, and landfills (Ahrens et al., 2011b). Perfluorinated acids were measured in Albany, New York air samples (gas mean concentration of 3.16 pg/m^3 and particulate phase mean concentration of 2.03 pg/m^3) (Kim and Kannan, 2007). In Minneapolis, Minnesota, PFOA in the particulate phase ranged from 1.6 pg/m^3 to 5.1 pg/m^3 and from 1.7 pg/m^3 to 16.1 pg/m^3 in the gas phase (MPCA, 2008). Even remote areas far from urban centers have previously reported PFOA concentrations in air samples; PFOA has been detected in Resolute Bay, Nunavut, Canada (Stock et al., 2007), as well as other Arctic environments (Butt et al., 2010).

The EPA's Toxics Release Inventory reported release data for PFOA in 2022 (EPA, 2024o). PFOA is not listed as a hazardous air pollutant under the Clean Air Act (EPA, 2024p). However, two states (New York and Michigan) have set enforceable air emissions limits. Ambient air is a possible source of exposure to PFOA for the general population; however, the contribution of air to total exposure is likely low. For example, De Silva et al. (2021) estimated that less than 1% of PFOA exposure to humans in the United States is from inhalation.

6.2.6 Summary and Recommended RSC for PFOA

As mentioned above, the scope of exposure sources considered for the draft recommended human health AWQC is limited to surface water used for drinking water and the consumption of freshwater/estuarine fish and shellfish (EPA, 2000a), consistent with previous human health AWQC (EPA, 2015). The EPA followed the Exposure Decision Tree approach to determine the RSC for PFOA (EPA, 2000a; see Figure 2).

To identify the population(s) of concern (Box 1, Figure 2), the EPA first identified potential subpopulations or life stages based on the PFOA exposure interval in the critical studies from which the critical effect was selected for RfD derivation in the PFOA toxicity assessment (EPA, 2024d). Since the critical effects are the most sensitive adverse health effects that were identified from the available data of sufficient quality, then the exposure intervals may be sensitive windows of exposure. Three co-critical effects were identified for PFOA in three human epidemiological studies (decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children, decreased infant birth weight, and increased total cholesterol in adults); however, the specific critical windows of exposure for each of the critical effects is not known. However, based on epidemiological study design, potentially sensitive life stages include women of childbearing age who may be or become pregnant, pregnant women and their developing fetuses, lactating women, and early childhood. Limited information was available regarding specific PFOA exposure in these life stages from different environmental sources. Therefore, the EPA considered exposures in the general U.S. population, ages 21+, which includes some of these potentially sensitive life stages (i.e., women of childbearing age, pregnant women and their developing fetuses, and lactating women).

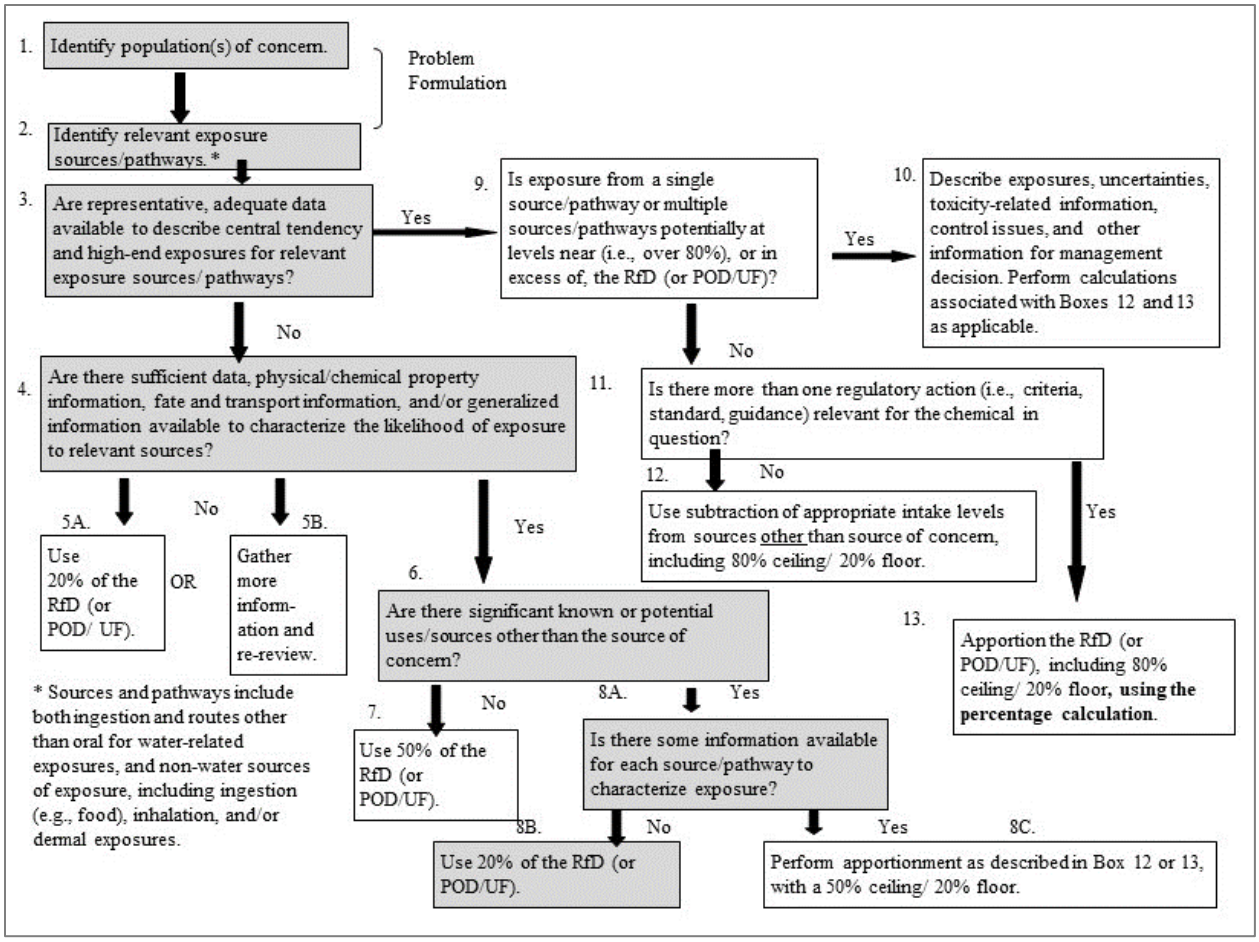


Figure 2. RSC exposure decision tree framework for PFOA; figure adapted from EPA (2000a) with gray boxes indicating key decision points for this chemical.

Second, the EPA identified PFOA-relevant exposure sources/pathways (Box 2, Figure 2) including dietary consumption, incidental oral, inhalation, or dermal exposure via dust, consumer products, and soil, and inhalation exposure via ambient air. Several of these may be potentially significant exposure sources.

Third, the EPA evaluated whether adequate data were available to describe the central tendencies and high-end exposures for all potentially significant exposure sources and pathways (Box 3, Figure 2). The EPA determined that there were inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources. For example, studies from the United States, Canada and Europe indicate that consumer products may be significant sources of exposure to PFOA. Although several studies report PFOA detections in consumer products, most examined very few samples (i.e., n = 1–5) of only a few types of media. Therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population.

Fourth, the agency determined whether there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4, Figure 2). Sufficient information for PFOA was available to characterize the likelihood of exposure. To determine if there are potential uses/source of PFOA other than AWQC-related sources (Box 6, Figure 2), the agency relied on the studies summarized in Section 6 (this document). There are potential other uses/sources of PFOA. PFOA has been detected in soils, dust in carpets and upholstered furniture in homes, offices, and vehicles. Incidental exposure from soils and dust is an important exposure route, particularly for small children because of their increase level of hand-to-mouth behaviors compared to adults. Also, the levels in soils and surface waters can affect the concentrations in local produce, meat/poultry, dairy products and particulates in the air. Based on this information, the next step was to determine if adequate information was available on PFOA to characterize each source/pathway of exposure (Box 8a, Figure 2). The EPA determined there is not enough information available on each source to make a quantitative characterization of exposure among exposure sources. Therefore, the data are insufficient to allow for quantitative characterization of the different exposure sources. The EPA's Exposure Decision Tree approach states that when there is insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20% (EPA, 2000a). Thus, the EPA recommends an **RSC of 20% (0.20)** for PFOA (Box 8b, Figure 2) for both the water plus organism AWQC as well as the organism only AWQC.

7 Criteria Derivation: Analysis

Table 3 summarizes the input parameters used to derive the draft recommended human health AWQC that are protective of exposure to PFOA from consuming drinking water and/or eating fish and shellfish (organisms) from inland and nearshore waters. The criteria calculations are presented below. These criteria recommendations are based on the 2000 Methodology (EPA, 2000a) and the toxicity and exposure assumptions described above (see Section 4, AWQC Input Parameters; Section 5, Selection of Toxicity Value; and Section 6, Relative Source Contribution Derivation).

Table 3. Input parameters for the human health AWQC for PFOA.

Input Parameter		Value
RfD		0.00000003 mg/kg-d
CSF		29,300 $[mg/kg\text{-}d]^{-1}$
RSC		0.20
BW		80.0 kg
DWI		2.3 L/d
FCR	TL 2	0.0076 kg/d
	TL 3	0.0086 kg/d
	TL 4	0.0051 kg/d
BAF	TL 2	22 L/kg
	TL 3	49 L/kg
	TL 4	31 L/kg

Notes: RfD = reference dose; CSF = cancer slope factor; RSC = relative source contribution; BW = bodyweight; DWI = drinking water intake; FCR = fish consumption rate; TL = trophic level; BAF = bioaccumulation factor.

7.1 AWQC for Noncarcinogenic Toxicological Effects

For consumption of water and organisms:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\text{DWI (L/d)} + \sum_{i=2}^{4} (\text{FCR}_i\ \text{(kg/d)} \times \text{BAF}_i\ \text{(L/kg)})}$$

$$= \frac{0.00000003\ \text{mg/kg-d} \times 0.20 \times 80.0\ \text{kg} \times 1{,}000\ \mu g/mg}{2.3\ \text{L/d} + ((0.0076\ \text{kg/d} \times 22\ \text{L/kg}) + (0.0086\ \text{kg/d} \times 49\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 31\ \text{L/kg}))}$$

$$= 0.0001575\ \mu g/L$$

$$= 0.0002\ \mu g/L \text{ (rounded)}$$

For consumption of organisms only:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\sum_{i=2}^{4} (\text{FCR}_i\ \text{(kg/d)} \times \text{BAF}_i\ \text{(L/kg)})}$$

$$= \frac{0.00000003\ \text{mg/kg-d} \times 0.20 \times 80.0\ \text{kg} \times 1{,}000\ \mu g/mg}{(0.0076\ \text{kg/d} \times 22\ \text{L/kg}) + (0.0086\ \text{kg/d} \times 49\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 31\ \text{L/kg})}$$

$$= 0.0006428\ \mu g/L$$

$$= 0.0006\ \mu g/L \text{ (rounded)}$$

7.2 AWQC for Carcinogenic Toxicological Effects

The EPA derives cancer-based HHC for contaminants that have been determined to be *Carcinogenic to Humans* or *Likely to Be Carcinogenic to Humans* (EPA, 2000a; EPA, 2000d). Since PFOA was determined to be *Likely to Be Carcinogenic to Humans* (EPA, 2024b,c), the EPA derived AWQC for carcinogenic toxicological effects.

Consumption of water and organisms:

$$\text{AWQC} = \frac{\text{RSD} \times \text{BW} \times 1{,}000^{f}}{\text{DWI} + \sum_{i=2}^{4} (\text{FCR}_i \times \text{BAF}_i)}$$

$$= \frac{(10^{-6} / 29{,}300)\ \text{mg/kg-d} \times 80.0\ \text{kg} \times 1{,}000\ \mu g/mg}{2.3\ \text{L/d} + ((0.0076\ \text{kg/d} \times 22\ \text{L/kg}) + (0.0086\ \text{kg/d} \times 49\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 31\ \text{L/kg}))}$$

$$= 0.000000896175\ \mu g/L$$

$$= 0.00000090\ \mu g/L \text{ (rounded)}$$

[f] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

For consumption of organisms only:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{g}}{\sum_{i=2}^{4} (FCR_i \times BAF_i)}$$

$$= \frac{(10^{-6} / 29{,}300)\ \text{mg/kg-d} \times 80.0\ \text{kg} \times 1{,}000\ \mu\text{g/mg}}{(0.0076\ \text{kg/d} \times 22\ \text{L/kg}) + (0.0086\ \text{kg/d} \times 49\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 31\ \text{L/kg})}$$

$$= 0.00000365659\ \mu\text{g/L}$$

$$= 0.0000036\ \mu\text{g/L (rounded)}$$

7.3 AWQC Summary for PFOA

The EPA derived the draft recommended AWQC for PFOA using both noncarcinogenic and carcinogenic toxicity endpoints. The human health AWQC for noncarcinogenic effects for PFOA are **0.0002 µg/L** (0.2 ng/L) for consumption of water and organisms and **0.0006 µg/L** (0.6 ng/L) for consumption of organisms only for the general population (≥ 21 years old) (Table 4). The EPA also evaluated the use of exposure factors relevant to sensitive subpopulations based on the critical effect(s) used to derive the noncarcinogenic RfD (Appendix B). For children 1 to < 3 years old, the criteria calculated for illustrative purposes based on noncarcinogenic effects are slightly lower than for the general population (≥ 21 years old), 0.0001 µg/L (0.1 ng/L) for consumption of water and organisms and 0.0005 µg/L (0.5 ng/L) for consumption of organisms only. The human health AWQC for carcinogenic effects (at a 10^{-6} cancer risk level) for PFOA are **0.00000090 µg/L** (0.0009 ng/L) for consumption of water and organisms and **0.0000036 µg/L** (0.0036 ng/L) for consumption of organisms only (Table 4). The EPA recommends the lower AWQC, based on the carcinogenic effects of PFOA, as the national recommended human health AWQC because they are protective of the general population, including potentially sensitive subpopulations.

Under the EPA's recently finalized Method 1633 (EPA, 2024q) for aqueous samples, the level of quantification (LOQ) representing the observed LOQs in the multi-laboratory validation study, range from 1 to 4 ng/L for PFOA. The pooled MDL for PFOA is 0.54 ng/L. The pooled MDL value is derived from the multi-laboratory validation study using MDL data from eight laboratories and represents the sensitivity that should be achievable in a well-prepared laboratory but may not represent the actual MDL used for data reporting or data quality assessments (EPA, 2024q). The MDLs and ranges presented here provide a reference for comparison of analytical concentrations and recommended criteria.

Table 4. Summary of the EPA's recommended human health AWQC for PFOA chemicals.

	Human Health AWQC for Carcinogenic Effects
Water and Organism	0.00000090 µg/L (0.0009 ng/L)
Organism Only	0.0000036 µg/L (0.0036 ng/L)

[g] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

8 Consideration of Noncancer Health Risks from PFAS Mixtures

The EPA recently released its final *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (referred to here as the PFAS mixtures framework; EPA, 2024r). The PFAS mixtures framework describes three flexible, data-driven approaches that facilitate practical component-based mixtures evaluation of two or more PFAS based on dose additivity, consistent with the EPA*'s Guidelines for the Health Risk Assessment of Chemical Mixtures* (EPA, 1986) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA, 2000c). The approaches described in the EPA PFAS mixtures framework may support interested federal, state, and Tribal partners, as well as public health experts and other stakeholders to assess the potential noncancer human health hazards and risks associated with PFAS mixtures. The EPA is providing an illustration of one approach which could be applied to PFAS mixture HHC derivation. The PFAS mixtures framework underwent peer review by the EPA Science Advisory Board (EPA, 2022b) and public review and the EPA responded to comments (EPA, 2024s). The public comment period ended on May 30, 2023. The public docket can be accessed at www.regulations.gov under Docket ID: EPA-HQ-OW-2022-0114.

Dose additivity means that the combined effect of the component chemicals in a mixture is equal to the sum of the individual doses or concentrations scaled for potency. As noted in the PFAS mixtures framework, exposure to a number of individual PFAS has been shown to elicit the same or similar profiles of adverse effects in various organs and systems. Many toxicological studies of PFAS as well as other classes of chemicals support the health-protective conclusion that chemicals that elicit the same or similar observed adverse effects following individual exposure should be assumed to act in a dose-additive manner when in a mixture unless data demonstrate otherwise (EPA, 2024r). Importantly, few studies have examined the toxicity of PFAS mixtures, particularly with component chemical membership and proportions that are representative of the diverse PFAS mixtures that occur in the environment. Mixtures assessments for chemicals that share similar adverse health effects, and therefore assume dose additivity, typically apply component-based assessment approaches.

The Hazard Index (HI) approach is one of the component-based mixtures assessment approaches described in the PFAS mixtures framework. In order to support states and Tribes interested in addressing potential noncancer risks of PFAS mixtures, the application of the HI approach for deriving HHC for mixtures is described below. States and authorized Tribes may choose to adopt this approach to derive HHC for PFAS mixtures. Use of the HI approach to assess risks associated with PFAS mixtures was supported by the EPA Science Advisory Board (EPA, 2022b).

In the HI approach (see PFAS mixtures framework; EPA, 2024r), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (EPA, 1986). The HQs for the component chemicals are then summed to derive a mixture-specific HI (for the specified exposure route/medium). Since the HI is unitless, the E and the RfV inputs to the HI formula must be expressed in the same dose units (e.g., mg/L) (Eq. 5). For example, in the context of the

human health criteria, HQs for each individual PFAS are calculated by dividing the measured ambient water concentration of each component PFAS (e.g., expressed as µg/L) by its corresponding human health criterion (e.g., expressed as µg/L), and the resulting component PFAS HQs are summed to yield the PFAS mixture HI (Eqs. 5–7). Either water-plus-organism or organism-only HHC can be used in the PFAS mixtures HI approach; however, the type of HHC selected for HI calculation should be consistent. Because cancer data are lacking for most PFAS, the HI approach is currently recommended for PFAS HHC based on noncancer effects.

A hypothetical example is included below to illustrate using the HI approach to derive an HHC for a mixture of three PFAS. A PFAS mixture HI exceeding 1 indicates that co-occurrence of two or more PFAS in a mixture in ambient water exceeds the health-protective level(s), indicating potential health risks. Some individual PFAS have HHC below the analytical MDLs (e.g., PFOA, PFOS). If one such PFAS is included as a component PFAS in the HI approach, then any detectable level of that component PFAS in surface water will result in a component HQ greater than 1, and thus, an HI greater than 1 for the PFAS mixture.

$$\mathrm{HI} = \sum_{i=1}^{n} \mathrm{HQ_i} = \sum_{i=1}^{n} \frac{\mathrm{E_i}}{\mathrm{HHC_i}} \quad \text{(Eq. 5)}$$

$$\mathrm{HI} = \mathrm{HQ_{PFAS_X}} + \mathrm{HQ_{PFAS_Y}} \quad \text{(Eq. 6)}$$

$$\mathrm{HI} = \left(\frac{[\mathrm{PFAS_{X,ambient\ water}}]}{[\mathrm{PFAS_{X,HHC}}]}\right) + \left(\frac{[\mathrm{PFAS_{Y,ambient\ water}}]}{[\mathrm{PFAS_{Y,HHC}}]}\right) \quad \text{(Eq. 7)}$$

Where:
HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS
HHC_i = human health criterion for component PFAS (i)
HQ_{PFAS} = hazard quotient for a given individual PFAS
$PFAS_X$ = Hypothetical PFAS
$PFAS_Y$ = Hypothetical PFAS
$[PFAS_{ambient\ water}]$ = concentration of a given PFAS in ambient water
$[PFAS_{HHC}]$ = water-plus-organism HHC or organism-only HHC for a given PFAS

9 Chemical Name and Synonyms

- Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1)
- PFOA
- 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid
- pentadecafluoro-1-octanoic acid
- pentadecafluoro-n-octanoic acid
- octanoic acid, pentadecafluoro-

AB Ex. 4

0104

- perfluorocaprylic acid
- pentadecafluorooctanoic acid
- perfluoroheptanecarboxylic acid

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Appendix A: Bioaccumulation Factor (BAF) Supporting Information

BAF Calculation Description for PFOA

The EPA used the decision framework presented in the *Technical Support Document, Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003) to identify procedures to derive national trophic level-specific BAFs for PFOA based on chemical's properties (e.g., ionization, hydrophobicity), metabolism, and biomagnification potential (see Figure 1 this document). The EPA followed the guidelines provided in Section 5.5 of the EPA's 2000 Methodology (EPA, 2000), to assess the occurrence of cationic and anionic forms of PFOA at typical environmental pH ranges. Based on the dissociation constant (pK_a) information provided in the Hazardous Substance Data Bank (HSDB) for PFOA, it was determined that ionization of PFOA was significant at typical environmental pH ranges (NCBI, 2023; EPA, 2024a,b).

As explained in Section 5.5 of EPA's 2000 Methodology (EPA, 2000), when a significant fraction of the total chemical concentration is expected to be present as the ionized species in water, procedures for deriving the national BAF rely on empirical (measured) methods (i.e., Procedures 5 and 6) in Figure 1. EPA followed the guidelines in Section 3.2.1 of the Technical Support Document, Volume 2, to evaluate the biomagnification potential of PFOA. Based on information in the peer-reviewed literature, it was determined that biomagnification of PFOA was unlikely (Houde et al. 2011; Du et al., 2021; Munoz et al., 2022). Based on the characteristics of PFOA, EPA selected Procedure 5 for deriving national BAF values for this chemical.

As described in Section 4.2.1, for a given procedure, the EPA selected the method that provided BAF estimates for all three TLs (TL 2–TL 4) in the following priority:

- BAF estimates using the BAF method (i.e., based on field-measured BAFs) if possible.
- BAF estimates using the laboratory BCF method if (a) the BAF method did not produce estimates for all three TLs and (b) the BCF method produced national-level BAF estimates for all three TLs.

The EPA was able to locate field-measured BAFs for TLs 2, 3, and 4 for PFOA from the peer-reviewed literature sources for which sufficient information was provided to determine the quality and usability of the data. Therefore, the EPA used the Field BAF method (EPA, 2003) to derive the national BAF values for this chemical.

Calculating Baseline BAFs

The EPA calculated baseline BAFs for PFOA using a procedure analogous to the baseline BAF calculation for nonionic organic chemicals to account for the physical and chemical properties of PFOA. Dissolved field measured BAFs were considered to be 100 percent bioavailable for the purposes of the baseline BAF calculation. Field measured BAFs reported in total concentrations were converted to dissolved BAFs using K_{poc} values (the equilibrium partition coefficient of the chemical between the particulate organic carbon [POC] phase and the freely dissolved phase of water), from the peer-reviewed literature; these BAF data converted from total to dissolved

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were added to the dissolved field measured BAF data set and used to calculate baseline BAFs for TLs 2, 3, and 4.

Methods for calculating baseline BAFs ($(Baseline\ BAF)_{TL\ n}$) involves normalizing the field-measured BAF, which are based on total concentrations in tissue and water, by the lipid content in the organism and the freely dissolved concentration in the study water (EPA, 2000, 2003). As described in ATSDR (2021), the partitioning of PFOA is related to protein binding properties (ATSDR, 2021). The EPA considered protein-normalizing measured BAF values in the baseline BAF equation. However, insufficient data were available from the scientific literature on protein content of aquatic organisms and on the binding efficiencies of PFOA to various proteins in aquatic organisms. Because of this lack of data on the relationship between protein content and PFOA bioaccumulation, attempts to normalize BAFs based on protein content would likely introduce greater uncertainty into BAF averages.

Consistent with the EPA's 2000 Methodology (EPA, 2000), a procedure analogous to the one used to adjust for the water-dissolved portions of a nonionic organic chemical is applied to measured BAFs for PFOA. As described in the EPA's (2003) Technical Support Document, Volume 2, the K_{poc} is approximately equal to the K_{ow} of a hydrophobic organic chemical. It is further described in the EPA's (2003) Technical Support Document, Volume 2, that K_{doc} (the equilibrium partition coefficient of the chemical between the dissolved organic carbon (DOC) phase and the freely dissolved phase of water) is directly proportional to the K_{ow} of a hydrophobic organic chemical, and that K_{doc} is less than the K_{ow}. Due to the unique physical-chemical properties of PFOA, K_{ow} cannot be reliably measured for these compounds and therefore cannot be used to estimate K_{poc} or K_{doc} (ATSDR, 2021).

Using the K_{oc} information in Higgins and Luthy (2006), the EPA determined that the K_{oc} values were applicable to POC but there is no indication that they would be applicable to DOC. Currently, information is not available on the partitioning of PFOA to DOC, nor on the bioavailability of PFOA partitioned to DOC. In addition, Higgins and Luthy (2006) included DOC-bound PFOA in the aqueous phase of their calculations. Thus, the amount of PFOA partitioned to DOC was presumed to be part of the aqueous fraction of the f_{fd} equation, resulting in the following formula (Equation 1):

$$f_{fd} = \frac{1}{[1 + (POC \cdot K_{oc})]} \qquad \text{(Eq. 1)}$$

Where:

- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved.
- POC = national default value of 0.5 mg/L (refer to page 5-44 of EPA's 2000 Methodology (EPA, 2000)) is used in baseline BAF calculations, unless this value is reported in the BAF source.
- K_{oc} = PFOA log K_{oc} = 2.06 (Higgins and Luthy, 2006).

Because the measured BAFs for PFOA are not adjusted for lipid or protein content, the baseline BAF equation (refer to Equation 5-10 on pages 5-24 and 5-25 of the EPA's 2000 Methodology [EPA, 2000]) is adjusted (as shown below in Equation 2) to determine the freely dissolved concentration of PFOA BAFs in water:

$$\text{Baseline BAF} = \frac{\text{Measured BAF}}{f_{fd}} - 1 \qquad \text{(Eq. 2)}$$

The EPA used this equation to calculate baseline BAFs from field measured BAFs based on total concentrations.

Dissolved PFOA Baseline BAFs

The EPA included results from several field BAF studies for PFOA reported as dissolved (i.e., filtered) concentrations in its baseline BAF calculations. Because these dissolved PFOA data are presumed to represent the freely-dissolved (non-particulate) fraction, the ffd term in Equation 2 is set to 1. Also, as described above, the measured BAFs for PFOA are not being adjusted for lipid or protein content to calculate baseline BAFs for PFOA. Thus, Equation 3 is used to calculate the freely dissolved concentration of PFOA for "baseline BAFs" using field-measured dissolved PFOA BAFs:

$$\text{Baseline BAF} = \text{Measured (dissolved) BAF} - 1 \qquad \text{(Eq. 3)}$$

Calculating National BAFs

Final baseline BAFs were used to compute national BAFs for PFOA. Equation 4 (an equation analogous to the equation used for nonionic organic chemicals for calculating national BAFs (see Equation 5-28 on Page 5-42 of the EPA's 2000 Methodology (EPA, 2000)) is used to convert the baseline BAF to a national BAF for each trophic level:

$$\text{National BAF}_{(\text{TL n})} = [(\text{Final Baseline BAF}^{fd})_{\text{TL n}} + 1] \cdot (f_{fd}) \qquad \text{(Eq. 4)}$$

Where:

- National BAF = national BAF (L/kg-tissue).
- $(\text{Final Baseline BAF})_{\text{TL n}}$ = mean baseline BAF for TL "n" (L/kg-lipid).
- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved.

In summary, for PFOA, the baseline BAFs are calculated using Equation 2 (for field BAFs calculated from total water concentrations) and Equation 3 (for field BAFs calculated from dissolved water concentrations) for each TL. National BAFs are then calculated from TL baseline BAFs using Equation 4.

National Trophic level BAF calculations:

$$\text{National BAF PFOA}_{(\text{TL 2})} = [(21.2)_{\text{TL 2}} + 1] \times (0.999942596)$$

= 22.2 L/kg

= 22 L/kg (rounded)

$$\text{National BAF PFOA}_{(\text{TL 3})} = [(47.9)_{\text{TL 3}} + 1] \times (0.999942596)$$
= 48.9 L/kg
= 49 L/kg (rounded)

$$\text{National BAF PFOA}_{(\text{TL 4})} = [(30.0)_{\text{TL 4}} + 1] \times (0.999942596)$$
= 30.9 L/kg
= 31 L/kg (rounded)

The corresponding values for TL 2, TL 3 and TL 4 were computed as 22.2 L/kg, 48.9 L/kg and 30.9 L/kg, respectively. Rounding the values to two significant figures yields national BAF values of 22, 49 and 31 L/kg for TLs 2, 3, and 4, respectively.

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Appendix B: Comparative Analysis for Potentially Sensitive Populations for PFOA

The EPA evaluated several exposure scenarios for PFOA to determine whether the national recommended criteria based on carcinogenic effects are sufficiently protective of potentially sensitive subpopulations related to the noncancer health effects. To accomplish this, the EPA considered four additional exposure scenarios, as supported by data from the EPA *Exposure Factors Handbook* (EFH; EPA, 2011) and the Human Health Methodology (EPA, 2000). Specifically, the EPA evaluated exposure parameters for "all ages" as well as four potentially sensitive life stages associated with the critical effects used to derive the PFOA chronic RfD, i.e., co-critical effects of decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children (PFOA concentration measured at age 5 and antibody concentration measured at age 7), decreased infant birth weight, and increased total cholesterol in adults. Based on this exposure interval in the critical study, the potentially sensitive subpopulations in humans include women of childbearing age who may be or become pregnant, pregnant women, lactating women, and early childhood (ages 1 to < 3 years and 3 to < 6 years) (EPA, 2024; Table B-1, this document). The age ranges for early childhood were selected because they are relevant to the exposure in the critical study (e.g., children were exposed through infancy to age five) and based on data availability (e.g., trophic level specific fish consumption rates).

For the body weight exposure parameter, a mean bodyweight of 75 kg for pregnant women (all trimesters) was identified in the EFH (2011, Ch. 8, Table 8-29). Representative body weights for the "all ages" scenario and lactating women populations were not specifically presented in the EFH (EPA, 2011). To address this data limitation, for this exercise, the EPA assumed that the average body weight for "all ages" was 71.6 kg based on the sum of the time-weighted averages of the mean male and female combined body weights from 1 year up to 80 years old from the NHANES (1999–2006) (Table 8-3; EPA, 2011). A body weight average of 67 kg for women of childbearing age was identified in the Human Health Methodology (EPA, 2000); however, this average is based on an older NHANES dataset (NHANES III; WESTAT 2000). More recent NHANES data (1999–2006) suggest that the mean body weight for women of childbearing age ranges from 65.9 kg for 16 to < 21-year-olds to 77.1 kg for 40 to < 50-year-olds (Table 8-5; EPA, 2011). Using these data, the EPA assumed a time-weighted average body weight of 73.4 kg for women of childbearing age (Table 8-5; EPA, 2011). The EPA also used this body weight for women of childbearing age as a proxy for lactating women, in the absence of other data. For children 1 to < 3 years, an average bodyweight of 11.4 kg for children 1 to < 2 years was used as a proxy for children 1 to < 3 years (EPA, 2011, Table 8-1). For children 3 to < 6 years, a mean of 18.6 kg was used (EPA, 2011, Table 8-1).

Drinking water intake values were available for all populations (Table B-1, this document).

The EPA encountered several data limitations for trophic level specific fish consumption rates for some of these potentially sensitive populations. The EPA's national criteria are typically derived using trophic-level specific fish consumption rates (FCRs), paired with trophic-level specific bioaccumulation factors (BAFs) to account for the potential bioaccumulation of some chemicals in aquatic food webs and the broad physiological differences between trophic levels which may influence bioaccumulation (EPA, 2000). Trophic level specific FCRs for women of

Table B-1. Comparison of noncancer-based HHC values for different candidate sensitive populations identified from the critical effect and study.

Population	Bodyweight (kg)	Drinking Water Intake (L/day)	Fish Consumption Rate (g/day)				Criteria (µg/L)	
			Total	TL 2	TL 3	TL 4	W + O	OO
General, adult (≥ 21 years)	80[a]	2.3[b]	22[c]	7.6[c]	8.6[c]	5.1[c]	0.0002	0.0006
Women of childbearing Age (13–49 years)	73.4[d]	2.1[e]	15.8[c]	5.6[c]	6.0[c]	2.9[c]	0.0002	0.0009
Children 1 to < 3 years	11.4[f]	0.507[g]	4.7[h]	1.2[h]	1.4[h]	1.2[h]	0.0001	0.0005
Children 3 to < 6 years	18.6[i]	0.588[j]	5.8[h]	1.7[h]	2.5[h]	1.1[h]	0.0001	0.0006
All Ages (Birth to 80 years)	71.6[k]	2.0[b]	19.3[l]	NA	NA	NA	ND	ND
Pregnant Women	75[m]	2.1[e]	10[n]	NA	NA	NA	ND	ND
Lactating Women	73.4[d]	2.7[e]	7.2[o, p]	NA	NA	NA	ND	ND

Notes: g/day = grams of fish consumed per day; L/day = liters of water per day; NA = not available; ND = not determined; OO = organism only; W + O = water plus organism.

[a] EPA, 2011, *Exposure Factors Handbook*, Ch. 8, Table 8-1, NHANES 1999–2006. Recommended mean bodyweight for adults.

[b] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010, community water, 90th percentile per capita rate.

[c] EPA, 2014; NHANES 2003–2010 survey data, 90th percentile per capita rate, freshwater and estuarine fish and shellfish edible portion, adults ≥ 21 years.

[d] Time weighted average of combined bodyweights for women ages 16 to < 50 years, NHANES 1999–2006 (EPA, 2011; Table 8-5).

[e] EPA, 2019, *Exposure Factors Handbook*; Update Ch. 3., Table 3-62, Community water, 90th percentile, per capita rate.

[f] EPA, 2011, *Exposure Factors Handbook*, Ch. 8, Table 8-1, NHANES 1999–2006. Recommended mean bodyweight ages 1 to < 2 years.

[g] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010, community water, 90th percentile per capita rate, age 1 to < 3 years.

[h] EPA, 2014. NHANES 2003–2010 survey data, 90th percentile per capita rate, freshwater and estuarine fish and shellfish edible portion, Tables 27a, 28a, 29a, ages 1 to < 3 years.

[i] EPA, 2011, *Exposure Factors Handbook*, Ch. 8, Table 8-1, NHANES 1999–2006. Recommended mean bodyweight ages 3 to < 6 years.

[j] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010, community water, 90th percentile per capita rate, ages 3 to < 6 years.

[k] Time weighted average of mean male and female combined body weights from 1 year up to 80 years, NHANES 1999–2006 (EPA, 2011; Table 8-3).

[l] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate; male and female, all ages included.

[m] EPA, 2011, *Exposures Factors Handbook*, Ch 8, mean, NHANES 1999–2006, Table 8-29

[n] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate pregnant females only.

[o] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate, breastfeeding females only.

[p] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports.

childbearing age and children were identified (Table B-1). However, trophic level specific FCRs are not available for three of the potentially sensitive life stages—all ages, pregnant women, or lactating women. Therefore, criteria could not be calculated for these three life stages. However, in these cases with available data, the total FCR for the alternative scenarios is lower than the FCR for the general population. Because bodyweights for all ages, pregnant women, and lactating women are similar to the general population (see above and Table B-1), the FCR is likely to be the main determinant of the criteria value, with a larger FCR resulting in a lower, more health protective criterion. Therefore, criteria based on the general population are expected to be protective of the identified potentially sensitive life stages (Table B-1). Separately, paired bodyweight adjusted FCRs are not available for specific trophic levels which precludes the use of body-weight adjusted DWI rates to derive ambient water quality criteria.

For illustrative purposes, the EPA calculated noncancer-based criteria based on the exposure parameters for women of childbearing age, children ages 1 to < 3 years, and children ages 3 to < 6 years. As demonstrated in Table B-1, criteria based on the exposure inputs for children 1 to < 3 years result in a slightly more health protective noncancer criteria as compared to the general population; however, the national recommended criteria for PFOA (0.00000060 µg/L W + O and 0.0000036 µg/L OO) are based on the carcinogenic toxicological endpoint (CSF), which results in the most health protective criteria overall. Therefore, the criteria based on carcinogenic effects of PFOA is protective of the noncancer-based criteria derived for the potentially sensitive populations and life stages.

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AMIGOS BRAVOS EXHIBIT 5

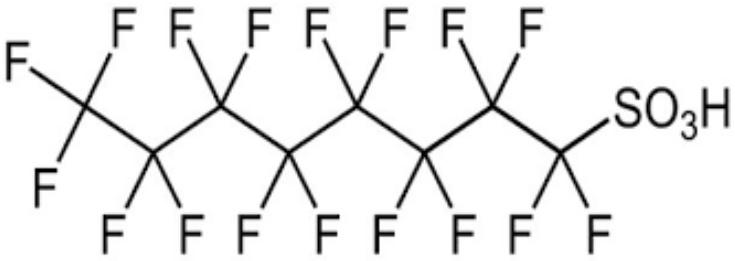
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DRAFT Human Health Ambient Water Quality Criteria:

Perfluorooctane Sulfonic Acid (PFOS) and Related Salts



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1 Introduction: Background and Scope

The U.S. Environmental Protection Agency's national recommended ambient water quality criteria (AWQC) for human health are scientifically derived numeric values that define ambient water concentrations that are expected to protect human health from the adverse effects of individual pollutants in ambient water.

Section 304(a)(1) of the Clean Water Act (CWA) requires the EPA to develop and publish, and from time-to-time revise, recommended criteria for the protection of water quality that accurately reflect the latest scientific knowledge. Water quality criteria for human health developed under section 304(a) are based solely on data and scientific judgments about the relationship between pollutant concentrations and human health effects. Section 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting pollutant concentrations in ambient water.

The EPA's recommended section 304(a) criteria provide technical information for states and authorized Tribes[a] to consider and use in adopting water quality standards that ultimately provide the basis for assessing water body health and controlling discharges of pollutants into waters of the United States. Under the CWA and its implementing regulations, states and authorized Tribes are required to adopt water quality criteria to protect the designated uses of waters (e.g., public water supply, aquatic life, recreational use, industrial use). The EPA's recommended water quality criteria do not substitute for the CWA or regulations, nor are they regulations themselves. Thus, the EPA's recommended criteria do not establish legal rights or obligations or impose legally binding requirements and are not final agency actions. States and authorized Tribes may adopt, where appropriate, other scientifically defensible water quality criteria that differ from these recommendations. EPA's water quality standards regulation at 40 CFR 131.20(a) requires states and authorized Tribes to consider any new or updated national section 304(a) recommended criteria as part of their triennial review process, and, if the state or authorized Tribe does not adopt new or revised criteria for parameters that correspond to those new or revised 304(a) criteria, to provide an explanation when it submits its triennial review to EPA. This requirement is to ensure that state or Tribal water quality standards reflect the current science and protect applicable designated uses.

The water quality criteria that are the subject of this document are draft national AWQC recommendations for human health issued under CWA section 304(a). Unless expressly indicated otherwise, all references to "human health criteria," "criteria," "water quality criteria," "ambient water quality criteria recommendations," or similar variants thereof are references to draft national AWQC recommendations for human health.

[a] Throughout this document, the term *states* means the 50 states, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term *authorized Tribe* or *Tribe* means an Indian Tribe authorized for treatment in a manner similar to a state under CWA section 518 for the purposes of section 303(c) water quality standards.

Perfluorooctane sulfonic acid (PFOS) is a member of the per- and polyfluoroalkyl substances (PFAS) class. PFAS are a large class of thousands of synthetic chemicals that have been in use in the United States and around the world since the 1940s (EPA, 2018). The ability for PFAS to withstand heat and repel water and stains makes them useful in a wide variety of consumer, commercial, and industrial products, and in the manufacturing of other products and chemicals. The current scientific evidence has shown the potential for harmful health effects after human exposure to certain PFAS. The persistence and resistance to hydrolysis, photolysis, metabolism, and microbial degradation of PFAS raise additional concerns about long-term exposure and human health effects.

The EPA developed the draft human health criteria (HHC) for PFOS to reflect the latest scientific information for input values including exposure factors (i.e., body weight [BW], drinking water intake [DWI] rate, and fish consumption rate [FCR]), bioaccumulation factors (BAFs), human health toxicity values (i.e., reference dose [RfD] or cancer slope factor [CSF]), and relative source contribution (RSC). The draft criteria are based on the EPA's current *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000a), which is referred to as the "2000 Methodology" in this document (EPA, 2000a).

2 Problem Formulation

Problem formulation provides a strategic framework for ambient water quality criteria development to systematically identify the major factors and chemical-specific scientific issues to be considered in the assessment (EPA, 2014a). The structure of this draft criteria document is intended to be consistent with general concepts of health assessments as described in the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a).

In developing AWQC, the EPA follows the assessment method outlined in the 2000 Methodology (EPA, 2000a). The 2000 Methodology describes different approaches for addressing water and nonwater exposure pathways to derive human health AWQC depending on the toxicological endpoint of concern, the toxicological effect (noncarcinogenic or carcinogenic), and whether toxicity is considered a linear or threshold effect. Water sources of human exposure include both consuming drinking water and eating fish or shellfish from inland and nearshore water bodies that have been contaminated with pollutants. For pollutants that exhibit a threshold of exposure before deleterious human health effects occur, as is the case for noncarcinogens and nonlinear carcinogens, the EPA applies an RSC. The RSC is the percentage of the total exposure to a contaminant that is attributed to the combination of drinking water and eating freshwater and estuarine fish and shellfish, where the remainder of exposure is allocated to other sources of oral exposure and other routes of exposure. The RSC is calculated by examining the data for other sources of exposure (e.g., air, food, soil) and pathways of exposure following the exposure decision tree for calculation of an RSC described in the 2000 Methodology (EPA, 2000a).

For carcinogenic substances for which the cancer slope factor was quantified using linear low-dose extrapolation, only the exposures from drinking water and fish ingestion are reflected in human health AWQC; that is, nonwater sources are not explicitly included, and no RSC is

applied (EPA, 2000a). This is because in these situations, AWQC are derived with respect to the *incremental* lifetime cancer risk posed by the presence of a substance in ambient water, rather than an individual's total risk from all exposure sources. Therefore, the resulting AWQC represents the ambient water concentration that is expected to increase an individual's lifetime risk of cancer from exposure to the pollutant by no more than one chance in one million (10^{-6}) for the general population (male and female adults, 21 years and older; referred to as "general population" herein), regardless of the additional lifetime cancer risk due to exposure, if any, to that substance from other sources. The EPA calculates AWQC at a 10^{-6} cancer risk level for the general population (EPA, 2000a). The 2000 Methodology recommends that states set human health criteria cancer risk levels for the target general population at either 10^{-5} or 10^{-6} and also notes that states and authorized Tribes can choose a more stringent risk level, such as 10^{-7}.

For substances that are carcinogenic, the EPA takes an integrated approach by considering both cancer and noncancer effects when deriving AWQC (EPA, 2000a,b). Where sufficient data are available, the EPA first derives separate AWQC for both carcinogenic and noncarcinogenic toxicity endpoints and then selects the lower (more health protective) of the two values for the recommended AWQC.

PFOS may exist in multiple forms, such as isomers or associated salts and each form may have a separate Chemical Abstracts Service registry number [CASRN] or no CASRN at all. Additionally, these compounds have various names under different classification systems. PFOS is a strong acid that is generally present as the sulfonate anion at typical environmental pH values. Therefore, the conclusions in this document apply to all isomers of PFOS, as well as nonmetal salts of PFOS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9. For the purpose of this assessment, "PFOS" will signify the ion, acid or any nonmetal salt of PFOS.

2.1 Uses and Sources of PFOS

PFAS are manufactured chemicals that have been widely used in industrial and consumer processes and products over the past several decades in the United States due to their repellant and surfactant properties. PFAS are persistent chemicals based on their physicochemical properties. Concerns about persistence of PFOS and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, metabolism, and microbial degradation.

PFOS has been used in a variety of products including surface treatments for soil and stain resistance, coating of paper, specialized applications such as firefighting foams and as a pesticide active ingredient for insect bait traps (NCBI, 2024). Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007, the EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (EPA, 2024a).

Point sources of PFOS to the aquatic environment include both industrial facilities and municipal wastewater treatment plants (WWTPs). Additional sources may include surface water runoff from industrial use sites such as metal plating facilities, areas that have received aqueous film-forming foam (AFFF) applications, landfills, and contaminated soils.

2.2 Environmental Fate and Transport in the Environment

PFOS has low volatility in the ionized form, but long-range transport occurs through particulates and through the atmospheric transport of PFOS precursors, which results in PFOS detections in remote locations (Benskin et al., 2012; Butt et al., 2010; Young et al., 2007). PFOS is water soluble and has been found in surface water, groundwater, and drinking water. The sorption of PFOS to sediments and soils varies based on the amount of organic carbon present and many other site-specific conditions; in sediments, the range of log(K_d)[b] values reported in literature is −1.4 to 6.2 (EPA, 2024b; Ahrens, 2011; Beach et al., 2006; Giesy et al., 2010; Higgins and Luthy, 2006). In the water column, and other environmental compartments, the PFOS anion is stable and resistant to hydrolysis, photolysis, volatilization and biodegradation (Beach et al., 2006; OECD, 2002; EPA, 2024a,c). The persistence of PFOS has been attributed to the strong carbon-fluorine (C-F) bond. PFOS is considered highly persistent in the aquatic environment (Ahrens, 2011). Due to the surfactant properties of PFOS, it forms three layers when added to octanol and water in a standard test system used to measure an n-octanol-water partition coefficient (K_{ow}), therefore direct measurement is not possible (Giesy et al., 2010; OECD, 2002). Additionally, PFOS is thought to bind to proteins rather than partitioning into lipids (Giesy et al., 2010; OECD, 2002; EPA, 2024a,c).

PFOS is not expected to volatilize from aqueous solution based on its vapor pressure; Henry's law constant is not measurable (i.e., predicted to be < 2.0×10^{-6}) (Beach et al., 2006). The Organization for Economic Co-Operation and Development (OECD) classified PFOS as a type 2, nonvolatile chemical that has a very low or possibly negligible volatility (Beach et al., 2006; Giesy et al., 2010; OECD, 2002). However, a potential source of PFOS is the indirect formation of PFOS through transformation of other PFAS, particularly volatile precursors (Butt et al. 2010; Wang et al. 2015; Young and Mabury, 2010).

2.3 Occurrence and Detection in Sources Relevant to Ambient Water Quality Criteria

PFOS has been detected in a variety of environmental matrices. The occurrence and detection of PFOS in sources relevant to ambient water quality criteria, including ambient water, fish and shellfish, is described below. Additional occurrence information for sources other than ambient water (e.g., air, food, soil) is summarized in Section 6.2 as part of the determination of the RSC.

2.3.1 Occurrence in Surface Water

Among the PFAS with established analytical methods for detection, PFOS is one of the dominant PFAS detected in ambient water both in the United States and worldwide (Ahrens, 2011; Benskin et al., 2012; Dinglasan-Panlilio et al., 2014; Nakayama et al., 2007; Remucal, 2019; Zareitalabad et al., 2013). Though it has a history of wide usage and is highly persistent in

[b] Log(Kd) is the logarithm of the equilibrium dissociation constant.

aquatic environments, current information on the distribution of PFOS in surface waters of the United States is somewhat limited; most published PFOS ambient water occurrence data focus on regions with known PFAS use or occurrence. These regions are primarily freshwater systems in eastern states, including the Mississippi River, Great Lakes, Cape Fear Drainage Basin, and waterbodies near Decatur, Alabama and in northern Georgia (Jarvis et al., 2021). Additional monitoring has been conducted in areas of known AFFF use.

Jarvis et al. (2021) found that concentrations of PFOS in global surface waters ranged over eight orders of magnitude, generally in picogram per liter (pg/L) to nanogram per liter (ng/L) concentrations, with reported maximum concentrations in the microgram per liter (µg/L) range (range: 0.074 ng/L–8,970,000 ng/L, arithmetic mean: 786.77 ng/L, geometric mean: 5.468 ng/L, median: 3.6 ng/L). Though these global calculated concentrations are not necessarily representative of PFOS concentrations in U.S. surface waters, the majority of PFOS concentrations reported across the U.S. (approximately 91%) are less than 300 ng/L.

2.3.2 Occurrence in Freshwater and Estuarine Fish and Shellfish

Studies have shown that PFOS bioaccumulates and biomagnifies with increasing trophic level in a variety of freshwater ecosystems (Kannan et al., 2005; Martin et al., 2004; Penland et al., 2020; Xu et al., 2014) and saltwater ecosystems (de Vos et al., 2008; Houde et al., 2006; Loi et al., 2011; Powley et al., 2008; Tomy et al., 2004) in North America, Europe, and Asia. PFOS is often the most abundant PFAS in aquatic organisms, and this high relative abundance is at least partially explained by the biotransformation of PFOS precursor chemicals into PFOS (Haukås et al., 2007; Kannan et al., 2005; Kelly et al., 2009; Martin et al., 2004; Tomy et al., 2004). Higher trophic level organisms have a greater capacity to metabolize PFOS precursor chemicals, which have been found in lower concentrations in increasing trophic level (Fang et al., 2014; Kannan et al., 2005; Martin et al., 2004).

Global distribution of PFAS, including PFOS, in tissues of aquatic species has been demonstrated in studies conducted in freshwater and marine environments across every continent, including remote regions far from direct sources, such as the high arctic, Antarctica, and oceanic islands (Giesy and Kannan, 2001; Houde et al., 2006).

The EPA collaborates with federal agencies, states, Tribes, and other partners to conduct freshwater fish contamination studies as part of a series of statistically based surveys to produce information on the condition of U.S. lakes, streams, rivers, and coastal waters (EPA, 2023a, 2024d). PFOS was detected in nearly all freshwater fish fillet samples collected during several national studies in rivers and the Great Lakes (Table 1). The 2022 National Lakes Assessment reported 86% of fish fillet samples had detectable PFOS, with a maximum concentration reported of 526 ng/g ww (EPA, 2024d).

Table 1. Summary of the EPA national fish tissue monitoring results for PFOS.

Reference	Most Commonly Sampled Species	Site Description	Results
2008–2009 National Rivers and Streams Assessment (NRSA) (Stahl et al., 2014)	Smallmouth bass Largemouth bass Channel catfish	162 urban river sites across the United States	PFOS was the most commonly detected out of 13 PFAS. PFOS was detected in 73% of fillet samples. Maximum detected concentration: 127 ng/g.
2013–2014 NRSA (EPA, 2020, 2023b)	Channel Catfish Largemouth bass Smallmouth bass	349 urban and nonurban river sites across the United States.	PFOS was the most commonly detected out of 13 PFAS. PFOS was detected in 99% of fillet samples. Maximum detected concentration: 283 ng/g.
2018–2019 NRSA (EPA, 2023a,c)	Channel catfish Smallmouth bass Largemouth bass	290 urban and nonurban river sites across the United States	PFOS was the most commonly detected out of 33 PFAS. PFOS detected in 91% of fillet samples. Maximum detected: concentration: 131 ng/g.
2010 National Coastal Condition Assessment (NCCA) Great Lakes Human Health Fish Tissue Study (Stahl et al., 2014)	Lake trout Smallmouth bass Walleye	157 nearshore sites along the U.S. shoreline of the Great Lakes	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 100% of fillet samples. Maximum detected concentration: 80 ng/g; median 15 ng/g.
2015 NCCA Great Lakes Human Health Fish Tissue Study (EPA, 2021, 2024c)	Lake Whitefish Yellow Perch Lake Trout Walleye	152 nearshore sites along the U.S. shoreline of the Great Lakes	PFOS was the most commonly detected PFAS (out of 13 PFAS). PFOS was detected in 100% of fillet samples. Maximum detected concentration: 64 ng/g; median 11 ng/g.
2022 National Lakes Assessment (EPA, 2024d)	Largemouth Bass Rainbow Trout Bluegill Yellow Perch Black Crappie	413 sampled lakes within the contiguous U.S. (excluding The Great Lakes, Great Salt Lake and lakes which are tidally influenced).	PFOS was the most commonly detected PFAS (out of 40 PFAS). PFOS was detected in 86% of fillet samples. Maximum detected concentration: 526 ng/g; median 3.17 ng/g.

Guo et al. (2012) measured PFOS in lake trout muscle tissue in Canadian waters of Lake Superior, Huron, Erie, and Ontario. Average PFOS concentrations correlated with increased watershed urbanization, and were 0.85 ng/g, 8.3 ng/g, 27 ng/g, and 46 ng/g wet weight (ww), respectively. Delinsky et al. (2010) measured PFOS in bluegill, black crappie, and pumpkinseed muscle tissue in (59 lakes in Minnesota, including four lakes in the Minneapolis–St. Paul metropolitan area. PFOS was detected in muscle tissues of fish collected in 13 of the 59 lakes, and concentrations ranged from 1.08 ng/g ww to 52.4 ng/g ww in lakes where it was detected. In the four lakes in the Minneapolis–St. Paul metropolitan area, PFOS concentrations in fish muscle tissues ranged from 4.39 ng/g ww to 47.3 ng/g ww.

Penland et al. (2020) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin–Pee Dee River, in North Carolina and South Carolina in 2015. PFOS was measured in whole body tissues of snails (6.47 ng/g ww) but was not detected in whole body tissues of Asian clam, unionid mussels, or crayfish. The highest concentrations in invertebrates were measured in aquatic insect whole body samples (132.8 ng/g ww) which was hypothesized to result from dietary uptake of aquatic biofilms. PFOS was measured in muscle tissue of all 11 sampled fish species and ranged from 11.42 ng/g ww in channel catfish to 37.36 ng/g ww in whitefin shiner. The highest PFOS concentration that Penland et al. (2020) measured was 482.9 ng/g ww, from the eggs of a redhorse fish sample.

Houde et al. (2006) measured whole body PFOS in six fish species in Charleston Harbor, South Carolina, and whole body PFOS in zooplankton and five fish species in Sarasota Bay, Florida. Charleston Harbor was the more developed of the two sites and had higher overall PFOS concentrations. Average PFOS concentrations in Charleston Harbor ranged from 19 ng/g in pinfish to 92 ng/g in spot. In Sarasota Bay, PFOS concentrations averaged 0.2 ng/g in zooplankton, and ranged from 3.1 ng/g in pigfish to 8.8 ng/g in spotted seatrout, suggesting trophic biomagnification.

Ruffle et al. (2020) analyzed freshwater finfish and shellfish from four regions of the United States and seven countries with significant imports to the United States. The highest PFOS concentrations (1.2 ng/g ww to 19.1 ng/g ww) were detected in whitefish, walleye, and yellow perch from the Great Lakes region.

PFOS concentrations in aquatic biota tend to be higher in areas with known PFAS manufacturing, industrial use, and/or application of AFFF, which also tend to be more populated areas and where recreational and subsistence fishing is more common. Several states have developed fish consumption advisories for PFOS (e.g., Alabama, Wisconsin, Minnesota, Michigan) (EPA, 2024e).

3 Criteria Formulas: Analysis Plan

Human health AWQC for toxic pollutants may be necessary to protect designated uses of water bodies related to ingestion of water (i.e., public water supply or source water protection) and ingestion of freshwater/estuarine fish and shellfish. *See* CWA 303(c)(2)(A)–(B). Although the AWQC are based on chronic health effects data (both cancer and noncancer effects), the criteria are intended to also be protective against adverse effects that may reasonably be

expected to occur as a result of elevated acute or short-term exposures (EPA, 2000a). Human health AWQC are expected to provide adequate protection not only for the general population over a lifetime of exposure, but also for sensitive life stages and subpopulations who, because of high water or fish intake rates, or because of biological sensitivities, have an increased risk of receiving a dose that would elicit adverse effect (EPA, 2000a).

The derivation of human health AWQC requires information about both the toxicological endpoints of concern from exposure to water pollutants and human exposure pathways for those pollutants. The EPA considers only the following two primary pathways of human exposure to pollutants present in a particular water body when deriving human health 304(a) AWQC: (1) direct ingestion of drinking water obtained from the water body and (2) consumption of fish and shellfish obtained from the water body.

The equations for deriving human health AWQC are presented as Equations (Eqs.) 1 and 2 for noncancer and nonlinear carcinogenic effects, and Eqs. 3 and 4 for linear carcinogenic effects. The EPA derives two separate recommended human health AWQC based on 1) the consumption of both water and aquatic organisms (Eq. 1), called "water + organism"; and 2) the consumption of freshwater/estuarine fish and shellfish alone (Eq. 2), called "organism only." The use of one criterion over the other depends on the designated use of a particular water body or water bodies (i.e., drinking water source and/or fishable waters). The EPA recommends applying organism only AWQC (Eq. 2) to a water body where the designated use includes supporting fishable uses under section 101(a) of the CWA but the water body is not a drinking water supply source (e.g., nonpotable estuarine waters that support fish or shellfish for human consumption) (EPA, 2000a).

The EPA recommends including the drinking water exposure pathway for ambient surface waters where drinking water is a designated use for the following reasons: (1) drinking water is a designated use for surface waters under the CWA, and therefore, criteria are needed to ensure that this designated use can be protected and maintained; (2) although they are rare, some public water supplies provide drinking water from surface water sources without treatment; (3) even among the majority of water supplies that do treat surface waters, existing treatments might not be effective for reducing levels of particular contaminants; and (4) in consideration of the agency's goals of pollution prevention, ambient waters should not be contaminated to a level where the burden of achieving health objectives is shifted away from those responsible for pollutant discharges and placed on downstream users that must bear the costs of upgraded or supplemental water treatment (EPA, 2000a).

The equations for deriving the criteria values are as follows (EPA, 2000a):

Equations for Noncancer and Nonlinear Carcinogen HHC:
Consumption of water and organisms:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{DWI + \sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 1)}$$

For consumption of organisms only:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{\sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 2)}$$

Where:

AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)
RfD = reference dose, expressed in milligrams per kilogram-day (mg/kg-d)
RSC = relative source contribution, unitless
BW = body weight, expressed in kg
DWI = drinking water intake, expressed in L/d
$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4
FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d
BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

Equations for Linear Carcinogens HHC:
Consumption of water and organisms:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{c}}{DWI + \sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 3)}$$

For consumption of organisms only:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{c}}{\sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 4)}$$

Where:

AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)
RSD = RSD = risk specific dose; the cancer risk level (i.e., a target risk for the population; 1 in 1 million or 10^{-6}) divided by the cancer slope factor (i.e., incidence of cancer relative to dose in units of $[mg/kg/day]^{-1}$), expressed in milligrams per kilogram-day (mg/kg-d)
BW = body weight, expressed in kg

[c] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

DWI = drinking water intake, expressed in L/d

$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4

FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d

BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

The EPA rounds AWQC to the number of significant figures in the least precise parameter as described in the 2000 Methodology (EPA, 2000a, Section 2.7.3). The EPA used a rounding procedure that is consistent with the 2000 Methodology (EPA, 2000a) and the 2015 HHC update (https://www.epa.gov/wqc/human-health-water-quality-criteria-and-methods-toxics).

4 AWQC Input Parameters

4.1 Exposure Factor Inputs

National recommended HHC establish ambient concentrations of pollutants in waters of the United States which, if not exceeded, will protect the general population from adverse health impacts from those pollutants due to consumption of aquatic organisms (i.e., freshwater and estuarine fish and shellfish) and water (EPA, 2000a). It is the EPA's longstanding practice to set national recommended HHC at a level intended to be adequately protective of a human exposure over a lifetime (EPA, 2000a). To accomplish this, the EPA uses a combination of median values, mean values, and percentile estimates for the HHC inputs consistent with the EPA's 2000 Methodology. The EPA's assumptions afford an overall level of protection targeted at the high end of the general adult population (i.e., the target population or the criteria-basis population) (EPA, 2000a). This approach is reasonably conservative and appropriate to meet the goals of the CWA and the 304(a) criteria program (EPA, 2000a). If the EPA determines that another population or life stage (e.g., pregnant women and their fetuses, young children) is the target population, then exposure parameters for that target population or life stage could be considered in the derivation of the criteria (EPA, 2000a). Potentially sensitive life stages for PFOS are explored further in a comparative analysis in Appendix B.

4.1.1 Body Weight

The BW for the general adult population including males and females, ages 21 years and older, was selected for the PFOS HHC, consistent with the population selected in the agency's most recent major update to existing 304(a) HHC (EPA, 2015) and the EPA's 2000 Methodology (EPA, 2000a). The EPA used the mean weight for adults ages 21 and older of 80.0 kg, based on National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2006 as reported in Table 8.1 of the EPA's *Exposure Factors Handbook* (EPA, 2011), the EPA's most recent publication of body weight exposure factors.

4.1.2 Drinking Water Intake Rate

For adults ages 21 and older, the EPA used an updated DWI of 2.3 L/d, rounded from 2.345 L/d. This DWI was estimated using the Food Commodity Intake Database consumption calculator (http://fcid.foodrisk.org[d]) which is based on NHANES 2005–2010 data used to develop the EPA's *Exposure Factors Handbook Update to Chapter 3, Ingestion of Water and Other Select Liquids* (EPA, 2019, Section 3.3.1.1). This rate represents the per capita estimate of combined direct and indirect community water[e] ingestion at the 90th percentile for adults, males and females, ages 21 and older. The EPA selected the per capita rate for the updated DWI because it represents the average daily dose estimates; that is, it includes both people who drank water during the survey period and those who did not, which is appropriate for a national-scale assessment such as the development of CWA section 304(a) national human health criteria development (EPA, 2019, Section 3.2.1). The updated DWI of 2.3 L/d reflects the latest scientific knowledge in accordance with CWA 304(a)(1).

The EPA's selection of the DWI of 2.3 L/d is consistent with the 2000 Methodology's selection of a rate based on per capita community water ingestion at the 86th percentile for adults surveyed in the U.S. Department of Agriculture's *1994–1996 Continuing Survey of Food Intake by Individuals (CSFII)* analysis (EPA, 2000a, Section 4.3.2.1).

4.1.3 Fish Consumption Rate

The FCR used for the general adult population is 22.0 g/d, or 0.0220 kg/d (EPA, 2014b, Table 9a). This FCR represents the 90th percentile per capita consumption rate of fish from inland and nearshore waters for U.S. adults ages 21 years and older based on NHANES data from 2003–2010. The 95% confidence interval (CI) of the 90th percentile per capita FCR is 19.1 g/d and 25.4 g/d, respectively.

As recommended in the 2000 Methodology, the EPA used TL-specific FCRs to better represent human dietary consumption of fish. An organism's trophic position in the aquatic food web can have an important effect on the magnitude of bioaccumulation of certain chemicals. The TL-specific FCRs are numbered 2, 3, and 4, and they account for different categories of fish and shellfish species based on their position in the aquatic food web: TL 2 accounts for benthic filter feeders; TL 3 accounts for forage fish; and TL 4 accounts for predatory fish (EPA, 2000a).

The EPA used the following TL-specific FCRs to derive the AWQC: TL 2 = 7.6 g/d (0.0076 kg/d) (95% CI [6.4, 9.1] g/d); TL 3 = 8.6 g/d (0.0086 kg/d) (95% CI [7.2, 10.2] g/d); and TL 4 = 5.1 g/d (0.0051 kg/d) (95% CI [4.0, 6.4] g/d). Each TL-specific FCR represents the 90th percentile per capita consumption rate of fish and shellfish from inland and nearshore waters from that particular TL for U.S. adults ages 21 years and older (EPA, 2014b, Tables 16a,

[d] Nutrition Examination Survey/What We Eat in America (NHANES/WWEIA) food intake and FCID recipes to estimate food commodity consumption for the purposes of pesticide dietary exposure assessment, as well as consumption estimates for EPA's *Exposure Factors Handbook* (EFH) users (University of Maryland, 2024).

[e] *Community water* includes direct and indirect use of tap water for household uses and excludes bottled water and other sources (EPA, 2019, Section 3.3.1.1). *Direct ingestion* is defined as direct consumption of water as a beverage, while *indirect ingestion* includes water added during food preparation (e.g., cooking, rehydration of beverages) but not water intrinsic to purchased foods (EPA, 2019, Section 3.1).

17a, and 18a). The sum of these three TL-specific FCRs is 21.3 g/d, which is within the 95% CI of the overall FCR of 22.0 g/d. The EPA recommends using the TL-specific FCRs when deriving AWQC; however, the overall FCR (22.0 g/d) may be used if a simplified approach is preferred.

4.2 Bioaccumulation Factor (BAF)

4.2.1 Approach

Several attributes of the bioaccumulation process are important to understand when deriving national BAFs for use in developing national recommended section 304(a) AWQC. First, the term *bioaccumulation* refers to the uptake and retention of a chemical by an aquatic organism from all surrounding media, such as water, food, and sediment. The term *bioconcentration* refers to the uptake and retention of a chemical by an aquatic organism from water only. In some cases, experiments conducted in a lab that measure *bioconcentration* can be used to estimate the degree of *bioaccumulation* expected in natural conditions. However, for many chemicals, particularly those that are highly persistent and hydrophobic, the magnitude of bioaccumulation by aquatic organisms can be substantially greater than the magnitude of bioconcentration. In these cases, an assessment of bioconcentration alone underestimates the extent of accumulation in aquatic biota. Accordingly, the EPA guidelines presented in the 2000 Methodology (EPA, 2000a) emphasize using, when possible, measures of *bioaccumulation* as opposed to measures of *bioconcentration* (EPA, 2000a).

The EPA estimated BAFs for the draft PFOS AWQC using the 2000 Methodology (EPA, 2000a) and the associated *Technical Support Document Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003). Specifically, these documents provide a framework for identifying alternative procedures to derive national TL-specific BAFs for a chemical based on the chemical's properties (e.g., ionization and hydrophobicity), metabolism, and biomagnification potential (EPA, 2000a, 2003). As described in the 2000 Methodology, the purpose of the EPA's national BAF is to represent the long-term, average bioaccumulation potential of a chemical in aquatic organisms that are commonly consumed by humans throughout the United States (EPA, 2000a). The EPA evaluated results from field BAF and laboratory bioconcentration factor (BCF) studies on aquatic organisms commonly consumed by humans in the United States for use in developing national trophic-level BAFs. National BAFs are not intended to reflect fluctuations in bioaccumulation over short periods (e.g., a few days) because human health AWQC are generally designed to protect humans from long-term (lifetime) exposures to waterborne chemicals (EPA, 2003).

The EPA followed the approach described in Figure 3-1 of the Technical Support Document, Volume 2 (EPA, 2003). The EPA used the best available data to classify each chemical according to this framework, and to derive the most appropriate BAFs following the 2000 Methodology (EPA, 2000a) and Technical Support Document, Volume 2 (EPA, 2003). Best available data consisted of peer-reviewed literature sources, government reports, and professional society proceedings, when sufficient information was provided to indicate the quality and usability of the data.

The framework provides six procedures to calculate a national BAF based on the pollutant's physical and chemical properties (see Figure 1, Procedures 1–6). Each procedure contains a

hierarchy of the BAF derivation methods (listed below); however, this hierarchy should not be considered inflexible (EPA, 2000a). The four methods are:

1. **BAF Method.** This method calculates national TL-specific BAFs using water and fish and shellfish tissue concentration data obtained from field studies. Field-measured BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of that contaminant in the surrounding water.

   For nonionic organic chemicals, BAFs are normalized to allow a common basis for averaging BAFs from several studies by adjusting for the water-dissolved portions of the chemical.

   In order to calculate representative TL-specific national BAFs used to calculate national recommended 304(a) criteria, the EPA averaged multiple field BAFs using a geometric mean of the normalized BAFs, first by species and then by TL, to calculate the TL baseline BAFs.

2. **BSAF Method.** This method uses biota-sediment accumulation factors (BSAFs) to estimate bioaccumulation. While BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of the contaminant in water, BSAFs divide the concentration in the organism by the concentration in surrounding sediments. BSAFs are useful when calculating site-specific criteria for compounds that are highly hydrophobic—these compounds have the potential to cause bioaccumulation in aquatic organisms even when concentrations in the water column are below detection limits.

3. **BCF Method.** This method estimates BAFs from laboratory-measured BCFs. Experiments designed to calculate BCFs aim to measure bioconcentration resulting from an organism's exposure to contaminated water. Unlike BAFs measured in the field, BCF experiments do not capture bioaccumulation from other routes of exposure or biomagnification (the increase in bioaccumulation at higher levels of the food chain). However, BCFs may be used to estimate bioaccumulation if a contaminant's chemical and physical properties indicate that the compound is likely to primarily accumulate in the organism via the water exposure route, and there is no evidence that the contaminant biomagnifies in the food chain. If insufficient field-collected data are available to calculate a national BAF, then the EPA may also estimate bioaccumulation using laboratory measured BCFs and a food chain multiplier term, which accounts for biomagnification.

   A similar process to the one described in the BAF method description (above) for normalizing of water-dissolved portions of the chemical and particulate organic carbon content is used for calculating national BAFs from laboratory-measured BCF data. Ionic organic chemicals are normalized, then multiplied by the food chain multiplier if biomagnification is likely to occur. All available BCFs are averaged using a geometric mean across species and then across TL to compute baseline BAFs.

4. **K_{ow} Method.** This method predicts BAFs based on a chemical's octanol-water partition coefficient (K_{ow}), with or without adjustment using a food chain multiplier, as described in Section 5.4 of the Technical Support Document, Volume 2 (EPA, 2003).

4.2.2 Data Selection and Evaluation

The EPA conducted a systematic literature search in October 2022 of publicly-available literature sources to determine whether they contained information relevant to calculating national BAFs for human health AWQC, using the 2000 Methodology and Technical Support Document, Volume 2 (EPA, 2000a, 2003). The literature search for reporting the bioaccumulation of PFOS was implemented by developing a series of chemical-based search terms, consistent with the process for derivation of BAFs used in the development of the EPA's Final Aquatic Life Criteria for PFOA (EPA, 2024f) and PFOS (EPA, 2024g) and described in Burkhard (2021). These terms included chemical names and Chemical Abstracts Service Registry Number (CASRN or CAS), synonyms, tradenames, and other relevant chemical forms (i.e., related compounds). Databases searched were Current Contents, ProQuest CSA, Dissertation Abstracts, Science Direct, Agricola, TOXNET, and UNIFY (database internal to the EPA's ECOTOX database). The literature search (including literature published through the first two quarters of 2020) yielded > 37,000 citations that were further refined by excluding citations on analytical methods, human health, terrestrial organisms, bacteria, and where PFOS was not a chemical of study (Burkhard, 2021). The citations meeting the search criteria were reviewed for reported BAFs and/or reported concentrations in which BAFs could be calculated. Data from papers that met the inclusion and data quality screening criteria described below were extracted into the chemical dataset for PFOS.

Specifically, studies were evaluated for inclusion in the dataset used for calculating national BAFs for PFOS using the following evaluation criteria:

- Only BAF studies that included units for tissue, water, and/or BAFs were included.
- Mesocosm, microcosm, and model ecosystem studies were not selected for use in calculating BAFs.
- BAF studies in which concentrations in tissue and/or water were below the minimum level of detection were excluded.
- Only studies performed using freshwater or brackish water were included; high salinity values were excluded.
- Studies of organisms (e.g., damselfly, goby) and tissues (e.g., fish bladder) not commonly consumed by humans or not used as surrogate species for those commonly consumed by humans were excluded. Information on the ecology, physiology, and biology of the organism was used to determine whether an organism is a reasonable surrogate of a commonly consumed organisms.
- Studies in which the BAFs were not found to be at steady state were excluded.
- For pooled samples, averaging BAF data from multiple locations was only considered acceptable if corresponding tissue and water concentrations were available from matching locations (e.g., a BAF would not have been calculated using water and tissue samples collected from eight separate locations with tissue concentrations collected from only six of these corresponding locations). In addition to the evaluation criteria listed above, PFOS bioaccumulation data were also evaluated using five study quality criteria outlined in Burkhard 2021 (Table 2).

Table 2. Bioaccumulation factor (BAF) study quality criteria based on Burkhard (2021).

Criteria	1	2	3
Number of water samples collected	> 3 samples	2–3 samples	1 sample
Number of organism samples collected	> 3 samples	2–3 samples	1 sample
Temporal coordination of water and biota samples	Concurrent collection of samples	Collected within a 1-year time frame	Collected > 1-year time frame
Spatial coordination of water and biota samples	Collected from same locations	Collected from reasonably close locations (1 kilometer (km)–2 km)	Significantly different sampling locations
General experimental design	Assigned a default value of zero for studies in which tissues from individual species were identified and analyzed		Assigned a value of 3 for studies in which tissues were from mixed species or reported as a taxonomic group.

Notes: The scores for each BAF were totaled and used to determine the overall confidence ranking for each individual BAF. The sum of quality values for the five criteria listed in Table 2 were classified as high quality (total score of 4 or 5), medium quality (total score of 5 or 6) or low quality (total score ≥ 7). Only high and medium quality data were included in final national BAFs calculations.

As noted in Burkhard (2021), study quality determinations based on temporal and spatial coordination were subjective and based on best professional judgement. In the absence of adequate quantifiable information regarding sample location (site coordinates for both water and tissue collection locations) or temporal coordination (specific dates of sample collection), BAF data were given a score of 2 or 3 for these categories.

4.2.3 BAFs for PFOS

Following the decision framework presented in Figure 1, the EPA selected one of the four methods to develop a national-level BAF for this chemical. Because PFOS is an organic chemical that predominantly exists in an anionic form in water (EPA, 2024a,e; NCBI, 2024), the BSAF and K_{ow} methods would not be applicable. The EPA selected the BAF estimate using the BAF method (i.e., based on a field-measured BAF) because sufficient field measured BAF data were available for PFOS.

The national-level BAF equation adjusts the TL baseline BAFs for nonionic organic chemicals by national default values for lipid content, as well as dissolved and particulate organic carbon content. The partitioning of PFOS is related to protein binding properties (EPA, 2016a,b); therefore, the EPA did not normalize measured BAF values for PFOS using lipid content when calculating baseline and national BAFs. The EPA selected the recommended 50th percentile dissolved and particulate organic carbon content for the national-level default values which is consistent with the goal of national BAFs (i.e., as central tendency estimates), as described in Section 6.3 of the Technical Support Document, Volume 2 (EPA, 2003). Adjustment for water-dissolved portions of PFOS is applied to TL baseline BAFs (EPA, 2000a) (see Appendix A).

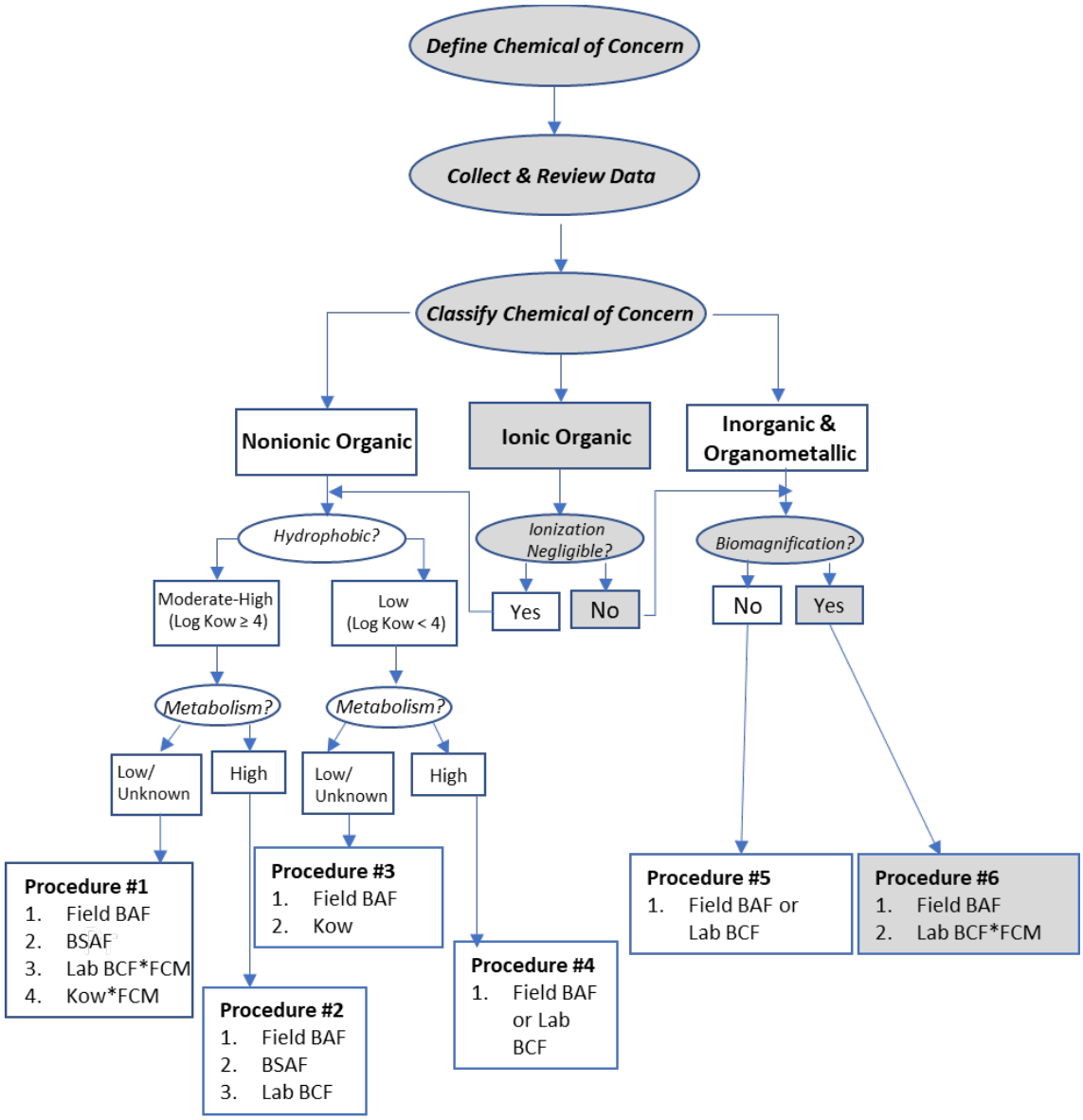


Figure 1. Application of the BAF framework for PFOS; gray boxes indicate steps followed based on available information for PFOS (EPA, 2000a).

The EPA followed the framework described in the Technical Support Document, Volume 2 (EPA, 2003), also presented in Figure 1 to select a procedure for estimating national BAFs for PFOS. Based on the characteristics of this chemical, the EPA selected Procedure 6 for deriving a national BAF value. PFOS has the following characteristics:

- Ionic organic chemicals, with ionization not negligible (EPA, 2024e; NCBI, 2024).
- Biomagnification likely (de Vos et al., 2008; EPA, 2016a; Houde et al., 2006; Kannan et al., 2005; Loi et al., 2011; Martin et al., 2004; Penland et al., 2020; Powley et al., 2008; Tomy et al., 2004; Xu et al., 2014).

The EPA was able to locate peer-reviewed, field-measured BAFs for TLs 2, 3, and 4 from the sources evaluated for which sufficient information was provided to indicate the quality and usability of the data; therefore, the EPA included only field BAF studies. The EPA used the BAF method to derive the national BAF values for PFOS:

- TL 2 = 420 L/kg
- TL 3 = 1,700 L/kg
- TL 4 = 860 L/kg

5 Selection of Toxicity Value

5.1 Approach

The EPA considered all available final toxicity values for both noncarcinogenic and carcinogenic toxicological effects after oral exposure to develop AWQC for PFOS. As described in the 2000 Methodology (EPA, 2000a), where data are available, the EPA derives AWQC for both noncarcinogenic and carcinogenic effects and selects the more protective value for the recommended AWQC (See Section 7, Criteria Derivation: Analysis).

For noncarcinogenic toxicological effects, the EPA uses chronic-duration oral reference values (RfVs; RfDs or equivalent) to derive human health AWQC. An RfV is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA, 2002). An RfV may be derived from an animal toxicological study or a human epidemiological study, from which a point of departure (POD; i.e., a no-observed-adverse-effect level [NOAEL], lowest-observed-adverse-effect level [LOAEL], or benchmark dose [BMD]) can be derived. To derive the RfV, uncertainty factors are applied to the POD to reflect the limitations of the data in accordance with the EPA human health risk assessment methodology (EPA, 2002, 2014a, 2022a).

For carcinogenic toxicological effects, the EPA uses an oral CSF, when applicable and available, to derive human health AWQC. The oral CSF is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime oral exposure to a stressor. This value may also be derived from animal toxicological studies or human epidemiological studies.

In developing AWQC, the EPA conducts a systematic search of peer-reviewed, publicly available final toxicity assessments to obtain the toxicity value(s) (RfV and/or CSF) for use in developing AWQC. The EPA identified toxicological assessments by systematically searching the following EPA program offices, other national and international programs, and state programs in April 2024:

- EPA, Office of Research and Development
    - Integrated Risk Information System (IRIS) program (EPA, 2024h)
    - Provisional Peer-Reviewed Toxicity Values (PPRTV) (EPA, 2024i)
    - ORD Human Health Toxicity Values (EPA, 2024j)

- EPA, Office of Pesticide Programs (EPA, 2024k)
- EPA, Office of Pollution Prevention and Toxics (EPA, 2024l)
- EPA, Office of Water (EPA, 2024m)
    - Drinking Water Health Effects Support Documents
    - Toxicity Assessments
- U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR, 2024)
- Health Canada (HC, 2023)
- California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (CalEPA, 2024)

After identifying and documenting all available final toxicity values, the EPA followed a systematic process to consider the identified toxicity values and select the toxicity value(s) to derive the AWQC for noncarcinogenic and carcinogenic effects. The EPA selected IRIS toxicity values to derive the draft AWQC if *any* of the following conditions were met:

1. The EPA's IRIS toxicological assessment was the only available source of a toxicity value.
2. The EPA's IRIS toxicological assessment was the most current source of a toxicity value.
3. The toxicity value from a more current toxicological assessment from a source other than the EPA's IRIS program was based on the same principal study and was numerically the same as an older toxicity value from the EPA IRIS program.
4. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not include the relevant toxicity value (chronic-duration oral RfV or CSF).
5. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not introduce new science (e.g., the toxicity value was not based on a newer principal study) or use a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

The EPA selected the toxicity value from a peer-reviewed, publicly available source other than the EPA IRIS program to derive the draft AWQC if *any* of the following conditions were met:

1. The chemical is currently used as a pesticide, and the EPA Office of Pesticide Programs had a toxicity value that was used in pesticide registration decision-making.
2. A toxicological assessment from a source other than the EPA's IRIS program was the only available source of a toxicity value.
3. A more current toxicological assessment from a source other than the EPA's IRIS program introduced new science (e.g., the toxicity value was based on a newer principal study) or used a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

5.2 Toxicity Value for PFOS

5.2.1 Reference Dose

After following the approach outlined in Section 5.1, the EPA identified the *Final Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* (EPA, 2024a). This document is the most recent toxicity assessment identified for PFOS and used the best available science in the evaluation of noncancer risk. The EPA did not identify any other assessments that presented newer scientific information (i.e., unique RfVs) for PFOS.

The EPA's final human health toxicity assessment for PFOS (EPA, 2024a) considered all publicly available human epidemiological, animal toxicological, mechanistic and toxicokinetic evidence relevant to studies that evaluated health effects after oral PFOS exposure. Overall, the available *evidence indicates* that PFOS exposure is likely to cause hepatic, immunological, cardiovascular, and developmental effects in humans, given sufficient exposure conditions (e.g., at levels in humans as low as 0.57 to 5.0 ng/mL and doses in animals as low as 0.0017 to 0.4 mg/kg/day). These judgments are based on data from epidemiological studies of infants, children, adolescents, pregnant individuals, and non-pregnant adults, as well as short-term (28-day), subchronic (90-day), developmental (gestational), and chronic (2-year) oral-exposure studies in rodents.

PODs were developed following EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012) and converted to external POD human equivalent doses (POD_{HED}s) using pharmacokinetic modeling. Consistent with the recommendations presented in *A* Review *of the Reference Dose and Reference Concentration Processes* (EPA, 2002), the EPA applied uncertainty factors (UFs) to POD_{HED}s to address intraspecies variability, interspecies variability, extrapolation from a lowest observed adverse effect level (LOAEL) to no observed adverse effect level (NOAEL), extrapolation from a subchronic to a chronic exposure duration, and database deficiencies. The EPA derived and considered multiple candidate RfDs from both epidemiological and animal toxicological studies across the four noncancer health outcomes that the EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental).

Decreased infant birth weight (Wikström et al., 2019) and increased total cholesterol in adults (Dong et al., 2019) were selected as the co-critical effects for the overall oral **RfD of 1×10^{-7} mg/kg/day** (EPA, 2024a). This RfD was derived by applying a total UF of 10 to account for intraspecies variability (UF_H). Critical effects observed during developmental periods (decreased birthweight) represent effects in susceptible subpopulations. The RfD based on these effects is considered protective of effects resulting from lifetime exposures to PFOS, as well as short-term risk assessment scenarios, as the observed developmental endpoints can potentially result from a short-term exposure during critical periods of development.

5.2.2 Cancer Slope Factor

Consistent with EPA's Guidelines for Carcinogen Risk Assessment (EPA, 2005a), the EPA's *Final Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* (EPA, 2024a) reviewed the available data and conducted a weight of evidence evaluation across the human epidemiological and animal toxicological studies and concluded that PFOS is *Likely*

to Be Carcinogenic to Humans via the oral route of exposure. Epidemiological studies provided evidence of bladder, prostate, liver, kidney, and breast cancers in humans, although evidence was limited or mixed for some cancer types. Animal toxicological studies supported findings from human studies. Bioassays conducted in Sprague-Dawley rats reported hepatocellular tumors, pancreatic islet cell tumors, and thyroid follicular cell tumors after chronic oral exposure. Some studies observed multisite tumorigenesis (liver and pancreas) in male and female rats. PFOS exposure is associated with multiple key characteristics of carcinogenicity (Smith et al., 2016). Available mechanistic data suggest that multiple modes of action (MOAs) play a role in pancreatic and hepatic tumorigenesis associated with PFOS exposure in animal models (EPA, 2024a).

To derive a CSF for PFOS, the EPA followed agency guidelines and methodologies for risk assessment in deriving CSFs for PFOS (EPA, 2005a, 2012, 2024a). EPA conducted benchmark dose modeling and used a similar pharmacokinetic modeling approach as described for the derivation of noncancer RfDs. Data from human epidemiological studies were not suitable for CSF derivation (see EPA, 2024a for further details). The EPA used multistage models to derive and consider multiple candidate CSFs from an animal toxicological study in multiple sexes, tissue types, and organ systems (i.e., liver and pancreas). Multistage cancer models were used to predict the doses at which the selected BMR for tumor incidence would occur. BMDLs for each tumor type served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type.

The oral slope factor of 39.5 (mg/kg/day)$^{-1}$ for combined hepatocellular adenomas and carcinomas in female rats from Butenhoff et al. (2012) and Thomford (2002) was selected as the basis of the overall CSF for PFOS. Per EPA's *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005a,b), age-dependent adjustment factors were not applied during CSF derivation, as a mutagenic mode of action (MOA) was not found for PFOS in available studies, and insufficient evidence was available to assess susceptibility to cancer following PFOS exposure during early life.

6 Relative Source Contribution) Derivation

6.1 Approach

The EPA applies an RSC to the RfD when calculating an AWQC based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to AWQC-related sources (EPA, 2000a). The purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the AWQC), when combined with other identified sources of exposure (e.g., diet excluding freshwater and estuarine fish and shellfish, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to consumption of ambient water (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with ambient water) and fish and shellfish from inland and nearshore

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waters relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. The EPA considers any potentially significant exposure source and route when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's 2000 Methodology (EPA, 2000a). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population, depending on the available data.

An RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (EPA, 2000a). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (EPA, 2000a). The EPA's approach recommends a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies.

The EPA's Exposure Decision Tree approach states that when there are insufficient environmental monitoring and/or exposure intake data to permit quantitative derivation of the RSC, the recommended RSC is 20%. In the case of AWQC development, this means that 20% of the exposure equal to the RfD is allocated to the consumption of ambient water and fish and shellfish from inland and nearshore waters and the remaining 80% is reserved for other potential sources, such as diet (excluding fish and shellfish from inland and nearshore waters), air, consumer products, etc. This 20% RSC can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (EPA, 2000a). Applying a lower RSC (e.g., 20%) is a more health protective approach to public health and results in a lower AWQC.

To derive an RSC for PFOS, the EPA evaluated the exposure information identified through conducting prior systematic literature searches performed as part of the EPA's final human health toxicity assessment for PFOS (EPA, 2024e). To identify information on PFOS exposure routes and sources to inform RSC determination, the EPA considered primary literature published between 2003–2020 that was collected by the EPA's Office of Research and Development as part of an effort to evaluate evidence for pathways of human exposure to eight PFAS, including PFOS. This search was not date-limited and spanned information collected across the Web of Science, PubMed, and ToxNet/ToxLine (now ProQuest) databases. An

updated literature search was conducted and captured relevant literature published through March 2021. Literature captured by this search is housed in the EPA's HERO database (https://hero.epa.gov/). To supplement the primary literature database, the EPA also searched the following gray literature sources in February 2022 for information related to relative exposure of PFOS for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans. The full description of methods used to identify and screen relevant literature is available in the EPA's *Final Appendix: Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* (EPA, 2024e). The following description in Section 6.2 is a summary of the information provided in the Appendix of the final PFOS toxicity assessment.

6.2 Summary of Potential Exposure Sources of PFOS Other Than Water and Freshwater and Estuarine Fish/Shellfish

6.2.1 Dietary Sources

A number of studies support food ingestion as a major source of exposure to PFOS for the general population based on early studies that modeled the relative contributions of various sources among the general populations of North America and Europe (Fromme et al., 2009; Trudel et al., 2008; Vestergren and Cousins, 2009). The exposure to adults in western countries is typically estimated to be about 1 ng/kg-d (Domingo and Nadal, 2017; East et al., 2021). The dominance of the food ingestion pathway is attributed to bioaccumulation in food from environmental emissions, relatively large amounts of foods being consumed, and high GI uptake (Trudel et al., 2008). However, the estimates are highly uncertain due to limited data availability, relatively low detection frequencies, and relatively large differences in composition of diets across geographic locations (Domingo and Nadal, 2017; EFSA, 2020).

There is currently no comprehensive, nationwide Total Diet Study (TDS) for PFOS that can be used to draw conclusions about the occurrence and potential risk of PFOS in the U.S. food supply for the general population. In 2021, the FDA released PFAS testing results from their first survey of nationally distributed processed foods, including several baby foods, collected for the TDS (FDA, 2021a). Results of the survey showed that 164 of the 167 foods tested had no detectable levels of PFAS measured. Three food samples (fish sticks, canned tuna, and protein powder) had detectable levels of PFAS, including PFOS (FDA, 2021a). In another recent FDA study, PFOS was detected in one sample (baked cod: 98 ppt) out of 94 food samples collected nationally (FDA, 2021b). In a 2019 national survey of produce, meats, dairy and grain products, PFOS was detected in three of the 179 food samples tested (two samples of tilapia, one sample of ground turkey) (FDA, 2020a,b). PFOS was also detected in produce samples (collard greens and lettuce) in a 2018 focused study near a PFAS production plant in the Fayetteville, North Carolina area (FDA, 2018). PFOS was below the lower limit of quantification (LOQ; 4 ng/L) in all 30 samples analyzed in a study of domestic and imported carbonated water and noncarbonated bottled water (FDA, 2016). The sample size in all of these studies is limited, and thus, the results cannot be used to draw definitive conclusions about the levels of PFAS in the U.S. food supply more generally (FDA, 2023). In a 2010 study of 31 types of food collected from 5 grocery stores in Texas, PFOS was not detected in any of the samples (Schecter et al., 2010).

As a component of a scientific evaluation on the risks to human health related to PFAS in food, the European Food Safety Authority (EFSA) conducted an exposure assessment using consumption data from the EFSA Comprehensive Food Consumption Database and 69,433 analytical results for 26 PFAS in 1,528 samples of food and beverages obtained from 16 European countries (EFSA, 2020). Samples were collected between the years 2000 and 2016 (74% after 2008), mainly from Norway, Germany, and France. With 92% of the analytical results below the limit of detection (LOD) or LOQ, lower bound dietary exposure estimates were obtained by assigning zero to values below LOD/LOQ. Median chronic dietary exposures of PFOS for children and adults were estimated as 1.02 ng/kg-d and 0.58 ng/kg-d, respectively. The most important contributors for PFOS were "fish and other seafood[f]," "eggs and egg products," and "meat and meat products." It is unclear whether the contribution from food contact material is reflected in the data. The authors determined diet to be the major source of PFAS exposure for most of the population but noted that dust ingestion and indoor air inhalation may provide substantial contributions for some individuals.

The 2020 EFSA report highlighted a recent study of aggregate exposure to PFAS from diet, house dust, indoor air, and dermal contact among Norwegian adults (Poothong et al., 2020). Dietary exposures were estimated for 61 study participants using food diaries and data on concentrations from an extensive Norwegian database of concentrations in 68 different food and drinks (including drinking water). For PFOS, dietary intake was by far the greatest contributor to aggregate exposure (contributing 95% of total estimated PFOS intake), but intake from ingestion of house dust represented the dominant pathway for some of the top 20% most highly exposed individuals. The authors reported a significant positive correlation between the observed and modeled serum concentrations for PFOS ($r = 0.29$, $p < 0.05$). The correlation existed despite the model underestimating serum concentrations of PFOS by a factor of 4, which was attributed to the long half-life and decreased exposure over recent years. While the authors did not separately quantify intake from food and drinking water, an earlier article from the same research group (Papadopoulou et al., 2017) reported measured concentrations in duplicate diets with median estimated intake of PFOS approximately 150 times higher from solid food than from liquids.

De Felip et al. (2015) investigated correlations of blood concentrations of PFOS with dietary intake among Italian women. They estimated daily intake of PFOS based on the reported food consumption frequencies of specific food items and found strongly significant correlations of blood levels with consumption of beef, pork, and vegetables ($p < 0.01$), and moderate correlation with consumption of fish ($p < 0.05$).

Zafeiraki et al. (2019) analyzed samples of marine species of fish caught in Dutch waters or purchased at Dutch markets between 2012 and 2018. Of the 16 PFAS that were analyzed, PFOS was generally detected at a higher frequency and concentration across the tested species. Shrimp and seabass had the highest average concentrations of PFOS (each over 4 ng/g ww), but

[f] Some dietary studies use the term "seafood" to indicate fish and shellfish from ocean, freshwater, or estuarine water bodies. Information about the water bodies assessed in individual studies is reported in the articles.

was also detected in cod, mackerel, sole and common dab (100% detection, ranging from 1.10 to 2.5 ng/g ww).

In marine fish and shellfish samples collected for the FDA 2021–2022 seafood survey, Young et al. (2022), analyzed concentrations of 20 PFAS, including PFOS, in eight of the most highly consumed seafood products in the United States. PFOS was detected most frequently (100% of samples; n = 10) and at the highest average concentrations (422.9 ppt) in clams. The study also reported detections in crab (45.5% of samples; n = 11; 151.6 ppt average concentration in samples with detections), tuna (50% of samples; n = 10; 86.8 ppt average concentration in samples with detections), tilapia (20% of samples; n = 10; 57.5 ppt average concentration in samples with detections), and cod (60% of samples; n = 10; 62.5 ppt average concentration in samples with detections). In this study, PFOS was not detected above the method detection limits (MDLs) (39 ppt or 45 ppt) in salmon, shrimp, or pollock.

Based on the National Oceanic and Atmospheric Administration's (NOAA's) National Status and Trends Data Portal, PFOS concentrations (in ww) were not detected in mussels, oysters, and fish liver samples. However, PFOS was detected in marine fish fillet samples, up to 75.1 ppb (NOAA, 2024).

6.2.2 Food Contact Materials

The FDA has authorized the use of PFAS in food contact substances due to their non-stick and grease, oil, and water-resistant properties since the 1960s. There are four categories of products that may contain PFAS (FDA, 2020a,b):

- Nonstick cookware: PFAS may be used as a coating to make cookware nonstick.
- Gaskets, O-Rings, and other parts used in food processing equipment: PFAS may be used as a resin in forming certain parts used in food processing equipment that require chemical and physical durability.
- Processing aids: PFAS may be used as processing aids for manufacturing other food contact polymers to reduce build-up on manufacturing equipment.
- Paper/paperboard food packaging: PFAS may be used as grease-proofing agents in fast-food wrappers, microwave popcorn bags, take-out paperboard containers, and pet food bags to prevent oil and grease from foods from leaking through the packaging.

Paper products used for food packaging are often treated with PFAS for water and grease resistance. In previous testing, sandwich wrappers, french fry boxes, and bakery bags were all been found to contain PFAS (Schreder and Dickman, 2018). Older generation PFAS (e.g., PFOA, PFOS) were manufactured and used in products for decades, and the bulk of the information available on PFAS toxicity relates to the older compounds. However, because newer-generation PFAS are more mobile than their predecessors, they migrate more readily into food. In 2016, the FDA deauthorized the remaining uses of long-chain "C8" PFAS in food packaging, which are therefore, no longer used in food contact applications sold in the United States (FDA, 2020a,b). An analysis of popcorn bags, snack bags, and sandwich bags purchased in 2018 from international vendors and grocery stores in the United States found no evidence of PFOS at

concentrations above the LOD (0.63 ng/g paper) (Monge Brenes et al., 2019). The authors presented these results as evidence of a reduction in PFOS concentrations in microwave packaging between 2005 and 2018. In an analysis of microwave popcorn bags from around the world, Zabaleta et al. (2017) reported no measurable concentrations of any perfluorosulfonic acid (PFSA), including PFOS, in any of the samples. In a second study, Zabaleta et al. (2020) looked at PFAS in 25 paper- and paperboard packaging materials primarily collected in Spain. Again, no PFSAs, including PFOS, were found above the level of detection. The packaging materials with the largest number of detectable analytes was a popcorn bag from China and the inside paper lining from three individual pet food products, which contained a spectrum of C3 to C10 perfluorinated carboxylates. Similarly, in an analysis of 52 European products collected between 2014 and 2016, Borg and Ivarrson (2017) reported that PFSAs were rarely detected in the samples; PFOS was the only PFSA detected and was only present in one sample, a microwave popcorn bag. Kotthoff et al. (2015) reported PFOS was the most frequently and abundantly detected PFAS in paper-based cooking materials.

6.2.3 Consumer Product Uses

An early investigation of consumer exposure to PFOS by Trudel et al. (2008) used mechanistic modeling together with information on product-use habits to estimate exposures from mill-treated carpets and impregnated clothing. The authors concluded that contact with consumer products represents less than 1% of total exposure to PFOS, but also pointed out that because carpets have a relatively long lifetime, the exposure is expected to continue long after cessation of use of PFOS in carpet treatments. Liu et al. (2014) also investigated trends in PFAS content of household goods between 2007 and 2011. They reported a decrease in the availability of consumer products that contain PFOS but were still able to find products that contained PFOS.

In contrast, Kotthoff et al. (2015) reported broad detection of PFOS in a 2010 sampling effort that collected 115 European consumer products, which included carpets, leather, outdoor materials, and others. PFOS was detected in all but two sample types, often at the highest median concentration compared to other PFSAs. PFOS has also been detected in textile samples of outdoor apparel from Europe and Asia (Gremmel et al., 2016; van der Veen et al., 2020). PFOS was detected in one-third of the jackets tested by Gremmel et al. (2016) at relatively low concentrations ranging from 0.01 $\mu g/m^2$–0.59 $\mu g/m^2$.

6.2.4 Indoor Dust

Several studies suggest that PFOS and its precursors in indoor dust may be an important exposure source for some individuals (Shoeib et al., 2011; Gebbink et al., 2015; Gleason et al., 2017; Poothong et al., 2020). PFOS is generally a dominant ionic PFAS constituent in household dust, frequently occurring above detection limits and at relatively high concentrations in all or most samples (Shoeib et al., 2011; Kim et al., 2019; Wu et al., 2015; Poothong et al., 2020; Makey et al., 2017; Byrne et al., 2017; Fraser et al., 2013).

PFOS was measured at the second highest concentrations (geometric mean concentrations ranging from 29.0 ng/g to 34.6 ng/g) and frequencies (ranging from 85% to 87% detected) in dust sampled from Californian households. Similarly, PFOS was found at the highest levels

(mean concentration of 3.06 ng/g) of 15 PFAS measured in dust samples taken from households in Seoul, Republic of Korea (Kim et al., 2019). One study of Alaska Natives noted that PFOS was the predominant compound in dust samples (Byrne et al., 2017).

6.2.5 Ambient Air

Air concentrations of PFOS in the atmosphere vary widely across the globe. Areas near wastewater treatment facilities, waste incinerators, and landfills can be sources of PFOS to air (Ahrens et al., 2011). In an urban area in Albany, New York, perfluorinated acids were measured in air samples in both the gas and particulate phase in May and July 2006 (Kim and Kannan, 2007). PFOS in the gas phase had a mean concentration of 1.70 pg/m^3 (range: 0.94 pg/m^3–3.0 pg/m^3) and in the particulate phase had a mean concentration of 0.64 g/m^3 (range: 0.35 pg/m^3–1.16 pg/m^3). However, at Lake Ontario, concentrations of PFOS in the particulate phase measured in air samples over the lake were higher (Boulanger et al., 2005). The mean concentration of PFOS at Lake Ontario was 6.4 ± 3.3 pg/m^3. In an urban area in Minneapolis, Minnesota, PFOS was measured in both the particulate and gas phase (MPCA, 2008). PFOS in the particulate phase ranged from 2.1 pg/m^3 to 7.9 pg/m^3 and the gas phase ranged from 1.8 pg/m^3 to 5.0 pg/m^3 across the five samples.

The EPA's Toxics Release Inventory reported release data for PFOS in 2022 (EPA, 2024n). PFOS is not listed as a hazardous air pollutant under the Clean Air Act (EPA, 2024o). In Canada, PFOS air concentrations measured in 2009 showed widespread distribution with remote sites having similar concentrations to urban sites (ECCC, 2018). Using passive samplers, PFOS concentrations were detected in Toronto, Ontario (8 pg/m^3), an agricultural site in Saskatchewan (5 pg/m^3), Whistler, British Columbia (4 pg/m^3), and Alert, N Nunavut (2 pg/m^3) (ECCC, 2018).

Other reported concentrations of PFOS in air samples from Sydney, Florida (3.4 pg/m^3), Tudor Hill, Bermuda (6.1 pg/m^3), Malin Head, Ireland (3.3 pg/m^3), and Hilo, Hawaii (6.6 pg/m^3) are similar to the concentrations reported in Canada (ECCC, 2018) and Japan (Sasaki et al., 2003). The annual geometric mean concentration of PFOS in air samples collected monthly from 2001 to 2002 in the town of Oyamazaki and Fukuchiyama City were 5.3 pg/m^3 and 0.6 pg/m^3, respectively (Sasaki et al., 2003).

Across Europe, PFOS air concentrations were reported to be variable. In the particulate phase, PFOS concentrations ranged from < 1.8 pg/m^3 to 46 pg/m^3 (Martin et al., 2004). Most locations had low concentrations (~1 pg/m^3–2 pg/m^3) to less than the reported MDL and included Hazelrigg, United Kingdom, Kjeller, Norway, and Mace Head, Ireland (Barber et al., 2007). The highest concentrations were reported in Manchester, United Kingdom. Similarly, high concentrations (150 pg/m^3) were reported in Paris, France (ECCC, 2018).

Even in the Arctic, PFOS, its precursors, and degradation products, have been detected in air samples in Resolute Bay, Nunavut, Canada, during the summer of 2004 (Stock et al., 2007). PFOS in the filter samples were 1–2 orders of magnitude greater than other compounds, with a mean concentration of 5.9 pg/m^3. These concentrations are greater than PFOS concentrations measured in the particle phase of air samples measured in Zeppelinstasjonen, Svalbard, Norway (Butt et al., 2010). PFOS was measured in September and December 2006 and August and

December 2007, with mean concentrations of 0.11 pg/m^3 (range: 0.03 pg/m^3–0.50 pg/m^3) and 0.18 pg/m^3 (range: 0.02 pg/m^3–0.97 pg/m^3), respectively.

6.2.6 Summary and Recommended RSC for PFOS

As mentioned above, the scope of exposure sources considered for the draft recommended human health AWQC is limited to surface water used for drinking water and the consumption of freshwater/estuarine fish and shellfish (EPA, 2000a), consistent with previous human health AWQC (EPA, 2015). The EPA followed the Exposure Decision Tree approach to determine the RSC for PFOS (EPA, 2000a; see Figure 2).

To identify the population(s) of concern (Box 1, Figure 2), the EPA first identified potential subpopulations or lifestages based on the PFOS exposure intervals in the two co-critical studies from which the co-critical effects were selected for RfD derivation in the PFOS toxicity assessment (EPA, 2024a). Since the critical effects are the most sensitive adverse health effect that were identified from the available data of sufficient quality, then the exposure interval may be a sensitive window of exposure. Two co-critical effects were identified for PFOS (decreased infant birth weight and increased total cholesterol in adults) based on two human epidemiological studies; however, the specific critical window of exposure for each of the critical effects is not known. Based on epidemiological study design, the potentially sensitive life stages include women of childbearing age who may be or become pregnant, and pregnant women and their developing fetuses. However, limited information was available regarding specific PFOS exposure in these two potentially sensitive life stages from different environmental sources. Therefore, the EPA considered exposures in the general U.S. population, ages 21 years and older, which includes these two life stages.

Second, the EPA identified PFOS relevant exposure sources/pathways (Box 2, Figure 2), including dietary consumption, incidental oral, inhalation, or dermal exposure via dust, consumer products, and soil, and inhalation exposure via ambient air. Several of these may be potentially significant exposure sources.

Third, the EPA evaluated whether adequate data were available to describe the central tendencies and high-end exposures for all potentially significant exposure sources and pathways (Box 3, Figure 2). The EPA determined that there were inadequate quantitative data to describe the central tendencies and high-end estimates for all relevant exposure pathways. For example, studies from the U.S. indicate that dust may be a significant source of exposure to PFOS. Although several studies report PFOS detections in consumer products, most examined samples from specific locations that may not be nationally representative. Therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population.

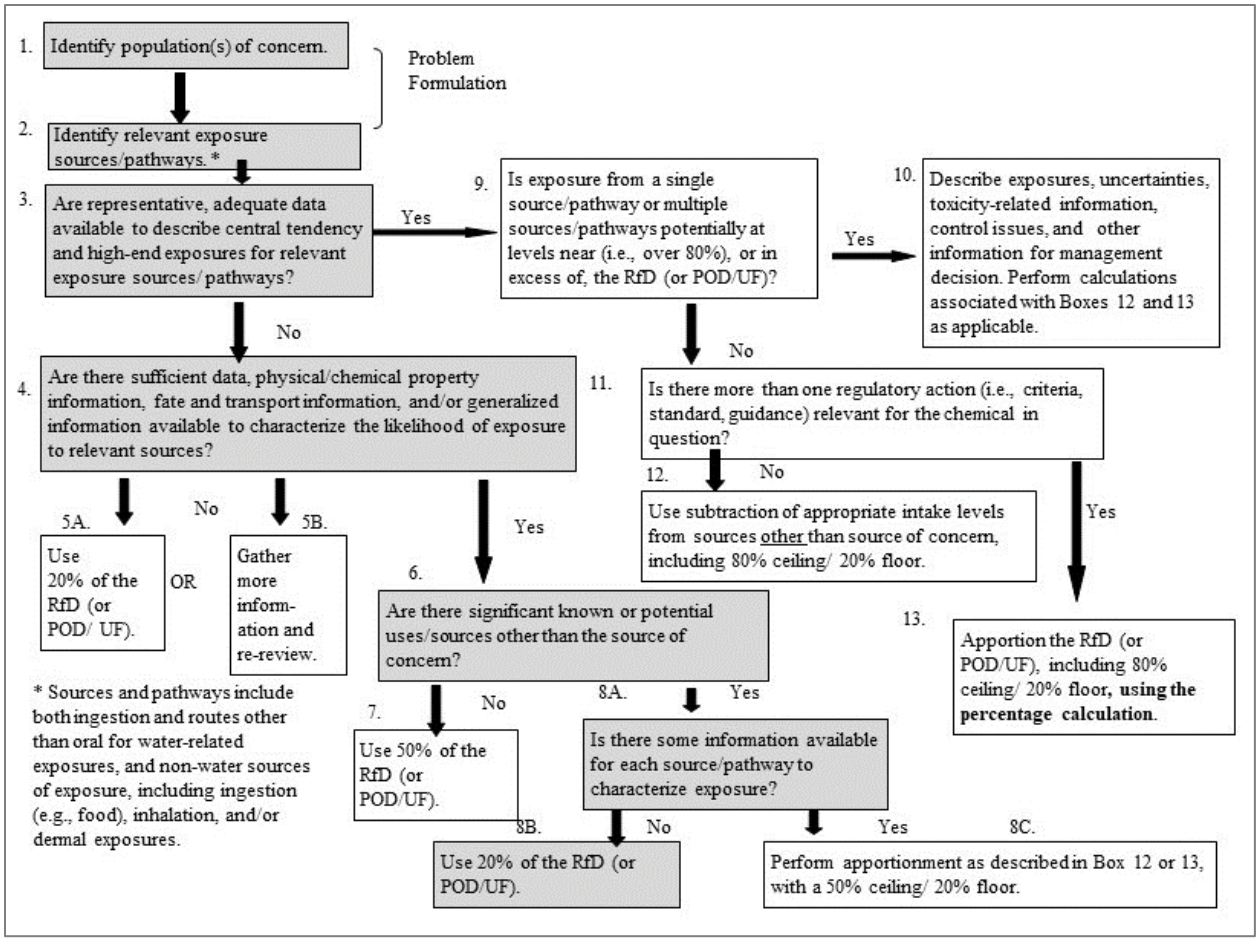


Figure 2. RSC exposure decision tree framework for PFOS; figure adapted from EPA (2000a) with gray boxes indicating key decision points for this chemical.

Fourth, the EPA determined whether there were sufficient data on the physical/chemical properties, fate and transport and generalized information characterizing the likelihood of exposure to PFOS from relevant sources (Box 4, Figure 2). Sufficient information for PFOS was available to characterize the likelihood of exposure. The agency relied on the studies summarized above to determine if there are potential uses/source of PFOS other than AWQC-related sources (Box 6, Figure 2). There are significant known or potential uses/sources of PFOS other than AWQC-related sources (Box 6, Figure 2). For example, diet from sources other than freshwater/estuarine fish and shellfish, such as marine fish, are potentially significant sources of PFOS. Based on this information, the next step was to determine if adequate information was available on PFOS to characterize each source/pathway of exposure (Box 8a, Figure 2). The EPA determined there is not enough information available on each source to make a quantitative characterization of exposure among exposure sources, such as dust, air and consumer products. Therefore, the data are insufficient to allow for quantitative characterization of the different exposure sources. The EPA's Exposure Decision Tree approach states that when there is insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20% (U.S. EPA, 2000a). Thus, the

EPA recommend an RSC of 20% (0.20) for PFOS for both the water plus organism AWQC as well as the organism only AWQC (Box 8b, Figure 2).

7 Criteria Derivation: Analysis

Table 3 summarizes the input parameters used to derive the draft recommended human health AWQC that are protective of exposure to PFOS from consuming drinking water and/or eating fish and shellfish (organisms) from inland and nearshore waters. The criteria calculations are presented below. These criteria recommendations are based on the 2000 Methodology (EPA, 2000a) and the toxicity and exposure assumptions described above (see Section 4, AWQC Input Parameters; Section 5, Selection of Toxicity Value; and Section 6, Relative Source Contribution Derivation).

Table 3. Input parameters for the human health AWQC for PFOS.

Input Parameter		Value
RfD		1 x 10^{-7} mg/kg/day
CSF		39.5 $(mg/kg/day)^{-1}$
RSC		0.20
BW		80.0 kg
DWI		2.3 L/d
FCR	TL 2	0.0076 kg/d
	TL 3	0.0086 kg/d
	TL 4	0.0051 kg/d
BAF	TL 2	420 L/kg
	TL 3	1,700 L/kg
	TL 4	860 L/kg

Notes: RfD = reference dose; CSF = cancer slope factor; RSC = relative source contribution; BW = bodyweight; DWI = drinking water intake; FCR = fish consumption rate; TL = trophic level; BAF = bioaccumulation factor.

7.1 AWQC for Noncarcinogenic Toxicological Effects

For consumption of water and organisms:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\text{DWI (L/d)} + \sum_{i=2}^{4} (\text{FCR}_i\ (\text{kg/d}) \times \text{BAF}_i\ (\text{L/kg}))}$$

$$= \frac{0.0000001\ \text{mg/kg-d} \times 0.20 \times 80.0\ \text{kg} \times 1{,}000\ \mu g/mg}{2.3\ \text{L/d} + ((0.0076\ \text{kg/d} \times 420\ \text{L/kg}) + (0.0086\ \text{kg/d} \times 1{,}700\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 860\ \text{L/kg}))}$$

$$= 0.00006531\ \mu g/L$$

$$= 0.00006\ \mu g/L \text{ (rounded)}$$

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For consumption of organisms only:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\sum_{i=2}^{4} (FCR_i\ (kg/d) \times BAF_i\ (L/kg))}$$

$$= \frac{0.0000001\ mg/kg\text{-}d \times 0.20 \times 80.0\ kg \times 1{,}000\ \mu g/mg}{(0.0076\ kg/d \times 420\ L/kg) + (0.0086\ kg/d \times 1{,}700\ L/kg) + (0.0051\ kg/d \times 860\ L/kg)}$$

$$= 0.00007208\ \mu g/L$$

$$= 0.00007\ \mu g/L \text{ (rounded)}$$

7.2 AWQC for Carcinogenic Toxicological Effects

The EPA derives cancer-based HHC for contaminants that have been determined to be *Carcinogenic to Humans* or *Likely to Be Carcinogenic to Humans* (EPA, 2000a,c). Since PFOS was determined to be *Likely to Be Carcinogenic to Humans* (EPA, 2024a,e), the EPA derived AWQC for carcinogenic toxicological effects.

Consumption of water and organisms:

$$\text{AWQC} = \frac{\text{RSD} \times \text{BW} \times 1{,}000^{g}}{\text{DWI} + \sum_{i=2}^{4} (FCR_i \times BAF_i)}$$

$$= \frac{(10^{-6} / 39.5)\ mg/kg\text{-}d \times 80.0\ kg \times 1{,}000\ \mu g/mg}{2.3\ L/d + ((0.0076\ kg/d \times 420\ L/kg) + (0.0086\ kg/d \times 1{,}700\ L/kg) + (0.0051\ kg/d \times 860\ L/kg))}$$

$$= 0.00008267\ \mu g/L$$

$$= 0.000083\ \mu g/L \text{ (rounded)}$$

For consumption of organisms only:

$$\text{AWQC} = \frac{\text{RSD} \times \text{BW} \times 1{,}000^{g}}{\sum_{i=2}^{4} (FCR_i \times BAF_i)}$$

$$= \frac{(10^{-6} / 39.5)\ mg/kg\text{-}d \times 80.0\ kg \times 1{,}000\ \mu g/mg}{(0.0076\ kg/d \times 420\ L/kg) + (0.0086\ kg/d \times 1{,}700\ L/kg) + (0.0051\ kg/d \times 860\ L/kg)}$$

$$= 0.00009124\ \mu g/L$$

$$= 0.000091\ \mu g/L \text{ (rounded)}$$

[g] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

7.3 AWQC Summary for PFOS

The EPA derived the draft recommended AWQC for PFOS using both noncarcinogenic and carcinogenic toxicity endpoints. The human health AWQC for noncarcinogenic effects for PFOS are **0.00006 µg/L** (0.06 ng/L) for consumption of water and organisms and **0.00007 µg/L** (0.07 ng/L) for consumption of organisms only (Table 4). The human health AWQC for carcinogenic effects (at a 10^{-6} cancer risk level) for PFOS are **0.000083 µg/L** (0.083 ng/L) for consumption of water and organisms and **0.000091 µg/L** (0.091 ng/L) for consumption of organisms only (Table 4). EPA recommends the lower AWQC, based on the noncarcinogenic effects of PFOS, as the human health AWQC. The EPA evaluated the use of exposure factors relevant to sensitive subpopulations based on the critical effect(s) used to derive the RfD (Appendix B). Based on the results of this evaluation, the criteria based on exposure factors for the general adult (≥ 21 years of age) population are the most health protective.

Under the EPA's recently finalized Method 1633 (EPA, 2024p) for aqueous samples, the level of quantification (LOQ) representing the observed LOQs in the multi-laboratory validation study, range from 1 to 4 ng/L for PFOS. The pooled MDL for PFOS is 0.63 ng/L. The pooled MDL value is derived from the multi-laboratory validation study using MDL data from eight laboratories and represents the sensitivity that should be achievable in a well-prepared laboratory but may not represent the actual MDL used for data reporting or data quality assessments (EPA, 2024p). The MDLs and ranges presented here provide a reference for comparison of analytical concentrations and recommended criteria.

Table 4. Summary of the EPA's recommended human health AWQC for PFOS chemicals.

	Human Health AWQC for Noncarcinogenic Effects
Water and Organism	0.00006 µg/L (0.06 ng/L)
Organism Only	0.00007 µg/L (0.07 ng/L)

8 Consideration of Noncancer Health Risks from PFAS Mixtures

The EPA recently released its final *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (referred to here as the PFAS mixtures framework; EPA, 2024q). The PFAS mixtures framework describes three flexible, data-driven approaches that facilitate practical component-based mixtures evaluation of two or more PFAS based on dose additivity, consistent with the EPA*'s Guidelines for the Health Risk Assessment of Chemical Mixtures* (EPA, 1986) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA, 2000d). The approaches described in the EPA PFAS mixtures framework may support interested federal, state, and Tribal partners, as well as public health experts and other stakeholders to assess the potential noncancer human health hazards and risks associated with PFAS mixtures. The EPA is providing an illustration of one approach which could be applied to PFAS mixture HHC derivation. The PFAS mixtures framework underwent peer review by the EPA Science Advisory Board (EPA, 2022b) and public review and the EPA responded to comments (EPA, 2024r). The public comment period ended on May 30, 2023. The public docket can be accessed at www.regulations.gov under Docket ID: EPA-HQ-OW-2022-0114.

Dose additivity means that the combined effect of the component chemicals in a mixture is equal to the sum of the individual doses or concentrations scaled for potency. As noted in the PFAS mixtures framework, exposure to a number of individual PFAS has been shown to elicit the same or similar profiles of adverse effects in various organs and systems. Many toxicological studies of PFAS as well as other classes of chemicals support the health-protective conclusion that chemicals that elicit the same or similar observed adverse effects following individual exposure should be assumed to act in a dose-additive manner when in a mixture unless data demonstrate otherwise (EPA, 2024q). Importantly, few studies have examined the toxicity of PFAS mixtures, particularly with component chemical membership and proportions that are representative of the diverse PFAS mixtures that occur in the environment. Mixtures assessments for chemicals that share similar adverse health effects, and therefore assume dose additivity, typically apply component-based assessment approaches.

The Hazard Index (HI) approach is one of the component-based mixtures assessment approaches described in the PFAS mixtures framework. In order to support states and Tribes interested in addressing potential noncancer risks of PFAS mixtures, the application of the HI approach for deriving HHC for mixtures is described below. States and authorized Tribes may choose to adopt this approach to derive HHC for PFAS mixtures. Use of the HI approach to assess risks associated with PFAS mixtures was supported by the EPA Science Advisory Board (EPA, 2022b).

In the HI approach (see PFAS mixtures framework; EPA, 2024q), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (EPA, 1986). The HQs for the component chemicals are then summed to derive a mixture-specific HI (for the specified exposure route/medium). Since the HI is unitless, the E and the RfV inputs to the HI formula must be expressed in the same dose units (e.g., mg/L) (Eq. 5). For example, in the context of the human health criteria, HQs for each individual PFAS are calculated by dividing the measured ambient water concentration of each component PFAS (e.g., expressed as µg/L) by its corresponding human health criterion (e.g., expressed as µg/L), and the resulting component PFAS HQs are summed to yield the PFAS mixture HI (Eqs. 5-7). Either water-plus-organism or organism-only HHC can be used in the PFAS mixtures HI approach; however, the type of HHC selected for HI calculation should be consistent. Because cancer data are lacking for most PFAS, the HI approach is currently recommended for PFAS HHC based on noncancer effects.

A hypothetical example is included below to illustrate using the HI approach to derive an HHC for a mixture of three PFAS. A PFAS mixture HI exceeding 1 indicates that co-occurrence of two or more PFAS in a mixture in ambient water exceeds the health-protective level(s), indicating potential health risks. Some individual PFAS have HHC below the analytical MDLs (e.g., PFOA, PFOS). If one such PFAS is included as a component PFAS in the HI approach, then any detectable level of that component PFAS in surface water will result in a component HQ greater than 1, and thus, an HI greater than 1 for the PFAS mixture.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{HHC_i} \quad \text{(Eq. 5)}$$

$$HI = HQ_{PFAS_X} + HQ_{PFAS_Y} \quad \text{(Eq. 6)}$$

$$HI = \left(\frac{[PFAS_{X,ambient\ water}]}{[PFAS_{X,HHC}]}\right) + \left(\frac{[PFAS_{Y,ambient\ water}]}{[PFAS_{Y,HHC}]}\right) \quad \text{(Eq. 7)}$$

Where:

HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS
HHC_i = human health criterion for component PFAS (i)
HQ_{PFAS} = hazard quotient for a given individual PFAS
$PFAS_X$ = Hypothetical PFAS
$PFAS_Y$ = Hypothetical PFAS
$[PFAS_{ambient\ water}]$ = concentration of a given PFAS in ambient water
$[PFAS_{HHC}]$ = water-plus-organism HHC or organism-only HHC for a given PFAS

9 Chemical Name and Synonyms

- Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1)
- 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid
- perfluorooctane sulfonic acid
- heptadecafluoro-1-octane sulfonic acid
- PFOS acid

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Appendix A: Bioaccumulation Factor (BAF) Supporting Information

BAF Calculation Description for PFOS

EPA used the decision framework presented in the *Technical Support Document, Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003) to identify procedures to derive national trophic level-specific BAFs for PFOS based on that chemical's properties (e.g., ionization, hydrophobicity), metabolism, and biomagnification potential (see Figure 1). EPA followed the guidelines provided in Section 5.5 of EPA's 2000 Methodology (EPA, 2000), to assess the occurrence of cationic and anionic forms of PFOS at typical environmental pH ranges. Based on the dissociation constant (pK_a) information provided in the Hazardous Substance Data Bank (HSDB) sources for PFOS, it was determined that ionization of PFOS was significant at typical environmental pH ranges (NCBI, 2024; EPA, 2024).

As explained in Section 5.5 of EPA's 2000 Methodology (EPA, 2000), when a significant fraction of the total chemical concentration is expected to be present as the ionized species in water, procedures for deriving the national BAF rely on empirical (measured) methods (i.e., Procedures 5 and 6) in Figure 1. EPA followed the guidelines in Section 3.2.1 of the Technical Support Document, Volume 2, to evaluate the biomagnification potential of PFOS. Based on the information in the *ATSDR Toxicological Profile for Perfluoroalkyls* (ATSDR, 2021), it was determined that biomagnification was likely. Based on the characteristics of PFOS, EPA selected Procedure 6 for deriving national BAF values for this chemical.

As described in Section 4.1.1, for a given procedure, EPA selected the method that provided BAF estimates for all three TLs (TL 2–TL 4) in the following priority:

- BAF estimates using the BAF method (i.e., based on field-measured BAFs) if possible.
- BAF estimates using the BCF method if (a) the BAF method did not produce estimates for all three TLs and (b) the BCF method produced national-level BAF estimates for all three TLs.

The EPA was able to locate field-measured BAFs for TLs 2, 3, and 4 for PFOS from the peer-reviewed literature sources for which sufficient information was provided to determine the quality and usability of the data. Therefore, EPA used the Field BAF method (EPA, 2003) to derive the national BAF values for this chemical.

Calculating Baseline BAFs

EPA calculated baseline BAFs for PFOS using a procedure analogous to the baseline BAF calculation for nonionic organic chemicals to account for the physical and chemical properties of PFOS. Dissolved field measured BAFs were considered to be 100 percent bioavailable for the purposes of the baseline BAF calculation. Field measured BAFs reported in total concentrations were converted to dissolved BAFs using national default K_{poc} values (the equilibrium partition coefficient of the chemical between the particulate organic carbon [POC] phase and the freely dissolved phase of water), from the EPA's (2000) Methodology; these BAF data were converted

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from total to dissolved and then added to the dissolved field measured BAF data set and used to calculate baseline BAFs for TLs 2, 3, and 4.

Methods for calculating baseline BAFs ($(Baseline\ BAF)_{TL\ n}$) involves normalizing the field-measured BAF, which are based on total concentrations in tissue and water, by the lipid content in the organism and the freely dissolved concentration in the study water (EPA, 2000, 2003). As described in EPA's (2016) Drinking Water Health Advisory for PFOS, partitioning of PFOS is related to protein binding properties. The EPA considered protein-normalizing the measured BAF values in the baseline BAF equation; however, insufficient data were available from the scientific literature on protein content of aquatic organisms and on the binding efficiencies of PFOS to various proteins in aquatic organisms. Because of this lack of data on the relationship between protein content and PFOS bioaccumulation, attempts to normalize BAFs based on protein content would likely introduce greater uncertainty into BAF averages.

Consistent with EPA's 2000 Methodology (EPA, 2000), a procedure analogous to the one used to adjust for the water-dissolved portions of a nonionic organic chemical is applied to measured BAFs for PFOS. As described in EPA's (2003) Technical Support Document, Volume 2, the K_{poc} is approximately equal to the K_{ow} of a hydrophobic organic chemical. It is further described in EPA's (2003) Technical Support Document, Volume 2, that K_{doc} (the equilibrium partition coefficient of the chemical between the dissolved organic carbon (DOC) phase and the freely dissolved phase of water) is directly proportional to the K_{ow} of a hydrophobic organic chemical, and that K_{doc} is less than the K_{ow}. Due to the physical-chemical properties of PFOS, its K_{ow} cannot be reliably measured for these compounds and therefore cannot be used to estimate K_{poc} or K_{doc} (ATSDR, 2021; Hidalgo and Mora-Diez, 2016; EPA, 2003, 2024; Xiang et al. 2018)**.**

Using the K_{oc} information in the study of Higgins and Luthy (2006), EPA determined that the K_{oc} values were applicable to POC but there is no indication that they would be applicable to DOC. Currently, information is not available on the partitioning of PFOS to DOC, nor on the bioavailability of PFOS partitioned to DOC. In addition, Higgins and Luthy (2006) included DOC-bound PFOS in the aqueous phase of their calculations. Thus, the amount of PFOS partitioned to DOC was presumed to be part of the aqueous fraction of the f_{fd} equation, resulting in the following formula (Equation 1):

$$f_{fd} = \frac{1}{[1 + (POC \cdot K_{OC})]} \qquad \text{(Eq. 1)}$$

Where:

- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved.
- POC = national default value of 0.5 mg/L (refer to page 5-44 of EPA's 2000 Methodology (U.S. EPA, 2000)) is used in baseline BAF calculations, unless this value is reported in the BAF source.
- K_{oc} = PFOS log K_{oc} = 2.57 (Higgins and Luthy, 2006).

Because the measured BAFs for PFOS are not adjusted for lipid or protein content, the baseline BAF equation (refer to Equation 5-10 on pages 5-24 and 5-25 of EPA's 2000 Methodology [EPA, 2000]) is adjusted (as shown below in Equation 2) to determine the freely dissolved concentration of PFOS BAFs in water:

$$\text{Baseline BAF} = \frac{\text{Measured BAF}}{f_{fd}} - 1 \qquad \text{(Eq. 2)}$$

EPA used this equation to calculate baseline BAFs from field measured BAFs based on total concentrations.

Dissolved PFOS Baseline BAFs

EPA included results from several field BAF studies for PFOS reported as dissolved (i.e., filtered) concentrations in its baseline BAF calculations. Because these dissolved PFOS data are presumed to represent the freely-dissolved (non-particulate) fraction, the f_{fd} term in Equation 2 is set to 1. Also, as described above, the measured BAFs for PFOS are not being adjusted for lipid or protein content to calculate baseline BAFs for PFOS. Thus, Equation 3 is used to calculate the freely dissolved concentration of PFOS for "baseline BAFs" using field-measured dissolved PFOS BAFs:

$$\text{Baseline BAF} = \text{Measured (dissolved) BAF} - 1 \qquad \text{(Eq. 3)}$$

Calculating National BAFs

Final baseline BAFs were used to compute national BAFs for PFOS. Equation 4 (an equation analogous to the equation used for nonionic organic chemicals in EPA's 94 chemical criteria updates (EPA, 2015) for calculating national BAFs (see Equation 5-28 on Page 5-42 of EPA's 2000 Methodology [U.S. EPA, 2000]) is used to convert the baseline BAF to a national BAF for each trophic level:

$$\text{National BAF}_{(\text{TL n})} = [(\text{Final Baseline BAF}^{fd})_{\text{TL n}} + 1] \cdot (f_{fd}) \qquad \text{(Eq. 4)}$$

Where:

- National BAF = national BAF (L/kg-tissue)
- $(\text{Final Baseline BAF})_{\text{TL n}}$ = mean baseline BAF for TL "n" (L/kg-lipid)
- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved

In summary, for PFOS, the baseline BAFs are calculated using Equation 2 (for field BAFs calculated from total water concentrations) and Equation 3 (for field BAFs calculated from dissolved water concentrations) for each TL. National BAFs are then calculated from TL baseline BAFs using Equation 4.

National Trophic level BAF calculations:

$$\text{National BAF PFOS}_{(\text{TL 2})} = [(425.0)_{\text{TL 2}} + 1] \times (0.999814267)$$

= 425.9 L/kg

= 420 L/kg (rounded)

$$\text{National BAF PFOS}_{(\text{TL 3})} = [(1663.4)_{\text{TL 3}} + 1] \times (0.999814267)$$

= 1664.1 L/kg

= 1700 L/kg (rounded)

$$\text{National BAF PFOS}_{(\text{TL 4})} = [(859.2)_{\text{TL 4}} + 1] \times (0.999814267)$$

= 860.0 L/kg

= 860 L/kg (rounded)

The corresponding values for TL 2, TL 3 and TL 4 were computed as 425.9 L/kg, 1664.1 L/kg and 860.0 L/kg, respectively. Rounding the values to two significant figures yields national BAF values of 420, 1,700 and 860 L/kg for TLs 2, 3, and 4, respectively.

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Appendix B: Comparative Analysis for Potentially Sensitive Populations for PFOS

The EPA evaluated several exposure scenarios for PFOS to determine whether the national recommended criteria for the general population (male and female adults ≥ 21 years old) are sufficiently protective of potentially sensitive subpopulations. To accomplish this, the EPA considered three additional exposure scenarios, as supported by data from the EPA *Exposure Factors Handbook* (EFH; EPA, 2011) and the Human Health Methodology (EPA, 2000). Specifically, the EPA evaluated exposure parameters for "all ages" as well as two potentially sensitive life stages associated with the critical effects used to derive the chronic RfD, i.e., decreased infant birth weight and increased total cholesterol in adults as the co-critical effects. Based on this exposure interval in the critical study, the potentially sensitive subpopulations in humans include women of childbearing age who may be or become pregnant and pregnant women (EPA, 2024; Table B-1, this document).

For the body weight exposure parameter, a mean bodyweight of 75 kg for pregnant women (all trimesters) was identified in the EFH (2011, Ch. 8, Table 8-29). A representative body weight for the "all ages" scenario was not specifically presented in the EFH (EPA, 2011). To address this data limitation, for this exercise, the EPA assumed that the average body weight for "all ages" was 71.6 kg based on the sum of the time-weighted averages of the mean male and female combined body weights from 1 year up to 80 years old from the NHANES (1999–2006) (EPA, 2011, Table 8-3). A body weight average of 67 kg for women of childbearing age was identified in the Human Health Methodology (EPA, 2000); however, this average is based on an older NHANES dataset (NHANES III; WESTAT 2000). More recent NHANES data (1999–2006) suggest that the mean body weight for women of childbearing age ranges from 65.9 kg for 16 to < 21-year-olds to 77.1 kg for 40 to < 50-year-olds (EPA, 2011, Table 8-5). Using these data, the EPA assumed a time-weighted average body weight of 73.4 kg for women of childbearing age (EPA, 2011, Table 8-5).

Drinking water intake values were available for all populations (Table B-1, this document).

The EPA encountered several data limitations for trophic level specific fish consumption rates for some of these potentially sensitive populations. The EPA's national criteria are typically derived using trophic-level specific fish consumption rates (FCRs), paired with trophic-level specific bioaccumulation factors (BAFs) to account for the potential bioaccumulation of some chemicals in aquatic food webs and the broad physiological differences between trophic levels which may influence bioaccumulation (EPA, 2000). Trophic level specific FCRs for women of childbearing age were identified (Table B-1). However, trophic level specific FCRs are not available for two of the potentially sensitive life stages—all ages and pregnant women. Therefore, criteria could not be calculated for these two populations. However, in all cases with available data, the total FCR for the alternative scenarios is lower than the FCR for the general population. Because bodyweights are similar for all of the considered populations (see above and Table B-1), the FCR is likely to be the main determinant of the criteria value, with a larger FCR resulting in a lower, more health protective criterion. Therefore, criteria based on the general population are expected to be protective of the identified potentially sensitive life stages (Table B-1). Separately, paired bodyweight adjusted FCRs are not available for specific trophic levels which precludes the use of body-weight adjusted DWI rates to derive ambient water quality criteria.

Table B-1. Comparison of noncancer-based HHC values for different candidate sensitive populations identified from the critical effect and study.

Population	Bodyweight (kg)	Drinking Water Intake (L/day)	Fish Consumption Rate (g/day)				Criteria (µg/L)	
			Total	TL 2	TL 3	TL 4	W + O	OO
General, adult (≥ 21 years)	**80[a]**	**2.3[b]**	**22[c]**	**7.6[c]**	**8.6[c]**	**5.1[c]**	**0.00006**	**0.00007**
Women of childbearing Age (13–49 years)	73.4[d]	2.1[e]	15.8[c]	5.6[c]	6.0[c]	2.9[c]	0.00008	0.0001
All Ages (Birth to 80 years)	71.6[f]	2.0[b]	19.3[g]	NA	NA	NA	ND	ND
Pregnant Women	75[h]	2.1[e]	10[i]	NA	NA	NA	ND	ND

Notes: g/day = grams of fish consumed per day; L/day = liters of water per day; NA = not available; ND = not determined; OO = organism only; W + O = water plus organism.
Bold values indicate draft national recommended criteria.
Gray highlighting indicates most health protective HHC based on noncancer effects.
[a] EPA, 2011, *Exposure Factors Handbook*, Ch. 8, Table 8-1, NHANES 1999–2006. Recommended mean bodyweight for adults.
[b] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010, community water, 90th percentile per capita rate.
[c] EPA, 2014; NHANES 2003–2010 survey data, 90th percentile per capita rate, freshwater and estuarine fish and shellfish edible portion, adults ≥ 21 years.
[d] Time weighted average of combined bodyweights for women ages 16 to < 50 years, NHANES 1999–2006 (EPA, 2011; Table 8-5).
[e] EPA, 2019, *Exposure Factors Handbook*; Update Ch. 3., Table 3-62, Community water, 90th percentile, per capita rate.
[f] Time weighted average of mean male and female combined body weights from 1 year up to 80 years, NHANES 1999–2006 (EPA, 2011; Table 8-3).
[g] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate; male and female, all ages included.
[h] EPA, 2011, *Exposures Factors Handbook*, Ch 8, mean, NHANES 1999–2006, Table 8-29.
[i] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate pregnant females only.

For illustrative purposes, the EPA calculated criteria based on the exposure parameters for women of childbearing age. As demonstrated in Table B-1, criteria based on the exposure inputs for the general population result in the more health protective criteria and thus are protective of the potentially susceptible life stage of women of childbearing age (Table B-1). Overall, when bodyweight averages are similar, the resulting criteria are driven predominantly by the FCR; thus, a higher FCR results in a more health protective criteria.

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AMIGOS BRAVOS EXHIBIT 6

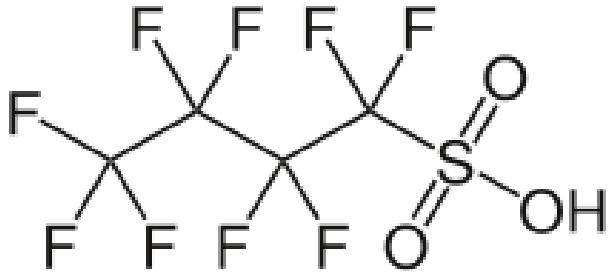
United States Environmental Protection Agency

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DRAFT Human Health Ambient Water Quality Criteria:

Perfluorobutane Sulfonic Acid (PFBS) and Related Salts



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1 Introduction: Background and Scope

The U.S. Environmental Protection Agency's national recommended ambient water quality criteria (AWQC) for human health are scientifically derived numeric values that define ambient water concentrations that are expected to protect human health from the adverse effects of individual pollutants in ambient water.

Section 304(a)(1) of the Clean Water Act (CWA) requires the EPA to develop and publish, and from time-to-time revise, recommended criteria for the protection of water quality that accurately reflect the latest scientific knowledge. Water quality criteria for human health developed under section 304(a) are based solely on data and scientific judgments about the relationship between pollutant concentrations and human health effects. Section 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting pollutant concentrations in ambient water.

The EPA's recommended section 304(a) criteria provide technical information for states and authorized Tribes[a] to consider and use in adopting water quality standards that ultimately provide the basis for assessing water body health and controlling discharges of pollutants into waters of the United States. Under the CWA and its implementing regulations, states and authorized Tribes are required to adopt water quality criteria to protect the designated uses of waters (e.g., public water supply, aquatic life, recreational use, industrial use). The EPA's recommended water quality criteria do not substitute for the CWA or regulations, nor are they regulations themselves. Thus, the EPA's recommended criteria do not establish legal rights or obligations or impose legally binding requirements and are not final agency actions. States and authorized Tribes may adopt, where appropriate, other scientifically defensible water quality criteria that differ from these recommendations. The EPA's water quality standards regulation at 40 CFR 131.20(a) requires states and authorized Tribes to consider any new or updated national section 304(a) recommended criteria as part of their triennial review process, and, if the state or authorized Tribe does not adopt new or revised criteria for parameters that correspond to those new or revised 304(a) criteria, to provide an explanation when it submits its triennial review to EPA. This requirement is to ensure that state or Tribal water quality standards reflect the current science and protect applicable designated uses.

The water quality criteria that are the subject of this document are draft national AWQC recommendations for human health issued under CWA section 304(a). Unless expressly indicated otherwise, all references to "human health criteria," "criteria," "water quality criteria," "ambient water quality criteria recommendations," or similar variants thereof are references to draft national AWQC recommendations for human health.

[a] Throughout this document, the term *states* means the 50 states, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term *authorized Tribe* or *Tribe* means an Indian Tribe authorized for treatment in a manner similar to a state under CWA section 518 for the purposes of section 303(c) water quality standards.

Perfluorobutane sulfonic acid (PFBS) is a member of the per- and polyfluoroalkyl substances (PFAS) class. PFAS are a large class of thousands of synthetic chemicals that have been in use in the United States and around the world since the 1940s (EPA, 2018). The ability for PFAS to withstand heat and repel water and stains makes them useful in a wide variety of consumer, commercial, and industrial products, and in the manufacturing of other products and chemicals. The current scientific evidence has shown the potential for harmful health effects after human exposure to certain PFAS. The persistence and resistance to hydrolysis, photolysis, metabolism, and microbial degradation of PFAS raise additional concerns about long-term exposure and human health effects.

The EPA developed the draft human health criteria (HHC) for PFBS to reflect the latest scientific information for input values, including exposure factors (i.e., body weight [BW], drinking water intake [DWI] rate, and fish consumption rate [FCR]), bioaccumulation factors (BAFs), human health toxicity values (i.e., reference dose [RfD]), and relative source contribution (RSC). The draft criteria are based on the EPA's current *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000a), which is referred to as the "2000 Methodology" in this document (EPA, 2000a).

2 Problem Formulation

Problem formulation provides a strategic framework for ambient water quality criteria development to systematically identify the major factors and chemical-specific scientific issues to be considered in the assessment (EPA, 2014a). The structure of this draft criteria document is intended to be consistent with general concepts of health assessments as described in the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a).

In developing AWQC, the EPA follows the assessment method outlined in the 2000 Methodology (EPA, 2000a). The 2000 Methodology describes different approaches for addressing water and nonwater exposure pathways to derive human health AWQC depending on the toxicological endpoint of concern, the toxicological effect (noncarcinogenic or carcinogenic), and whether toxicity is considered a linear or threshold effect. Water sources of human exposure include both consuming drinking water and eating fish or shellfish from inland and nearshore water bodies that have been contaminated with pollutants. For pollutants that exhibit a threshold of exposure below which deleterious human health effects are unlikely to occur, as is the case for noncarcinogens and nonlinear carcinogens, the EPA applies an RSC. The RSC is the percentage of the total exposure to a contaminant that is attributed to the combination of drinking water and eating freshwater and estuarine fish and shellfish, where the remainder of exposure is allocated to other sources of oral exposure and other routes of exposure. The RSC is calculated by examining the data for other sources (e.g., air, food, soil) and pathways of exposure following the exposure decision tree for calculation of an RSC described in the 2000 Methodology (EPA, 2000a).

For carcinogenic substances for which the cancer slope factor was quantified using linear low-dose extrapolation, only the exposures from drinking water and fish ingestion are reflected in the human health AWQC; nonwater sources are not explicitly included, and no RSC is applied

(EPA, 2000a). This is because in these situations, AWQC are derived with respect to the *incremental* lifetime cancer risk posed by the presence of a substance in ambient water, rather than an individual's total risk from all exposure sources. Therefore, the resulting AWQC represents the ambient water concentration that is expected to increase an individual's lifetime risk of cancer from exposure to the pollutant by no more than one chance in one million (10^{-6}) for the general population (male and female adults, 21 years and older; referred to as "general population" herein), regardless of the additional lifetime cancer risk due to exposure, if any, to that substance from other sources. The EPA calculates AWQC at a 10^{-6} cancer risk level for the general population (EPA, 2000a). The 2000 Methodology recommends that states set human health criteria cancer risk levels for the target general population at either 10^{-5} or 10^{-6} and also notes that states and authorized Tribes can choose a more stringent risk level, such as 10^{-7}.

For substances that are carcinogenic, the EPA takes an integrated approach by considering both cancer and noncancer effects when deriving AWQC (EPA, 2000a,b). Where sufficient data are available, the EPA first derives separate AWQC for both carcinogenic and noncarcinogenic toxicity endpoints and then selects the lower (more health protective) of the two values for the recommended AWQC.

PFBS may exist in multiple forms, such as isomers or associated salts and each form may have a separate Chemical Abstracts Service registry number (CASRN) or no CASRN at all. Additionally, these compounds have various names under different classification systems. PFBS and its related salts are members of the group of PFAS known as short-chain perfluoroalkane sulfonates. PFBS is an acid that is generally present as the sulfonate anion at typical environmental pH values. Therefore, the conclusions in this document apply to all isomers of PFBS, as well as nonmetal salts of PFBS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9. For purposes of this assessment, "PFBS" will signify the ion, acid, or any nonmetal salt of PFBS.

2.1 Uses and Sources of PFBS

PFAS are manufactured chemicals that have been widely used in industrial and consumer processes and products over the past several decades in the United States due to their repellant and surfactant properties. PFAS are persistent chemicals based on their physicochemical properties. Concerns about persistence of PFAS stem from the resistance of these compounds to hydrolysis, photolysis, metabolism, and microbial degradation.

PFBS has been used as a replacement chemical for perfluorooctane sulfonic acid (PFOS), a chemical that was voluntarily phased out (with some exceptions) by its primary U.S. manufacturer, 3M Company, by 2002 (3M, 2002; EPA, 2007). Prior to its use as a PFOS replacement, PFBS had been produced as a byproduct and was present in consumer products as an impurity (AECOM, 2019). Concerns arising in the early 2000s about the environmental persistence, bioaccumulation potential, and long half-lives of PFOS and other long-chained PFAS in humans resulted in their replacement with shorter-chain PFAS, such as PFBS, in consumer products and applications (EPA, 2021a,b). PFBS and other shorter-chain PFAS possess

the desired chemical properties of longer-chain PFAS, but have shorter half-lives in humans (EPA, 2021a,b).

Environmental releases of PFBS may result directly from the production and use of PFBS itself, production and use of PFBS-related substances for various applications, and/or from the degradation of PFBS precursors (i.e., substances that may form PFBS during use, as a waste, or in the environment). PFBS is used in the manufacture of paints, cleaning agents, and water- and stain-repellent products and coatings (EPA, 2021a,b). PFBS has also been used as a mist suppressant for chrome electroplating and has been found associated with the use of aqueous film-forming foam (AFFF) (EPA, 2021a,b). PFBS has been detected in dust, carpeting and carpet cleaners, floor wax, and food packaging (ATSDR, 2021; EPA, 2021a,b).

2.2 Environmental Fate and Transport in the Environment

The European Chemical Agency (ECHA) reports that PFBS is stable to hydrolysis, oxidation, and photodegradation in the atmosphere, and there have been no reports of abiotic degradation under environmental conditions (ECHA, 2019). The persistence of PFBS has been attributed to the strong carbon-fluorine (C-F) bond. PFBS has a high solubility in water (52.6 grams per liter [g/L] at 22.5–24 degrees Celsius (°C) for the potassium salt) and high mobility in the environment[b] (log K_{oc} 1.2 to 2.7) (ECHA, 2019).

The Norwegian Environment Agency conducted a literature review of physicochemical properties and environmental monitoring data for PFBS to assist an evaluation under Registration, Evaluation, Authorization and Restriction of Chemicals (Arp and Slinde, 2018). No studies were identified that observed degradation of PFBS under environmental conditions, including atmospheric photolysis. The review determined that the air-water partition coefficient (K_{aw}) for PFBS is too low to measure and that volatilization from water is negligible, but that the presence of PFBS in ambient air can result from direct emissions or transport of droplets in contaminated water. ECHA (2019) modeled photodegradation of PFBS in air and concluded that PFBS has the potential for long-range transport.

2.3 Occurrence and Detection in Sources Relevant to Ambient Water Quality Criteria

PFBS has been detected in a variety of environmental matrices. Studies describing the occurrence and detection of PFBS in sources relevant to ambient water quality criteria, including ambient water, fish, and shellfish, were identified through systematic literature searches of the peer reviewed and gray literature (see Section 6.2 below and Appendix B of EPA, 2024a for additional detail) and are described below. Additional occurrence information for sources other than ambient water (e.g., air, food, soil) is summarized in Section 6.2 as part of the determination of the RSC.

[b] A measure of mobility is the sediment or soil organic carbon-water partition coefficient (K_{oc}) with units of liters per kilogram (L/kg) and commonly expressed as log K_{oc}, which is unitless.

2.3.1 Occurrence in Surface Water

Studies evaluating the occurrence of PFBS in surface water in North America or Europe are summarized in Table A-1. Broadly, studies either targeted surface waters used as drinking water sources, surface waters known to be contaminated with PFAS (as reported by the study authors), or surface waters over a relatively large geographic area (i.e., statewide) with some or no known point sources of PFAS.

Zhang et al. (2016) identified major sources of surface water PFAS contamination by collecting samples from 37 rivers and estuaries in the northeastern United States (metropolitan New York area and Rhode Island). PFBS was detected at 82% of sites and the range of PFBS concentrations was nondetect (ND) to 6.2 nanograms per liter (ng/L). Appleman et al. (2014) collected samples of surface water that were impacted by wastewater effluent discharge in several states. PFBS was detected in 64% of samples from 11 sites with concentrations ranging from ND to 47 ng/L. Several other studies from North America (four from the United States and two from Canada) evaluated surface waters from sites for which authors did not indicate whether sites were associated with any specific, known PFAS releases (Nakayama et al., 2010; Pan et al., 2018; Subedi et al., 2015; Veillette et al., 2012; Yeung et al., 2017). Nakayama et al. (2010) also collected samples across several states, but no specific source of PFAS was identified. The detection frequency (DF) of PFBS in the Nakayama et al. (2010) study was 43% with median and maximum levels of 0.71 ng/L and 84.1 ng/L, respectively. As reported in EPA (2024b), Pan et al. (2018) sampled surface water sites in the Delaware River with 100% DF, though PFBS levels were relatively low (0.52 ng/L to 4.20 ng/L); Yeung et al. (2017) reported results for a creek (PFBS concentration of 0.02 ng/L) and a river (no PFBS detected) in Canada. Veillette et al. (2012) analyzed surface water from an Arctic lake and detected PFBS at concentrations ranging from 0.011 ng/L to 0.024 ng/L. Subedi et al. (2015) evaluated lake water potentially impacted by septic effluent from adjacent residential properties, and detected PFBS in only one sample at a concentration of 0.26 ng/L.

Additional available studies assessed surface water samples at U.S. sites contaminated with PFAS from nearby PFAS manufacturing facilities (ATSDR, 2021; Galloway et al., 2020; Newsted et al., 2017; Newton et al., 2017) or facilities that manufacture products containing PFAS (Lasier et al., 2011; Procopio et al., 2017; Zhang et al., 2016). A few of these studies identified potential point sources of PFAS contamination, including industrial facilities (e.g., textile mills, metal plating/coating facilities), airports, landfills, and wastewater treatment plants (WWTPs) (Galloway et al., 2020; Zhang et al., 2016). Among these sites, PFBS DFs (0% to 100%) and PFBS levels (ND to 336 ng/L) varied. In general, PFBS DFs that ranged from 0% to 3% were associated with samples collected upstream of PFAS point sources, and higher PFBS DFs (up to 100%) and PFBS concentrations were associated with samples collected downstream of point sources. An additional study (Lindstrom et al., 2011) sampled pond and stream surface water in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer, and the maximum and mean PFBS concentrations were 208 ng/L and 26.3 ng/L, respectively.

Another group of studies from the United States evaluated sites known to be contaminated from military installations with known or presumed AFFF use (Anderson et al., 2016; Nakayama et al., 2007; Post et al., 2013). The highest PFBS levels in ambient water reported among these available studies were from Anderson et al. (2016) who performed a national study of 40 AFFF-impacted sites across 10 military installations and reported a maximum PFBS concentration of 317,000 ng/L. Lescord et al. (2015) examined PFAS levels in Meretta Lake, a Canadian lake contaminated with runoff from an airport and military base, which are likely sources of PFAS from AFFF use. The authors reported a 70-fold greater mean PFBS concentration for the contaminated lake versus a control lake. In addition to AFFF, Nakayama et al. (2007) identified industrial sources, including metal-plating facilities and textile and paper production, as contributing to the total PFAS contamination in North Carolina's Cape Fear River Basin. Nakayama et al. (2007) reported a PFBS DF of 17% and PFBS concentrations ranging from ND to 9.41 ng/L at these sites.

The EPA identified additional studies evaluating surface water samples from sites in Europe with known or suspected PFAS releases associated with AFFF use (Dauchy et al., 2017; Gobelius et al., 2018; Mussabek et al., 2019) or fluorochemical manufacturing (Bach et al., 2017; Boiteux et al., 2017; Gebbink et al., 2017; Valsecchi et al., 2015). PFBS levels were comparable at the AFFF-impacted sites (< 300 ng/L overall). Of the four study sites potentially contaminated based on proximity to fluorochemical manufacturing sites, two (from studies conducted in France) did not have PFBS detections (Bach et al., 2017; Boiteux et al., 2017). PFBS levels were low at most sampling locations of the remaining two studies (up to approximately 30 ng/L) except for the site in River Brenta in Italy (maximum PFBS concentration of 1,666 ng/L) which is also impacted by nearby textile and tannery manufacturers (Valsecchi et al., 2015).

Eight studies in Europe evaluated areas close to urban areas, commercial activities, or industrial activities (e.g., textile manufacturing) (Boiteux et al., 2012; Eschauzier et al., 2012; Lorenzo et al., 2015; Rostkowski et al., 2009; Zhao et al., 2015) and/or wastewater effluent discharges (Labadie and Chevreuil, 2011; Lorenzo et al., 2015; Möller et al., 2010; Wilkinson et al., 2017). Among these sites, PFBS DFs varied (0 to 100%) and PFBS levels were less than 250 ng/L overall.

Ten studies conducted in Europe evaluated sites with no known fluorochemical source of contamination (Ahrens et al., 2009a,b; Barreca et al., 2020; Ericson et al., 2008; Eriksson et al., 2013; Loos et al., 2017; Munoz et al., 2016; Pan et al., 2018; Shafique et al., 2017; Wagner et al., 2013). Pan et al. (2018) analyzed surface water from sites in the United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). While none of the sites sampled were proximate to known sources of PFAS, but PFBS was detected in all three water bodies. Concentrations of PFBS ranged from 0.46 ng/L to 146 ng/L; the highest level (146 ng/L) was detected in the Rhine River and was more than 20 times greater than any maximum level found in the other water bodies. In the remaining nine studies, reported PFBS levels ranged from ND to 26 ng/L, except for one study in Italy that reported a PFBS DF of 39% and levels in the µg/L range at three out of 52 locations within the same river basin: Legnano (16,000 ng/L), Rho (15,000 ng/L), and Pero (3,400 ng/L) (Barreca et al., 2020).

2.3.2 Occurrence in Freshwater and Estuarine Fish and Shellfish

Based on the available data collected to date, PFBS has been rarely detected in freshwater and estuarine fish and shellfish in the U.S. Several large-scale sampling efforts have been conducted by the EPA and other agencies to determine PFAS levels in fish. In the EPA's 2013–2014 National Rivers and Streams Assessment (NRSA), PFBS was detected at concentrations above the method detection limit (MDL) (0.1 ng/g) but below the quantitation limit (1 ng/g), at 0.571 ng/g in a largemouth bass fish fillet sample collected from Big Black River, Mississippi; 0.475 ng/g in a smallmouth bass fillet composite collected from Connecticut River, New Hampshire; and 0.148 ng/g in a walleye fillet composite collected from Chenango River, New York (EPA, 2020). However, in the 2008–2009 NRSA, PFBS was not detected in any fish species sampled (Stahl et al., 2014). In the EPA's *2015 Great Lakes Human Health Fish Fillet Tissue Study*, PFBS was detected at a concentration of 0.36 ng/g in a smallmouth bass fillet composite collected from Lake Erie, New York (EPA, 2021c). In the National Rivers and Streams Assessment 2018–2019 (EPA, 2023) PFBS was a target chemical but was not detected in any of the fish samples analyzed. Note that PFBS was not a target chemical in the EPA's National Lake Fish Tissue Study (EPA, 2009) or the EPA's National Lakes Assessment 2017 (EPA, 2022a). PFBS was a target chemical for the National Lakes Assessment 2022 (EPA, 2024c), but was not detected in any of the fish samples analyzed (MDL 0.090 wet weight [ww]). More recently, PFBS has been detected in several estuarine species, including Irish pompano, silver porgy, grey snapper, and eastern oyster from the St. Lucie Estuary in the National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2024).

3 Criteria Formulas: Analysis Plan

Human health AWQC for toxic pollutants may be necessary to protect designated uses of water bodies related to ingestion of water (i.e., public water supply or source water protection) and ingestion of freshwater/estuarine fish and shellfish. *See* CWA 303(c)(2)(A)–(B). Although the AWQC are based on chronic health effects data (both cancer and noncancer effects), the criteria are intended to also be protective against adverse effects that may reasonably be expected to occur as a result of elevated acute or short-term exposures (EPA, 2000a). Human health AWQC are expected to provide adequate protection not only for the general population over a lifetime of exposure, but also for sensitive life stages and subpopulations who, because of high water- or fish intake rates, or because of biological sensitivities, have an increased risk of receiving a dose that would elicit adverse effect (EPA, 2000a).

The derivation of human health AWQC requires information about both the toxicological endpoints of concern from exposure to water pollutants and human exposure pathways for those pollutants. The EPA considers two primary pathways of human exposure to pollutants present in a particular water body when deriving human health 304(a) AWQC: (1) direct ingestion of drinking water obtained from the water body; and (2) consumption of fish and shellfish obtained from the water body.

The equations for deriving human health AWQC are presented as Equations (Eqs.) 1 and 2 for noncancer and non-linear carcinogenic effects, and Eqs. 3 and 4 for linear carcinogenic effects. The EPA derives two separate recommended human health AWQC based on 1) the consumption of both water and aquatic organisms (Eq. 1), called "water + organism"; and 2) the consumption of freshwater/estuarine fish and shellfish alone (Eq. 2), called "organism only." The use of one criterion over the other depends on the designated use of a particular water body or water bodies (i.e., drinking water source and/or fishable waters). The EPA recommends applying organism only AWQC (Eq. 2) to a water body where the designated use includes supporting fishable uses under section 101(a) of the CWA but the water body is not a drinking water supply source (e.g., non-potable estuarine waters that support fish or shellfish for human consumption) (EPA, 2000a).

The EPA recommends including the drinking water exposure pathway for ambient surface waters where drinking water is a designated use for the following reasons: (1) drinking water is a designated use for surface waters under the CWA, and therefore, criteria are needed to ensure that this designated use can be protected and maintained; (2) although they are rare, some public water supplies provide drinking water from surface water sources without treatment; (3) even among the majority of water supplies that do treat surface waters, existing treatments might not be effective for reducing levels of particular contaminants; and (4) in consideration of the agency's goals of pollution prevention, ambient waters should not be contaminated to a level where the burden of achieving health objectives is shifted away from those responsible for pollutant discharges and placed on downstream users that must bear the costs of upgraded or supplemental water treatment (EPA, 2000a).

The equations for deriving the criteria values are as follows (EPA, 2000a):

Equations for Noncancer and Nonlinear Carcinogen HHC:

Consumption of water and organisms:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{DWI + \sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 1)}$$

For consumption of organisms only:

$$AWQC = \frac{RfD \times RSC \times BW \times 1{,}000^{c}}{\sum_{i=2}^{4} (FCR_i \times BAF_i)} \quad \text{(Eq. 2)}$$

Where:
AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)
RfD = reference dose, expressed in milligrams per kilogram-day (mg/kg-d)
RSC = relative source contribution, unitless
BW = body weight, expressed in kg

[c] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

DWI = drinking water intake, expressed in L/d
$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4
FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d
BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

Equations for Linear Carcinogens HHC:

Consumption of water and organisms:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{d}}{DWI + \sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 3)}$$

For consumption of organisms only:

$$AWQC = \frac{RSD \times BW \times 1{,}000^{d}}{\sum_{i=2}^{4}(FCR_i \times BAF_i)} \quad \text{(Eq. 4)}$$

Where:
AWQC = ambient water quality criteria, expressed in micrograms per liter (µg/L)
RSD = RSD = risk specific dose; the cancer risk level (i.e., a target risk for the population; 1 in 1 million or 10^{-6}) divided by the cancer slope factor (i.e., incidence of cancer relative to dose in units of $[mg/kg/day]^{-1}$), expressed in milligrams per kilogram-day (mg/kg-d)
BW = body weight, expressed in kg
DWI = drinking water intake, expressed in L/d
$\sum_{i=2}^{4}$ = summation of values for aquatic trophic levels (TLs), where the letter *i* stands for the TLs to be considered, starting with TL 2 and proceeding to TL 4
FCR_i = fish consumption rate for aquatic TLs (i) 2, 3, and 4, expressed in kg/d
BAF_i = bioaccumulation factor for aquatic TLs (i) 2, 3, and 4, expressed in L/kg

The EPA rounds AWQC to the number of significant figures in the least precise parameter as described in the 2000 Methodology (EPA, 2000a, Section 2.7.3). The EPA used a rounding procedure that is consistent with the 2000 Methodology (EPA, 2000a) and the 2015 HHC update (https://www.epa.gov/wqc/human-health-water-quality-criteria-and-methods-toxics).

4 AWQC Input Parameters

4.1 Exposure Factor Inputs

National recommended HHC establish ambient concentrations of pollutants in waters of the United States which, if not exceeded, will protect the general population from adverse health impacts from those pollutants due to consumption of aquatic organisms (i.e., freshwater and estuarine fish and shellfish) and water (EPA, 2000a). It is the EPA's longstanding practice to set national recommended HHC at a level intended to be adequately protective of human exposure over a lifetime (EPA, 2000a). To accomplish this, the EPA uses a combination of median values,

[d] 1,000 µg/mg is used to convert the units of mass from milligrams to micrograms.

mean values, and percentile estimates for the HHC inputs consistent with the EPA's 2000 Methodology (EPA, 2000a). The EPA's assumptions afford an overall level of protection targeted at the high end of the general adult population (i.e., the target population or the criteria-basis population) (EPA, 2000a). This approach is reasonably conservative and appropriate to meet the goals of the CWA and the 304(a) criteria program (EPA, 2000a). If the EPA determines that another population or life stage (e.g., pregnant women and their fetuses, young children) is the target population, then exposure parameters for that target population or life stage could be considered in the derivation of the criteria (EPA, 2000a). Potentially sensitive life stages for PFBS are explored further in a comparative analysis in Appendix D.

4.1.1 Body Weight

The BW for the general adult population including males and females, ages 21 years and older, was selected for the PFBS HHC, consistent with the population selected in the agency's most recent major update to existing 304(a) HHC (EPA, 2015) and the EPA's 2000 Methodology (EPA, 2000a). The EPA used the mean weight for adults ages 21 and older of 80.0 kg, based on National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2006 as reported in Table 8.1 of the EPA's *Exposure Factors Handbook* (EPA, 2011), the EPA's most recent publication of body weight exposure factors.

4.1.2 Drinking Water Intake Rate

For adults ages 21 and older, the EPA used an updated DWI of 2.3 L/d, rounded from 2.345 L/d. This DWI was estimated using the Food Commodity Intake Database consumption calculator (http://fcid.foodrisk.org[e]) which is based on NHANES 2005–2010 data used to develop the EPA's *Exposure Factors Handbook Update to Chapter 3, Ingestion of Water and Other Select Liquids* (EPA, 2019, Section 3.3.1.1). This rate represents the per capita estimate of combined direct and indirect community water[f] ingestion at the 90th percentile for adults, males and females, ages 21 and older. The EPA selected the per capita rate for the updated DWI because it represents the average daily dose estimates; that is, it includes both people who drank water during the survey period and those who did not, which is appropriate for a national-scale assessment such as the development of CWA section 304(a) national human health criteria development (EPA, 2019, Section 3.2.1). The updated DWI of 2.3 L/d reflects the latest scientific knowledge in accordance with CWA 304(a)(1).

The EPA's selection of the DWI of 2.3 L/d is consistent with the 2000 Methodology's selection of a rate based on per capita community water ingestion at the 86th percentile for adults

[e] The FCID Consumption Calculator is an application that uses National Health and Nutrition Examination Survey/What We Eat in America (NHANES/WWEIA) food intake and FCID recipes to estimate food commodity consumption for the purposes of pesticide dietary exposure assessment, as well as consumption estimates for EPA's *Exposure Factors Handbook* (EFH) users (University of Maryland, 2024).

[f] *Community water* includes direct and indirect use of tap water for household uses and excludes bottled water and other sources (EPA, 2019, Section 3.3.1.1). *Direct ingestion* is defined as direct consumption of water as a beverage, while *indirect ingestion* includes water added during food preparation (e.g., cooking, rehydration of beverages) but not water intrinsic to purchased foods (EPA, 2019, Section 3.1).

surveyed in the U.S. Department of Agriculture's *1994–1996 Continuing Survey of Food Intake by Individuals (CSFII)* analysis (EPA, 2000a, Section 4.3.2.1).

4.1.3 Fish Consumption Rate

The FCR used for adults ages 21 years and older is 22.0 g/d, or 0.0220 kg/d (EPA, 2014b, Table 9a). This FCR represents the 90th percentile per capita consumption rate of fish from inland and nearshore waters for U.S. adults ages 21 years and older based on NHANES data from 2003–2010. The 95% confidence interval (CI) of the 90th percentile per capita FCR is 19.1 g/d and 25.4 g/d, respectively.

As recommended in the 2000 Methodology, the EPA used TL-specific FCRs to better represent human dietary consumption of fish. An organism's trophic position in the aquatic food web can have an important effect on the magnitude of bioaccumulation of certain chemicals. The TL-specific FCRs are numbered 2, 3, and 4, and they account for different categories of fish and shellfish species based on their position in the aquatic food web: TL 2 accounts for benthic filter feeders; TL 3 accounts for forage fish; and TL 4 accounts for predatory fish (EPA, 2000a).

The EPA used the following TL-specific FCRs to derive the AWQC: TL 2 = 7.6 g/d (0.0076 kg/d) (95% CI [6.4, 9.1] g/d); TL 3 = 8.6 g/d (0.0086 kg/d) (95% CI [7.2, 10.2] g/d); and TL 4 = 5.1 g/d (0.0051 kg/d) (95% CI [4.0, 6.4] g/d). Each TL-specific FCR represents the 90th percentile per capita consumption rate of fish and shellfish from inland and nearshore waters from that particular TL for U.S. adults ages 21 years and older (EPA, 2014b, Tables 16a, 17a, and 18a). The sum of these three TL-specific FCRs is 21.3 g/d, which is within the 95% CI of the overall FCR of 22.0 g/d. The EPA recommends using the TL-specific FCRs when deriving AWQC; however, the overall FCR (22.0 g/d) may be used if a simplified approach is preferred.

4.2 Bioaccumulation Factor (BAF)

4.2.1 Approach

Several attributes of the bioaccumulation process are important to understand when deriving national BAFs for use in developing national recommended section 304(a) AWQC. First, the term *bioaccumulation* refers to the uptake and retention of a chemical by an aquatic organism from all surrounding media, such as water, food, and sediment. The term *bioconcentration* refers to the uptake and retention of a chemical by an aquatic organism from water only. In some cases, experiments conducted in a lab that measure *bioconcentration* can be used to estimate the degree of *bioaccumulation* expected in natural conditions. However, for many chemicals, particularly those that are highly persistent and hydrophobic, the magnitude of bioaccumulation by aquatic organisms can be substantially greater than the magnitude of bioconcentration. In these cases, an assessment of bioconcentration alone underestimates the extent of accumulation in aquatic biota. Accordingly, the EPA guidelines presented in the 2000 Methodology (EPA, 2000a) emphasize using, when possible, measures of *bioaccumulation* as opposed to measures of *bioconcentration* (EPA, 2000a).

The EPA estimated BAFs for this draft PFBS AWQC using the 2000 Methodology (EPA, 2000a) and the associated *Technical Support Document, Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003). Specifically,

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these documents provide a framework for identifying alternative procedures to derive national TL-specific BAFs for a chemical based on the chemical's properties (e.g., ionization and hydrophobicity), metabolism, and biomagnification potential (EPA, 2000a, 2003). As described in the 2000 Methodology, the purpose of the EPA's national BAF is to represent the long-term, average bioaccumulation potential of a chemical in aquatic organisms that are commonly consumed by humans throughout the United States (EPA, 2000a). The EPA evaluated results from field BAF and laboratory bioconcentration factor (BCF) studies on aquatic organisms commonly consumed by humans in the United States for use in developing national trophic-level BAFs. National BAFs are not intended to reflect fluctuations in bioaccumulation over short periods (e.g., a few days) because human health AWQC are generally designed to protect humans from long-term (lifetime) exposures to waterborne chemicals (EPA, 2003).

The EPA followed the approach described in Figure 3-1 of the Technical Support Document, Volume 2 (EPA, 2003). The EPA used the best available data to classify each chemical according to this framework, and to derive the most appropriate BAFs following the 2000 Methodology (EPA, 2000a) and Technical Support Document, Volume 2 (EPA, 2003). Best available data consisted of peer-reviewed literature sources, government reports, and professional society proceedings, when sufficient information was provided to indicate the quality and usability of the data.

The framework provides six procedures to calculate a national BAF based on the pollutant's physical and chemical properties (see Figure 1, Procedures 1–6). Each procedure contains a hierarchy of the BAF derivation methods (listed below); however, this hierarchy should not be considered inflexible (EPA, 2000a). The four methods are:

1. **BAF Method.** This method calculates national TL-specific BAFs using water and fish and shellfish tissue concentration data obtained from field studies. Field-measured BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of that contaminant in the surrounding water.

   For nonionic organic chemicals, BAFs are normalized to allow a common basis for averaging BAFs from several studies by adjusting for the water-dissolved portions of the chemical.

   In order to calculate representative TL-specific national BAFs used to calculate national recommended 304(a) criteria, the EPA averaged multiple field BAFs using a geometric mean of the normalized BAFs, first by species and then by TL, to calculate the TL baseline BAFs.

2. **BSAF Method.** This method uses biota-sediment accumulation factors (BSAFs) to estimate bioaccumulation. While BAFs are calculated by dividing the concentration of a contaminant in an organism by the concentration of the contaminant in water, BSAFs divide the concentration in the organism by the concentration in surrounding sediments. BSAFs are useful when calculating site-specific criteria for compounds that are highly hydrophobic—these compounds have the potential to cause bioaccumulation in aquatic organisms even when concentrations in the water column are below detection limits.

3. **BCF Method.** This method estimates BAFs from laboratory-measured BCFs. Experiments designed to calculate BCFs aim to measure bioconcentration resulting from an organism's exposure to contaminated water. Unlike BAFs measured in the field, BCF experiments do not capture bioaccumulation from other routes of exposure or biomagnification (the increase in bioaccumulation at higher levels of the food chain). However, BCFs may be used to estimate bioaccumulation if a contaminant's chemical and physical properties indicate that the compound is likely to primarily accumulate in the organism via the water exposure route, and there is no evidence that the contaminant biomagnifies in the food chain. If insufficient field-collected data are available to calculate a national BAF, then the EPA may also estimate bioaccumulation using laboratory measured BCFs and a food chain multiplier term, which accounts for biomagnification.

   A similar process to the one described in the BAF method description (above) for normalizing of water-dissolved portions of the chemical and particulate organic carbon content is used for calculating national BAFs from laboratory-measured BCF data. Ionic organic chemicals are normalized, then multiplied by the food chain multiplier if biomagnification is likely to occur. All available BCFs are averaged using a geometric mean across species and then across TL to compute baseline BAFs.

4. **K_{ow} Method.** This method predicts BAFs based on a chemical's octanol-water partition coefficient (K_{ow}), with or without adjustment using a food chain multiplier, as described in Section 5.4 of the Technical Support Document, Volume 2 (EPA, 2003).

4.2.2 Data Selection and Evaluation

The EPA conducted a systematic literature search in June 2023 of publicly available literature sources to determine whether they contained information relevant to calculating national BAFs for human health AWQC, using the 2000 Methodology and Technical Support Document, Volume 2 (EPA, 2000a, 2003). Initially, bioaccumulation data published in Burkhard (2021) was reviewed for inclusion. Burkhard (2021) evaluated bioaccumulation literature through mid-2020. To supplement this literature, a second literature search was conducted to identify additional bioaccumulation data published from 2020 through June 2023. The literature search for reporting the bioaccumulation of PFBS was implemented by developing a series of chemical-based search terms (see Appendix B) consistent with the process for derivation of BAFs used in the development of the EPA's Final Aquatic Life Criteria for perfluorooctanoic acid (PFOA) (EPA, 2024d) and PFOS (EPA, 2024e) and described in Burkhard (2021). These terms included chemical names and Chemical Abstracts Service Registry Numbers (CASRNs or CAS), synonyms, tradenames, and other relevant chemical forms (i.e., related compounds) (see Section 8). Databases searched were Current Contents, ProQuest CSA, Dissertation Abstracts, Science Direct, Agricola, Scopus, PubMed, Google Scholar, TOXNET, and UNIFY (database internal to the EPA's ECOTOX database). The supplemental literature search yielded > 10,000 results and the citation list that were further refined by excluding citations on analytical methods, human health, terrestrial organisms, bacteria, and where PFBS was not a chemical of study. The citations meeting the search criteria were reviewed for reported BAFs and/or reported concentrations in which BAFs could be calculated. Data from papers that met the inclusion and data quality screening criteria described below were extracted into the chemical dataset for PFBS.

Specifically, studies were evaluated for inclusion in the dataset used for calculating national BAFs for PFBS using the following evaluation criteria:

- Only BAF studies that included units for tissue, water, and/or BAFs were included.
- Mesocosm, microcosm, and model ecosystem studies were not selected for use in calculating BAFs.
- BAF studies in which concentrations in tissue and/or water were below the minimum level of detection were excluded.
- Only studies performed using freshwater or brackish water were included; high salinity values were excluded.
- Studies of organisms (e.g., damselfly, goby) and tissues (e.g., fish bladder) not commonly consumed by humans or not used as surrogate species for those commonly consumed by humans were excluded. Information on the ecology, physiology, and biology of the organism was used to determine whether an organism is a reasonable surrogate of a commonly consumed organisms.
- Studies in which the BAFs were not found to be at steady state were excluded.
- Initially, for pooled samples, averaging BAF data from multiple locations was only considered acceptable if corresponding tissue and water concentrations were available from matching locations (e.g., a BAF would not have been calculated using water and tissue samples collected from eight separate locations with tissue concentrations collected from only six of these corresponding locations). After further review, water samples averaged from samples collected between tissue sampling sites, were considered acceptable as these water samples were determined to be from the same overall spatial area of the study.

In addition to the evaluation criteria listed above, PFBS bioaccumulation data were also evaluated using five study quality criteria outlined in Burkhard (2021) to further evaluate BAF literature for inclusion in the national BAF calculation (Table 1).

As noted in Burkhard (2021), study quality determinations based on temporal and spatial coordination were subjective. In the absence of adequate quantifiable information regarding sample location (site coordinates for both water and tissue collection locations) or temporal coordination (specific dates of sample collection), additional follow-up with study authors was used to determine final quality values.

Table 1. Bioaccumulation factor (BAF) study quality criteria based on suggested criteria in Burkhard (2021).

Criteria	1	2	3
Number of water samples collected	> 3 samples	2–3 samples	1 sample
Number of organism samples collected	> 3 samples	2–3 samples	1 sample
Temporal coordination of water and biota samples	Concurrent collection of samples	Collected within a 1-year time frame	Collected > 1-year time frame
Spatial coordination of water and biota samples	Collected from same locations	Collected from reasonably close locations (1 kilometer [km]–2 km)	Significantly different sampling locations
General experimental design	Assigned a default value of zero for studies in which tissues from individual species were identified and analyzed		Assigned a value of 3 for studies in which tissues were from mixed species or reported as a taxonomic group.

Notes: The scores for each BAF were totaled and used to determine the overall confidence ranking for each individual BAF. The sum of quality values for the five criteria listed in Table 1 were classified as high quality (total score of 4 or 5), medium quality (total score of 5 or 6) or low quality (total score ≥ 7). Only high and medium quality data were included in final national BAFs calculations.

4.2.3 BAFs for PFBS

Following the decision framework presented in Figure 1, the EPA selected one of the four methods to develop a national-level BAF for this chemical. Because PFBS is an organic chemical that predominantly exist in an anionic form in water (ATSDR, 2021; EPA, 2021a,b, 2024a), the BSAF and K_{ow} methods would not be applicable. The EPA selected the BAF estimate using the BAF method (i.e., based on a field measured BAF) because sufficient field measured BAF data were available for PFBS.

The national-level BAF equation adjusts the TL baseline BAFs for nonionic organic chemicals by national default values for lipid content, as well as dissolved and particulate organic carbon content. The partitioning of PFBS is related to protein binding properties (ATSDR, 2021; ECHA, 2019); therefore, the EPA did not normalize measured BAF values for PFBS using lipid content when calculating baseline and national BAFs. The EPA selected the recommended 50th percentile dissolved and particulate organic carbon content for the national-level default values which is consistent with the goal of national BAFs (i.e., as central tendency estimates), as described in Section 6.3 of the Technical Support Document, Volume 2 (EPA, 2003). Adjustment for water-dissolved portions of PFBS is applied to TL baseline BAFs (EPA, 2000a) (see Appendix B).

The EPA followed the framework described in the Technical Support Document, Volume 2 (EPA, 2003), also presented in Figure 1, to select a procedure for estimating national BAFs for PFBS.

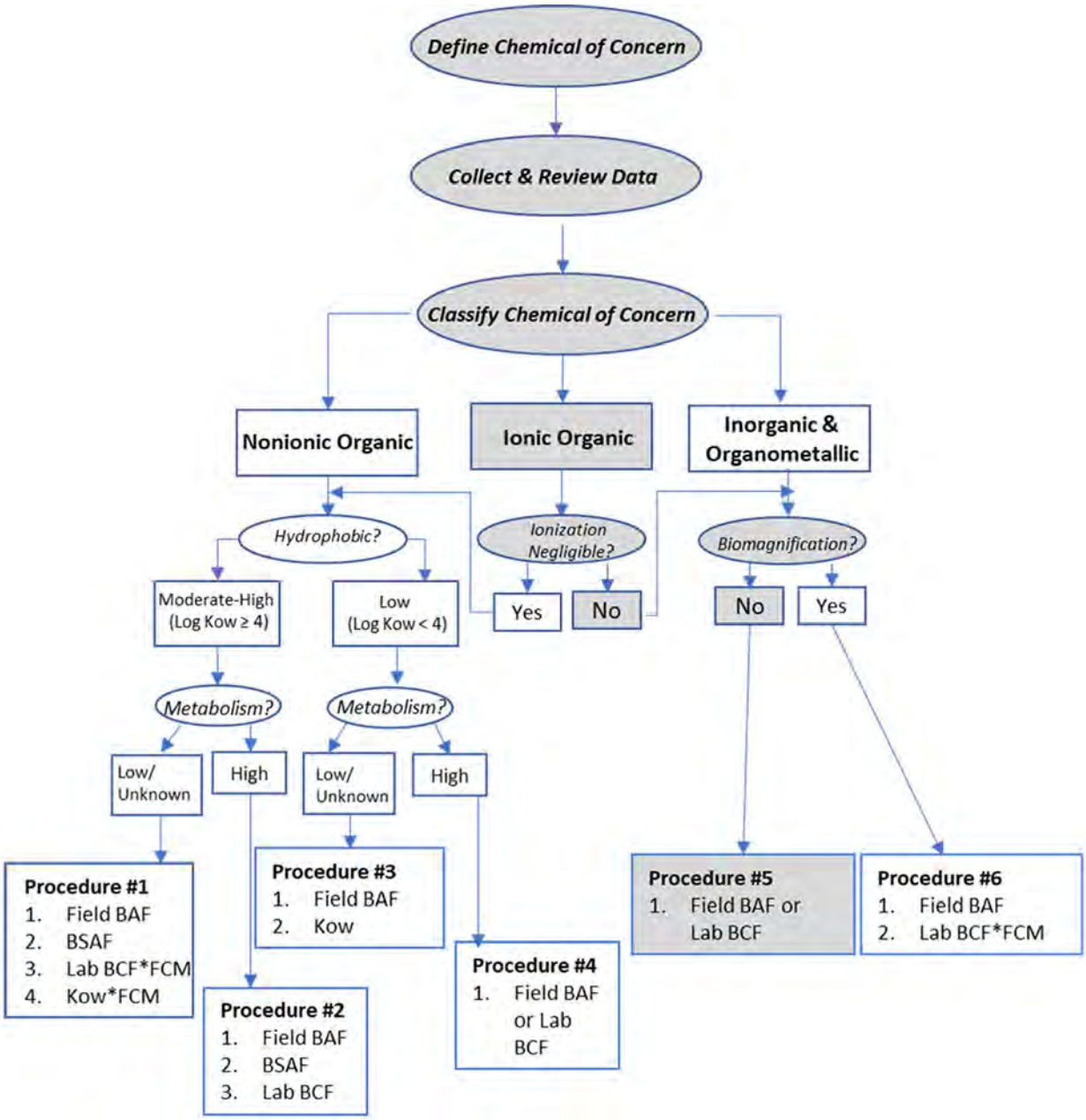


Figure 1. Application of the BAF framework for PFBS; gray boxes indicate steps followed based on available information for PFBS (EPA, 2000a).

Based on the characteristics for PFBS, the EPA selected Procedure 5 for deriving a national BAF value. PFBS has the following characteristics:

- Ionic organic chemicals, with ionization not negligible (ATSDR, 2021; EPA, 2021a,b, 2024a).
- Biomagnification unlikely (Loi et al., 2011).

The EPA was able to locate peer-reviewed, field-measured BAFs for TLs 2, 3, and 4 from the sources evaluated for which sufficient information was provided to indicate the quality and usability of the data; therefore, the EPA included only field BAF studies. The EPA used the BAF method to derive the national BAF values for PFBS:

- TL 2 = 360 L/kg
- TL 3 = 290 L/kg
- TL 4 = 870 L/kg

5 Selection of Toxicity Value

5.1 Approach

The EPA considered all available final toxicity values for both noncarcinogenic and carcinogenic toxicological effects after chronic oral exposure to develop AWQC for PFBS. As described in the 2000 Methodology (EPA, 2000a), where data are available, the EPA derives AWQC for both noncarcinogenic and carcinogenic effects and selects the more protective value for the recommended AWQC. (See Section 7, Criteria Derivation: Analysis.)

For noncarcinogenic toxicological effects, the EPA uses a chronic-duration oral reference values (RfVs; RfDs or equivalent) to derive human health AWQC. An RfV is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA, 2002). An RfV may be derived from an animal toxicological study or a human epidemiological study, from which a point of departure (POD; i.e., a no-observed-adverse-effect level [NOAEL], lowest-observed-adverse-effect level [LOAEL], or benchmark dose [BMD]) can be derived. To derive the RfV, uncertainty factors are applied to the POD to reflect the limitations of the data in accordance with the EPA human health risk assessment methodology (EPA, 2014a, 2021b, 2024a).

For carcinogenic toxicological effects, the EPA uses an oral CSF, when applicable and available, to derive human health AWQC. The oral CSF is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime oral exposure to a stressor. This value may also be derived from animal toxicological studies or human epidemiological studies.

In developing AWQC, the EPA conducts a systematic search of peer-reviewed, publicly available final toxicity assessments to obtain the toxicity value(s) (RfV and/or CSF) for use in developing AWQC. The EPA identified toxicological assessments by systematically searching websites of the following EPA program offices, other national and international programs, and state programs in January 2024:

- EPA, Office of Research and Development
    - Integrated Risk Information System (IRIS) program (EPA, 2024f)
    - Provisional Peer-Reviewed Toxicity Values (PPRTV) (EPA, 2024g)
    - ORD Human Health Toxicity Values (EPA, 2024h)

- EPA, Office of Pesticide Programs (EPA, 2024i)
- EPA, Office of Pollution Prevention and Toxics (EPA, 2024j)
- EPA, Office of Water (EPA, 2024k)
    - Drinking Water Health Effects Support Documents
    - Toxicity Assessments
- U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR, 2024)
- Health Canada (HC, 2023)
- California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (CalEPA, 2024)

After identifying and documenting all available final toxicity values, the EPA followed a systematic process to consider the identified toxicity values and select the toxicity value(s) to derive the AWQC for noncarcinogenic and carcinogenic effects. The EPA selected IRIS toxicity values to derive the draft AWQC if *any* of the following conditions were met:

1. The EPA's IRIS toxicological assessment was the only available source of a toxicity value.
2. The EPA's IRIS toxicological assessment was the most current source of a toxicity value.
3. The toxicity value from a more current toxicological assessment from a source other than the EPA's IRIS program was based on the same principal study and was numerically the same as an older toxicity value from the EPA IRIS program.
4. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not include the relevant toxicity value (chronic-duration oral RfD or CSF).
5. A more current toxicological assessment from a source other than the EPA's IRIS program was available, but it did not introduce new science (e.g., the toxicity value was not based on a newer principal study) or use a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

The EPA selected the toxicity value from a peer-reviewed, publicly available source other than the EPA IRIS program to derive the draft AWQC if *any* of the following conditions were met:

1. The chemical is currently used as a pesticide, and the EPA Office of Pesticide Programs had a toxicity value that was used in pesticide registration decision-making.
2. A toxicological assessment from a source other than the EPA's IRIS program was the only available source of a toxicity value.
3. A more current toxicological assessment from a source other than the EPA's IRIS program introduced new science (e.g., the toxicity value was based on a newer principal study) or used a more current modeling approach compared to an older toxicological assessment from the EPA's IRIS program.

5.2 Toxicity Value for PFBS

5.2.1 Reference Dose

After following the approach outlined in Section 5.1, the EPA identified the *Provisional Peer-Reviewed Toxicity Values for Perfluorobutane Sulfonic Acid (PFBS) and Related Compound Potassium Perfluorobutane Sulfonate* (EPA, 2021a), developed by the EPA's PPRTV program. The EPA identified a second human health assessment, *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)* (EPA, 2021b). These documents are identical and were identified as the most recent toxicity assessment(s) for PFBS, which use the best available science in the evaluation of noncancer risk. The EPA did not identify any other assessments that presented newer scientific information (i.e., unique RfVs) for PFBS.

The EPA's final human health toxicity assessment for PFBS (EPA, 2021a,b) considered all publicly available human epidemiology, animal toxicology, and mechanistic studies that evaluated health effects after PFBS exposure. The assessment identified associations between PFBS exposure and thyroid, developmental, and kidney health effects based on toxicology studies in animals. Limited evidence from human epidemiology studies was identified; findings for thyroid or kidney health effects was equivocal, and no studies evaluating developmental effects were identified. Human epidemiology and animal toxicology studies evaluated other health effects following PFBS exposure including effects on the reproductive system, liver, and lipid and lipoprotein homeostasis, but the evidence did not support clear associations between exposure and effect (EPA, 2021a,b). The most sensitive noncancer effect observed from sufficient quality studies was an adverse developmental effect on thyroid activity, specifically decreased serum total thyroxine, in newborn mice (postnatal day [PND] 1) born to mothers that had been orally exposed to K^+PFBS throughout gestation (Feng et al., 2017; EPA, 2021a,b).

To develop the chronic RfD for PFBS[g], the EPA derived a human equivalent dose (HED) of 0.095 mg/kg-d from BMD modeling of the critical effect in mice. The EPA then applied a composite uncertainty factor (UF) of 300 (i.e., 10× for intraspecies variability [UF_H], 3× for interspecies differences [UF_A], and 10× for database deficiencies [UF_D] to yield the chronic oral **RfD of 3×10^{-4} mg/kg-d** (EPA, 2021a,b).

5.2.2 Cancer Slope Factor

Under the 2005 EPA *Guidelines for Carcinogen Risk Assessment* (EPA, 2005), the PFBS toxicity assessment determined that there is *Inadequate Information to Assess Carcinogenic Potential* for PFBS (EPA, 2021a,b). Therefore, these most recent assessment did not derive a CSF for PFBS (EPA, 2021a,b).

[g] Data for K^+PFBS were used to derive the chronic RfD for the free acid (PFBS), resulting in the same value (3×10^{-4}), after adjusting for differences in molecular weight between K^+PFBS (338.19) and PFBS (300.10) (EPA, 2021a,b).

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6 Relative Source Contribution (RSC) Derivation

6.1 Approach

The EPA applies an RSC to the RfD when calculating an AWQC based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to AWQC-related sources (EPA, 2000a). The purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the AWQC), when combined with other identified sources of exposure (e.g., diet excluding freshwater and estuarine fish and shellfish, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to consumption of ambient water (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with ambient water) and fish and shellfish from inland and nearshore waters relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. The EPA considers any potentially significant exposure source and route when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's 2000 Methodology (EPA, 2000a). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population, depending on the available data.

An RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (EPA, 2000a). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (EPA, 2000a). The EPA's approach recommends a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies.

The EPA's Exposure Decision Tree approach states that when there are insufficient environmental monitoring and/or exposure intake data to permit quantitative derivation of the RSC, the recommended RSC is 20%. In the case of AWQC development, this means that 20% of the exposure equal to the RfD is allocated to the consumption of ambient water and fish and shellfish from inland and nearshore waters and the remaining 80% is reserved for other potential sources, such as diet (excluding fish and shellfish from inland and nearshore waters), air, consumer products, etc. This 20% RSC can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant,

the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (EPA, 2000a). Applying a lower RSC (e.g., 20%) is a more health protective approach to public health and results in a lower AWQC.

To derive an RSC for PFBS, the EPA evaluated the exposure information identified through conducting prior systematic literature searches performed as part of the EPA's *Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS* (EPA, 2024a), which included available information on all exposure sources and routes for PFBS. To identify information on PFBS exposure routes and sources to inform RSC determination, the EPA considered primary literature published between 2003–2020 that was collected by the EPA's Office of Research and Development as part of an effort to evaluate evidence for pathways of human exposure to eight PFAS, including PFBS. The full description of methods for peer-reviewed journal articles is available in the EPA's *Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS* (EPA, 2024a). In order to consider more recently published information on PFBS exposure, the EPA incorporated the results of a date-unlimited gray literature search that was conducted in February 2022 and 2024 as well as an ad hoc process to identify relevant and more recently published peer-reviewed scientific literature. The literature resulting from the search and screening process included only final (not draft) documents and articles that were then reviewed to inform the PFBS RSC. The following description in Section 6.2 is a summary of the information provided in the Appendix of the final MCLG document three individual PFAS, including PFBS (EPA, 2024a).

6.2 Summary of Potential Exposure Sources of PFBS Other Than Water and Freshwater and Estuarine Fish/Shellfish

6.2.1 Dietary Sources

PFBS was included in a suite of individual PFAS selected as part of PFAS-targeted reexaminations of samples collected for the U.S. Food and Drug Administration's Total Diet Study (FDA, 2020a,b, 2021a,b; EPA, 2024a); however, it was not detected in any of the food samples tested. It should be noted that the FDA indicated that the sample sizes were limited and that the results should not be used to draw definitive conclusions about PFAS levels or presence in the general food supply (FDA, 2023). PFBS was detected in cow milk samples collected from a farm with groundwater known to be contaminated with PFAS, as well as in produce (collard greens) collected from an area near a PFAS production plant, in FDA studies of the potential exposure of the U.S. population to PFAS (FDA 2018, 2021c). Maximum residue levels for PFBS were not found in the Global MRL Database (Bryant Christie, Inc., 2024).

In addition to efforts by the FDA, 34 peer-reviewed studies conducted in North America (n = 7), Europe (n = 26), and across multiple continents (n = 1) analyzed PFBS in food items obtained from home, recreational, or commercial sources (see Table C-1 in the Appendix). Food types evaluated include fruits and vegetables, grains, meat, seafood, dairy, and fats/other (e.g., eggs, spices, and oils), with seafood showing the highest levels of PFBS detected. PFBS was not detected in any of the eight studies that analyzed human milk for PFAS (not shown in Table C-1)—one in the United States (von Ehrenstein et al., 2009) and seven in Europe (Abdallah et al., 2020;

Beser et al., 2019; Cariou et al., 2015; Kärrman et al., 2007, 2010; Lankova et al., 2013; Nyberg et al., 2018). Some PFBS dietary studies use the term "seafood" to indicate fish and shellfish from ocean, freshwater, or estuarine water bodies. Information about the water bodies assessed in individual studies is reported in the articles.

Of the studies conducted in North America, four U.S. studies (Blaine et al., 2014; Byrne et al., 2017; Schecter et al., 2010; Scher et al., 2018) found PFBS in at least one food item. Locations and food sources varied in these studies. In Schecter et al. (2010), PFBS was detected in cod samples but not in any of the other foods collected from Texas grocery stores. Scher et al. (2018) detected PFBS in plant parts (leaf and stem samples) analyzed from garden produce collected at homes in Minnesota within a groundwater contamination area (GCA) impacted by a former 3M PFAS production facility (PFBS concentrations ranged from ND to 0.065 ng/g). The authors suggested that the PFBS detections in plant parts were likely associated with PFAS present in irrigation water that had accumulated in produce. Blaine et al. (2014) found PFBS in radish, celery, tomato, and peas that were grown in soil amended with industrially impacted biosolids. They also found PFBS in these crops grown in soil that had received municipal biosolid applications over 20 years. In unamended control soil samples, PFBS was only detected in radish root with an average value of 22.36 ng/g (Blaine et al., 2014). In a similar study conducted by Blaine et al. (2013), PFBS was found in lettuce, tomato, and corn grown in industrially impacted biosolids-amended soils in greenhouses. Young et al. (2012) analyzed 61 raw and retail milk samples from 17 states for PFAS, but PFBS was not detected.

Several peer-reviewed publications that examined PFBS concentrations in marine fish and shellfish are also available. Schecter et al. (2010) detected PFBS in cod samples, averaging 0.12 ng/g ww. In two additional studies from North America, PFBS was not detected in samples of farmed and wild-caught seafood (Chiesa et al., 2019; Young et al., 2013). Vassiliadou et al. (2015) detected PFBS in raw shrimp (from Greek markets) but did not detect PFBS in either fried shrimp, raw hake (from Greek fishing sites), or fried hake.

The European Food Safety Authority reported the presence of PFBS in various food and drink items, including fruits, vegetables, cheese, and bottled water (EFSA, 2012). For average adult consumers, the estimated exposure ranges for PFBS were 0.03–1.89 ng/kg bw-d (minimum) to 0.10–3.72 ng/kg bw-d (maximum) (EFSA, 2012). Of 27 studies conducted in Europe, 12 found PFBS in at least one food type (Table C-1). Eight of the 12 studies included food samples obtained solely from markets (D'Hollander et al., 2015; Domingo et al., 2012; Eschauzier et al., 2013; Hlouskova et al., 2013; Pérez et al., 2014; Scordo et al., 2020; Surma et al., 2017; Sznajder-Katarzyńska et al., 2019). Across studies, PFBS detections were found in marine fish and shellfish; other animal products such as meat, dairy, and eggs; fruits and vegetables; tap water-based beverages such as coffee; sweets; and spices.

Papadopoulou et al. (2017) analyzed duplicate diet samples with PFBS detected in only one solid food sample (ND–0.001 ng/g; DF 2%; food category unspecified). Eriksson et al. (2013) evaluated foods that were farmed or freshly caught in the Faroe Islands, and only detected PFBS in cow milk (0.019 ng/g ww) and packaged dairy milk (0.017 ng/g ww) samples among the

products analyzed. In eight of the European studies where PFBS was not detected, foods were primarily obtained from commercial sources, but wild-caught seafood was also included.

In summary, in Europe and North America, PFBS has been detected in multiple food types, including fruits, vegetables, meats, marine fish and shellfish, and other fats. Although several U.S. studies have evaluated PFBS in meats, fats/oils, fruits, vegetables, and other non-seafood food types, many of these sampling efforts were localized to specific cities or markets and/or used relatively small sample sizes. Broader-scale sampling efforts will be helpful in determining the general levels of PFBS contamination in these food types, as well as the impact of known PFAS contamination sources on PFBS concentrations in foods.

6.2.2 Food Contact Materials

PFBS is not authorized for use in food packaging in the United States; however, PFBS has been detected in food packaging materials in the few available studies that investigate this potential route of exposure (ATSDR, 2021; EPA, 2021a,b). In one report from the United States, PFBS was detected in fast-food packaging (7/20 samples) although the concentrations detected were not reported (Schaider et al., 2017).

The EPA identified five peer-reviewed studies in Europe (conducted in Poland, Norway, Greece, Czech Republic, and Germany) which analyzed the occurrence of PFBS in food packaging or food contact materials (FCMs), such as baking papers and fast-food boxes and wrappers. Surma et al. (2015) measured levels of 10 perfluorinated compounds in three different brands of common FCMs commercially available in Poland, including wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3). PFBS was detected in one brand of baking paper at 0.02 picograms per square centimeter (pg/cm^2), but PFBS was not detected at or below the limit of quantitation in all other FCMs. Vestergren et al. (2015) analyzed paper plates (n = 2), paper cups (n = 1), baking covers (n = 1), and baking molds (n = 1) purchased from retail stores in Tromsø and Trondheim, Norway. PFBS was detected in one paper plate at 6.9 pg/cm^2.

The remaining three studies did not detect PFBS in FCMs. Zafeiraki et al. (2014) analyzed FCMs made of paper, paperboard, or aluminum foil collected from a Greek market. PFBS was not detected in any of the samples of beverage cups (n = 8), ice cream cups (n = 1), fast-food paper boxes (n = 8), fast-food wrappers (n = 6), paper materials for baking (n = 2), microwave bags (n = 3), or aluminum foil bags/wrappers (n = 14). Vavrous et al. (2016) analyzed 15 samples of paper FCMs acquired from a market in the Czech Republic. FCMs included paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220 °C (n = 3), and paper filters for coffee preparation (n = 3). PFBS was not detected in any samples. Kotthoff et al. (2015) analyzed 82 samples for perfluoroalkane sulfonate (PFSA) and perfluoroalkyl carboxylic acid (PFCA) compounds in 10 consumer products including individual paper-based FCMs (n = 33) from local retailers in Germany in 2010. PFBS was not detected in paper-based FCMs.

Overall, the few available studies conducted in the United States and Europe indicate that PFBS may be present in food packaging materials; however, further research is needed to understand which packaging materials generally contain PFBS at the highest concentrations and with the greatest frequency. There are also uncertainties related to data gaps on topics that may influence whether food packaging is a significant PFBS exposure source in humans, including differences in transfer efficiency from different packaging types directly to humans or indirectly through foodstuffs.

6.2.3 Consumer Product Uses

Several studies examined a range of consumer products and found multiple PFAS, including PFBS, at various levels (Bečanová et al., 2016; Favreau et al., 2016; Gremmel et al., 2016; Kotthoff et al., 2015; Liu et al., 2014; Schultes et al., 2018; van der Veen et al., 2020; Vestergren et al., 2015; Zheng et al., 2020). Two of the studies collected consumer products in the United States, five purchased consumer products in Europe, and two studies did not report the purchase location(s) of the consumer products that were tested.

Zheng et al. (2020) determined the occurrence of ionic and neutral PFAS in items collected from childcare environments in the United States. Nap mats (n = 26; 20 polyurethane foam, 6 vinyl cover samples) were collected from seven Seattle childcare centers. PFBS was detected in 5% of nap mat samples at a maximum concentration of 0.04 ng/g. Liu et al. (2014) analyzed the occurrence of PFAS in commonly used consumer products (carpet, commercial carpet-care liquids, household carpet/fabric-care liquids, treated apparel, treated home textiles, treated nonwoven medical garments, floor waxes, membranes for apparel, and thread-sealant tapes) purchased from retail outlets in the United States. PFBS was detected in 100% of commercial carpet/fabric-care liquids samples (n = 2) at concentrations of 45.8 ng/g and 89.6 ng/g, in 75% of household carpet/fabric-care liquids and foams samples (n = 4) at concentrations up to 911 ng/g, in one treated apparel samples (n = 2) at a concentration of 2 ng/g, in the single treated floor wax and stone/wood sealant sample (143 ng/g, n = 2), and in the single apparel membrane sample (30.7 ng/g, n = 2). PFBS was not detected in treated home textile and upholstery (n = 2) or thread-sealant tapes and pastes (n = 2).

van der Veen et al. (2020) examined the effects of weathering on PFAS content in durable water-repellent clothing collected from six suppliers in Sweden (one pair of outdoor trousers, seven jackets, four fabrics for outdoor clothes, and one pair of outdoor overalls). Two pieces of each of the 13 fabrics were cut. One piece of each fabric was exposed to elevated ultraviolet radiation, humidity, and temperature in an aging device for 300 hours (assumed lifespan of outdoor clothing); the other was not aged. Both pieces of each fabric were analyzed for ionic PFAS (including PFBS) and volatile PFAS. In general, aging of outdoor clothing resulted in increased perfluoroalkylated acid levels of 5-fold or more. For eight of 13 fabrics, PFBS was not detected before or after aging. For three fabrics, PFBS was detected before and after aging, increasing approximately 3- to 14-fold in the aged fabric (i.e., from 43 to 140 micrograms per square meter [$\mu g/m^2$], 45 to 350 $\mu g/m^2$, and 9.6 to 130 $\mu g/m^2$ respectively for the three fabrics). For the remaining two fabrics, PFBS was not detected prior to aging but was detected afterward at concentrations of 0.57 and 1.7 $\mu g/m^2$, respectively. The authors noted

that possible explanations for this could be weathering of precursor compounds (e.g., fluorotelomer alcohols) to PFAAs such as PFBS or increased extractability due to weathering.

Kotthoff et al. (2015) analyzed 82 samples for PFSA and PFCA compounds in outdoor textiles (n = 3), gloves (n = 3), carpets (n = 6), cleaning agents (n = 6), impregnating sprays (n = 3), leather (n = 13), wood glue (n = 1), ski wax (n = 13), and awning cloth (n = 1). Individual samples were bought from local retailers or collected by coworkers of the involved institutes or local clubs in Germany. The age of the samples ranged from a few years to decades. PFBS was detected in outdoor textiles (level not provided), carpet samples (up to 26.8 µg/m^2), ski wax samples (up to 3.1 micrograms per kilogram [µg/kg]), leather samples (up to 120 µg/kg), and gloves (up to 2 µg/kg). Favreau et al. (2016) analyzed the occurrence of 41 PFAS in a wide variety of liquid products (n = 132 consumer products, 194 total products), including impregnating agents, lubricants, cleansers, polishes, AFFFs, and other industrial products purchased from stores and supermarkets in Switzerland. PFBS was not detected in impregnation products (n = 60), cleansers (n = 24), or polishes (n = 18). PFBS was detected in 13% of a miscellaneous category of products (n = 23) that included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances, with mean and maximum concentrations of 998 and 2,992 parts per million (ppm), respectively (median = ND).

The remaining two European studies from Norway (Vestergren et al., 2015) and Sweden (Schultes et al., 2018) did not detect PFBS in the consumer products analyzed. Vestergren et al. (2015) analyzed furniture textile, carpet, and clothing samples (n = 40) purchased from retail stores in Tromsø and Trondheim, Norway, while Schultes et al. (2018) determined levels of 39 PFAS in 31 cosmetic products collected in Sweden. Both studies found measurable concentrations of at least one PFAS; however, PFBS was not detected in any of the samples.

Of the two studies for which purchase location(s) were not specified, Gremmel et al. (2016) determined levels of 23 PFAS in 16 new outdoor jackets since it has been shown that outdoor jackets emit PFAS to the air as well as into water during washing. The jackets were selected based on factors such as fabric and origin of production (primarily Asia, with some origins not specified). PFBS (concentration of 0.51 µg/m^2) was only detected in one large hardshell jacket made of 100% polyester that was polyurethane-coated and finished with Teflon® (production origin unknown). Bečanová et al. (2016) analyzed 126 samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment [EEE], and plastics); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, facade materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) for 15 target PFAS, including PFBS. The condition (new versus used) and production year of the samples varied; the production year ranged from 1981 to 2010. The origin(s) of production were not specified. PFBS was detected in 31/55, 9/54, 7/10, and 6/7 household equipment, building materials, car interior, and WEEE samples, respectively. The highest level was 11.4 µg/kg found in a used 1999 screen associated with WEEE.

In summary, in the few studies available from North America and Europe, PFBS was detected in a wide range of consumer products including clothing, household textiles and products, children's products, and commercial/industrial products. However, there is some uncertainty in these results as the number and types of products tested in each study were often limited in terms of sample size. While there is evidence indicating PFBS exposure may occur through the use of or contact with consumer products, more research is needed to understand the DF and concentrations of PFBS that occur in specific products, as well as how the concentrations of PFBS change in these products with age or weathering.

6.2.4 Indoor Dust

Dust ingestion may be an important exposure source of PFAS including PFBS (ATSDR, 2021), though it should be noted that dust exposure may also occur via inhalation and dermal routes. The EPA identified several studies conducted in the United States, Canada, various countries in Europe, and across multiple continents analyzed PFBS in dust of indoor environments (primarily in homes, but also schools, childcare facilities, offices, and vehicles; see Table C-2). Most of the studies sampled dust from areas not associated with any known PFAS activity or release. PFBS concentrations in dust measured in these studies ranged from ND to 170 ng/g with three exceptions: two studies (Kato et al., 2009; Strynar and Lindstrom, 2008) reported maximum PFBS concentrations greater than 1,000 ng/g in dust from homes and daycare centers, and a third study (Huber et al., 2011) reported a PFBS concentration of 1,089 ng/g in dust from a storage room that had been used to store "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years."

Of the two available studies that measured PFBS in dust from vehicles, one (in the United States) detected no PFBS (Fraser et al., 2013) and the other (in Ireland) reported a DF of 75% and PFBS concentrations ranging from ND to 170 ng/g (Harrad et al., 2019).

One U.S. study, Scher et al. (2019) evaluated indoor dust from 19 homes in Minnesota within a GCA impacted by the former 3M PFAS production facility. House dust samples were collected from both interior living rooms and entryways to the yard. The DFs for PFBS were 16% and 11% for living rooms and entryways, respectively, and a maximum PFBS concentration of 58 ng/g was reported for both locations.

Haug et al. (2011) indicated that house dust concentrations are likely influenced by a number of factors related to the building (e.g., size, age, floor space, flooring type, ventilation); the residents or occupants (e.g., number of people, housekeeping practices, consumer habits such as buying new or used products); and the presence and use of certain products (e.g., carpeting, carpet or furniture stain-protective coatings, waterproofing sprays, cleaning agents, kitchen utensils, clothing, shoes, cosmetics, insecticides, electronic devices). In addition, the extent and use of the products affects the distribution patterns of PFAS in dust of these buildings.

At this time, there is uncertainty regarding the extent of human exposure to PFBS through indoor dust compared with other exposure pathways.

6.2.5 Air

PFAS have been released to air from WWTPs, waste incinerators, and landfills (EPA, 2016). ATSDR (2021) noted that PFAS have been detected in particulates and in the vapor phase in air and can be transported long distances via the atmosphere; they have been detected at low concentrations in areas as remote as the Arctic and ocean waters. However, the EPA's Toxic Release Inventory did not report release data for PFBS in 2020 (EPA, 2024l). In addition, PFBS is not listed as a hazardous air pollutant (EPA, 2024m).

6.2.5.1 Indoor Air

No studies from the U.S. reporting levels of PFBS in indoor air were identified from the peer-reviewed or gray literature. However, the EPA identified studies from Europe that are summarized below. These three studies were conducted in Norway (Barber et al., 2007), Spain (Jogsten et al., 2012), and Ireland (Harrad et al., 2019).

In Norway, neutral and ionic PFAS were analyzed in four indoor air samples collected from homes in Tromsø (Barber et al., 2007). PFBS levels were below the limit of quantitation. The authors noted that measurable amounts of other ionic PFAS were found in indoor air samples, but levels were not significantly elevated above levels in outdoor air. In Spain, Jogsten et al. (2012) collected indoor air samples (n = 10) from selected homes in Catalonia and evaluated levels of 27 perfluorinated chemicals (PFCs). PFBS was not detected.

In Ireland, Harrad et al. (2019) measured eight target PFAS in air from cars (n = 31), home living rooms (n = 34), offices (n = 34), and school classrooms (n = 28). PFBS was detected in all four indoor microenvironments, at DFs of 53%, 90%, 41%, and 54% in samples from homes, cars, offices, and classrooms, respectively. The mean (maximum) concentrations were 22 (270) picograms per cubic meter (pg/m^3) in homes, 54 (264) pg/m^3 in cars, 37 (313) pg/m^3 in offices, and 36 (202) pg/m^3 in classrooms.

There is some evidence from European studies indicating PFBS exposure via indoor air. However, further research is needed to understand the DF and concentrations of PFBS that occur in indoor environments in the United States.

6.2.5.2 Ambient Air

Similar to studies on indoor PFBS air concentrations, no studies from the U.S. reporting levels of PFBS in ambient air were identified from the peer-reviewed or gray literature. Four studies conducted across Europe (Barber et al., 2007; Beser et al., 2011; Harrad et al., 2020; Jogsten et al., 2012) and one study conducted in Canada (Ahrens et al., 2011) analyzed ambient air samples for PFBS. Two of the studies (Barber et al., 2007; Harrad et al., 2020) found detectable levels of PFBS in outdoor air. Barber et al. (2007) collected air samples from four field sites in Europe (one semirural site [Hazelrigg] and one urban site [Manchester] in the United Kingdom, one rural site from Ireland, and one rural site from Norway) for analysis of neutral and ionic PFAS. Authors did not indicate whether any of the sites had a history of local PFAS-related activities (e.g., AFFF usage, PFAS manufacturing or use). PFBS was detected in the particle phase of outdoor air samples during one of the two sampling events in Manchester at 2.2 pg/m^3 and one of the two sampling events in Hazelrigg at 2.6 pg/m^3. PFBS was not detected above the

method quantification limit at the Ireland and Norway sites. Harrad et al. (2020) measured PFBS in air near 10 Irish municipal solid waste landfills located in nonindustrial areas. Air samples were collected upwind and downwind of each landfill. PFBS was detected in more than 20% of the samples, with mean concentrations (ranges) at downwind and upwind locations of 0.50 (< 0.15–1.4) pg/m^3 and 0.34 (< 0.15–1.2) pg/m^3, respectively. Beser et al. (2011) and Jogsten et al. (2012) did not detect PFBS in ambient air samples in Spain. Beser et al. (2011) analyzed fine airborne particulate matter (PM 2.5) in air samples collected from five stations located in Alicante province, Spain (three residential, one rural, one industrial) to determine levels of 12 ionic PFAS. PFBS was below the method quantification limit at all five locations. Jogsten et al. (2012) did not detect PFBS in ambient air samples collected outside homes in Catalonia, Spain.

In the one study identified from North America, Ahrens et al. (2011) determined levels of PFAS in air around a WWTP and two landfill sites in Canada. PFBS was not detected in any sample above the MDL.

PFBS has been detected in Artic air in one study, with a DF of 66% and mean concentration of 0.1 pg/m^3 (Arp and Slinde, 2018; Wong et al., 2018).

As with exposure to PFBS via indoor air, there is some evidence from European studies indicating PFBS is present in some ambient air samples. Further research is needed to understand the DF and concentrations of PFBS that occur in ambient environments in the United States.

6.2.6 Soil

PFBS can be released into soil from manufacturing facilities, industrial uses, fire/crash training sites, and biosolids containing PFBS (ATSDR, 2021; EPA, 2021a,b). The EPA identified 16 studies that evaluated the occurrence of PFBS and other PFAS in soil, with studies conducted in the United States, Canada, and Europe (see Table C-3). Two U.S. studies and two Canadian studies (Blaine et al., 2013; Cabrerizo et al., 2018; Dreyer et al., 2012; Venkatesan and Halden, 2014) were conducted in areas not reported to be associated with any known PFAS release or were experimental studies conducted at research facilities. At these sites, PFBS levels were low (≤ 0.10 ng/g) or below detection limits in non-amended or control soils. Two U.S. studies by Scher et al. (2018, 2019) evaluated soils at homes in Minnesota within and outside of a GCA impacted by a former 3M PFAS production facility; for sites within the GCA, one of the studies reported a DF of 10% and a 90th percentile PFBS concentration of 0.02 ng/g, and the other reported a DF of 9% and a maximum PFBS concentration of 0.017 ng/g. For sites outside of the GCA, the DF was 17% and the maximum PFBS concentration was 0.031 ng/g. Three U.S. studies and one Canadian study analyzed soils potentially impacted by AFFF used to fight fires—one at U.S. Air Force installations with historic AFFF use (Anderson et al., 2016), two at former fire training sites (Eberle et al., 2017; Nickerson et al., 2020), and another at the site of a train derailment and fire in Canada (Mejia-Avendaño et al., 2017). In these four studies, DFs ranged from 35% to 100%. PFBS concentrations in the study of the U.S. Air Force installations ranged from ND–79 ng/g, and PFBS concentrations ranged from ND–58.44 ng/g at one fire training site

(Nickerson et al., 2020). The study of the other fire training site measured PFBS pretreatment (0.61–6.4 ng/g) and posttreatment (0.07–0.83 ng/g) (Eberle et al., 2017). The DFs and range of PFBS concentrations measured in soils at the site of the train derailment were 75% DF and ND–3.15 ng/g, respectively, for the AFFF run-off area (measured in 2013, the year of accident) and 36% DF and ND–1.25 ng/g, respectively, at the burn site and adjacent area (measured in 2015) (Mejia-Avendaño et al., 2017).

Of the six European studies, one study (Harrad et al., 2020) analyzed soil samples collected upwind and downwind of 10 municipal solid waste landfills in Ireland and found PFBS levels to be higher in soils from downwind locations. Based on the overall study findings, however, the authors concluded there was no discernible impact of the landfills on concentrations of PFAS in soil surrounding these facilities. Grønnestad et al. (2019) investigated soils from a skiing area in Norway to elucidate exposure routes of PFAS into the environment from ski products, such as ski waxes. The authors found no significant difference in mean total PFAS in soil samples from the Granåsen skiing area and the Jonsvatnet reference area but noted that the skiing area samples were dominated by long-chain PFAS (C8–C14; ≥ 70%) and the reference area samples were dominated by short-chain PFAS (> 60%), which included PFBS. A study in Belgium (Groffen et al., 2019) evaluated soils collected at a 3M fluorochemical plant in Antwerp and at four sites located at increasing distances from the plant. PFBS levels were elevated at the plant site and decreased with increasing distance from the plant. The other three studies analyzed soil samples from areas near firefighting training sites in Norway and France and reported PFBS concentrations varying from ND to 101 ng/g dry weight (Dauchy et al., 2019; Hale et al., 2017; Skaar et al., 2019).

A U.S. study of biosolid samples from 94 WWTPs across 32 states and the District of Columbia detected PFBS in 60% of samples at a mean concentration (range) of 3.4 (2.5–4.8) ng/g (Venkatesan and Halden, 2013). PFBS has been detected in drinking water wells, food types, and plant samples from soils or fields that have received biosolids applications that were industrially impacted (Blaine et al., 2013, 2014; Lindstrom et al., 2011).

In summary, results of some available studies suggest that proximity to a PFAS production facility or a site with historical AFFF use or firefighting is correlated with increased PFBS soil concentrations compared to soil from sites not known to be impacted by PFAS. However, few available studies examined PFBS concentrations in soils not known to have nearby sources of PFBS. Additional research is needed that quantifies ambient levels of PFBS in soils in the United States.

6.2.7 Summary and Recommended RSC for PFBS

As mentioned above, the scope of exposure sources considered for the draft recommended human health AWQC is limited to surface water used for drinking water and the consumption of freshwater/estuarine fish and shellfish (EPA, 2000a), consistent with previous human health AWQC (EPA, 2015). The EPA followed the Exposure Decision Tree approach to determine the RSC for PFBS (EPA, 2000a; see Figure 2).

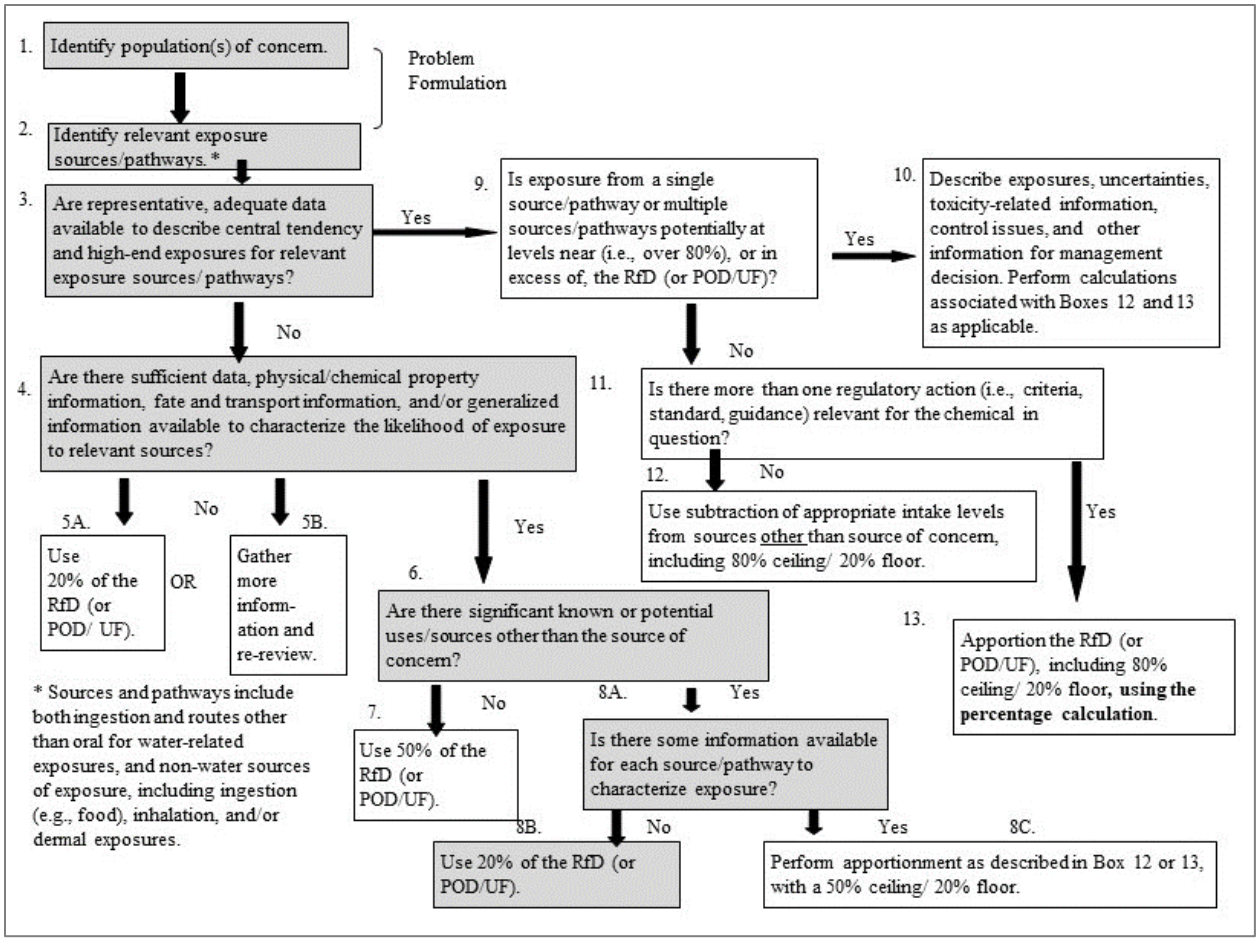


Figure 2. RSC exposure decision tree framework for PFBS; figure adapted from EPA (2000a) with gray boxes indicating key decision points for this chemical.

To identify the population(s) of concern (Box 1, Figure 2), the EPA first identified potentially sensitive subpopulations or life stages based on the PFBS exposure interval in the critical study from which the critical effect (adverse developmental effect on thyroid activity) was selected for RfD derivation in the PFBS toxicity assessment (EPA, 2021a,b). Since the critical effect is the most sensitive adverse health effect that was identified from the available data of sufficient quality, then the exposure interval may be a sensitive window of exposure. The exposure interval of the critical study in rodents corresponds to the following two potentially sensitive human life stages: women of childbearing age who may be or become pregnant; and pregnant women and their developing fetus. However, limited information was available regarding specific PFBS exposure in these two life stages from different environmental sources. Therefore, the EPA considered exposures in the general U.S. population, ages 21 years of age and older, which includes these two life stages.

Second, the EPA identified PFBS relevant exposure sources/pathways (Box 2, Figure 2), including nonfish (except marine) dietary consumption, incidental oral consumption via dust, consumer products, and soil or dermal exposure via soil, consumer products, and dust, and

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inhalation exposure via indoor or ambient air. Several of these may be potentially significant exposure sources.

Third, the EPA evaluated whether adequate data were available to describe the central tendencies and high-end exposures for all potentially significant exposure sources and pathways (Box 3, Figure 2). The EPA determined that there were inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources. For example, studies from Canada and Europe indicate that indoor and ambient air may be a significant source of exposure to PFBS. At the time of the literature search, the EPA was unable to identify studies assessing PFBS concentrations in indoor or ambient air samples from the United States and therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population.

Fourth, the agency determined whether there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4, Figure 2). Sufficient information on PFBS was available to characterize the likelihood of exposure. The agency relied on the studies summarized above to determine if there are potential uses/sources of PFBS other than AWQC-related sources (Box 6, Figure 2). There are significant known or potential uses/sources of PFBS other than AWQC-related sources. Based on this information, the next step was to determine if adequate information was available on PFBS to characterize each source/pathway of exposure (Box 8a, Figure 2). The EPA determined there is not enough information available on each source to make a quantitative characterization of exposure among exposure sources. For example, there are several studies from the U.S. indicating that PFBS may occur in dust sampled from various microenvironments (e.g., homes, offices, daycare centers, vehicles). However, the majority of studies sampled in only one location and few studies examined dust samples outside of the home (e.g., one study from the U.S. assessed PFBS occurrence in dust sampled from vehicles). Additionally, though several studies from around the U.S. measured PFBS concentrations in dust from houses, the detection frequencies in these studies varied widely (from 3% to 59%) and may be a result of uncertainties including home characteristics, behaviors of the residents, and the presence or absence of PFBS-containing materials or products (Haug et al., 2011). Therefore, it is not possible to determine whether dust, food sources other than freshwater/estuarine fish and shellfish, or consumer products, may be major or minor contributors to total PFBS exposure. Therefore, the data are insufficient to allow for quantitative characterization of the different exposure sources. The EPA's Exposure Decision Tree approach states that when there is insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20% (EPA, 2000a). Thus, the EPA recommends an RSC of **20% (0.20)** for PFBS for AWQC for both the water plus organism AWQC as well as the organism only AWQC (Box 8b, Figure 2).

7 Criteria Derivation: Analysis

Table 2 summarizes the input parameters used to derive the draft recommended human health AWQC that are protective of exposure to PFBS from consuming drinking water and/or eating

Table 2. Input parameters for the human health AWQC for PFBS.

Input Parameter		Value
RfD		0.0003 mg/kg-d
CSF		No data
RSC		0.20
BW		80.0 kg
DWI		2.3 L/d
FCR	TL 2	0.0076 kg/d
	TL 3	0.0086 kg/d
	TL 4	0.0051 kg/d
BAF	TL 2	360 L/kg
	TL 3	290 L/kg
	TL 4	870 L/kg

Notes: RfD = reference dose; CSF = cancer slope factor; RSC = relative source contribution; BW = bodyweight; DWI = drinking water intake; FCR = fish consumption rate; TL = trophic level; BAF = bioaccumulation factor.

fish and shellfish (organisms) from inland and nearshore waters. The criteria calculations are presented below. These criteria recommendations are based on the 2000 Methodology (EPA, 2000a) and the toxicity and exposure assumptions described above (see Section 4, AWQC Input Parameters; Section 5, Selection of Toxicity Value; and Section 6, Relative Source Contribution Derivation).

7.1 AWQC for Noncarcinogenic Toxicological Effects

For consumption of water and organisms:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\text{DWI (L/d)} + \sum_{i=2}^{4} (\text{FCR}_i\ (\text{kg/d}) \times \text{BAF}_i\ (\text{L/kg}))}$$

$$= \frac{0.0003\ \text{mg/kg-d} \times 0.20 \times 80.0\ \text{kg} \times 1{,}000\ \mu g/mg}{2.3\ \text{L/d} + ((0.0076\ \text{kg/d} \times 360\ \text{L/kg}) + 0.0086\ \text{kg/d} \times 290\ \text{L/kg}) + (0.0051\ \text{kg/d} \times 870\ \text{L/kg}))}$$

$$= 0.4011\ \mu g/L$$

$$= 0.4\ \mu g/L \text{ (rounded)}$$

For consumption of organisms only:

$$\text{AWQC } (\mu g/L) = \frac{\text{RfD (mg/kg-d)} \times \text{RSC} \times \text{BW (kg)} \times 1{,}000\ (\mu g/mg)}{\sum_{i=2}^{4} (FCR_i\ (kg/d) \times BAF_i\ (L/kg))}$$

$$= \frac{0.0003\ mg/kg\text{-}d \times 0.20 \times 80.0\ kg \times 1{,}000\ \mu g/mg}{(0.0076\ kg/d \times 360\ L/kg) + (0.0086\ kg/d \times 290\ L/kg) + (0.0051\ kg/d \times 870\ L/kg)}$$

$$= 0.4965\ \mu g/L$$

$$= 0.5\ \mu g/L \text{ (rounded)}$$

7.2 AWQC for Carcinogenic Toxicological Effects

The PFBS toxicity assessments determined that there is *Inadequate Information to Assess Carcinogenic Potential* for PFBS (EPA, 2021a,b; see Section 5, Selection of Toxicity Value). The EPA derives cancer-based HHC for contaminants that have been determined to be *Carcinogenic to Humans* or *Likely to Be Carcinogenic to Humans* (EPA, 2000a,c). Therefore, the EPA did not derive AWQC for carcinogenic toxicological effects.

7.3 AWQC Summary for PFBS

The EPA derived the draft recommended AWQC for PFBS using a noncarcinogenic toxicity endpoint. The human health AWQC for noncarcinogenic effects for PFBS are **0.4 µg/L** (400 ng/L) for consumption of water and organisms and **0.5 µg/L** (500 ng/L) for consumption of organisms only (Table 3). The EPA evaluated the use of exposure factors relevant to sensitive subpopulations based on the critical effect(s) used to derive the RfD (Appendix D). Based on the results of this evaluation, the criteria based on exposure factors for the general adult (≥ 21 years of age) population are the most health protective.

Under the EPA's recently finalized Method 1633 (EPA, 2024n) for aqueous samples, the level of quantification (LOQ) representing the observed LOQs in the multi-laboratory validation study, range from 1 to 4 ng/L for PFBS. The pooled MDL for PFBS is 0.37 ng/L. The pooled MDL value is derived from the multi-laboratory validation study using MDL data from eight laboratories and represents the sensitivity that should be achievable in a well-prepared laboratory but may not represent the actual MDL used for data reporting or data quality assessments (EPA, 2024n). The MDLs and ranges presented here provide a reference for comparison of analytical concentrations and recommended criteria.

Table 3. Summary of the EPA's recommended human health AWQC for PFBS chemicals.

	Human Health AWQC
Water and Organism	0.4 µg/L (400 ng/L)
Organism Only	0.5 µg/L (500 ng/L)

8 Consideration of Noncancer Health Risks from PFAS Mixtures

The EPA recently released its final *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* (referred to here as the PFAS mixtures framework; EPA, 2024o). The PFAS mixtures framework describes three flexible, data-driven approaches that facilitate practical component-based mixtures evaluation of two or more PFAS based on dose additivity, consistent with the EPA*'s Guidelines for the Health Risk Assessment of Chemical Mixtures* (EPA, 1986) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA, 2000d). The approaches described in the EPA PFAS mixtures framework may support interested federal, state, and Tribal partners, as well as public health experts and other stakeholders to assess the potential noncancer human health hazards and risks associated with PFAS mixtures. The EPA is providing an illustration of one approach which could be applied to PFAS mixture HHC derivation. The PFAS mixtures framework underwent peer review by the EPA Science Advisory Board (EPA, 2022b) and public review and the EPA responded to comments (EPA, 2024p). The public comment period ended on May 30, 2023. The public docket can be accessed at www.regulations.gov under Docket ID: EPA-HQ-OW-2022-0114.

Dose additivity means that the combined effect of the component chemicals in a mixture is equal to the sum of the individual doses or concentrations scaled for potency. As noted in the PFAS mixtures framework, exposure to a number of individual PFAS has been shown to elicit the same or similar profiles of adverse effects in various organs and systems. Many toxicological studies of PFAS as well as other classes of chemicals support the health-protective conclusion that chemicals that elicit the same or similar observed adverse effects following individual exposure should be assumed to act in a dose-additive manner when in a mixture unless data demonstrate otherwise (EPA, 2024o). Importantly, few studies have examined the toxicity of PFAS mixtures, particularly with component chemical membership and proportions that are representative of the diverse PFAS mixtures that occur in the environment. Mixtures assessments for chemicals that share similar adverse health effects, and therefore assume dose additivity, typically apply component-based assessment approaches.

The Hazard Index (HI) approach is one of the component-based mixtures assessment approaches described in the PFAS mixtures framework. In order to support states and Tribes interested in addressing potential noncancer risks of PFAS mixtures, the application of the HI approach for deriving HHC for mixtures is described below. States and authorized Tribes may choose to adopt this approach to derive HHC for PFAS mixtures. Use of the HI approach to assess risks associated with PFAS mixtures was supported by the EPA Science Advisory Board (EPA, 2022b).

In the HI approach (see PFAS mixtures framework; EPA, 2024o), a hazard quotient (HQ) is calculated as the ratio of human exposure (E) to a human health-based toxicity value (e.g., reference value [RfV]) for each mixture component chemical (i) (EPA, 1986). The HQs for the component chemicals are then summed to derive a mixture-specific HI (for the specified exposure route/medium). Since the HI is unitless, the E and the RfV inputs to the HI formula must be expressed in the same dose units (e.g., mg/L) (Eq. 5). For example, in the context of the

human health criteria, HQs for each individual PFAS are calculated by dividing the measured ambient water concentration of each component PFAS (e.g., expressed as µg/L) by its corresponding human health criterion (e.g., expressed as µg/L), and the resulting component PFAS HQs are summed to yield the PFAS mixture HI (Eqs. 5-7). Either water-plus-organism or organism-only HHC can be used in the PFAS mixtures HI approach; however, the type of HHC selected for HI calculation should be consistent. Because cancer data are lacking for most PFAS, the HI approach is currently recommended for PFAS HHC based on noncancer effects.

A hypothetical example is included below to illustrate using the HI approach to derive an HHC for a mixture of three PFAS. A PFAS mixture HI exceeding 1 indicates that co-occurrence of two or more PFAS in a mixture in ambient water exceeds the health-protective level(s), indicating potential health risks. Some individual PFAS have HHC below the analytical MDLs (e.g., PFOA, PFOS). If one such PFAS is included as a component PFAS in the HI approach, then any detectable level of that component PFAS in surface water will result in a component HQ greater than 1, and thus, an HI greater than 1 for the PFAS mixture.

$$\mathrm{HI} = \sum_{i=1}^{n} \mathrm{HQ_i} = \sum_{i=1}^{n} \frac{\mathrm{E_i}}{\mathrm{HHC_i}} \quad \text{(Eq. 5)}$$

$$\mathrm{HI} = \mathrm{HQ_{PFBS}} + \mathrm{HQ_{PFAS_X}} + \mathrm{HQ_{PFAS_Y}} \quad \text{(Eq. 6)}$$

$$\mathrm{HI} = \left(\frac{[\mathrm{PFBS_{ambient\ water}}]}{[\mathrm{PFBS_{HHC}}]}\right) + \left(\frac{\mathrm{PFAS_{x,ambient\ water}}}{[\mathrm{PFAS_{x,HHC}}]}\right) + \left(\frac{[\mathrm{PFAS_{Y,ambient\ water}}]}{[\mathrm{PFAS_{Y,HHC}}]}\right) \quad \text{(Eq. 7)}$$

Where:

HI = hazard index
n = the number of component (i) PFAS
HQ_i = hazard quotient for component (i) PFAS
E_i = human exposure for component (i) PFAS
HHC_i = human health criterion for component PFAS (i)
HQ_{PFAS} = hazard quotient for a given individual PFAS
$PFAS_X$ = Hypothetical PFAS
$PFAS_Y$ = Hypothetical PFAS
$[PFAS_{ambient\ water}]$ = concentration of a given PFAS in ambient water
$[PFAS_{HHC}]$ = water-plus-organism HHC or organism-only HHC for a given PFAS

9 Chemical Name and Synonyms

- Perfluorobutane Sulfonic Acid (CASRN 375-73-5)
- Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)
- PFBS
- K^+PFBS
- 1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid
- 1-Perfluorobutanesulfonic acid

- Nonafluoro-1-butanesulfonic acid
- Nonafluorobutanesulfonic acid
- Perfluorobutanesulfonic acid
- 1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid
- Perfluorobutanesulfonate
- Perfluorobutane sulfonate
- 1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-
- 1-Butanesulfonic acid, nonafluoro-
- Perfluoro-1-butanesulfonate
- Perfluorobutylsulfonate

10 References

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Appendix A: Summary of Supporting Literature for Surface Water Occurrence

Table A-1. Compilation of studies describing PFBS occurrence in surface water.

Study	Location	Site Details	PFBS Results
North America			
Anderson et al. (2016)	United States (national)	Ten U.S. Air Force installations with historic AFFF release	DF 80.00%, median (range) = 106 (ND–317,000) ng/L
Appleman et al. (2014)	United States (Wisconsin, Oklahoma, Alaska, California, Alabama, Colorado, Ohio, Nevada, Minnesota, New Jersey)	Raw surface waters from 11 sites, some impacted by upstream wastewater effluent discharge	DF[a] 64% (n = 25); range = ND–47 ng/L (MRL = 0.3)
Bradley et al. (2020)	United States (Lake Michigan)	Untreated Lake Michigan water from treatment plant intake (4 sites)	DF 29%, range = ND–0.5 ng/L
Galloway et al. (2020)	United States (Ohio and West Virginia; Ohio River Basin)	Rivers and tributaries 58 km upstream to 130 km downwind of a fluoropolymer production facility, some sample locations potentially impacted by local landfills	DF NR, range[a] = ND–28.0 ng/L
Lasier et al. (2011)	United States (Georgia; Coosa River watershed)	Upstream (sites 1 and 2) and downstream (sites 3–8) of a land-application site where effluents from carpet manufacturers (suspected of producing wastewaters containing perfluorinated chemicals) are processed at a WWTP and the treated WWTP effluent is sprayed onto the site. Site 4 was downstream of a manufacturing facility for latex and polyurethane backing material.	Upstream Sites 1 and 2: DF 0% Downstream Site 3: DF NR, mean = 205 ng/L Site 4: DF NR, mean = 260 ng/L Site 5: DF NR, mean = 125 ng/L Site 6: DF NR, mean = 134 ng/L Site 7: DF NR, mean = 122 ng/L Site 8: DF NR, mean = 105 ng/L
Lescord et al. (2015)	Canada (Resolute Bay, Nunavut)	One lake (Meretta) contaminated with runoff from an airport, which is a known source of PFAS; one control lake (9 Mile)	Meretta: DF NR, mean = 4.9 ng/L 9 Mile: DF NR, mean = 0.07 ng/L

Study	Location	Site Details	PFBS Results
Lindstrom et al. (2011)	United States (Alabama)	32 surface water samples (ponds and streams) from areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 63%, range = ND–208 ng/L
Nakayama et al. (2007)	United States (North Carolina; Cape Fear River Basin)	80 sampling sites in river basin; some sites near industrial areas and Fort Bragg and Pope Air Force Base with suspected use of AFFF at the Air Force Base	DF 62%, mean (range) = 2.58 (ND–9.41) ng/L
Nakayama et al. (2010)	United States (Illinois, Iowa, Minnesota, Missouri, Wisconsin; Upper Mississippi River Basin and Missouri River Basin)	88 sampling sites from tributaries and streams	DF 43%, median (range) = 0.71 (ND–84.1) ng/L
Newsted et al. (2017)	United States (Minnesota; Upper Mississippi River Pool 2)	Upstream and downstream of 3M Cottage Grove facility outfall, which is a source of PFAS	Upstream: DF[a] 3%, point = 4.2 ng/L Downstream: DF[a] 67%, range = ND–336.0 ng/L
Newton et al. (2017)	United States (Decatur, Alabama; Tennessee River)	6 sites upstream and 3 sites downstream of fluorochemical manufacturing facilities	Upstream: DF 0% Downstream: DF[a] 100%, mean[a] (range) = 69 (10–160) ng/L
Post et al. (2013)	United States (New Jersey)	6 rivers and 6 reservoirs from public drinking water system intakes, some sites may include nearby small industrial park and civil-military airport	DF 17%, range = ND–6 ng/L
Procopio et al. (2017)	United States (New Jersey; Metedeconk River Watershed)	Downstream of suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs	DF[a] 5%, range = ND–100 ng/L
Subedi et al. (2015)	United States (New York; Skaneateles Lake)	Lake water along the shoreline of residences that use an enhanced treatment unit for onsite wastewater treatment	DF[a] 4% (n = 28); single detection value = 0.26 ng/L

Study	Location	Site Details	PFBS Results
Veillette et al. (2012)	Canada (Ellesmere Island, Nunavut)	A lake near the northwest coast with no known sources of PFAS	DF[a] 100%, mean (range) = 0.016 (0.011–0.024) ng/L
Yeung et al. (2017)	Canada (Ontario; Mimico Creek, Rouge River)	Two water samples at each of the sites	Mimico Creek: point = 0.020 ng/L Rouge River: DF 0%
Zhang et al. (2016)	United States (Rhode Island, New York Metropolitan Region)	Rivers and creeks, some sampling locations downstream from industrial activities, airport, textile mills, and WWTP. PFAS are used for water resistant coating in textiles.	DF[a] 85%, range = ND–6.181 ng/L
Europe			
Ahrens et al. (2009a)	Germany (Elbe River)	Sampling sites in Hamburg city (sites 16–18) and from Laurenburg to Hamburg (sites 19–24)	Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.6 (1.1–2.5) ng/L Laurenburg to Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.1 (0.53–1.5) ng/L
Ahrens et al. (2009b)	Germany (Elbe River)	Sampling locations 53 to 122 km (sites 1 to 9)[c] upstream of estuary mouth of Elbe River	DF NR; range of mean (for different locations) = 1.8–3.4 ng/L
Bach et al. (2017)	France (southern)	Upstream and downstream from discharge point that receives wastewater from an industrial site with two fluoropolymer manufacturing facilities	Upstream: DF 0% Downstream: DF 0%
Barreca et al. (2020)	Italy (Lombardia Region)	Rivers and streams with no known fluorochemical sources	DF[a] 39%, range = ND–16,000 ng/L
Boiteux et al. (2012)	France (national)	Rivers; some locations may have upstream industrial sources	DF 1%, range = ND–5 ng/L
Boiteux et al. (2017)	France (northern)	River samples from upstream and downstream of an industrial WWTP that processes raw sewage from fluorochemical manufacturing facility	Upstream: DF 0% Downstream: DF 0%
Dauchy et al. (2017)	France (unspecified)	Samples collected near 3 sites (B, C, D) impacted by the use of firefighting foams	Site B: DF 0% Site C: DF 0% Site D: DF[a] 30%, range = ND–138 ng/L

Study	Location	Site Details	PFBS Results
Ericson et al. (2008)	Spain (Tarragona Province; Ebro River, Francolí River, Cortiella River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Ebro site 1: DF 0% Ebro site 2: DF 0% Francolí: DF 0% Cortiella: DF 0%
Eriksson et al. (2013)	Denmark (Faroe Islands)	Lakes Leitisvatn, Havnardal, Kornvatn, and Á Mýranar with no known point sources of any fluorochemical facilities	Leitisvatn: DF 0% Havnardal Lake: DF 0% Kornvatn Lake: DF 0% Á Mýranar: DF 0%
Eschauzier et al. (2012)	The Netherlands (Amsterdam; Lek Canal, tributary of Rhine River)	Downstream of an industrial point source in the German part of the Lower Rhine	DF[a] 100%, mean (range) = 35 (31–42) ng/L
Gebbink et al. (2017)	The Netherlands (Dordrecht)	Upstream and downstream of Dordrecht fluorochemical production plant; two control sites	Control sites: DF[a] 100%, mean[a] (range) = 17 (12–22) ng/L Upstream: DF[a] 100%, mean[a] (range) = 19.7 (18–21) ng/L Downstream: DF[a] 100%, mean[a] (range) = 21 (16–27) ng/L
Gobelius et al. (2018)	Sweden (national)	Sampling locations selected based on potential vicinity of PFAS hot spots and importance as a drinking water source area, some sites include firefighting training sites at airfields and military areas	DF[a] 29%, range = ND–299 ng/L
Labadie and Chevreuil (2011)	France (Paris; River Seine)	Urban stretch of the River Seine during a flood cycle, sampling location under the influence of two urban WWTPs and two major combined sewer overflow outfalls	DF 100%, mean (range) = 1.3 (0.6–2.6) ng/L
Loos et al. (2017)	Austria, Bulgaria, Croatia, Moldova, Romania, Serbia, Slovakia (Danube River and tributaries)	Some sampling locations downstream of major cities	DF 94%, mean (range) = 1.6 (ND–3.7) ng/L

Study	Location	Site Details	PFBS Results
Lorenzo et al. (2015)	Spain (Guadalquivir River Basin, Ebro River Basin)	Guadalquivir sampling locations included downstream of WWTPs, near industrial areas, near a military camp, or through major cities; Ebro sampling locations included nearby ski resorts and downstream of WWTP and industrial areas	Guadalquivir: DF 8%, mean (range) = 10.1 (ND–228.3) ng/L Ebro: DF 0%
Möller et al. (2010)	Germany (Rhine River watershed)	Upstream and downstream of Leverkusen, where effluent of a WWTP treating industrial wastewater was discharged; other major rivers and tributaries	Rhine upstream Leverkusen: DF 100%, mean (range) = 3.19 (0.59–6.58) ng/L Rhine downstream Leverkusen: DF 100%, mean (range) = 45.4 (15.0–118) ng/L River Ruhr: DF 100%, mean (range) = 7.08 (2.87–11.4) ng/L River Moehne: point = 31.1 ng/L Other tributaries: DF 100%, mean (range) = 2.84 (0.22–6.82) ng/L
Munoz et al. (2016)	France (Seine River)	Two sites downstream of Greater Paris and one site unaffected by the Greater Paris region	DF 70%, range = ND–3.1 ng/L
Mussabek et al. (2019)	Sweden (Luleå)	Samples from lake and pond near a firefighting training facility at the Norrbotten Air Force Wing known to use PFAS-containing AFFF	Lake: DF NR, mean = 200 ng/L Pond: DF NR, mean = 150 ng/L
Rostkowski et al. (2009)	Poland (national)	Rivers, lakes, and streams in northern and southern Poland, some southern locations near chemical industrial activities	North: DF[a] 60%, range = ND–10 ng/L South: DF[a] 73%, range = ND–16.0 ng/L
Shafique et al. (2017)	Germany (Leipzig, Pleiße-Elster River, Saale River, and Elbe River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Pleiße-Elster: DF NR, mean = 1.2 ng/L Saale: DF NR, mean = 7.5 ng/L Elbe: DF NR, mean = 4.3 ng/L

Study	Location	Site Details	PFBS Results
Valsecchi et al. (2015)	Italy (Po River Basin, Brenta River Basin, Adige River Basin, Tevere River Basin, and Arno River Basin)	Two river basins (Po and Brenta) which receive discharges from two chemical plants that produce fluorinated polymers and intermediates; three river basins (Adige, Tevere, Arno) with no known point sources of any fluorochemical facilities	Po: DF[a] 56%, range = ND–30.4 ng/L Brenta: DF[a] 100%, mean[a] (range) = 707 (23.1–1,666) ng/L Adige: DF[a] 20%, range = ND–4.3 ng/L Tevere: DF 0% Arno: DF[a] 58%, range = ND–31.4 ng/L
Wagner et al. (2013)	Germany (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean[b] (range[b]) = 18 (9–26) ng/L
Wilkinson et al. (2017)	England (Greater London and southern England; Hogsmill River, Chertsey Bourne River, Blackwater River)	50 m upstream and 250 m and 1,000 m downstream from WWTP effluent outfalls	Upstream: DF NR, mean = 20.4 ng/L Downstream 250 m: DF NR, mean = 40.3 ng/L Downstream 1,000 m: DF NR, mean = 41.1 ng/L
Zhao et al. (2015)	Germany (Elbe River and lower Weser River)	Some sampling sites near Hamburg city and industrial plants	Elbe: DF 100%, mean (range) = 7.4 (0.24–238) ng/L Weser: DF 100%, mean (range) = 1.41 (0.75–1.85) ng/L
Multiple Continents			
Pan et al. (2018)	United States (Delaware River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 2.19 (0.52–4.20) ng/L
	United Kingdom (Thames River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 5.06 (3.26–6.75) ng/L
	Germany and the Netherlands (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 21.9 (0.46–146) ng/L
	Sweden (Mälaren Lake)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 1.43 (0.75–1.92) ng/L

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; km = kilometer; m = meter; ND = not detected; ng/L = nanogram per liter; NR = not reported; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; WWTP = wastewater treatment plant; µg/L = microgram per liter.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] For Wagner et al. (2013), PFBS concentrations were calculated using the fluorine concentrations reported in Table 4 from the study.
[c] Freshwater locations determined as sites with conductivity < 1.5 milliSiemens/cm.

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Appendix B: Bioaccumulation Factor (BAF) Supporting Information

Search Strings used for literature review of PFBS bioaccumulation data:

("375-73-5" OR "29420-49-3" OR "45187-15-3" OR "Perfluorobutane Sulfonic Acid" OR PFBS OR "Potassium Perfluorobutane Sulfonate" OR "K+PFBS" OR "nonafluorobutane-1-sulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutane sulfonic Acid" OR "Perfluro-1-butanesulfonate" OR "1-Perfluorobutanesulfonic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutanesulfonyl fluoride" OR "Nonafluorobutane-1-sulfonatato" OR "Nonafluorobutanesulfonic acid" OR "Perfluorobutanesulfonic acid" OR "Perfluorobutane sulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonyl fluoride" OR "1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonyl fluoride" OR "Perfluorobutanesulfonate" OR "Perfluorobutane Sulfonate" OR "Perfluorobutanesulfonyl fluoride" OR "1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-" OR "1-butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluror, potassium salt" OR "1-Butanesulfonic acid, nonafluoro-" OR "Perfluoro-1-butanesulfonate" OR "Perfluorobutylsulfonate" OR "Perfluoro-1-butanesulfonyl fluoride" OR "Potassium;1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate" OR "Potassium nonafluoro-1-butanesulfonate" OR "Ammonium nonafluorobutane-1-sulfonate" OR "Ammonium perfluorobutanesulfonate" OR "Potassium perfluorobutanesulfonate" OR "Potassium perfluorobutane sulfonate" OR "Potassium nonafluorobutane-1-sulfonate" OR "Potassium nonafluoro-1-butanesulfonate" OR "Potassium PFBS" OR "PFBuS" OR "C539348" OR "1FV02N6NVO" OR "DTXSID5030030" OR "FC-98" OR "EFTOP FBSA" OR "UNII-1FV02N6NVO" OR "SCHEMBL23932" OR "CHEMBL1198521" OR "HSDB 8294" OR "CHEBI:132446" OR "CS-B0899" OR "MFCD01320794" OR "AKOS015852768" OR "NCI60_006096" OR "FT-0676348" OR "FT-0676859" OR "N0709" OR "D77221" OR "Q410426") AND ("Bioaccumulation Factor" OR "Bioconcentration Factor" OR bcf OR baf OR bioaccumulation OR bioconcentration OR uptake OR depuration OR accumulation)

BAF Calculation Description for PFBS

The EPA used the decision framework presented in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000), Technical Support Document, Volume 2: Development of National Bioaccumulation Factors* (Technical Support Document, Volume 2) (EPA, 2003) to identify procedures to derive national trophic level-specific BAFs for PFBS based on that chemical's properties (e.g., ionization, hydrophobicity), metabolism, and biomagnification potential (see Figure 1). The EPA followed the guidelines provided in Section 5.5 of EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (EPA's 2000 Methodology) (EPA, 2000), to assess the occurrence of cationic and anionic forms of PFBS at typical environmental pH ranges. PFBS is a nonionic organic chemical (with ionization significant at typical environmental pH ranges) (EPA, 2021a,b).

As explained in Section 5.5 of EPA's 2000 Methodology (EPA, 2000), when a significant fraction of the total chemical concentration is expected to be present as the ionized species in water, procedures for deriving the national BAF rely on empirical (measured) methods (i.e., Procedures 5 and 6). The EPA followed the guidelines in Sections 3.2.1 and 3.2.2 of the Technical Support Document, Volume 2, to evaluate the biomagnification potential of PFBS. Based on the information in Loi et al. (2011), it was determined that biomagnification was unlikely. Based on the characteristics of PFBS, the EPA selected Procedure 5 for deriving national BAF values for this chemical.

As described in Section 4.2.1, for a given procedure, the EPA selected the method that provided BAF estimates for all three TLs (TL 2–TL 4) in the following priority:

- BAF estimates using the BAF method (i.e., based on field-measured BAFs) if possible.
- BAF estimates using the BCF method if (a) the BAF method did not produce estimates for all three TLs and (b) the BCF method produced national-level BAF estimates for all three TLs.

The EPA was able to locate field-measured BAFs for TLs 2, 3, and 4 for PFBS from the peer-reviewed literature sources for which sufficient information was provided to determine the quality and usability of the data. Therefore, the EPA used the BAF method (EPA, 2003) to derive the national BAF values for this chemical.

Calculating Baseline BAFs

As described in Section 4.2.3, the national-level BAF equation adjusts the TL baseline BAFs for nonionic organic chemicals by national default values for lipid content, as well as dissolved and particulate organic carbon content. However, the partitioning of PFBS is related to protein binding properties (ATSDR, 2021; ECHA, 2019). The EPA considered protein-normalizing the measured BAF values in the baseline BAF equation; however, insufficient data were available from the scientific literature on protein content of aquatic organisms and on the binding efficiencies of PFBS to various proteins in aquatic organisms. Because of this lack of data on the relationship between protein content and PFBS bioaccumulation, attempts to normalize BAFs based on protein content would likely introduce greater uncertainty into BAF averages.

Consistent with the EPA's 2000 Methodology (EPA, 2000), a procedure analogous to the one used to adjust for the water-dissolved portions of a nonionic organic chemical is applied to the measured BAFs for PFBS. As described in EPA's (2003) Technical Support Document, Volume 2, the K_{poc} (the equilibrium partition coefficient of the chemical between the particulate organic carbon [POC] phase and the freely dissolved phase of water) is approximately equal to the K_{ow} of a hydrophobic organic chemical. It is further described in the EPA's (2003) Technical Support Document, Volume 2, that K_{doc} (the equilibrium partition coefficient of the chemical between the dissolved organic carbon [DOC] phase and the freely dissolved phase of water) is directly proportional to the K_{ow} of a hydrophobic organic chemical, and that K_{doc} is less than the K_{ow}. The log K_{oc} for PFBS provided in ATSDR (2021) is 2.06 (as determined from a groundwater aquifer study) and was used in the national BAF calculations to adjust for the water-dissolved portions of a nonionic organic chemical. The EPA determined that the K_{oc} values were applicable to POC but there is no indication that they would be applicable to DOC. Thus, the amount of PFBS partitioned to DOC was presumed to be part of the aqueous fraction of the f_{fd} equation, resulting in the following formula (Eq. 1):

$$f_{fd} = \frac{1}{[1 + (POC \cdot K_{OC})]} \quad \text{(Eq. 1)}$$

Where:

- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved.
- POC = national default value of 0.5 mg/L (refer to page 5-44 of the EPA's 2000 Methodology [EPA, 2000]) is used in baseline BAF calculations, unless this value is reported in the BAF source.
- K_{oc} = PFBS log K_{oc}; log k_{oc} = 2.06 (ATSDR, 2021).

Because the measured BAFs for PFBS are not adjusted for lipid or protein content, the baseline BAF equation (refer to Eq. 5-10 on pages 5-24 and 5-25 of the EPA's 2000 Methodology [EPA, 2000]) is adjusted (as shown below in Eq. 2) to determine the freely dissolved PFBS in water:

$$\text{Baseline BAF} = \frac{\text{Measured BAF}}{f_{fd}} - 1 \quad \text{(Eq. 2)}$$

The EPA used this equation to calculate baseline BAFs from field measured BAFs based on total concentrations.

Dissolved PFBS Baseline BAFs

The EPA included results from several field BAF studies for PFBS reported as dissolved (i.e., filtered) concentrations in its baseline BAF calculations. Because these dissolved PFBS data are presumed to represent the freely-dissolved (non-particulate) fraction, the ffd term in Eq. 2 is set to 1. Also, as described above, the measured BAFs for PFBS are not being adjusted for lipid or protein content to calculate baseline BAFs for PFBS. Thus, Eq. 3 is used to calculate the freely dissolved concentration of PFBS for "baseline BAFs" using field-measured dissolved PFBS BAFs:

$$\text{Baseline BAF} = \text{Measured (dissolved) BAF} - 1 \quad \text{(Eq. 3)}$$

Calculating the National BAFs

Final baseline BAFs were used to compute national BAFs for PFBS. Eq. 4 (an equation analogous to the equation used for nonionic organic chemicals in the EPA's 2015 Updated Human Health criteria for calculating national BAFs (see Eq. 5-28 on Page 5-42 of the EPA's 2000 Methodology [EPA, 2000]) is used to convert the baseline BAF to a national BAF for each trophic level:

$$\text{National BAF}_{(\text{TL n})} = [(\text{Final Baseline BAF}^{\text{fd}})_{\text{TL n}} + 1] \cdot (\text{f}_{\text{fd}}) \qquad \text{(Eq. 4)}$$

Where:

- National BAF = national BAF (L/kg-tissue).
- (Final Baseline BAF)$_{\text{TL n}}$ = mean baseline BAF for TL "n" (L/kg-lipid).
- f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved.

In summary, for PFBS, the baseline BAFs are calculated using Equation 2 (for field measured BAFs calculated from total water concentrations) and Equation. 3 (for field BAFs calculated from dissolved water concentrations) for each TL. National BAFs are then calculated from TL baseline BAFs using Equation 4 as shown below.

National Trophic level BAF calculations:

$$\text{National BAF PFBS}_{(\text{TL 2})} = [(355.9)_{\text{TL 2}} + 1] \times (0.9999)$$
= 356.9 L/kg
= 360 L/kg (rounded)

$$\text{National BAF PFBS}_{(\text{TL 3})} = [(285.6)_{\text{TL 3}} + 1] \times (0.9999)$$
= 286.6 L/kg
= 290 L/kg (rounded)

$$\text{National BAF PFBS}_{(\text{TL 4})} = [(866.9)_{\text{TL 4}} + 1] \times (0.9999)$$
= 867.8 L/kg
= 870 L/kg (rounded)

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Appendix C: Supporting Literature for Deriving the Relative Source Contribution

Table C-1. Compilation of studies describing PFBS occurrence in food.

Study	Location and Source	Food Types	Results
North America			
Blaine et al. (2013)	United States (Midwestern) Greenhouse and field studies, unamended controls	Fruits and vegetables, grain	ND in corn, lettuce, tomato in unamended soil
Blaine et al. (2014)	United States (Midwestern) Greenhouse study, unamended controls	Fruits and vegetables	**Radish root: DF NR, mean = 22.36 ng/g** ND in celery shoot, pea fruit
Byrne et al. (2017)	United States (Alaska) Upstream/downstream of former defense site (Suqi River)	Seafood	**Blackfish: DF 48%, range = ND–59.2 ng/g ww** Highest concentration was upstream
Schecter et al. (2010)	United States (Texas) Grocery stores	Dairy, fruits and vegetables, grain, meat, seafood[h], fats/other	**Cod: DF NR, mean = 0.12 ng/g ww** ND in salmon, canned sardines, canned tuna, fresh catfish fillet, frozen fish sticks, tilapia, cheeses (American, mozzarella, Colby, cheddar, Swiss, provolone, and Monterey jack), butter, cream cheese, frozen yogurt, ice cream, whole milk, whole milk yogurt, potatoes, apples, cereals, bacon, canned chili, ham, hamburger, roast beef, sausages, sliced chicken breast, sliced turkey, canola oil, margarine, olive oil, peanut butter, eggs
Scher et al. (2018)	United States (Minnesota) Home gardens Near former 3M PFAS production facility, homes within and outside a GCA	Fruits and vegetables	Within GCA: **Leaf: DF 6%, max = 0.061 ng/g** **Stem: DF 4%, max = 0.065 ng/g** ND in floret, fruit, root, seed Outside GCA: ND
Young et al. (2012)	United States (17 states) Retail markets	Dairy	ND in retail cow's milk
Young et al. (2013)	United States (Maryland, Mississippi, Tennessee, Florida, New York, Texas, Washington, D.C.) Retail markets	Seafood	ND in crab, shrimp, striped bass, farm raised catfish, farm raised salmon

[h] Some PFBS dietary studies use the term "seafood" to indicate fish and shellfish from ocean, freshwater, or estuarine water bodies. Information about the water bodies assessed in individual studies is reported in the articles.

Study	Location and Source	Food Types	Results
Europe			
Barbosa et al. (2018)	Belgium, France, the Netherlands, Portugal Various markets	Seafood	ND in raw and steamed fish (*P. platessa, M. australis, M. capenis, K. pelamis*, and *M. edulis*)
D'Hollander et al. (2015)	Belgium, Czech Republic, Italy, Norway PERFOOD study; items from 3 national retail stores of different brands and countries of origin	Fruit, cereals, sweets, salt	**Sweets: DF[a] 25%, range = ND–0.0016 ng/g** **Fruit: DF[a] 19%, range = ND–0.067 ng/g** ND in cereals, salt
Domingo et al. (2012)	Spain (Catalonia) Local markets, small stores, supermarkets, big grocery stores	12 food categories	**Vegetables: DF NR, mean = 0.013 ng/g fw** **Fish and seafood: DF NR, mean = 0.054 ng/g fw** ND in meat and meat products, tubers, fruits, eggs, milk, dairy products, cereals, pulses, industrial bakery, oils
Ericson et al. (2008)	Spain Local markets, large supermarkets, grocery stores	18 food categories	ND in all categories: veal, pork, chicken, lamb, white fish, seafood, tinned fish, blue fish, whole milk, semi-skimmed milk, dairy products, vegetables, pulses, cereals, fruits, oil, margarine, and eggs
Eriksson et al. (2013)	Denmark Farm, dairy farm, fish from Faroe Shelf area	Dairy, fruits and vegetables, seafood	**Milk:** **Farmer (Havnardal): point = 0.019 ng/g ww** **Dairy (Faroe Island): point = 0.017 ng/g ww**; ND or NQ in 4 samples ND in yogurt, creme fraiche, potatoes, farmed salmon, wild-caught cod, wild-caught saithe
Eschauzier et al. (2013)	The Netherlands (Amsterdam) Cafés, universities, supermarkets	Fats/other	**Brewed coffee (manual): mean (range) = 1.6 (1.3–2.0) ng/L** **Brewed coffee (machine): mean (range) = 2.9 (ND–9.8) ng/L** **Cola: mean (range) = 7.9 (ND–12) ng/L**
Falandysz et al. (2006)	Poland Gulf of Gdañsk, Baltic Sea south coast	Meat, seafood	ND in eider duck, cod

Study	Location and Source	Food Types	Results
Gebbink et al. (2015)	Sweden Major grocery chain stores, market basket samples	12 food categories	ND in all categories: dairy products, meat products, fats, pastries, fish products, egg, cereal products, vegetables, fruit, potatoes, sugar and sweets, soft drinks
Herzke et al. (2013)	Belgium, Czech Republic, Italy, Norway PERFOOD study: items from 3 national retail stores of different brands per location	Vegetables	ND for all vegetables
Hlouskova et al. (2013)	Belgium, Czech Republic, Italy, Norway Several national supermarkets	Pooled milk/dairy products, meat, fish, hen eggs	**DF 5%, mean (range) = 0.00975 (0.006–0.012) ng/g**
Hölzer et al. (2011)	Germany Fish from Lake Möhne and river Möhne, contaminated with PFCs from use of polluted soil conditioner on agricultural lands; retail trade, wholesale trade, supermarkets, and producers	Seafood	Lake Möhne /River Möhne: ND in cisco, eel, perch, pike, and roach Trade/markets: ND in eel, pike/perch, and trout
Jogsten et al. (2009)	Spain (Catalonia) Local markets, large supermarkets, grocery stores	Fruits and vegetables, meat, seafood, fats/other	ND in lettuce, raw, cooked, and fried meat (veal, pork, and chicken), fried chicken nuggets, black pudding, lamb liver, pate of pork liver, foie gras of duck, “Frankfurt” sausages, home-made marinated salmon, and common salt
Jörundsdóttir et al. (2014)	Iceland Collected during biannual scientific surveys, commercially produced	Seafood	ND in anglerfish, Atlantic cod, blue whiting, lemon sole, ling, lumpfish, plaice, and pollock
Lankova et al. (2013)	Czech Republic Retail market	Fats/other	ND in infant formula
Noorlander et al. (2011)	The Netherlands Several Dutch retail store chains with nationwide coverage	15 food categories	ND in all categories: flour, fatty fish, lean fish, pork, eggs, crustaceans, bakery products, vegetables/fruit, cheese, beef, chicken/poultry, butter, milk, vegetable oil, and industrial oil

Study	Location and Source	Food Types	Results
Papadopoulou et al. (2017)	Norway A-TEAM project: food and drinks collected by participants as duplicate diet samples	Solid foods (11 food categories), liquid foods (5 drinks)	**Solid foods (unspecific food category): DF 2%, range = ND–0.001 ng/g** ND in liquid foods (coffee, tea and cocoa, milk, water, alcoholic beverages and soft drinks)
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Various supermarkets and retail stores	8 food categories	Categories included cereals, pulses and starchy roots, tree-nuts, oil crops and vegetable oils, vegetables and fruits, meat and meat products, milk, animal fats, dairy products, and eggs, fish and seafood, and others such as candies or coffee **Spain: DF 3.2%, range = ND–13 ng/g (primarily fish, oils)** **Serbia: DF 5.2%, range = ND–0.460 ng/g (primarily meat and meat products, cereals)**
Rivière et al. (2019)	France Based on results of national consumption survey	Seafood, fats/other	ND in infant food, vegetables, nonalcoholic beverages, dairy-based desserts, milk, mixed dishes, fish, ultra-fresh dairy products, meat, poultry and game
Scordo et al. (2020)	Italy Supermarkets	Fruits	**Olives: DF[a] 100%, mean[a] (range) = 0.294 (0.185–0.403) ng/g dw** ND in strawberries
Surma et al. (2017)	Spain, Slovakia Source NR	Fats/other	**Spices: ND–1.01 ng/g** Spain: Detected in anise, star anise, fennel, coriander, cinnamon, peppermint, parsley, thyme, laurel, cumin, and oregano ND in white pepper, cardamon, clove, nutmeg, allspice, vanilla, ginger, garlic, black paper, and hot pepper (mild and hot) Slovakia: ND in anise, star anise, white pepper, fennel, cardamom, clove, coriander, nutmeg, allspice, cinnamon, vanilla, and ginger
Sznajder-Katarzyńska et al. (2018)	Poland Markets	Fruits and vegetables	ND in apples, bananas, cherries, lemons, oranges, strawberries, beetroots, carrots, tomatoes, potatoes, and white cabbage

Study	Location and Source	Food Types	Results
Sznajder-Katarzyńska et al. (2019)	Poland Markets	Dairy	**All dairy: sum PFBS = 0.04 ng/g** **Butter: range = 0.01–0.02 ng/g** ND in camembert-type cheese, cottage cheese, milk, natural yogurt, sour cream, kefir (bonny clabber)
Vassiliadou et al. (2015)	Greece Local fish markets, mariculture farm, fishing sites	Seafood	**Hake: raw mean = 0.45 ng/g ww, fried mean = 0.83 ng/g ww** **Shrimp: raw mean = 1.37 ng/g ww** ND in raw, fried, and grilled anchovy, bogue, picarel, sand smelt, sardine, squid, striped mullet, raw and fried mussel, fried shrimp, and grilled hake
Zafeiraki et al. (2016a)	Greece, the Netherlands Home and commercially produced	Fats/other	ND in chicken eggs
Zafeiraki et al. (2016b)	The Netherlands Local markets and slaughterhouses	Meat	ND for horse, sheep, cow, pig, and chicken liver
Multiple Continents			
Chiesa et al. (2019)	United States (Pacific Ocean) Wholesale fish market	Seafood	ND in wild-caught salmon
	Canada Wholesale fish market	Seafood	ND in wild-caught salmon
	Norway Wholesale fish market	Seafood	ND in farm salmon
	Scotland Wholesale fish market	Seafood	ND in wild-caught and farm salmon

Notes: DF = detection frequency; dw = dry weight; fw = fresh weight; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; ng/L = nanogram per liter; NR = not reported; PFAS = per- and polyfluoroalkyl substances; NQ = not quantified; µg/L = microgram per liter; ww = wet weight.
Bold indicates detected levels of PFBS in food.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

Table C-2. Compilation of studies describing PFBS occurrence in indoor dust.

Study	Location	Site Details	Results
North America			
Byrne et al. (2017)	United States (St. Lawrence Island, Alaska)	Homes (49)	DF 16%, median = ND; 95th percentile = 1.76 ng/g
Fraser et al. (2013)	United States (Boston, Massachusetts)	Homes (30); offices (31); vehicles (13)	Homes: DF 3% (single detection), range = ND–4.98 ng/g Offices: DF 10%, range = ND–12.0 ng/g Vehicles: DF 0%
Knobeloch et al. (2012)	United States (Great Lakes Basin, Wisconsin)	Homes (39)	DF 59%, median (range) = 1.8 (ND–31) ng/g
Kubwabo et al. (2005)	Canada (Ottawa)	Homes (67)	DF 0%
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility; 19 homes within the GCA	Entryway: DF 11%, median (range) = ND (ND–58 ng/g) Living room: DF 16%, median (range) = ND (ND–58 ng/g)
Strynar and Lindstrom (2008)	United States (Cities in North Carolina and Ohio)	Homes (102) and daycare centers (10); samples had been collected in 2000–2001 during EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study	DF 33%, mean (range) = 41.7 (ND–1,150) ng/g
Zheng et al. (2020)	United States (Seattle, Washington and West Lafayette, Indiana)	Childcare facilities (20 samples from 7 facilities in Seattle and 1 in West Lafayette)	DF 90%, mean (range) = 0.34 (ND–0.86) ng/g
Europe			
de la Torre et al. (2019)	Spain (unspecified), Belgium (unspecified), Italy (unspecified)	Homes (65)	Spain: DF 52%, median (range) = 0.70 (ND–12.0) ng/g Belgium: DF 27%, median (range) = 0.40 (ND–56.7) ng/g Italy: DF 18%, median (range) = 0.40 (ND–11.6) ng/g
D'Hollander et al. (2010)	Belgium (Flanders)	Homes (45); offices (10)	Homes: DF 47%, median = 0 ng/g dw Offices: DF NR, median = 0.2 ng/g dw
Giovanoulis et al. (2019)	Sweden (Stockholm)	Preschools (20)	DF 0%

Study	Location	Site Details	Results
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick counties)	Homes (32); offices (33); cars (31); classrooms (32)	Homes: DF 81%, mean (range) = 17 (ND–110) ng/g Offices: DF 88%, mean (range) = 19 (ND–98) ng/g Cars: DF 75%, mean (range) = 12 (ND–170) ng/g Classrooms: DF 97%, mean (range) = 17 (ND–49) ng/g
Haug et al. (2011)	Norway (Oslo)	Homes (41)	DF 22%, mean (range) = 1.3 (0.17–9.8) ng/g
Huber et al. (2011)	Norway (Tromsø)	Homes (7; carpet, bedroom, sofa); one office; one storage room that had been used for storage of "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years"	All homes: DF NR, median = 1.1 ng/g Living room: DF[a] 57%, range = ND–10.6 ng/g Carpet, bedroom, sofa: DF 0% Office: point = 3.8 ng/g Storage room: point = 1,089 ng/g
Jogsten et al. (2012)	Spain (Catalonia)	Homes (10)	DF 60%, range = ND–6.5 ng/g
Padilla-Sánchez and Haug (2016)	Norway (Oslo)	Homes (7)	DF 14% (single detection), range = ND–3 ng/g
Winkens et al. (2018)	Finland (Kuopio)	Homes (63 children's bedrooms)	DF 12.7%, median (range) = ND (ND–13.5) ng/g
Multiple Continents			
Karásková et al. (2016)	United States (unspecified)	Homes (14)	DF 60%, mean (range) = 1.4 (ND–2.6) ng/g
	Canada (unspecified)	Homes (15)	DF 55%, mean (range) = 1.6 (ND–5.8) ng/g
	Czech Republic (unspecified)	Homes (12)	DF 37.5%, mean (range) = 3.6 (ND–14.4) ng/g
Kato et al. (2009)	United States (Atlanta, Georgia), Germany (unspecified), United Kingdom (unspecified), Australia (unspecified)	Homes (39)	DF 92.3%, median (range) = 359 (ND–7,718) ng/g

Notes: DF = detection frequency; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; NR = not reported; dw = dry weight.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

Table C-3. Compilation of studies describing PFBS occurrence in soil.

Study	Location	Site Details	Results
North America			
Anderson et al. (2016)	United States (unspecified)	Ten U.S. Air Force installations with historic AFFF release, surface and subsurface soils	Surface soil: DF 35%, median (range) = 0.775 (ND–52.0) ng/g Subsurface soil: DF 35%, median (range) = 1.30 (ND–79.0) ng/g
Blaine et al. (2013)	United States (Midwestern)	Urban and rural full-scale field study control (nonamended) soil	Urban control: DF NR, mean = 0.10 ng/g Rural control: DF NR, mean = ND
Cabrerizo et al. (2018)	Canada (Melville and Cornwallis Islands)	Catchment areas of lakes	DF 100%, mean[a] (range) = 0.0024 (0.0004–0.0083) ng/g dw
Dreyer et al. (2012)	Canada (Ottawa, Ontario)	Mer Bleue Bog Peat samples (core samples)	Detected once at 0.071 ng/g in 1973 sample and not considered for further evaluation
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Firefighting training site, pre- and posttreatment	Pretreatment: DF 60%, range = 0.61–6.4 ng/g Posttreatment: DF 100%, range = 0.07–0.83 ng/g
Mejia-Avendaño et al. (2017)	Canada (Lac-Mégantic, Quebec)	Site of 2013 Lac-Mégantic train accident (oil and AFFF runoff area [sampled 2013], burn site and adjacent area [sampled 2015])	Background: DF NR, mean = 0.035 ng/g dw 2013: DF 75%, mean range = ND–3.15 ng/g dw 2015: DF 36%, mean range = ND–1.25 ng/g dw
Nickerson et al. (2020)	United States (unspecified)	Two AFFF-impacted soil cores from former fire-training areas	Core E: DF[a] 91%, range = ND–27.37 ng/g dw Core F: DF 100%, range = 0.13–58.44 ng/g dw
Scher et al. (2018)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within and outside a GCA	Within GCA: DF 9%, median (range) = ND (ND–0.17 ng/g) Outside GCA: DF 17%, median (range) = ND (ND–0.031 ng/g)
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within a GCA	DF 10%, median (p90) = ND (0.02) ng/g
Venkatesan and Halden (2014)	United States (Baltimore, Maryland)	Control (nonamended) soil from Beltsville Agricultural Research Center	DF 0%

Study	Location	Site Details	Results
Europe			
Dauchy et al. (2019)	France (unspecified)	Firefighting training site, samples collected in 6 areas collected up to 15-m depth; in areas 2 and 6, foams used more intensely and/or before concrete slab was built	Areas 1, 3, 4, and 5 combined: DF[a] 0–10%, range = ND–7 ng/g dw, across all depths Area 2: DF[a] 35%, range = ND–82 ng/g dw, across all depths Area 6: DF[a] 55%, range = ND–101 ng/g dw, across all depths
Groffen et al. (2019)	Belgium (Antwerp)	3M perfluorochemical plant and 4 sites with increasing distance from plant	Plant: DF 92%, mean (range) = 7.84 (ND–33) ng/g dw Vlietbos (1 km from plant): DF 90%, mean (range) = 2.79 (ND–7.04) ng/g dw 2.3 km, 3 km, 11 km from plant: DF 0%
Grønnestad et al. (2019)	Norway (Granåsen, Jonsvatnet)	Granåsen (skiing area); Jonsvatnet (reference site)	Skiing area: DF 0%[b] Reference area: DF 70%, mean (range) = 0.0093 (ND–0.0385 ng/g dw)
Harrad et al. (2020)	Ireland (multiple cities)	10 landfills, samples collected upwind and downwind	Downwind: DF NR, mean (range) = 0.0059 (ND–0.044) ng/g dw Upwind: DF NR, mean (range) = 0.0011 (ND–0.0029) ng/g dw
Hale et al. (2017)	Norway (Gardermoen)	Firefighting training site	DF 0%
Skaar et al. (2019)	Norway (Ny-Ålesund)	Research facility near firefighting training site	Background: DF 0% Contaminated: DF 100%, mean[a] (range) = 4.9 (2.64–7.13) ng/g dw

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; dw = dry weight; GCA = groundwater contamination area; km = kilometer; ND = not detected; ng/g = nanogram per gram; NR = not reported; PFAS = per- and polyfluoroalkyl substances; p90 = 90th percentile.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] Grønnestad et al. (2019) reported a DF = 10% but a range, mean, and standard deviation of < LOQ.

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Appendix D: Comparative Analysis for Potentially Sensitive Populations for PFBS

The EPA evaluated several exposure scenarios for PFBS to determine whether the national recommended criteria for the general population, male and female adults ≥ 21 years old, are sufficiently protective of potentially sensitive subpopulations. To accomplish this, the EPA considered three additional exposure scenarios, as supported by data from the EPA *Exposure Factors Handbook* (EFH; EPA, 2011) and the Human Health Methodology (EPA, 2000). Specifically, the EPA evaluated exposure parameters for "all ages" as well as two potentially sensitive life stages associated with the critical effect used to derive the PFBS chronic RfD, i.e., adverse developmental effect on thyroid activity, specifically decreased serum total thyroxine, in newborn mice (postnatal day [PND] 1) born to mothers that had been orally exposed to K+PFBS throughout gestation (EPA, 2021a,b). Based on this exposure interval in the critical study, potentially sensitive subpopulations in humans include women of childbearing age who may be or become pregnant and pregnant women (Table D-1).

For the body weight exposure parameter, a mean bodyweight of 75 kg for pregnant women (all trimesters) was identified in the EFH (2011, Ch. 8, Table 8-29). A representative body weights for the "all ages" scenario was not specifically presented in the EFH (EPA, 2011). To address this data limitation, for this exercise, the EPA assumed that the average body weight for "all ages" was 71.6 kg based on the sum of the time-weighted averages of the mean male and female combined body weights from 1 year up to 80 years old from the NHANES (1999–2006) (Table 8-3; EPA, 2011). A body weight average of 67 kg for women of childbearing age was identified in the Human Health Methodology (EPA, 2000); however, this average is based on an older NHANES dataset (NHANES III; WESTAT 2000). More recent NHANES data (1999–2006) suggest that the mean body weight for women of childbearing age ranges from 65.9 kg for 16 to < 21-year-olds to 77.1 kg for 40 to < 50-year-olds (Table 8-5; EPA, 2011). Using these data, the EPA assumed a time-weighted average body weight of 73.4 kg for women of childbearing age (Table 8-5; EPA, 2011).

Drinking water intake values were available for all populations (Table D-1).

The EPA encountered several data limitations for trophic level specific fish consumption rates for some of these potentially sensitive populations. The EPA's national criteria are typically derived using trophic-level specific fish consumption rates (FCRs), paired with trophic-level specific bioaccumulation factors (BAFs) to account for the potential bioaccumulation of some chemicals in aquatic food webs and the broad physiological differences between trophic levels which may influence bioaccumulation (EPA, 2000). Trophic level specific FCRs for women of childbearing age were identified (Table D-1). However, trophic level specific FCRs are not available for two of the potentially sensitive life stages: all ages and pregnant women. Therefore, criteria could not be calculated for these life stages. However, in all cases with available data, the total FCR for the alternative scenarios is lower than the FCR for the general population. Because bodyweights are similar for all of the considered populations (see above and Table D-1), the FCR is likely to be the main determinant of the criteria value, with a larger FCR resulting in a lower, more health protective criterion. Therefore, criteria based on the general population are expected to be protective of the identified potentially sensitive life

Table D-1. Comparison of noncancer-based HHC values for different candidate sensitive populations identified from the critical effect and study.

Population	Bodyweight (kg)	Drinking Water Intake (L/day)	Fish Consumption Rate (g/day)				Criteria (µg/L)	
			Total	TL 2	TL 3	TL 4	W + O	OO
General, adult (≥ 21 years)	**80[a]**	**2.3[b]**	**22[c]**	**7.6[c]**	**8.6[c]**	**5.1[c]**	**0.4**	**0.5**
Women of childbearing Age (13–49 years)	73.4[d]	2.1[e]	15.8[c]	5.6[c]	6.0[c]	2.9[c]	0.6	0.8
All Ages (Birth to 80 years)	71.6[f]	2.0[b]	19.3[g]	NA	NA	NA	ND	ND
Pregnant Women	75[h]	2.1[e]	10[i]	NA	NA	NA	ND	ND

Notes: g/day = grams of fish consumed per day; L/day = liters of water per day; NA = not available; ND = not determined; OO = organism only; W + O = water plus organism.
Bold values indicate draft national recommended criteria.
Gray highlighting indicates most health protective HHC based on noncancer effects.
[a] EPA, 2011, *Exposure Factors Handbook*, Ch. 8, Table 8-1, NHANES 1999–2006.Recommended mean bodyweight for adults.
[b] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010, community water, 90th percentile per capita rate.
[c] EPA, 2014; NHANES 2003–2010 survey data, 90th percentile per capita rate, freshwater and estuarine fish and shellfish edible portion, adults ≥ 21 years.
[d] Time weighted average of combined bodyweights for women ages 16 to < 50 years, NHANES 1999–2006 (EPA, 2011; Table 8-5).
[e] EPA, 2019, *Exposure Factors Handbook*; Update Ch. 3., Table 3-62, Community water, 90th percentile, per capita rate.
[f] Time weighted average of mean male and female combined body weights from 1 year up to 80 years, NHANES 1999–2006 (EPA, 2011; Table 8-3).
[g] Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate; male and female, all ages included.
[h] EPA, 2011, Exposures Factors Handbook, Ch 8, mean, NHANES 1999–2006, Table 8-29
i Estimated using the FCID calculator (University of Maryland, 2024; https://fcid.foodrisk.org/), NHANES 2005–2010; freshwater and estuarine fish and shellfish combined, 90th percentile per capita rate pregnant females only.

stages (Table D-1). Separately, paired bodyweight adjusted FCRs are not available for specific trophic levels which precludes the use of body-weight adjusted DWI rates to derive ambient water quality criteria.

For illustrative purposes, the EPA calculated criteria based on the exposure parameters for women of childbearing age. As demonstrated in Table D-1, criteria based on the exposure inputs for the general population result in more health protective criteria and thus are protective of the potentially susceptible life stage of women of childbearing age (Table D-1). Overall, when bodyweight averages are similar, the resulting criteria are driven predominantly by the FCR; thus, a higher FCR results in a more health protective criteria.

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AMIGOS BRAVOS EXHIBIT 7

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Acute Freshwater Aquatic Life Benchmarks for Eight Data-Limited PFAS: PFBA, PFHxA, PFNA, PFDA, PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA

September 2024

U.S. Environmental Protection Agency Office of Water,
Office of Science and Technology, Health and Ecological Criteria Division,
Ecological Risk Assessment Branch
Washington, D.C.

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Project Team

Technical Analysis Leads:
Mike Elias, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Ecological Risk Assessment Branch Division, Washington, DC (Lead)*

Kathryn Gallagher, Branch Chief, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Ecological Risk Assessment Branch Division, Washington, DC

Interspecies Correlation Estimate Model Contributors:
Crystal Lilavois, Office of Research and Development, Center for Environmental Measuring and Modeling, Gulf Ecosystem Measurement and Modeling Division, Gulf Breeze, FL

Sandy Raimondo, Office of Research and Development, Center for Environmental Measuring and Modeling, Gulf Ecosystem Measurement and Modeling Division, Gulf Breeze, FL

Reviewers:
Colleen Flaherty, Division Director, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC

***Please contact Mike Elias at Elias.mike@epa.gov for any questions.**

Acronyms

AWQC	National Recommended Ambient Water Quality Criteria
CAS	Chemical Abstracts Service
CWA	Clean Water Act
DER	Data Evaluation Record
ECOTOX	ECOTOXicology database
EC_x	Effect concentration at x percent level
EPA	U.S. Environmental Protection Agency
FAV	Final Acute Value
7:3 FTCA	2H,2H,3H,3H-Pefluorodecanoic acid
8:2 FTUCA	2H-Perfluoro-2-decenoic acid
GMAV	genus mean acute value
hpf	hours post fertilization
ICE	Interspecies Correlation Estimation
LC_x	Lethal concentration at x percent level
LOECs	Lowest Observed Effect Concentrations
MDRs	minimum data requirements
MSE	Mean square error
NAMS	New Approach Methods
NOECs	No Observed Effect Concentrations
OCSPP	Office of Chemical Safety and Pollution Prevention
ORD	Office of Research and Development
OW	Office of Water
PFAS	per- and polyfluorinated substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroalkyl carboxylic acid or perfluoroheptanecarboxylic acid
PFOS	Perfluorooctane sulfonic acid or perfluorooctane sulfonate
SMAV	Species Mean Acute Value
SOP	standard operating procedure
SSD	species sensitivity distribution
U.S.	United States
web-ICE	Web-based Interspecies Correlation Estimation
WWTPs	wastewater treatment plants

Notices

This document provides information that states and authorized tribes may consider in their water quality protection programs under the Clean Water Act (CWA) to protect freshwater aquatic life from the acute effects of eight individual per- and polyfluoroalkyl substances (PFAS) substances: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). Toxicity data for these eight PFAS are limited relative to recommended data requirements traditionally used to develop ambient water quality criteria (AWQC) for the protection of aquatic life. Therefore, the United States Environmental Protection Agency (EPA) derived acute PFAS aquatic life freshwater benchmark values under Section 304(a)(2) of the CWA using the best available data, as informational values that states and Tribes may consider in their water quality protection programs, including establishing their water quality criteria. While this document contains the EPA's scientific analyses regarding benchmark values for ambient freshwater concentrations of these eight PFAS protective of aquatic life, this document does not substitute for the CWA or the EPA's regulations; nor is this document or the benchmarks values for the PFAS it presents a regulation itself. Thus, this document does not establish or affect legal rights or obligations, or impose legally binding requirements on the EPA, states, Tribes, or the regulated community. It cannot be finally determinative of the issues addressed. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

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Foreword

This document presents acute freshwater benchmark values for aquatic life in ambient water based upon consideration of all available toxicity information relating to the acute effects of eight individual per- and polyfluoroalkyl substances (PFAS) substances on freshwater aquatic organisms: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). The EPA developed this document to provide information that states and authorized Tribes may consider in their water quality protection programs.

Deborah G. Nagle
Director, Office of Science and Technology

Executive Summary

Per- and polyfluoroalkyl substances (PFASs) are organic, human-made chemical compounds composed of an alkyl chain bound to multiple fluorine atoms. There are estimated to be over 3,000 PFAS present in the global marketplace, that are used in a variety of specialized applications, including surface coatings and a variety of other industrial and commercial products (Wang et al. 2017). In addition to Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS), for which the U.S. Environmental Protection Agency (EPA) has recently developed Aquatic Life Ambient Water Quality Criteria (U.S. EPA 2024b,c), the EPA recognizes that there may be thousands of other PFAS present in the environment. The EPA (2024b,c) provides a detailed discussion of the sources, occurrence, fate and transport of PFOA, PFOS and other PFAS in the environment.

The EPA has developed acute freshwater aquatic life acute ambient water quality benchmarks for eight selected polyfluorinated substances. The eight chemicals for which acute benchmarks were developed are: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). These eight chemicals were selected based on interest by stakeholders and aquatic toxicity data availability.

The Clean Water Act (CWA) supports the protection of U.S. waters by helping to ensure that aquatic species have clean water to live in. States, Tribes, or the EPA may establish concentration limits of pollutants that will ensure that fish and other aquatic species can live, grow, and reproduce. CWA Section 304(a) directs the EPA to develop and publish water quality criteria that reflect the latest scientific knowledge.

National recommended ambient water quality criteria for the protection of aquatic life establish chemical concentrations (magnitude) that are averaged over a given time period (duration) and should not be exceeded more than once during a specified time period (frequency). The EPA's 304(a)(1) criteria recommendations generally follow the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (hereafter "Aquatic Life Criteria Guidelines" (U.S. EPA 1985). The Aquatic Life Criteria Guidelines recommend toxicity data for a minimum of eight families of aquatic animals be used in developing aquatic life criteria to protect aquatic ecosystems as a whole.

Aquatic life benchmarks, developed under 304(a)(2) of the CWA, are informational values that the EPA generates when there are limited high quality toxicity data available and data gaps exist for several families of aquatic organisms. Aquatic life benchmarks provide information that states and Tribes may consider in their water quality protection programs. In developing aquatic life benchmarks, data gaps may be filled using new approach methods (NAMs), such as computer-based toxicity estimation tools (e.g., the EPA's Web-ICE; Version 4.0) or other new approach methods intended to reduce reliance on animal-based testing (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical), including the use of read-across estimates based on other chemicals with similar structures. The EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's water quality standards.

Because direct empirical data are more limited for the eight PFAS evaluated within this document and do not fulfill MDRs as described by the Aquatic Life Criteria Guidelines (U.S. EPA 1985), the EPA developed acute protective benchmarks for these eight PFAS using available freshwater species empirical test data in conjunction with the application of the EPA's

peer-reviewed web-based Interspecies Correlation Estimate (ICE) tool (Web-ICE; Version 4.0; https://www.epa.gov/webice/) (Raimondo et al. 2010). ICE models support the derivation of acute values, and do not currently support the derivation of chronic values. This document accordingly focuses on the derivation of acute aquatic life benchmarks. These acute aquatic life benchmarks, developed using the direct empirical and ICE (estimated) data for these chemicals, were calculated by applying methods described in the EPA Aquatic Life Criteria Guidelines (U.S. EPA 1985).

In addition to addressing data limitations through NAMs, the EPA also developed application factors to account for species that are known to show markedly greater sensitivity to PFOA and PFOS. Specifically, Soucek et al. (2023) and Razak et al. (2023) indicated the mayfly, *Neocloeon triangulifer,* and the cladoceran, *Moina micrura,* are markedly more sensitive to PFOA and PFOS relative to other species tested. The EPA accordingly derived the adjustment factors from the available PFOA and PFOS data and applied them to the eight PFAS benchmarks to account for greater sensitivity of these species. The inclusion of the application factors protect the many untested species in the environment, some of which may be similarly sensitive to these PFAS as the mayfly and cladoceran species noted above. Development of these PFAS benchmarks reflects goals in the EPA's PFAS Strategic Roadmap and aligns with the Agency's intention to reduce the use of additional animal testing through application of NAMs. They provide information to states and Tribes regarding protective values for aquatic life for these eight data-limited PFAS.

This document describes the process used and results of a systematic review of available direct empirical toxicity data for aquatic organisms identified via the EPA's literature search for the eight selected PFAS substances, inclusive of their anionic, acid, and ammonium salt forms.

The derived acute benchmark values are summarized for each of the eight evaluated PFAS in **Table Ex-1**. These concentrations are expected to be protective of 95% of freshwater genera exposed to the listed PFAS under short term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. However, because only limited toxicity test data were available for these eight chemicals and aquatic toxicity was estimated for some taxa to develop these benchmarks, the values are somewhat less certain than national recommended Aquatic Life AWQC developed using more robust empirical data sets (i.e., those that meet the MDRs according to the EPA's Aquatic Life Criteria Guidelines). Further, while the eight MDRs were fulfilled for six of the PFAS chemicals when direct empirical and ICE data were combined, only seven of the eight MDRs were fulfilled for PFBS and only six of the eight MDRs were fulfilled for PFHxS when data were combined, and there is considered to be greater uncertainty associated with these benchmark values.

Table Ex-1. Acute Freshwater Aquatic Life Benchmarks for Eight PFAS.

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude[1]	5.3	4.8	0.65	0.50	5.0	0.21	0.037	0.012
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

[1] Values expressed as mg/L, or ppm.

The EPA additionally explored the application of a data "binning" approach, used by Giddings et al. (2019) for pyrethroids, as an exploratory approach for deriving protective acute aquatic life values for grouped perfluoroalkyl carboxylic acids and grouped perfluoroalkyl sulfonic acids. This approach is based on combining chemicals with similar modes of action to provide more robust datasets to support value derivation in data-limited situations. The EPA followed this methodology to explore the calculation of protective benchmark values for six of

the PFAS, based on the combined perfluoroalkyl carboxylic acid (PFBA, PFHxA, PFNA, PFDA) and perfluoroalkyl sulfonic acid (PFBS, PFHxS) groupings, that were used to support value derivation.

1 BACKGROUND

This document describes the development of acute water quality benchmarks to protect freshwater aquatic life from acute toxic effects of eight PFAS substances and their related forms:

- Perfluorobutanoic acid (PFBA)(CAS# 375224, 45048622, 2218544)
- Perfluorohexanoic acid (PFHxA)(CAS# 307244, 92612527)
- Perfluorononanoic acid (PFNA)(CAS# 375951)
- Perfluorodecanoic acid (PFDA)(CAS# 335762)
- Perfluorobutanesulfonic acid (PFBS)(CAS# 375735, 108427527, 29420493)
- Perfluorohexanesulfonic acid (PFHxS)(CAS# 355464, 108427538, 3871996, 82382125)
- 2H-Perfluoro-2-decenoic acid (8:2 FTUCA)(CAS# 70887842)
- 2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)(CAS# 812704)

These eight chemicals were selected based on interest by stakeholders and aquatic toxicity test data availability. Notably, the U.S. Department of Defense (DOD) published a set of draft Ecological Screening Values (Grippo et al. 2021) for six of these substances (PFBA, PFHxA, PFNA, PFDA, and PFHxS) based on stated concerns about the historic release of these substances at DOD facilities. The ECOTOXicology Knowledgebase (ECOTOX; https://cfpub.epa.gov/ecotox/), a curated source of high-quality toxicity data for aquatic life, terrestrial plants, and wildlife, was also searched to identify PFAS, besides PFOA and PFOS, that have some of the greatest numbers of empirical studies available to support benchmark development.

In addition to PFOA and PFOS, for which the EPA has recently developed final Aquatic Life AWQC, the EPA is aware of thousands of other PFAS chemicals that may be present in the environment. The above eight chemicals were selected to represent a range of PFAS that are present in aquatic ecosystems and of concern to stakeholders (e.g., states, Tribes, DOD. Another important consideration for selection was the availability of both acute direct empirical and ICE model toxicity data for these chemicals.

The EPA typically follows the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA 1985), which requires toxicity data for a minimum of eight families be available to fulfill taxonomic MDRs to calculate freshwater Aquatic Life AWQC. The MDR groups represent taxa with different ecological, trophic, taxonomic and functional characteristics in aquatic ecosystems, and are intended to collectively represent the diversity and range of sensitivity of species within an aquatic community. The MDRs, as defined by the Aquatic Life Criteria Guidelines, consist of the following representative taxa:

a) Family Salmonidae in the class Osteichthyes
b) Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
c) Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
d) Planktonic crustacean (e.g., cladoceran, copepod, etc.)
e) Benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
f) Insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
g) Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
h) Family in any order of insect or any phylum not already represented

For some contaminants, particularly those with newer or novel chemistries and emerging contaminants, these MDRs often cannot be met, and an alternative approach is necessary to provide information to states and Tribes under CWA Section 304(a). In this case, empirical acute data for these eight PFAS fulfill some but not all of these MDRs. Accordingly, the EPA applied the peer reviewed Interspecies Correlation Estimation (ICE) models (Raimondo et al. 2010, 2024) to estimate acute toxicity data to fulfill MDRs for which direct empirical toxicity data are not available. ICE models support the derivation of acute values, and do not currently support the derivation of chronic values and/or the values associated with potential bioaccumulative effects**.**

This document accordingly focuses on the derivation of acute aquatic life benchmarks. The EPA has previously used this same approach to derive final acute estuarine/marine benchmarks for PFOA and PFOS (U.S. EPA 2024b,c).

As noted above, the EPA used the acute empirical test data for these chemicals supplemented with the ICE values derived for the missing MDRs to derive acute freshwater benchmark recommendations for aquatic life using calculation procedures provided in the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The EPA also developed application factors to account for species that are known to show markedly greater sensitivity to PFOA and PFOS. Specifically, Soucek et al. (2023) and Razak et al. (2023) indicated the mayfly, *Neocloeon triangulifer,* and the cladoceran, *Moina micrura,* are markedly more sensitive to PFOA and PFOS relative to other species tested. The EPA accordingly derived the adjustment factors from the available PFOA and PFOS data and applied them to the eight PFAS benchmarks to account for greater sensitivity of these species. The inclusion of the application factors protect the many untested species in the environment, some of which may be similarly sensitive to these PFAS as the mayfly and cladoceran species noted above. The above approaches are consistent with the Aquatic Life Criteria Guidelines "good science" clause, the EPA's interest in providing useful information to states and Tribes regarding protective benchmark values for aquatic life, and also the EPA's intention to reduce the use of animal testing via application of NAMS in toxicity assessment (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing).

The EPA additionally explored the application of a data "binning" approach, used by Giddings et al. (2019), to derive protective aquatic life values for grouped carboxylic acid PFASs and grouped sulfonic acid PFAS. This approach, which is detailed in **Appendix G**, is based on

combining chemicals with similar modes of action to provide more robust datasets to support value derivation in data-limited situations.

2 INTRODUCTION TO ICE

Interspecies Correlation Estimation (ICE) models are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals that represent the relationship of inherent sensitivity between those species (**Figure 2-1**). ICE models are derived from an extensive, standardized database of acute toxicity values by pairing each species with every other species for which acceptable toxicity data are available (Raimondo et al. 2010, 2024). ICE models are used to predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis).

ICE models have been developed from a broad range of chemicals (e.g., metals and other inorganics, pesticides, solvents, and reactive chemicals) and across a wide range of toxicity values and have been validated as accurate predictors of acute toxicity when model criteria parameters are followed (Raimondo et al. 2024). The Web ICE application (v4.0, https://www3.epa.gov/webice/) contains approximately 4,800 statistically-significant ICE models for aquatic animal and plant species.

Validation and uncertainty analyses of ICE models are founded in both leave-one-out cross validation and direct comparison of measured and predicted acute values (Raimondo et al. 2010, 2024; Willming et al. 2016). Acute toxicity predictions have high accuracy when values are derived from models with robust parameters (e.g., mean square error and R^2 of regression between the tested and untested species), and variability of prediction accuracy is consistent with that of empirical test data (Raimondo et al. 2024). **Box 1** below outlines the parameter measures used to define acceptable ICE models for evaluation. Models identified as acceptable based on

the parameter measures identified in **Box 1** were further evaluated for use as discussed in **Sections 4.2** and **5.1**.

ICE models have undergone extensive peer review and their use has been supported through external peer review for development of aquatic life benchmark values for saltwater and estuarine species exposed to PFOA and PFOS (U.S. EPA 2024b,c). The application of ICE-predicted values to develop protective aquatic life values by multiple independent, international groups confirms that values developed from ICE-generated species sensitivity distributions (SSDs) provide a level of protection that is consistent with using measured laboratory data (Dyer et al. 2008; Feng et al. 2013; Fojut et al. 2012a,b; Palumbo et al. 2012; Wu et al. 2015, 2016; Wang et al. 2020; Zhang et al. 2017). A recent review of ICE models additionally supports their use in regulatory applications based on the reliability of underlying data, model transparency, statistical robustness, predictive reliability, proof of principle, applicability to probabilistic approaches, and reproducibility of model accuracy by numerous independent research teams (Bejarano and Wheeler 2020).

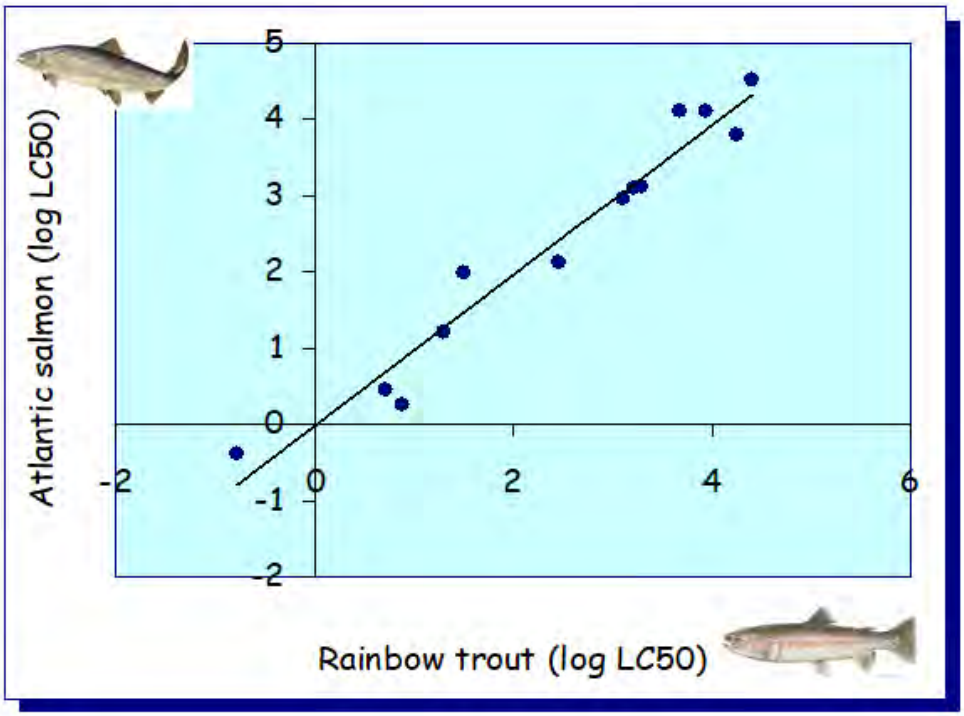


Figure 2-1. Example of ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested with both species to develop a log-linear model.

Box 1. ICE Model User Parameter Criteria for Robust Predictions (Willming et al. 2016, Raimondo et al. 2024):

- Low mean squared error (MSE) (<~ 0.95)
- High R^2 value (>~0.6)
- High slope (>~0.6; 0.66 – 1.33 when µg/L input is beyond model domain)
- Confidence interval range of 2 orders of magnitude between lower and upper limit
- For models between vertebrates and invertebrates, using those with lower MSE or MOA-specific models (not available for PFAS) has been recommended for listed species predictions

3 APPLICATION OF ICE WITH SELECTED PFAS

ICE models for freshwater species were used to predict acute toxicity of the eight PFAS from measured sensitivity of surrogate species listed in **Table 3-1**. For the application of ICE models, the type and number of predicted species available to fulfill missing MDRs in the selected PFAS acute criteria dataset was based on the number of models available within ICE for the surrogate species (**Table 3-1**). It is important to note, not all available ICE models met the prediction robustness criteria outlined in Box 1 for quantitative use. Accordingly, an evaluation of the predictions was conducted following the acquisition of all available predicted values from all available surrogate species for each compound.

Table 3-1. Measured Surrogate Species Sensitivity Values and Number of Available ICE Models for Eight PFAS.
The number of freshwater ICE models for each surrogate excludes models that predict values for species for which measured acute toxicity data are already available. Available ICE models are presented in Appendix E.

Chemical	Taxa	Surrogate Species	Acute Toxicity (mg/L)	Number of Freshwater ICE Models
PFBA	Vertebrate	*Danio rerio*	13,779	18 (5 Juveniles, 13 Embryo)
	Invertebrate	*Daphnia magna*	5,251	69
PFHxA	Invertebrate	*Daphnia magna*	1,048	69
	Vertebrate	*Lithobates catesbeianus*	1,105	8
PFNA	Invertebrate	*Chydorus sphaericus*	28	2
	Invertebrate	*Daphnia magna*	85	69
	Vertebrate	*Xenopus laevis*	336	4
PFDA	Invertebrate	*Chydorus sphaericus*	41	2
	Invertebrate	*Daphnia magna*	120	69
	Invertebrate	*Daphnia pulicaria*	150	2
	Vertebrate	*Oncorhynchus mykiss*	32	78
	Vertebrate	*Xenopus laevis*	77	4
	Invertebrate	*Daphnia magna*	2,183	69
	Vertebrate	*Lepomis macrochirus*	6,452	76
	Vertebrate	*Pimephales promelas*	1,938	74

Chemical	Taxa	Surrogate Species	Acute Toxicity (mg/L)	Number of Freshwater ICE Models
PFHxS	Vertebrate	*Danio rerio*	23	18 (5 Juveniles, 13 Embryo)
	Vertebrate	*Lithobates catesbeianus*	1,105	8
8:2 FTUCA	Invertebrate	*Daphnia magna*	3	69
	Vertebrate	*Oncorhynchus mykiss*	81	78
7:3 FTCA	Invertebrate	*Daphnia magna*	1	69
	Vertebrate	*Oncorhynchus mykiss*	32	78

PFAS acute values (typically reported as mg/L) can be beyond the range of values used to calibrate the ICE model (ICE database toxicity range $1x10^{-4}$ and $1x10^{8}$ μg/L) such that the input PFAS value of the surrogate would be outside the model domain. In situations where the input PFAS value is beyond the model range, a user can either:

- Enter the measured toxicity value (LC_{50}/ EC_{50}) into the ICE model as μg/L and allow the regression to **extrapolate** beyond the model range; or
- "**Scale**" the toxicity value by entering the measured LC_{50} value as mg/L, bringing the input value within the model range.

Because it is recognized that extrapolating beyond the model range can result in large confidence intervals around the prediction and can result in the acceptance of fewer ICE models based on acceptability parameters (see **Box 1**), a "scaled" approach was considered by Raimondo et al. (2024) as an alternative approach to predicting species sensitivity to place the input value within the model domain. The scaled approach underwent detailed evaluation by Raimondo et al. (2024), who compared the two approaches and reported that ICE models predict toxicity with the same level of accuracy using scaled and extrapolated input values, with smaller confidence intervals for the scaled approach (allowing acceptance of more ICE models). The first ("extrapolated") approach was selected as the primary approach for deriving the benchmark

values. The "scaled" approach was applied as an alternative approach and serves as an additional analysis to compare SSDs and HC5s with those based on extrapolated values and are summarized in **Section 5.10** and presented in detail in **Appendix F**.

Figure 3-1 provides an example of the approach taken to calculate a toxicity value when an LC_{50} or EC_{50} value falls beyond the ICE model range, through the use of either an "extrapolation" or "scaled" approach. The data plots and calculations below provide an illustrative example using an EC_{50} value for *Daphnia magna* for PFBS.

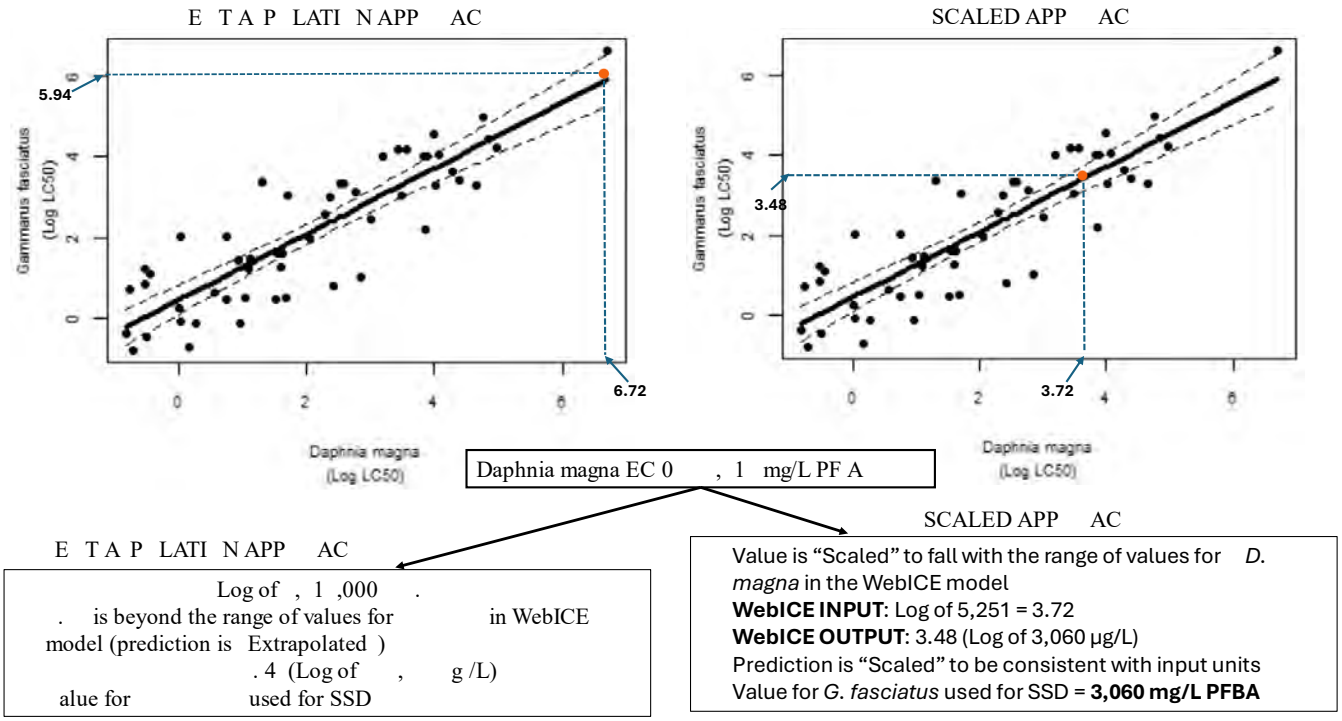


Figure 3-1. Example Calculation for *Daphnia magna* PFBA Toxicity Data Based on Extrapolation and Scaled Approaches.

4 EFFECTS ANALYSIS FOR AQUATIC LIFE

4.1 Summary and Evaluation of Available Empirical Toxicity Test Data

The EPA reviewed and considered all relevant empirical toxicity test data for the eight PFAS. Information available for all relevant species and genera were reviewed to identify data from acceptable tests that meet data quality standards. All available empirical studies relating to the acute toxicological effects of each PFAS on aquatic life were considered in the derivation of these freshwater acute benchmarks. Empirical data for possible inclusion in the derivation of the benchmarks were obtained from published literature reporting acute exposures of the selected PFAS that were associated primarily with mortality/survival. The development of benchmarks included the review of a range of forms of each PFAS substance for which data were available, as summarized in **Table 4-1**. **Figure 4-1** presents representative chemical structures for these PFAS. With the exception of 8:2 FTUCA, all of these PFAS can be analyzed using the EPA Method 1633 (U.S. EPA 2024a).

Table 4-1. Summary Table of PFAS Forms and Associated CAS Numbers Identified for the Evaluation of Available Empirical Data.

PFAS	Form	Chemical Formula	CAS Number
Perfluorobutanoic acid (PFBA)	PFBA	$C_4HF_7O_2$	375224
	PFBA Ion	$C_4F_7O_2^-$	45048622
	PFBA-Na	$C_4F_7NaO_2^-$	2218544
Perfluorohexanoic Acid (PFHxA)	PFHxA	$C_6HF_{11}O_2$	307244
	PFHxA Ion	$C_6F_{11}O_2^-$	92612527
Perfluorononanoic acid (PFNA)	PFNA	$C_9HF_{17}O_2$	375951
Perfluorodecanoic Acid (PFDA)	PFDA	$C_{10}HF_{19}O_2$	335762
Perfluorobutanesulfonic acid (PFBS)	PFBS	$C_4HF_9O_3S$	375735
	Tetrabutylammonium perfluorobutanesulfonate	$C_{20}H_{36}F_9NO_3S$	108427527
	PFBS.K	$C_4F_9KO_3S$	29420493

PFAS	Form	Chemical Formula	CAS Number
Perfluorohexanesulfonic acid (PFHxS)	PFHxS	$C_6HF_{13}O_3S$	355464
	Perfluorohexanesulfonate	$C_6F_{13}O_3S^-$	108427538
	PFHxS.K	$C_6F_{13}KO_3S$	3871996
	PFHxS.Na	$C_6F_{13}NaO_3S$	82382125
2H-Perfluoro-2-decenoic acid (8:2 FTUCA)	8:2 FTUCA	$C_{10}H_2F_{16}O_2$	70887842
2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)	7:3 FTCA	$C_{10}H_5F_{15}O_2$	812704

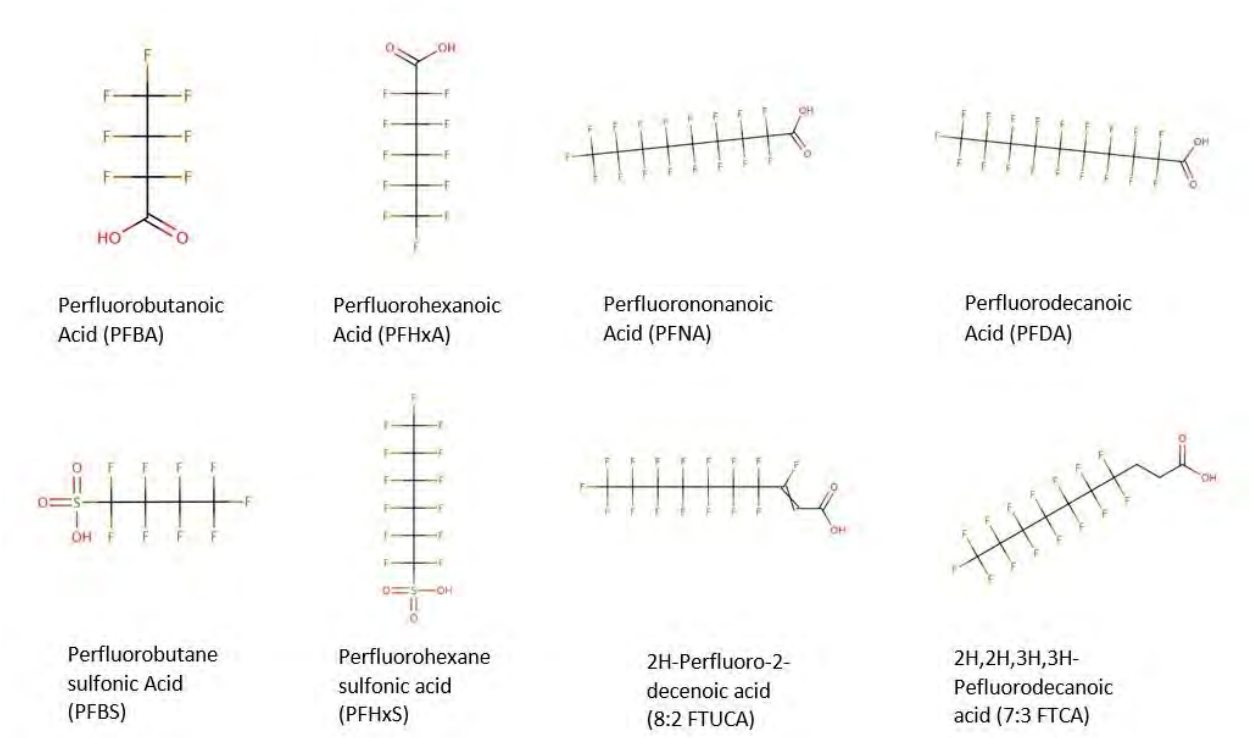


Figure 4-1. Chemical Structures of Selected PFAS.

Empirical studies available for the eight PFAS were identified using ECOTOX. Toxicity studies accessed from the ECOTOX database were further evaluated. Studies were evaluated for data quality as described by the EPA W's data quality standard operating procedure (SOP), and

consistent with W 's data quality review approach (U.S. EPA 1985), and the EPA's f fice of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016). The EPA completed a Data Evaluation Record (DER) for each species empirical toxicity test from the studies identified by ECOTOX for the eight PFAS undergoing evaluation. This in-depth review ensured the studies used for derivation would result in robust, scientifically-defensible benchmarks. Studies that did not fully meet the data quality objectives outlined in the EPA SOP were not considered for inclusion in the benchmark derivation, including some studies with other PFAS exposures. Further, only single chemical toxicity tests (i.e., not tests with chemical mixtures) were considered for possible inclusion in benchmark derivation. When available, both controlled laboratory experiments and field observations/studies were evaluated for inclusion.

Due to the EPA's interest in using all available quality data, particularly for these data-limited chemicals, toxicity studies were considered for possible inclusion regardless of the test species residential status in North America. Use of non-North American residential species is also consistent with other published aquatic life criteria (U.S. EPA 2018). Non-North American resident species also help to serve as taxonomically-related surrogate organisms for untested resident species.

Toxicity data from tests reporting either measured or nominal concentrations were considered for quantitative use in benchmark derivation. Toxicity tests used in many of the EPA's previous aquatic life criteria documents are typically based only on measured chemical concentrations. For PFOA and PFOS, the EPA examined the issue of whether nominal (unmeasured) and measured concentrations are in close agreement with each other (U.S. EPA 2024b,c). For both PFOA and PFOS, pairs of nominal and corresponding measured

concentrations were compared to one another through: (1) linear correlation analysis and; (2) an assessment of measured concentrations as a percent of its paired nominal concentration. For PFOA 83% of measured freshwater concentrations and 82% of the measured concentrations for PFOS fell within 20% of paired nominal concentrations, which represent the test acceptability threshold identified by the EPA's CS PP's Ecological Effects Test Guidelines. Based on the results for PFOA and PFOS (U.S. EPA 2024b,c), the EPA concluded that nominal test concentrations adequately represent actual PFAS exposures in standard acute laboratory-based toxicity tests and PFAS toxicity tests were not excluded from quantitative use in benchmark derivation on the basis of unmeasured test concentrations alone. Resultingly, renewal, and flow-through experiments were considered for inclusion for species mean acute values regardless of whether the PFAS concentrations were measured.

Available empirical acute data meeting the quality objectives and test requirements were utilized quantitatively in conjunction with ICE values in deriving benchmarks for aquatic life. The number of acute genus and species level mean values and MDRs fulfilled by the quantitatively acceptable empirical acute toxicity data available for each of the eight PFAS is summarized in **Table 4-2** and detailed in **Appendix A: Quantitatively Acceptable Freshwater Acute Toxicity Studies**. Studies that were determined to be qualitatively acceptable as supporting information, but not acceptable for quantitative use, are summarized in **Appendix C: Qualitative Freshwater Acute Toxicity Studies**. Studies that were not considered acceptable for quantitative or qualitative use are summarized in **Appendix D: Unused Freshwater Acute Toxicity Studies**.

Acute values were presented as reported by the study authors for each individual study. Author-reported toxicity values were included in each study summary included in corresponding

appendices, as applicable. The results of all toxicity values (e.g., individual toxicity test outcomes and species- and genus-mean values) are given to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value. The specific toxicity value utilized in the derivation of the corresponding benchmarks is stated for each study at the end of the summaries in **Appendix A**.

Table 4-2. Number of Acute Genus and Species Level Mean Values in the Empirical Toxicity Datasets and Freshwater Minimum Data Requirements Fulfilled per the Aquatic Life Criteria Guidelines for the Eight PFAS.

MDR	PFBA	PFHxA	PPFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Family Salmonidae in the class Osteichthyes	-	-	-	1	-	-	1	1
Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	1	-	-	-	3	1	-	-
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	-	2	1	1	-	2	-	-
Planktonic Crustacean	3	1	2	3	1	-	1	1
Benthic Crustacean	-	-	-	-	-	-	-	-
Insect	-	-	-	-	-	-	-	-
Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, or Mollusca)	-	-	-	-	-	-	-	-
Family in any order of insect or any phylum not already represented	1	1	-	-	-	-	-	-
Total number of empirical studies	**5**	**4**	**3**	**5**	**4**	**3**	**2**	**2**
MDRs fulfilled with empirical data	**3**	**3**	**2**	**3**	**2**	**2**	**2**	**2**

4.2 Prediction of Species Sensitivity Using ICE Models

Values for the selected PFAS were first predicted with Web-ICE v4.0 for all possible species. The resulting ICE models are summarized in **Appendix E.** These ICE models were then evaluated using the acceptance parameters (e.g., MSE, R^2, slope) summarized in **Box 1**, provided

above under **Section 2**. Only ICE models based on freshwater species were considered for use. Models that met these acceptance parameters were used to derive species toxicity data, which were used in conjunction with empirical toxicity data to support the derivation of the benchmark values. The ICE models selected for use are summarized in each of the chemical-specific discussions presented in **Section 5**.

5 DERIVATION OF ACUTE WATER QUALITY BENCHMARKS FOR SELECTED PFAS

5.1 Approach

5.1.1 Use of Empirical and ICE Data

The following section describes the process used to derive the water quality benchmarks for each of the eight PFAS. The first step encompassed development of the acute toxicity dataset used to support the derivation of each benchmark, which involved combining available empirical test data with ICE-derived toxicity data in order to both meet the eight MDRs, as described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985), and provide as robust of a dataset as possible for benchmark derivation. The following approach was used to develop the dataset for the derivation of each acute PFAS benchmark:

- All acceptable empirical data (evaluated as described in **Section 4.1** and summarized in **Appendix A** and **B**) were used and prioritized for benchmark derivation. Accordingly, if both acceptable empirical and ICE-estimated LC_{50}/ EC_{50} values were available for a species, then only the empirical data were averaged to derive that SMAV, based on the greater certainty assumed to be associated with quantitatively-acceptable empirical test data.
- ICE-derived toxicity data were derived from acceptable ICE models (as described in **Section 4.2** and summarized in **Appendix E**), and used to derive toxicity data for each PFAS, with the objective of fulfilling gaps in the MDRs and creating an overall more robust acute dataset for benchmark derivation. As noted, ICE-estimated LC_{50}/EC_{50} values were used to derive SMAVs, except when empirical data were available. If multiple acceptable ICE models were available for a given species (based on prediction by

multiple surrogates) then only the model with the closest taxonomic relationship was used to support deriving the SMAV.

Following development of the toxicity database, values were then calculated using the same basic procedure as described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) for the derivation of acute criteria. Please refer to the Aquatic Life Criteria Guidelines for a detailed description of this approach. The primary difference between the derivation of aquatic life criteria versus the derivation of protective acute benchmarks in this document is in the composition of the constituent dataset, which consists of both empirical test and ICE-derived acute toxicity data, since the eight MDRs could not be fulfilled for these compounds using the available empirical test data.

Consistent with the Aquatic Life Criteria Guidelines (U.S. EPA 1985), acceptable empirical data were then used in conjunction with ICE estimated LC_{50} values to derive a "preliminary" value as follows:

- Species mean acute values (SMAVs) were derived based on the availability of multiple acceptable empirical or the selected ICE-estimated values by combining the accepted data into species-level groupings and calculating the means for each grouping.
- Genus Mean Acute Values (GMAVs) were then calculated by combining the SMAVs (comprised of both empirical data and ICE-estimated values) into genus-level groupings and calculating the means for each grouping.
- Sensitivity Distributions (SD) were developed from the GMAVs
- The four GMAVs closest to the 5th percentile of the distribution were used to calculate the Final Acute Value (FAV).

- The FAV is then divided by two, which is intended to estimate a minimal effect level and provide an acute value that is protective of nearly all individuals in the distribution (U.S. EPA 1985).

5.1.2 Application Factor to Adjust for Additional Unrepresented Sensitive Species Data

It was noted that no acceptable empirical data and limited ICE-estimated toxicity data were available for some taxa. This is particularly notable as recent investigations with both PFOA and PFOS indicate the mayfly, *Neocloeon triangulifer* (Soucek et al. 2023) and the cladoceran, *Moina micrura* (Razak et al. 2023) are among the most acutely sensitive of any species tested to these substances amongst the acceptable data, as determined by the EPA:

- For PFOA, the author-reported acute EC_{50} (i.e., 0.4747 mg/L) for *M. micrura* from a new study (Razak et al. 2023) was determined to be acceptable for quantitative use and was averaged as a geometric mean with other quantitatively acceptable acute *Moina* data, yielding the most sensitive genus mean acute value (8.885 mg/L). The EPA-calculated acute LC_{50} (i.e., 13.045 mg/L) for *N. triangulifer* from Soucek et al. (2023) was acceptable for quantitative use and was the second most sensitive genus mean acute value in the final PFOA acute freshwater criterion.
- For PFOS, the EPA-calculated acute LC_{50} (i.e., 0.07617 mg/L) for *N. triangulifer* was acceptable for quantitative use and was the most sensitive genus mean acute value in the final PFOS acute freshwater criterion. The new author-reported acute LC_{50} (i.e., 0.5496 mg/L) for *M. micrura* (Razak et al. 2023) was acceptable for quantitative use and was averaged as a geometric mean with other *Moina* data to yield the second most sensitive genus mean acute value (3.075 mg/L) in the final acute PFOS criterion.

These data were not available when the draft freshwater PFOA and PFOS acute aquatic life criteria were released (U.S. EPA 2022a,b), but were incorporated into the final freshwater acute

criteria released for these compounds (U.S. EPA 2024b,c), significantly lowering the derived freshwater acute values for these substances:

- For PFOA, the final acute freshwater criterion is 3.1 mg/L, compared to the draft acute criterion of 49 mg/L, and the addition of the acute value for *N. triangulifer* along with the addition of the sensitive *M. micrura* data decreased the criterion by a factor of 15.8.
- For PFOS, the final acute freshwater criterion is 0.071 mg/L, compared to the draft acute criterion of 3.0 mg/L, and the addition decreased the criterion by a factor of 42.3.

Based on the limited available data and to account for this marked difference in sensitivity, the EPA applied an application factor to the calculation of each PFAS benchmark value, to account for the absence of empirical insect, and other sensitive invertebrate, data and the markedly greater sensitivity of the mayfly *N. triangulifer*, and *M. micrura.* The following application factors were used:

- For PFAS with carboxylate groups (PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, and 7:3 FTCA), an application factor of 15.8 was used based on the difference in draft and final PFOA acute criterion values when the additional sensitive species data were added.
- For PFAS with sulfonate groups (PFBS and PFHxS), an application factor of 42.3 was similarly used.

These application factors were used to convert the "preliminary" benchmark value to a final benchmark value. **Table 5-1** provides a summary of the application factors developed to adjust for insect sensitivity.

Table 5-1. Application Factors Applied to Adjust for Additional Unrepresented Sensitive Species Data.

Chemical	Draft Acute Freshwater Criterion (mg/L)	Final Acute Freshwater Criterion (mg/L)	Application Factor	PFAS Applied To
PFOA	49	3.1	15.8	PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, 7:3 FTCA
PFOS	3.0	0.071	42.3	PFBS, PFHxS

Final benchmark values were calculated by dividing the value derived from the sensitivity distribution using procedures presented in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) by the relevant application factors, to derive benchmark values protective of the aquatic community, including sensitive aquatic insects. The following sections detail the derivation of the acute benchmark values for each of the eight PFAS.

5.2 Derivation of Acute Water Benchmark for Perfluorobutanoic Acid (PFBA)

Quantitatively acceptable empirical data for PFBA were available for five species comprising four genera and fulfilling three MDR groups (**Tables 4-2** and **5-3**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 22 ICE models were acceptable for use. Of these, three ICE models were not selected for use because empirical data were available for the three genera (i.e., models for *Daphnia pulex*, *Chydorus sphaericus* and *Danio rerio*). One other model was not used because there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Pimephales promelas*). A total of 18 ICE models were selected for use, resulting in ICE models predicting 18 SMAVs, representing 18 genera and seven MDRs (**Tables 5-2** and **5-3**).

The combined empirical and ICE data resulted in 22 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-3**. GMAVs for the four most sensitive genera were within a factor of eight of each other (**Table 5-4**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFBA is 166.1 mg/L (**Table 5-4**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus*, GMAV=110 mg/L). The FAV was divided by two to obtain a preliminary value of 83.03 mg/L PFBA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 5.3 mg/L PFBA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-1**).

Table 5-2. Acceptable Models for ICE-estimated Species Sensitivity to PFBA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	5,251**	0.0002	5,000	**878.8** (199.6-3,869.5)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	5,251	0.0003	8,694.5	**2,248.7** (1,108.2-4,562.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	5,251	0.0001	46,278.3	**959.0** (508.0-1,810.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	5,251	0.0003	46,278.3	**4,100.3** (2,125.4-7,909.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	5,251**	0.0002	4,894.7	4,297.8 (2,058.5-8,973.1)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	5,251	0.0002	46,500	1,174.4[b] (655.0-2,105.8)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	13,779	0.023	54,579	**11,531.9** (6,189.6-21,485.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**2,013.5** (809.4-5,008.9)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	13,779**	7.797	649.3	**85,148.8** (12,990.4-558,127.6)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	0.145	304.5	**19,384.5** (4,544.6-82,682.6)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	5,251	0.37	14,500	**12,920.8** (3,394.0-49,189.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	5,251**	0.011	281.6	**7,457.2** (1,357.9-40,952.1)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,748.6** (862.3-3,545.6)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	5,251	0.0001	14,500	**677.7** (396.2-1,159.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**3,490.7** (670.8-18,164.6)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**3,740.1** (1,061.7-13,176.1)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	5,251**	0.014	4,894.7	**881.4** (169.6-4,582.0)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**2,852.6** (949.6-8,569.8)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,759.2** (742.4-4,168.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	5,251**	0.009	977.6	1,522.7 (748.6-3,097.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,528.3** (497.6-4,694.2)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	5,251	0.0001	46,500	1,391.4 (413.4-4,683.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-3. Ranked PFBA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**110.0**	**110.0**	**0.04**
2	A	Rainbow trout	*Oncorhynchus mykiss*	677.7	677.7	0.09
3	E	Amphipod	*Gammarus fasciatus*	878.8**	878.8	0.13
4	G	Threeridge	*Amblema plicata*	881.4**	881.4	0.17
5	B	Bluegill	*Lepomis macrochirus*	959.0	959.0	0.22
6	G	Western pearlshell	*Margaritifera falcata*	1,528	1,528	0.26
7	G	Paper pondshell	*Utterbackia imbecillis*	1,749	1,749	0.30
8	G	Washboard	*Megalonaias nervosa*	1,759	1,759	0.35
9	G	Fatmucket	*Lampsilis siliquoidea*	2,013	2,013	0.39
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2,249	2,249	0.43
11	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2,853	2,853	0.48
12	G	Swamp lymnaea	*Lymnaea stagnalis*	3,491	3,491	0.52
13	G	Tadpole physa	*Physella gyrina*	3,740	3,740	0.57
14	D	Daphnid	*Ceriodaphnia dubia*	4,100	4,100	0.61
15	**D**	**Daphnid**	***Chydorus sphaericus***	**>4,281**	**>4,281**	**0.65**
16	**D**	**Daphnid**	***Daphnia magna***	**4,741**	**4,741**	**0.70**
	D	**Daphnid**	***Daphnia pulicaria***	**>1,006[a]**		
17	E	Oriental river shrimp	*Macrobrachium nipponense*	7,457**	7,457	0.74
18	B	Fathead minnow	*Pimephales promelas*	11,532	11,532	0.78
19	F	Midge	*Paratanytarsus parthenogeneticus*	12,921	12,921	0.83
20	**B**	**Zebrafish**	***Danio rerio***	**13,779**	**13,779**	**0.87**
21	B	Goldfish	*Carassius auratus*	19,385**	19,385	0.91
22	C	Flagfish	*Jordanella floridae*	85,149**	85,149	0.96

a Not used in GMAV calculation, because values represents a greater than low value

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-4. PFBA Protective Aquatic Life Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=22 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**110.0**	4.70	22.09	0.043	0.209
2	*Oncorhynchus*	677.8	6.52	42.49	0.087	0.295
3	*Gammarus*	878.8	6.78	45.95	0.130	0.361
4	*Amblema*	881.4	6.78	45.99	0.174	0.417
		Σ (Su):	**24.78**	**156.53**	**0.43**	**1.28**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 125.08 S = slope
L = 2.612 L = X-axis intercept
A = 5.112 A = lnFAV
FAV = 166.1
FAV/2 = 83.03 mg/L (Preliminary Value)
Adjustment = 83.03 / 15.8 = 5.255 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **5.3 mg/L PFBA** (rounded to two significant figures)

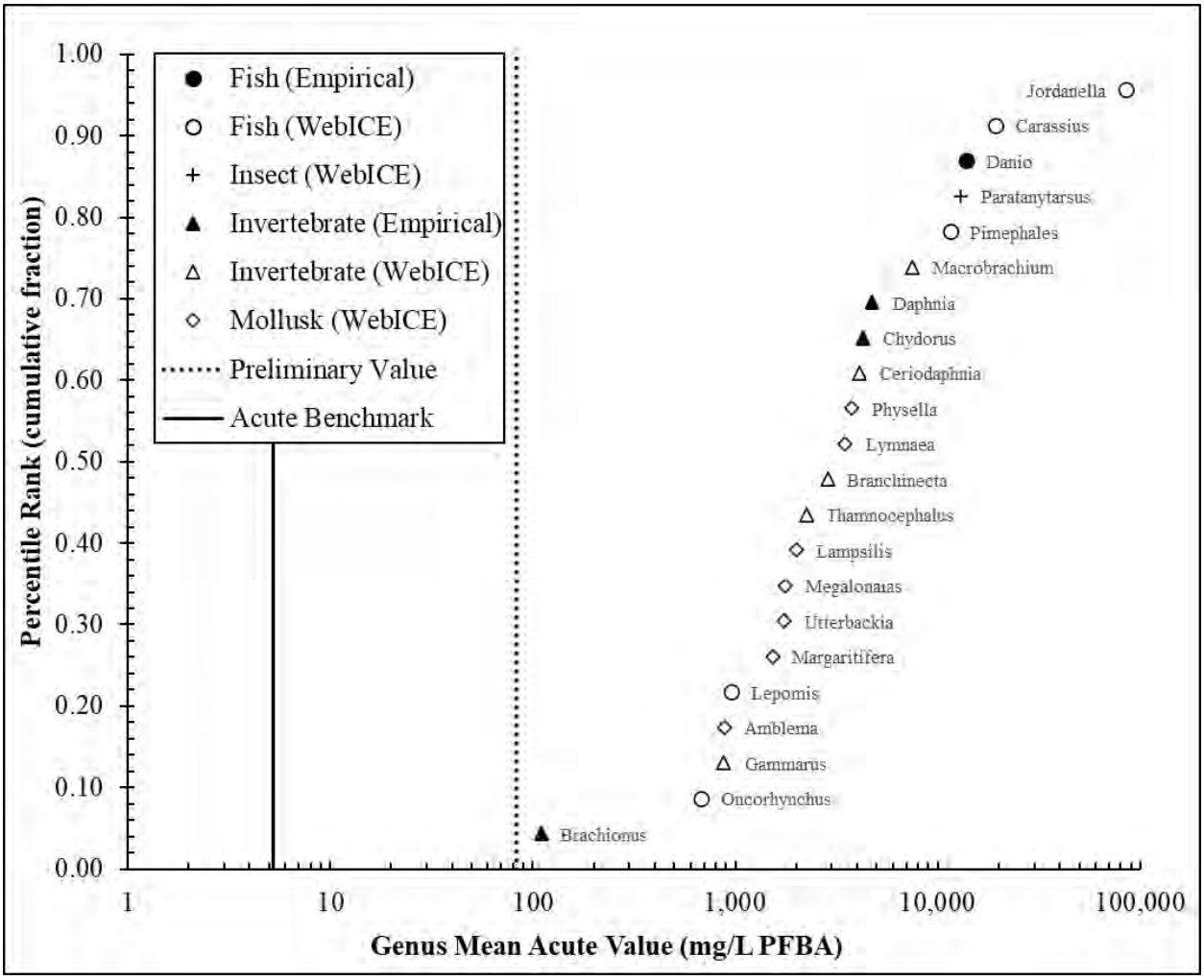


Figure 5-1. Ranked Acute PFBA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.3 Derivation of Acute Water Benchmark for Perfluorohexanoic Acid (PFHxA)

Quantitatively acceptable empirical data for PFHxA were available for four species comprising three genera and fulfilling three MDR groups (**Tables 4-2** and **5-6**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 28 ICE models were accepted for use. Of these, two ICE models were not selected for use because empirical data were available for the genus (i.e., models for *Daphnia magna* and *Daphnia pulex*). Three other ICE models were not used because

there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus; Daphnia magna* predicting for *Pimephales promelas*; and *Daphnia magna* predicting for *Oncorhynchus mykiss*). A total of 23 ICE models were selected for use, resulting in ICE models predicting 23 SMAVs representing, 21 genera, and 6 MDRs (**Tables 5-5** and **5-6**).

The combined empirical and ICE data resulted in 24 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-6**. GMAVs for the four most sensitive genera were within a factor of 2.4 of each other (**Table 5-7**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxA is 150.4 mg/L (**Table 5-7**), which is lower than all of the GMAVs except for one tested species, the rotifer, *Brachionus calyciflorus*, GMAV=140 mg/L). The FAV was divided by two to obtain a preliminary value of 75.21 mg/L PFHxA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 4.8 mg/L PFHxA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-2**).

Table 5-5. Acceptable Models for ICE-estimated Species Sensitivity of PFHxA.
Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	1,048	0.0002	5,000	**234.7** (64.5-853.7)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	1,048	0.0003	8,694.5	**517.3** (280.8-953.1)
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	0.003	233	**1,576.9** (395.3-6,289.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	1,048	0.0001	46,278	303.5[b] (176.0-523.2)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**719.8** (362.4-1,429.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1,048	0.0003	46,278	**785.1** (446.2-1,381.6)
Daphnid (Daphnia magna)*	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	424.1 (68.5-2,627.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	1,048	0.0002	4,894.7	841.4 (444.8-1,591.6)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	1,048	0.0002	46,500	384.7[b] (235.8-627.7)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**1,083.6** (468.5-2,506.4)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**470.8** (220.2-1,006.8)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**1,027.7** (405.9-2,602.1)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	1,048	0.37	14,500	**2,676.6** (917.7-7,806.3)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	18.6	20,900	**805.1** (394.5-1,643.4)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	1,048**	0.042	166.8	**404.3** (69.6-2,349.3)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1,048**	0.011	281.6	**1,172.2** (290.4-4,732.5)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**418.5** (231.7-755.9)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	1,048	0.0001	14,500	214.2[b] (135.8-337.8)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**713.5** (200.3-2,541.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**762.0** (188.8-3,075.8)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**794.5** (277.3-2,276.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	1,048	0.014	4,894.7	**191.3** (48.0-761.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**623.8** (247.0-1,575.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**372.3** (181.1-765.2)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	1,048**	0.009	977.6	**329.4** (183.8-590.5)
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1,048	166.8	4,894.7	**686.2** (127.8-3,684.7)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**370.2** (145.8-940.1)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	1,048	0.0001	46,500	**481.7** (172.4-1,346.1)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-6. Ranked PFHxA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**140.0**	**140.0**	**0.04**
2	G	Threeridge	*Amblema plicata*	191.3	191.3	0.08
3	E	Amphipod	*Gammarus fasciatus*	234.7	234.7	0.12
4	D	Water flea	*Chydorus sphaericus*	329.4**	329.4	0.16
5	G	Western pearlshell	*Margaritifera falcata*	370.2	370.2	0.20
6	G	Washboard	*Megalonaias nervosa*	372.3	372.3	0.24
7	G	Paper pondshell	*Utterbackia imbecillis*	418.5	418.5	0.28
8	B	Zebrafish	*Danio rerio-embryo*	481.7	481.7	0.32
9	G	Wavyrayed lampmussel	*Lampsilis fasciola*	686.2	507.4	0.36
	G	Neosho mucket	*Lampsilis rafinesqueana*	404.3**		
	G	Fatmucket	*Lampsilis siliquoidea*	470.8		
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	517.3	517.3	0.40
11	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	623.8	623.8	0.44
12	A	Rainbow trout	*Oncorhynchus mykiss*	713.5	713.5	0.48
13	B	Channel catfish	*Ictalurus punctatus*	719.8	719.8	0.52
14	G	Swamp lymnaea	*Lymnaea stagnalis*	762.0	762.0	0.56
15	D	Daphnid	*Ceriodaphnia dubia*	785.1	785.1	0.60
16	G	Tadpole physa	*Physella gyrina*	794.5	794.5	0.64
17	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.68
18	**C**	**Bullfrog**	***Lithobates catesbeianus***	**1,105**	**915.2**	**0.72**
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
19	B	Goldfish	*Carassius auratus*	1,028	1,028	0.76
20	**D**	**Daphnia**	***Daphnia magna***	**1,048**	**1,048**	**0.80**
21	B	Fathead minnow	*Pimephales promelas*	1,084	1,084	0.84
22	E	Oriental river shrimp	*Macrobrachium nipponense*	1,172**	1,172	0.88
23	B	Bluegill	*Lepomis macrochirus*	1,577**	1,577	0.92
24	F	Midge	*Paratanytarsus parthenogeneticus*	2,677	2,677	0.96

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-7. PFHxA Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=24 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	***Brachionus***	**140.0**	4.94	24.42	0.040	0.200
2	*Amblema*	191.3	5.25	27.60	0.080	0.283
3	*Gammarus*	234.7	5.46	29.79	0.120	0.346
4	*Chydorus*	329.4	5.80	33.61	0.160	0.400
		Σ (Su):	**21.45**	**115.42**	**0.40**	**1.23**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 17.41 S = slope
L = 4.080 L = X-axis intercept
A = 5.013 A = lnFAV
FAV = 150.4
FAV/2 = 75.21 mg/L (Preliminary Value)
Adjustment = 75.21 / 15.8 = 4.760 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **4.8 mg/L PFHxA** (rounded to two significant figures)

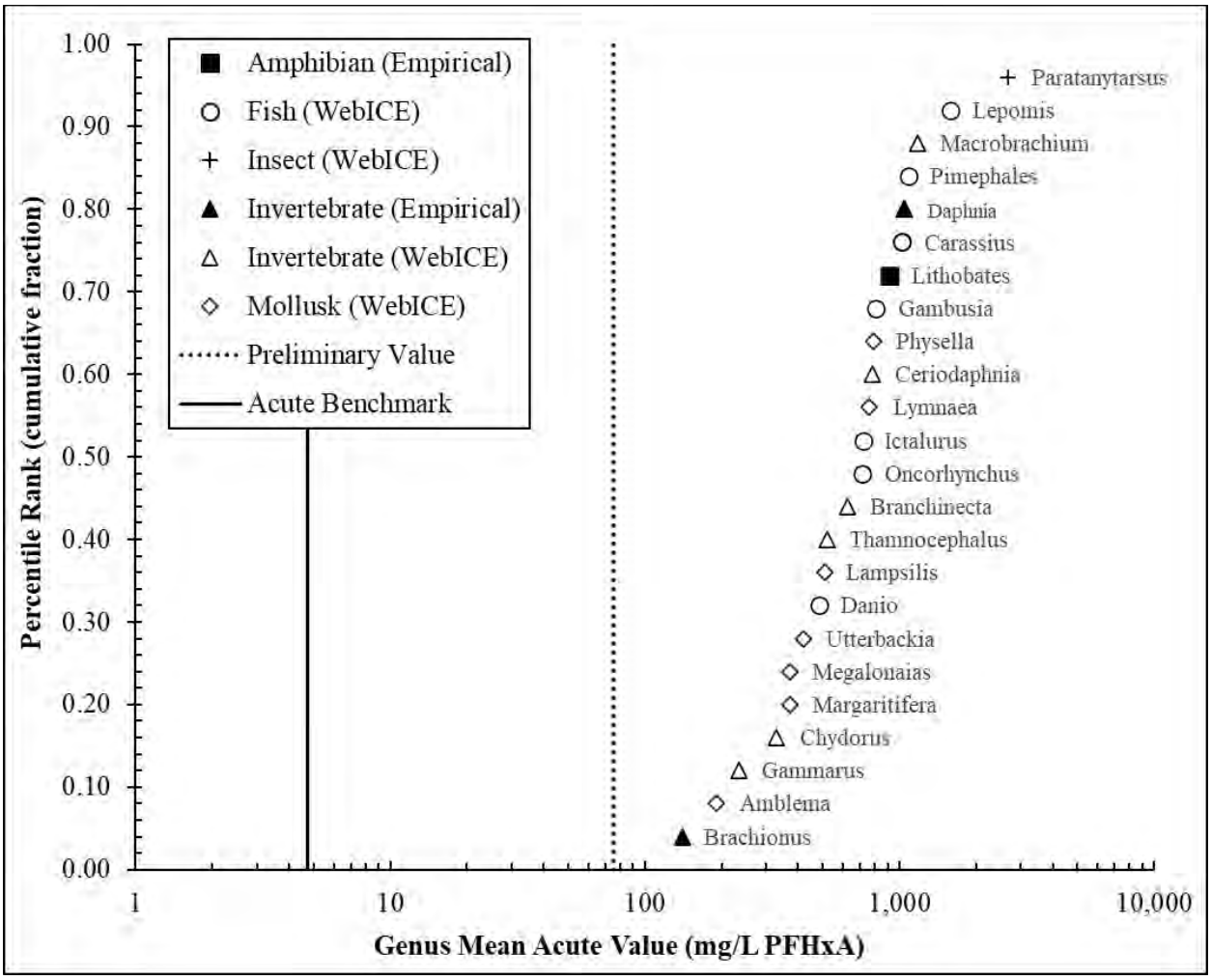


Figure 5-2. Ranked Acute PFHxA GMAVs Used for the Aquatic Life Acute Benchmark Calculations.

5.4 Derivation of Acute Water Benchmark for Perfluorononanoic Acid (PFNA)

Quantitatively acceptable empirical data for PFNA were available for three species comprising three genera and fulfilling two MDR groups (**Tables 4-2** and **5-9**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 29 ICE models were accepted for use. Of these, four ICE models were not selected for use because empirical data were available for two genera (i.e., models for *Daphnia magna*, *Daphnia pulex*, *Daphnia pulicaria* and *Chydorus sphaericus*). One other ICE model was

not used because there was an acceptable model for a more closely taxonomically-related model where the measured surrogate EC_{50} value fell within the ICE model range (i.e., *Daphnia magna* predicting for *Danio rerio*). A total of 24 ICE models were selected for use resulting in ICE models predicting 24 SMAVs representing 22 genera and 7 MDRs (**Tables 5-8** and **5-9**).

The combined empirical and ICE data resulted in 25 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-9**. GMAVs for the four most sensitive genera were within a factor of 2.0 of each other (**Table 5-10**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFNA is 20.58 mg/L (**Table 5-10**), which is lower than all of the GMAVs except for the one ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=17.58 mg/L). The FAV was divided by two to obtain a preliminary value of 10.29 mg/L PFNA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.65 mg/L PFNA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFNA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-3**).

Table 5-8. Acceptable Models for ICE-estimated Species Sensitivity to PFNA.
Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	84.51	0.0002	5,000	**29.8** (10.9-81.6)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	84.51**	0.0001	68.3	**57.2** (12.4-263.1)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	84.51	0.0003	8,694.5	**52.1** (32.2-84.2)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	84.51	0.0001	46,278	**50.3** (33.3-75.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	84.51	0.0003	46,278	**59.4** (38.2-92.1)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	27.84	0.007	462.0	76.4 (48.0-121.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	84.51	0.0002	4,894.7	65.8 (40.3-107.5)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	84.51	0.014	281.6	84.2 (18.0-392.8)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	84.51**	0.0002	7.2	**39.7** (8.4-187.6)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	84.51	0.0002	46,500	**67.3** (47.1-96.2)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**48.6** (27.6-85.6)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	84.51	0.0003	166.8	**1,261.8** (217.7-7314.9)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	84.51	0.37	14,500	**228.8** (101.9-513.5)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	84.51	0.042	166.8	**42.9** (12.1-152.7)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	84.51	0.003	281.6	**181.9** (43.1-768.2)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	84.51	0.011	281.6	**65.1** (24.3-174.3)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**44.8** (28.6-70.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	84.51	0.042	545.9	**54.4** (13.3-222.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	84.51	0.0001	14,500	**35.4** (25.2-49.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**70.7** (24.0-207.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**70.6** (31.3-159.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	84.51	0.014	4,894.7	**17.6** (6.1-50.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**58.0** (28.6-117.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**32.9** (18.5-58.5)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	84.51	0.009	977.5	30.1 (19.3-47.1)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	84.51	0.009	281.6	**44.3** (8.8-223.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**40.4** (19.8-82.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	84.51**	0.0001	50.0	104.2[c] (15.4-703.8)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	84.51	0.0001	46,500	**91.8** (42.1-200.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-9. Ranked PFNA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFNA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	17.58	17.58	0.04
2	**D**	**Daphnid**	***Chydorus sphaericus***	**27.84**	**27.84**	**0.08**
3	G	Washboard	*Megalonaias nervosa*	32.90	32.90	0.12
4	A	Rainbow trout	*Oncorhynchus mykiss*	35.40	35.40	0.15
5	D	Daphnid	*Simocephalus serrulatus*	39.74**	39.74	0.19
6	G	Western pearlshell	*Margaritifera falcata*	40.41	40.41	0.23
7	E	Amphipod	*Gammarus fasciatus*	29.82	41.28	0.27
	E	Amphipod	*Gammarus pseudolimnaeus*	57.16**		
8	D	Water flea	*Moina macrocopa*	44.31	44.31	0.31
9	G	Paper pondshell	*Utterbackia imbecillis*	44.82	44.82	0.35
10	G	Neosho mucket	*Lampsilis rafinesqueana*	42.93	45.69	0.38
	G	Fatmucket	*Lampsilis siliquoidea*	48.63		
11	B	Bluegill	*Lepomis macrochirus*	50.29	50.29	0.42
12	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	52.08	52.08	0.46
13	G	Pheasantshell	*Ortmanniana pectorosa*	54.41	54.41	0.50
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	58.02	58.02	0.54
15	D	Daphnid	*Ceriodaphnia dubia*	59.35	59.35	0.58
16	E	Oriental river shrimp	*Macrobrachium nipponense*	65.10	65.10	0.62
17	B	Fathead minnow	*Pimephales promelas*	67.29	67.29	0.65
18	G	Tadpole physa	*Physella gyrina*	70.62	70.62	0.69
19	G	Swamp lymnaea	*Lymnaea stagnalis*	70.68	70.68	0.73
20	**D**	**Daphnid**	***Daphnia magna***	**84.51**	**84.51**	**0.77**
21	B	Zebrafish	*Danio rerio*	91.85	91.85	0.81
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	181.9	181.9	0.85
23	F	Midge	*Paratanytarsus parthenogeneticus*	228.8	228.8	0.88
24	**C**	**Clawed frog**	***Xenopus sp.***	**335.8**	**335.8**	**0.92**
25	E	Isopod	*Asellus aquaticus*	1,262	1,262	0.96

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes

B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)

E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)

F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-10. PFNA Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=25 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	17.58	2.87	8.22	0.038	0.196
2	***Chydorus***	**27.84**	3.33	11.07	0.077	0.277
3	*Megalonaias*	32.90	3.49	12.20	0.115	0.340
4	*Oncorhynchus*	35.40	3.57	12.72	0.154	0.392
		Σ (Su):	**13.25**	**44.21**	**0.38**	**1.21**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 13.85 S = slope
L = 2.192 L = X-axis intercept
A = 3.024 A = lnFAV
FAV = 20.58
FAV/2 = 10.29 mg/L (Preliminary Value)
Adjustment = 10.29 / 15.8 = 0.6511 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.65 mg/L PFNA** (rounded to two significant figures)

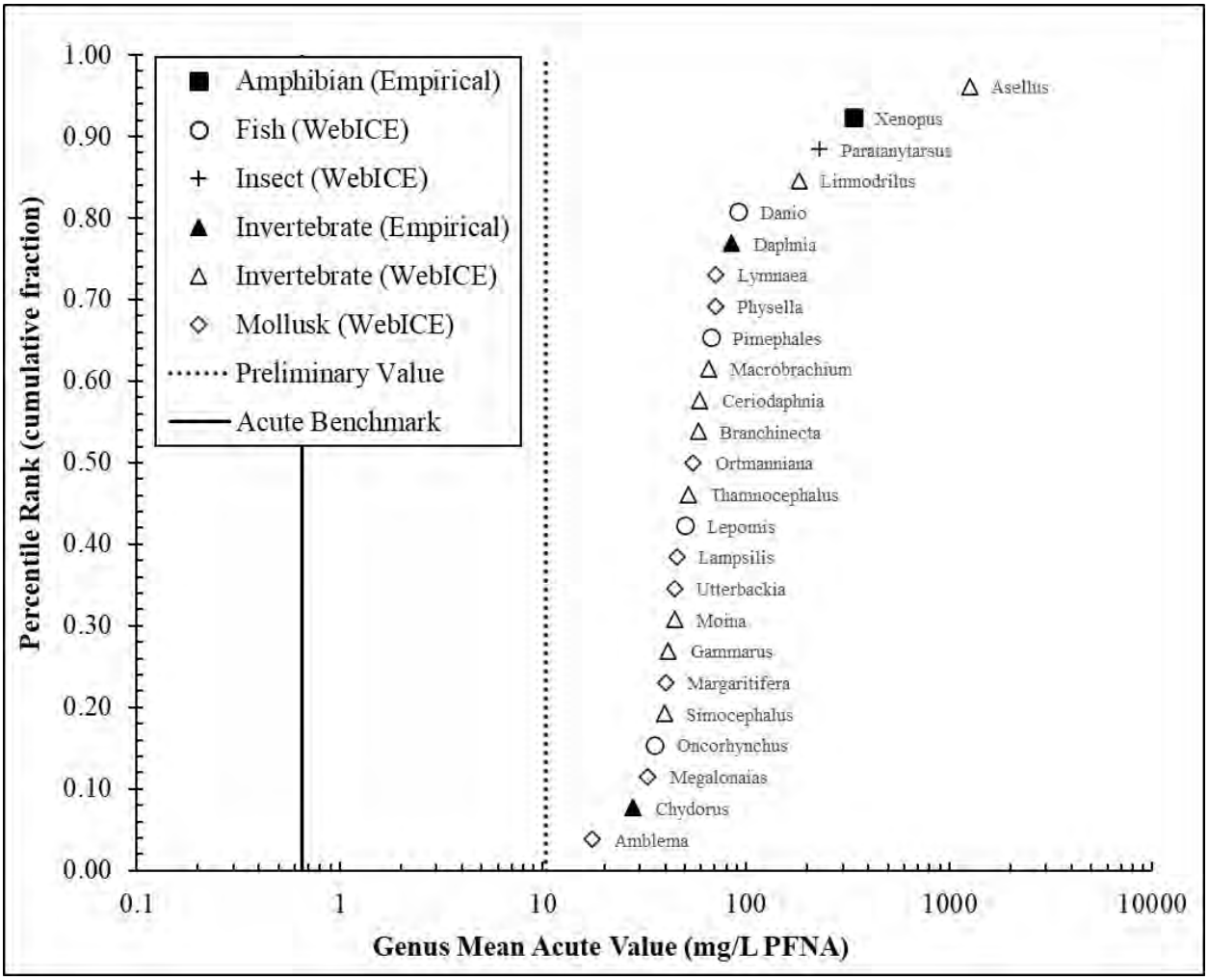


Figure 5-3. Ranked Acute PFNA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.5 Derivation of Acute Water Benchmark for Perfluorodecanoic Acid (PFDA)

Quantitatively acceptable empirical data for PFDA were available for five species comprising four genera and fulfilling three MDR groups (**Tables 4-2** and **5-12**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 61 ICE models were accepted for use. Of these, 11 ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia magna*). Three other ICE models were not used because

there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; and *Daphnia magna* predicting for *Danio rerio*). A total of 46 ICE models were selected for use resulting in ICE models predicting 46 SMAVs representing 38 genera and all eight MDRs (**Tables 5-11** and **5-12**).

The combined empirical and ICE data resulted in 42 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-12**. GMAVs for the four most sensitive genera were within a factor of 2.9 of each other (**Table 5-13**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFDA is 15.71 mg/L (**Table 5-13**), which is lower than all of the GMAVs except for the ICE-derived species values for the isopod, *Caecidotea brevicauda*, GMAV= 8.17 mg/L. The FAV was divided by two to obtain a benchmark value of 7.856 mg/L PFDA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.50 mg/L PFDA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFDA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-4**).

Table 5-11. Acceptable Models for ICE-estimated Species Sensitivity to PFDA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	119.7	0.0002	5,000	**39.7** (14.0-112.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	119.7**	0.0001	68.3	**78.6** (16.1-384.0)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.0041	1.625	39.4 (9.0-173.4)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.86	**31.0** (15.6-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	119.7	0.0003	8,694.5	**71.5** (43.6-117.5)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0001	8,341.5	**33.1** (27.6-39.6)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	119.7	0.0001	46,278	64.5[b] (42.0-99.0)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.1	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	96.7	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.9	**33.1** (19.8-55.3)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	**114.0** (33.0-394.0)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	0.003	724.4	40.0 (24.0-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	43.1	49.4 (38.4-63.6)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	0.71	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	119.7	0.0003	46,278	**84.8** (53.8-133.7)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	41.13	0.007	462.0	114.5 (70.9-184.8)
Daphnid (Daphnia magna)*	Daphnid (Daphnia pulicaria)	149.6	0.009	237.4	122.1 (27.4-544.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	119.7	0.0002	4,894.7	93.7 (56.2-156.0)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	119.7	0.014	281.6	122.0 (24.2-614.6)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	119.7**	0.0002	7.2	**55.1** (11.0-275.1)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	13,400	**46.7** (36.0-60.7)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	119.7	0.0002	46,500	85.6[b] (58.9-124.4)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**66.6** (37.0-119.8)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**62.2** (31.6-122.5)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	19.9	**45.8** (19.9-105.3)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	119.7	0.0003	166.8	**1,699.9** (273.7-10,558.5)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	82.0	**8.2** (1.9-35.7)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**18.0** (11.4-28.3)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	95.9	**19.5** (9.5-40.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	0.007	54.4	**52.1** (10.9-248.7)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	119.7	0.37	14,500	**321.4** (140.5-735.3)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	119.7	0.042	166.8	**58.5** (15.4-222.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	0.002	198.4	**64.7** (33.7-124.2)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	119.7	0.003	281.6	**232.8** (51.5-1,052.2)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	119.7	0.011	281.6	**97.1** (34.6-272.8)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**61.0** (38.3-97.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	119.7	0.042	545.9	**76.0** (17.4-332.2)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	119.7	0.0001	14,500	45.4 (31.9-64.7)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.1	**26.6** (5.3-134.0)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	0.03	95.9	**45.7** (11.2-185.6)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.0014	7.075	**73.0** (29.7-179.7)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.03	7.5	25.7 (4.0-164.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.03	9.7	**312.8** (88.4-1,106.8)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**98.2** (32.2-299.3)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**98.7** (42.7-227.9)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	119.7	0.014	4,894.7	**24.5** (8.2-73.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**80.6** (38.8-167.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**46.0** (25.6-82.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	119.7	0.009	977.6	41.9 (26.5-66.4)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	119.7	0.009	281.6	**56.9** (10.6-305.9)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**54.9** (26.3-114.5)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	16.2	**22.2** (5.1-95.7)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	8,341.5	**34.4** (16.8-70.4)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	119.7	0.0001	46,500	115.5[b] (51.4-259.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-12. Ranked PFDA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFDA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	E	Isopod	*Caecidotea brevicauda*	8.173	8.173	0.02
2	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.05
3	B	Yellow perch	*Perca flavescens*	22.20**	22.20	0.07
4	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.09
	A	Lake trout	*Salvelinus namaycush*	18.01		
5	G	Threeridge	*Amblema plicata*	24.46	24.46	0.12
6	B	Zebrafish	*Danio rerio*	24.56	24.56	0.14
7	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.16
8	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.19
9	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.21
10	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	**32.00**	**0.23**
11	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.26
	A	Brown trout	*Salmo trutta*	33.12		
12	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.28
13	B	Green sunfish	*Lepomis cyanellus*	45.79**	38.92	0.30
	B	Bluegill	*Lepomis macrochirus*	33.08		
14	**D**	**Daphnid**	***Chydorus sphaericus***	**41.13**	**41.13**	**0.33**
15	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.35
16	G	Washboard	*Megalonaias nervosa*	46.01	46.01	0.37
17	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.40
18	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.42
19	B	Medaka	*Oryzias latipes*	52.12	52.12	0.44
20	G	Western pearlshell	*Margaritifera falcata*	54.89	54.89	0.47
21	D	Daphnid	*Simocephalus serrulatus*	55.07**	55.07	0.49
22	E	Amphipod	*Gammarus fasciatus*	39.66	55.84	0.51
	E	Amphipod	*Gammarus pseudolimnaeus*	78.62**		
23	D	Water flea	*Moina macrocopa*	56.93	56.93	0.53
24	G	Paper pondshell	*Utterbackia imbecillis*	61.04	61.04	0.56
25	B	Goldfish	*Carassius auratus*	62.18	62.18	0.58
26	G	Neosho mucket	*Lampsilis rafinesqueana*	58.54	62.42	0.60
	G	Fatmucket	*Lampsilis siliquoidea*	66.56		
27	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	71.54	71.54	0.63
28	B	Bonytail	*Gila elegans*	72.68	72.68	0.65
29	B	Silver perch	*Bidyanus bidyanus*	73.03**	73.03	0.67
30	G	Pheasantshell	*Ortmanniana pectorosa*	76.02	76.02	0.70
31	**C**	**Clawed frog**	***Xenopus sp.***	**76.50**	**76.50**	**0.72**

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
32	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	80.58	80.58	0.74
33	D	Daphnid	*Ceriodaphnia dubia*	84.82	84.82	0.77
34	E	Oriental river shrimp	*Macrobrachium nipponense*	97.09	97.09	0.79
35	G	Swamp lymnaea	*Lymnaea stagnalis*	98.19	98.19	0.81
36	G	Tadpole physa	*Physella gyrina*	98.69	98.69	0.84
37	C	Bullfrog	*Lithobates catesbeianus*	58.75	106.0	0.86
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	312.8**		
38	B	Cape Fear shiner	*Notropis mekistocholas*	114.0**	114.0	0.88
39	**D**	**Daphnid**	***Daphnia magna***	**145.5**	**147.5**	**0.91**
	D	**Daphnid**	***Daphnia pulicaria***	**149.6**		
40	F	Midge	*Paratanytarsus dissimilis*	105.8	184.4	0.93
	F	Midge	*Paratanytarsus parthenogeneticus*	321.4		
41	H	Oligochaete	*Limnodrilus hoffmeisteri*	232.8	232.8	0.95
42	E	Isopod	*Asellus aquaticus*	1,700	1,700	0.98

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-13. PFDA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=42 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Caecidotea*	8.17	2.10	4.41	0.023	0.152
2	*Micropterus*	19.52	2.97	8.83	0.047	0.216
3	*Perca*	22.20	3.10	9.61	0.070	0.264
4	*Salvelinus*	23.80	3.17	10.05	0.093	0.305
		Σ (Su):	**11.34**	**32.90**	**0.23**	**0.94**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 57.22 S = slope
L = 1.063 L = X-axis intercept
A = 2.754 A = lnFAV
FAV = 15.71
FAV/2 = 7.856 mg/L (Preliminary Value)
Adjustment = 7.856 / 15.8 = 0.4972 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.50 mg/L PFDA** (rounded to two significant figures)

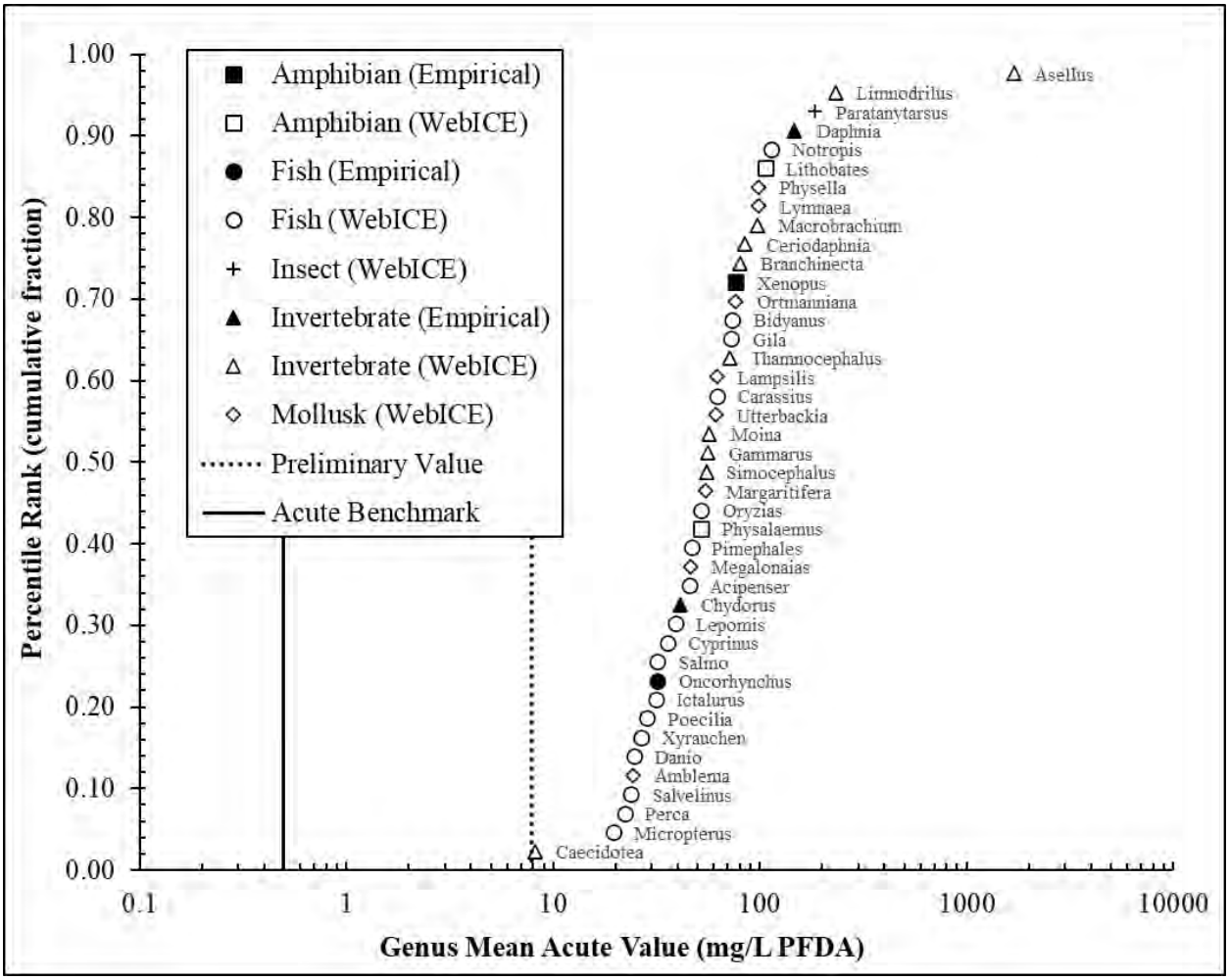


Figure 5-4. Ranked Acute PFDA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.6 Derivation of Acute Water Benchmark for Perfluorobutanesulfonic Acid (PFBS)

Quantitatively acceptable empirical data for PFBS were available for four species comprising four genera and fulfilling two MDR groups (**Tables 4-2** and **5-15**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 55 ICE models were acceptable for use. Of these, thirteen ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Lepomis macrochirus* and *Daphnia magna*). Three ICE models were not selected for use because

they were derived based on saltwater species surrogates (i.e., model for an amphipod, *Hyalella azteca*; daphnid, *Ceriodaphnia dubia*; and rainbow trout, *Oncorhynchus mykiss*). Four other models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lepomis macrochirus* predicting for *Cyprinus carpio*; *Lepomis macrochirus* predicting for *Carassius auratus*; *Daphnia magna* predicting for *Oncorhynchus mykiss*; and *Pimephales promelas* predicting for *Amblema plicata*). Two additional models (models for the bullfrog, *L hob e e be* and channel catfish, *I l r p*) were not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 33 ICE models were selected for use, resulting in ICE models predicting 30 SMAVs representing 26 genera and seven MDRs (**Tables 5-14** and **5-15**). An ICE model was not available to fulfill the MDR for a family in any order of insect or any phylum not already represented (**Tables 5-14** and **5-15**).

The combined empirical and ICE data resulted in 30 GMAVs that collectively fulfill seven MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-15**. Surrogate species EC_{50} values fell outside the ICE model range for 14 of the predicted SMAVs and the resultant GMAVs are less certain than GMAVs based on ICE models where surrogate EC_{50} values were in the range of the model. GMAVs for the four most sensitive genera were within a factor of 1.7 of each other (**Table 5-16**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFBS is 422.1 mg/L (**Table 5-16**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=383.6 mg/L). The FAV was

divided by two to obtain a preliminary value of 211.0 mg/L PFBS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 5.0 mg/L PFBS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-5**).

Table 5-14. Acceptable Models for ICE-estimated Species Sensitivity to PFBS.
Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	2,183	0.0002	5,000	**428.1** (107.9-1,697.9)
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	372**	<0.0001	193.4	449.4[a] (93.4-2,162.8)
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	6,452**	0.004	123.3	**14,523.6** (2,825.4-74,655)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	2,183	0.0003	8,694.5	**1,010** (525.1-1,942.9)
Bluegill (Lepomis macrochirus)*	Daphnid (Daphnia magna)	2,183	0.0001	46,278	512.5 (285.3-920.4)
Bluegill (Lepomis macrochirus)*	Fathead minnow (Pimephales promelas)	1,938	0.0002	50,154	751.0 (390.0-1,446)
Bluegill (Lepomis macrochirus)*	Mysid (Americamysis bahia)	372**	<0.0001	79.0	207.7 (107.0-403.2)
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	123.3	**3,505.7** (612.1-20,077)
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**1,791.4** (756.1-4,244.6)
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	201.0	3,420.6[c] (841.4-13,905)
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**1,617.3** (755.0-3,464.4)
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	517.8	1,863.7[c] (713.6-4,867.2)
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	150.8	**1,609.5** (248.6-10,421)
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	1,938**	0.005	133	**1,952.5** (498.7-7,644.4)
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	6,452**	0.0009	180	2,962.4[b] (569.2-15,418.0)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	1,938**	0.001	140.2	**602.6** (113.4-3,203.2)
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	180	**2,561.1** (548.4-11,961)
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	372	<0.0001	4,100	171.7 [a] (36.6-804.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	2,183	0.0003	46,278	**1,666.6** (909.0-3,055.6)
Daphnid (Daphnia magna)*	Mysid (Americamysis bahia)	372	<0.0001	11,000	243.9 (143.9-413.4)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	2,183	0.0002	4,894.7	1,768.1 (893.9-3,497.1)
Fathead minnow (Pimephales promelas)*	Mysid (Americamysis bahia)	372	<0.0001	11,000	158.6 (74.5-337.5)
Fathead minnow (Pimephales promelas)*	Daphnid (Daphnia magna)	2,183	0.0002	46,500	639.5 (375.7-1,088.5)
Fathead minnow (Pimephales promelas)*	Bluegill (Lepomis macrochirus)	6,452	0.0001	27,540	3,399.2 (1,631.9-7080.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**912.5** (399.0-2,086.8)
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**2,152.4** (1,075.2-4,309.0)
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	201	1,922.7 [b] (312.9-11,813.0)
Green sunfish (Lepomis cyanellus)*	Bluegill (Lepomis macrochirus)	6,452**	0.004	113	2,817.7 (563.5-14,089.5)
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	1,938**	0.001	140.2	**499.3** (131.8-1,892.4)
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	180	**687.3** (105.9-4,461.3)
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	6,452**	0.003	180	**320.8** (49.0-2,102.1)
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	123.3	**8,225.3** (2,938.4-23,024.8)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	1,938**	0.003	1,420	**1,013.6** (496.2-2,070.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	2,183	0.37	14,500	**5,481.6** (1,674.6-17,943.7)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	2,183**	0.042	166.8	**777.2** (115.1-5,248.1)
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	6,452**	0.002	6.969	**11,873.0** (8,879.5-15,875.6)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	2,183**	0.011	281.6	**2,722.2** (587.7-12,609.0)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**802.5** (422.2-1,525.5)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	2,183	0.0001	14,500	361.9[b] (221.2-592.1)
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	1,938	0.0002	26,500	**788.9** (505.7-1,230.8)
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	6,452	0.0001	7,100	**3,208.6** (2,275.7-4,524.0)
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	372**	<0.0001	113	110.0 [a] (58.8-205.9)
Redear sunfish (Lepomis microlophus)*	Bluegill (Lepomis macrochirus)	6,452**	0.017	4.5	9,763.6 (7,469.9-12,761.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,523.7** (337.4-6,881.2)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,608.6** (512.8-5,046.1)
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1,938	0.814	10,831	250.3[b] (39.4-1,592.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	2,183	0.014	4,894.7	**383.6** (85.6-1,717.6)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,246.4** (457.0-3,399.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**755.1** (345.4-1,650.5)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	2,183**	0.009	977.6	**661.4** (349.2-1,252.8)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**706.1** (255.8-1,948.9)
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	9.41	**3,426.2** (710.8-16,515)
Zebrafish (Danio rerio)*	Fathead minnow (Pimephales promelas)	1,938**	0.013	26.69	1,069.6 (242.1-4,726.4)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	2,183	0.0001	46,500	780.8 (257.2-2,370.6)
Zebrafish-embryo (Danio rerio-embryo)*	Fathead minnow (Pimephales promelas)	1,938	0.004	70,200	1,707.9 (1,061.5-2,748)

*Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-15. Ranked PFBS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	383.6	383.6	0.03
2	E	Amphipod	*Gammarus fasciatus*	428.1	428.1	0.06
3	B	Guppy	*Poecilia reticulata*	585.8**	585.8	0.10
4	D	Water flea	*Chydorus sphaericus*	661.4**	661.4	0.13
5	G	Western pearlshell	*Margaritifera falcata*	706.1	706.1	0.16
6	G	Washboard	*Megalonaias nervosa*	755.1	755.1	0.19
7	G	Paper pondshell	*Utterbackia imbecillis*	802.5	802.5	0.23
8	G	Neosho mucket	*Lampsilis rafinesqueana*	777.2**	842.1	0.26
	G	Fatmucket	*Lampsilis siliquoidea*	912.5		
9	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	1,010	1,010	0.29
10	B	Medaka	*Oryzias latipes*	1,014**	1,014	0.32
11	A	Brook trout	*Salvelinus fontinalis*	3,506**	1,060	0.35
	A	Lake trout	*Salvelinus namaycush*	320.8**		
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	1,246	1,246	0.39
13	A	Cutthroat trout	*Oncorhynchus clarkii*	1,242**	1,471	0.42
	A	Coho salmon	*Oncorhynchus kisutch*	1,610**		
	A	Rainbow trout	*Oncorhynchus mykiss*	1,591		
14	G	Swamp lymnaea	*Lymnaea stagnalis*	1,524	1,524	0.45
15	G	Tadpole physa	*Physella gyrina*	1,609	1,609	0.48
16	B	Channel catfish	*Ictalurus punctatus*	1,617	1,617	0.52
17	D	Daphnid	*Ceriodaphnia dubia*	1,667	1,667	0.55
18	C	Bullfrog	*Lithobates catesbeianus*	1,791	1,791	0.58
19	**B**	**Fathead minnow**	***Pimephales promelas***	**1,938**	**1,938**	**0.61**
20	B	Common carp	*Cyprinus carpio*	1,952**	1,952	0.65
21	B	Goldfish	*Carassius auratus*	2,152	2,152	0.68
22	**D**	**Daphnid**	***Daphnia magna***	**2,183**	**2,183**	**0.71**
23	E	Oriental river shrimp	*Macrobrachium nipponense*	2,722**	2,722	0.74
24	**B**	**Zebrafish**	***Danio rerio***	**>3,000**	**>3,000**	**0.77**
25	B	Yellow perch	*Perca flavescens*	3,426**	3,426	0.81
26	F	Midge	*Paratanytarsus parthenogeneticus*	5,482	5,482	0.84
27	**B**	**Bluegill**	***Lepomis macrochirus***	**6,452**	**6,452**	**0.87**
28	B	Largemouth bass	*Micropterus salmoides*	8,225**	8,225	0.90
29	B	Nile tilapia	*Oreochromis niloticus*	11,873**	11,873	0.94
30	A	Atlantic salmon	*Salmo salar*	14,524**	14,524	0.97

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-16. PFBS Protective Aquatic Life Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=30 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	383.6	5.95	35.40	0.032	0.180
2	*Gammarus*	428.1	6.06	36.72	0.065	0.254
3	*Hyalella*	585.8	6.37	40.61	0.097	0.311
4	*Ceriodaphnia*	661.4	6.49	42.18	0.129	0.359
		Σ (Su):	**24.88**	**154.90**	**0.32**	**1.10**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 11.02 S = slope
L = 5.303 L = X-axis intercept
A = 6.045 A = lnFAV
FAV = 422.1
FAV/2 = 211.0 mg/L (Preliminary Value)
Adjustment = 211.0 / 42.3 = 4.989 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **5.0 mg/L PFBS** (rounded to two significant figures)

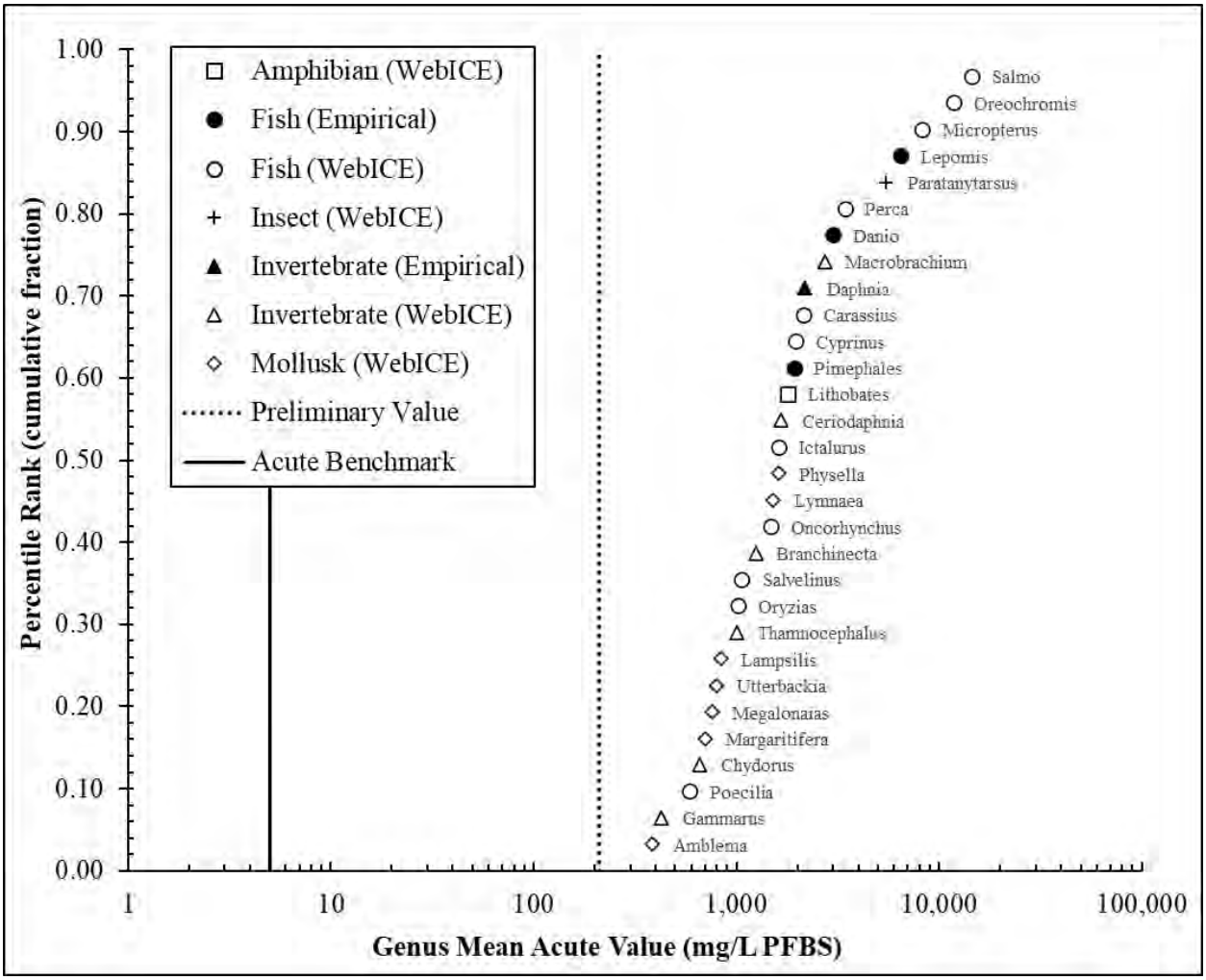


Figure 5-5. Ranked Acute PFBS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.7 Derivation of Acute Water Benchmark for Perfluorohexanesulfonic Acid (PFHxS)

Quantitatively acceptable empirical data for PFHxS were available for three species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-18**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 17 ICE models were acceptable for use. Of these, four models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lithobates catesbeianus* predicting for *Ictalurus punctatus*; *Lithobates catesbeianus* predicting

for *Pimephales promelas*; *Lithobates catesbeianus* predicting for *Carassius auratus*; and *Lithobates catesbeianus* predicting for *Oncorhynchus mykiss*). A total of 13 ICE models were selected for use, resulting in ICE models predicting 10 SMAVs representing 10 genera and five MDRs (**Tables 5-17** and **5-18**). ICE models were not available to fulfill two MDRs: an insect, and a family in a phylum other than Arthropoda or Chordata.

The combined empirical and ICE data resulted in 12 GMAVs that collectively fulfill six of the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-18**. GMAVs for the four most sensitive genera were within a factor of 1.2 of each other (**Table 5-19**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxS is 18.07 mg/L (**Table 5-19**), which is lower than all of the GMAVs. The FAV was divided by two to obtain a preliminary value of 9.035 mg/L PFHxS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 0.21 mg/L PFHxS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-6**). However, because only six of the eight MDRs were fulfilled for PFHxS (when both empirical test and ICE data were considered), there is considered to be greater uncertainty associated with this benchmark value compared with other PFAS benchmarks developed.

Table 5-17. Acceptable Models for ICE-estimated Species Sensitivity to PFHxS.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	0.003	233.0	**1,576.9** (395.3-6,289.9)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	304.5	**19.5** (9.2-41.0)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	719.8[b] (362.4-1,429.7)
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	22.5	0.003	36.91	**5.2** (1.2-22.7)
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**424.1** (68.5-2,627.9)
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	4.622	5,100.7	**114.2** (33.4-390.2)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.023	54,579	**18.3** (13.3-25.2)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	22.5	0.018	25.08	**26.0** (11.6-58.2)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	1,083.6[b] (468.5-2,506.4)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	22.5	7.797	649.3	**33.9** (19.7-58.4)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	304.5	**37.3** (23.2-59.9)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	1,027.7[b] (405.9-2,602.1)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	18.6	20,900	**805.1** (394.5-1,643.4)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	239.3	**63.2** (9.9-403.1)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.023	8,843.9	**9.6** (4.2-22.0)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	22.5	0.003	26.39	**38.3** (9.5-155.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	713.5[b] (200.3-2,541.4)

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-18. Ranked PFHxS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	A	Rainbow trout	*Oncorhynchus mykiss*	19.19	19.19	0.08
2	B	Channel catfish	*Ictalurus punctatus*	19.46	19.46	0.15
3	B	Fathead minnow	*Pimephales promelas*	21.79	21.79	0.23
4	**B**	**Zebrafish**	***Danio rerio***	**22.50**	**22.50**	**0.31**
5	B	Flagfish	*Jordanella floridae*	33.90	33.90	0.38
6	B	Goldfish	*Carassius auratus*	37.28	37.28	0.46
7	D	Daphnid	*Daphnia magna*	46.87	46.87	0.54
8	H	Oligochaete	*Limnodrilus hoffmeisteri*	63.23	63.23	0.62
9	E	Fairy shrimp	*Streptocephalus rubricaudatus*	114.2	114.2	0.69
10	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.77
11	**C**	**Bullfrog**	***Lithobates catesbeiana***	**1,105**	**915.2**	**0.85**
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
12	B	Bluegill	*Lepomis macrochirus*	1,577**	1,577	0.92

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-19. PFHxS Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=12 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)²**	**P=R/(N+1)**	**sqrt(P)**
1	*Oncorhynchus*	19.19	2.95	8.73	0.077	0.277
2	*Ictalurus*	19.46	2.97	8.81	0.154	0.392
3	*Pimephales*	21.79	3.08	9.50	0.231	0.480
4	***Danio***	**22.50**	3.11	9.69	0.308	0.555
		Σ (Su):	12.12	36.73	0.77	1.70

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 0.45 S = slope
L = 2.745 L = X-axis intercept
A = 2.894 A = lnFAV
FAV = 18.07
FAV/2 = 9.035 mg/L (Preliminary Value)
Adjustment = 9.035 / 42.3 = 0.2136 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **0.21 mg/L PFHxS** (rounded to two significant figures)

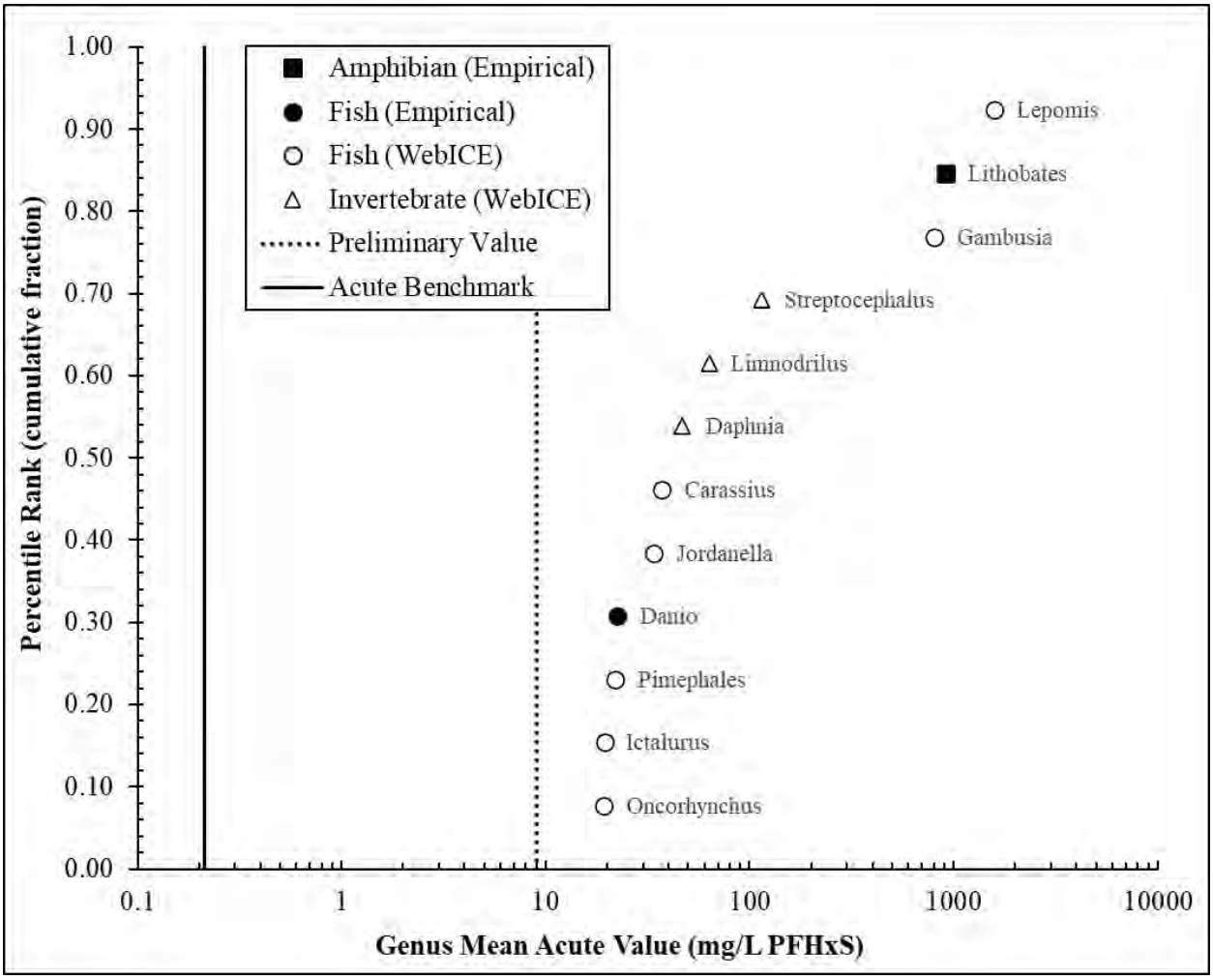


Figure 5-6. Ranked Acute PFHxS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.8 Derivation of Acute Water Benchmark for Hexadecafluoro-2-decenoic Acid (8:2 FTUCA)

Quantitatively acceptable empirical data for 8:2 FTUCA were available for two species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-21**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 66 ICE models were accepted for use. Of these, nine ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia pulex*). Four other models were not used because there were

acceptable models for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; *Daphnia magna* predicting for *Danio rerio*). A total of 53 ICE models were selected for use, resulting in ICE models predicting 52 SMAVs representing 43 genera and all eight MDRs (**Tables 5-20** and **5-21**).

The combined empirical and ICE data resulted in 45 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV is summarized in **Table 5-21**. GMAVs for the four most sensitive genera were within a factor of 1.8 of each other (**Table 5-22**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 8:2 FTUCA is 1.171 mg/L (**Table 5-22**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=0.79 mg/L). The FAV was divided by two to obtain a preliminary value of 0.5853 mg/L 8:2 FTUCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.037 mg/L 8:2 FTUCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 8:2 FTUCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-7**).

There is a visible gap in the data between the water flea (*Miona macrocopa*, SMAV = 4.2 mg/L) and bryozoan (*Pectinatella magnifica*, SMAV = 15.2 mg/L) for these ICE-generated values (see **Figure 5-7**). Although the exact reason is unclear for this large gap in values, it is noted that the lower portion of the curve is primarily composed of the phyla Mollusca and Arthropoda, while the upper portion of the curve is primarily composed of the phylum Chordata.

The distinct difference in sensitivity indicated by the gap in these data is thus likely to result from a greater estimated sensitivity of most invertebrates than vertebrates to 8:2 FTUCA. Additionally, the ICE estimates for the lower portion of the curve are based primarily on the surrogate *Daphnia magna*, while the ICE estimates for the upper portion of the curve are based primarily on the surrogate *Oncorhynchus mykiss*. However, these differences fall within the range of differences in acute response frequently observed between species. For example, within the PFOA and PFOS acute criteria datasets, there was single species sensitivity that spanned over two orders of magnitude, and the variation in acute toxicity values is frequently an order of magnitude and can be as high as three orders of magnitude (Hrovat et al. 2009, Schür et al. 2023, Raimondo et al. 2024). It is, therefore, not unexpected to see the difference in sensitivity observed between these species.

Table 5-20. Acceptable Models for ICE-estimated Species Sensitivity to 8:2 FTUCA.
Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	3.2	0.0008	166.8	**28.6** (7.0-116.1)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	3.2	0.0002	5,000	**2.0** (1.0-4.1)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	3.2	0.0001	68.3	**2.9** (1.0-7.8)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	81**	0.004	1.625	109.4 (20.3-589.1)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	95.86	**79.3** (36.6-171.6)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	3.2	0.0003	8,694.5	**2.6** (1.8-3.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	3.2	0.0001	46,278	4.9[b] (3.7-6.4)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	81	0.0001	8,341.5	**79.0** (64.3-97.1)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	96.69	**79.4** (44.1-142.9)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	95.86	**83.8** (47.0-149.4)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	3.2**	0.014	0.232	**15.2** (3.5-66.6)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	13,400	**132.5** (51.0-344.2)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	81**	0.004	1.625	**339.1** (82.8-1,388.7)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	13,400	**67.4** (39.8-114.0)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	81	0.003	724.4	102.9 (57.1-185.4)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	43.07	128.3 (96.6-170.5)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	198.4	**75.2** (31.0-182.2)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	198.4	65.5 (42.9-100.0)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	81	0.7099	155.5	**91.1** (48.4-171.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	3.2	0.0003	46,278	**2.1** (1.5-2.9)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	3.2**	0.0001	0.646	3.1 (0.8-12.3)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	3.2	0.009	10.36	4.9 (1.0-23.7)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	3.2	0.0002	4,894.7	2.4 (1.7-3.3)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	3.2	0.014	281.6	2.6 (0.7-9.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	3.2	0.0002	7.2	**1.8** (0.7-5.2)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	3.2	0.0001	166.8	**1.7** (0.2-12.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	3.2	0.0002	46,500	7.0[b] (5.4-9.0)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	13,400	**107.7** (79.8-145.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.5** (1.6-4.1)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	13,400	**132.7** (62.2-283.3)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	3.2	0.014	166.8	**1.9** (0.4-9.4)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	19.93	**105.8** (41.7-269)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	198.4	**54.9** (12.3-243.7)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	3.2	0.0003	166.8	**76.6** (23.0-255.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	82	**16.8** (3.2-89.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	198.4	**41.3** (24.5-69.7)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	95.86	**46.8** (20.6-106.2)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	81**	0.007	54.4	**121.6** (20.2-733.2)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	1,330	**241.0** (72.2-804.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	3.2	0.37	14,500	**9.3** (3.5-24.7)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	3.2	0.0003	58	**1.3** (0.3-6.2)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	3.2	0.042	166.8	**2.3** (1.1-5.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	81	0.002	198.4	**125.5** (62.3-252.8)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	3.2	0.003	281.6	**17.9** (6.9-46.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	3.2	0.0001	4,894.7	**31.6** (7.4-134.7)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	3.2	0.011	281.6	**1.5** (0.7-3.4)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.5** (1.6-3.7)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	3.2	0.042	545.9	**2.3** (0.8-7.0)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	3.2	0.0001	14,500	3.4 (2.7-4.3)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.003	43.07	**54.8** (8.5-352.2)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	81	0.028	95.86	**134.5** (26.7-677.1)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.0014	7.075	**200.3** (73.6-544.8)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.028	9.7	**1,023.9** (231.9-4,520.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**3.2** (1.2-8.4)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**3.0** (1.4-6.7)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	3.2	0.014	4,894.7	**0.8** (0.3-2.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.6** (1.4-4.9)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**1.4** (0.8-2.6)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	3.2	0.009	977.6	**1.3** (0.8-2.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	3.2	0.009	281.6	**4.2** (1.3-13.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.3** (1.1-4.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	16.24	**51.2** (10.0-262.6)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	3.2	0.0001	50	8.3[b] (2.5-28.0)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	229.1	**34.6** (11.3-106.0)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	3.2	0.0001	46,500	10.6[b] (5.7-19.9)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	8,341.5	**65.2** (29.0-146.3)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-21. Ranked 8:2 FTUCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 8:2 FTUCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.7894	0.7894	0.02
2	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1.327	1.327	0.04
3	D	Water flea	*Chydorus sphaericus*	1.344	1.344	0.07
4	G	Washboard	*Megalonaias nervosa*	1.403	1.403	0.09
5	E	Oriental river shrimp	*Macrobrachium nipponense*	1.518	1.518	0.11
6	D	Daphnid	*Simocephalus serrulatus*	1.848	1.795	0.13
	D	Daphnid	*Simocephalus vetulus*	1.743		
7	G	Green floater	*Lasmigona subviridis*	1.900	1.900	0.15
8	D	Daphnid	*Ceriodaphnia dubia*	2.066	2.066	0.17
9	G	Western pearlshell	*Margaritifera falcata*	2.268	2.268	0.20
10	G	Pheasantshell	*Ortmanniana pectorosa*	2.347	2.347	0.22
11	E	Amphipod	*Gammarus fasciatus*	2.040	2.412	0.24
	E	Amphipod	*Gammarus pseudolimnaeus*	2.853		
12	G	Neosho mucket	*Lampsilis rafinesqueana*	2.325	2.430	0.26
	G	Fatmucket	*Lampsilis siliquoidea*	2.540		
13	G	Paper pondshell	*Utterbackia imbecillis*	2.454	2.454	0.28
14	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2.632	2.632	0.30
15	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2.645	2.645	0.33
16	G	Tadpole physa	*Physella gyrina*	3.035	3.035	0.35
17	**D**	**Daphnid**	***Daphnia magna***	**3.200**	**3.200**	**0.37**
18	G	Swamp lymnaea	*Lymnaea stagnalis*	3.210	3.210	0.39
19	D	Water flea	*Moina macrocopa*	4.192	4.192	0.41
20	H	Bryozoan	*Pectinatella magnifica*	15.21**	15.21	0.43
21	E	Isopod	*Caecidotea brevicauda*	16.82	16.82	0.46
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	17.89	17.89	0.48
23	E	Amphipod	*Crangonyx pseudogracilis*	28.57	28.57	0.50
24	H	Oligochaete	*Tubifex tubifex*	31.57	31.57	0.52
25	B	Largemouth bass	*Micropterus salmoides*	46.83	46.83	0.54
26	B	Zebrafish	*Danio rerio*	47.47	47.47	0.57
27	F	Midge	*Paratanytarsus dissimilis*	241.0	47.45	0.59
	F	Midge	*Paratanytarsus parthenogeneticus*	9.343		
28	B	Yellow perch	*Perca flavescens*	51.18**	51.18	0.61
29	B	Razorback sucker	*Xyrauchen texanus*	54.83**	54.83	0.63

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
30	B	Guppy	*Poecilia reticulata*	54.85	54.85	0.65
31	A	Brook trout	*Salvelinus fontinalis*	79.36	57.25	0.67
	A	Lake trout	*Salvelinus namaycush*	41.30		
32	B	Channel catfish	*Ictalurus punctatus*	67.38	67.38	0.70
33	B	Common carp	*Cyprinus carpio*	75.18	75.18	0.72
34	E	Isopod	*Asellus aquaticus*	76.56	76.56	0.74
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**81.00**	**81.00**	**0.76**
36	A	Atlantic salmon	*Salmo salar*	79.29	81.49	0.78
	A	Brown trout	*Salmo trutta*	83.76		
37	C	Cuvier's foam froglet	*Physalaemus cuvieri*	91.14	91.14	0.80
38	B	Green sunfish	*Lepomis cyanellus*	105.8**	91.43	0.83
	B	Bluegill	*Lepomis macrochirus*	78.99		
39	B	Fathead minnow	*Pimephales promelas*	107.7	107.7	0.85
40	B	Medaka	*Oryzias latipes*	121.6**	121.6	0.87
41	B	Goldfish	*Carassius auratus*	132.7	132.7	0.89
42	B	Shortnose sturgeon	*Acipenser brevirostrum*	134.5	134.5	0.91
43	B	Silver perch	*Bidyanus bidyanus*	200.3**	200.3	0.93
44	C	Bullfrog	*Lithobates catesbeianus*	132.5	257.2	0.96
	C	Northern leopard frog	*Lithobates pipiens*	125.5		
	C	Southern leopard frog	*Lithobates sphenocephalus*	1,024**		
45	B	Cape Fear shiner	*Notropis mekistocholas*	339.1**	339.1	0.98

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-22. 8:2 FTUCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=45 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	0.7894	-0.24	0.06	0.022	0.147
2	*Palaemonetes*	1.327	0.28	0.08	0.043	0.209
3	*Chydorus*	1.344	0.30	0.09	0.065	0.255
4	*Megalonaias*	1.403	0.34	0.11	0.087	0.295
		Σ (Su):	**0.68**	**0.34**	**0.22**	**0.91**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 18.39 S = slope
L = -0.801 L = X-axis intercept
A = 0.158 A = lnFAV
FAV = 1.171
FAV/2 = 0.5853 mg/L (Preliminary Value)
Adjustment = 0.5853 / 15.8 = 0.03705 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.037 mg/L 8:2 FTUCA** (rounded to two significant figures)

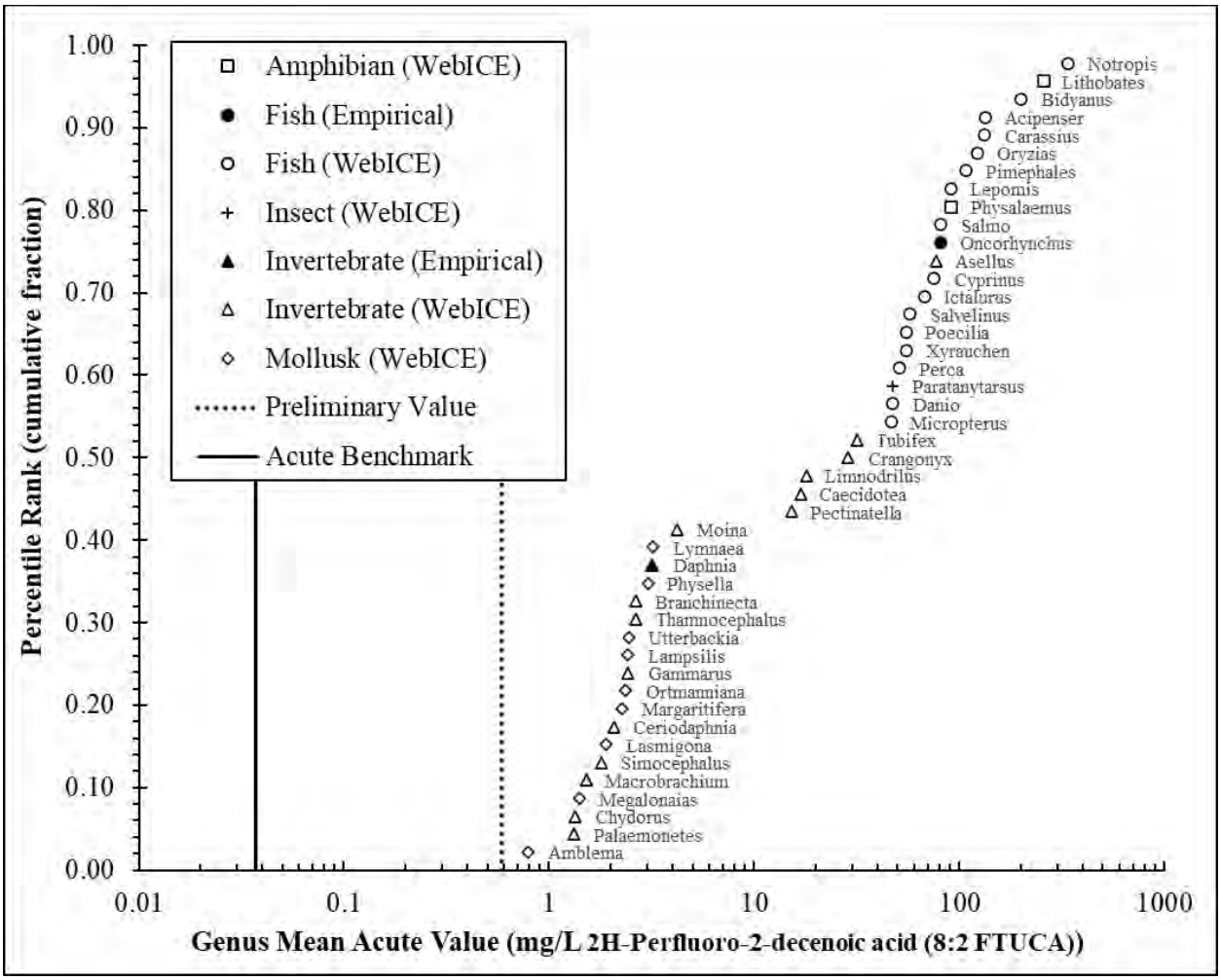


Figure 5-7. Ranked Acute 8:2 FTUCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.9 Derivation of Acute Water Benchmark for Pentadecafluorodecanoic Acid (7:3 FTCA)

Quantitatively acceptable empirical data for 7:3 FTCA were available for two species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-24**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 70 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia pulex*). Four other models were not used because there were

acceptable models for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; *Daphnia magna* predicting for *Danio rerio*). A total of 56 ICE models were selected for use, resulting in ICE models predicting 55 SMAVs representing 46 genera and all eight MDRs (**Tables 5-23** and **5-24**).

The combined empirical and ICE data resulted in 48 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-24**. GMAVs for the four most sensitive genera were within a factor of 1.7 of each other (**Table 5-25**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 7:3 FTCA is 0.3727 mg/L (**Table 5-25**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=0.2519 mg/L). The FAV was divided by two to obtain a preliminary value of 0.1864 mg/L 7:3 FTCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.012 mg/L 7:3 FTCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 7:3 FTCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-8**).

There is a gap in the data between the water flea (*Moina macrocopa*, SMAV = 1.76 mg/L) and midge (*Chironomus tentans*, SMAV = 4.63 mg/L) for these ICE-generated values (see **Figure 5-8**). This trend was also noted for 8:2 FTUCA, the other fluorotelomer evaluated. Although it is unclear the exact reason for this gap in values, it is noted that the lower portion of the curve is primarily composed of the phyla Mollusca and Arthropoda, while the upper portion

of the curve is primarily composed of the phylum Chordata. The distinct difference in sensitivity indicated by the gap in these data is thus likely to result from a greater estimated sensitivity of most invertebrates than vertebrates to 7:3 FTCA. Additionally, and as for 8:2 FTUCA, the ICE estimates for the lower portion of the curve are based primarily on the surrogate *Daphnia magna*, while the ICE estimates for the upper portion of the curve are based primarily on the surrogate *Oncorhynchus mykiss*. However, these differences fall within the range of differences in acute response frequently observed between species. For example, within the PFOA and PFOS acute criteria datasets, there was single species sensitivity that spanned over two orders of magnitude, and the variation in acute toxicity values is frequently an order of magnitude and can be as high as three orders of magnitude (Hrovat et al. 2009, Schür et al. 2023). It is, therefore, not unexpected to see the difference in sensitivity observed between these species.

Table 5-23. Acceptable Models for ICE-estimated Species Sensitivity to 7:3 FTCA.
Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.9592	0.0008	166.8	**9.5** (2.8-32.4)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.9592	0.0002	5,000	**0.8** (0.4-1.4)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.9592	0.0001	68.3	**0.9** (0.4-2.2)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	39.4 (9.0-173.4)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.9	**31.0** (15.6-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.9592	0.0003	8,694.5	**0.9** (0.6-1.3)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.9592	0.0001	46,278	2.1[b] (1.6-2.7)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.86	**33.1** (19.8-55.3)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	0.9592**	0.014	0.232	**5.3** (1.9-15.4)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	**114.0** (33.0-394.0)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**31.6** (19.9-50.1)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	0.003	724.4	40.0 (24.0-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	43.07	49.4 (38.4-63.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	0.9592	0.0003	46,278	**0.6** (0.4-0.8)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	0.9592**	0.0001	0.646	1.0 (0.3-3.2)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	0.9592	0.0089	10.36	1.1 (0.3-4.3)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	0.9592	0.0002	4,894.7	0.7 (0.5-0.9)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	0.9592	0.014	281.6	0.7 (0.2-3.1)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.9592	0.0002	7.2	**0.6** (0.3-1.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.9592	0.0001	166.8	**0.7** (0.1-3.8)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.9592	0.0002	46,500	3.0[b] (2.3-3.9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	13,400	**46.7** (36.0-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.9** (0.5-1.5)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.9592	0.014	166.8	**0.8** (0.2-3.5)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	19.93	**45.8** (19.9-105.3)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.9592	0.0003	166.8	**27.3** (9.3-80.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	82.0	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**18.0** (11.4-28.3)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	0.9592	0.0008	4.369	**131.2** (18.7-920.2)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	0.007	54.4	**52.1** (10.9-248.7)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.9592	0.0003	472	**4.6** (0.7-32.5)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.9592	0.37	14,500	**2.9** (0.9-9.1)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.9592	0.0003	58	**0.5** (0.1-1.9)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.9592	0.042	166.8	**0.8** (0.4-1.6)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	0.002	198.4	**64.7** (33.7-124.2)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.9592	0.003	281.6	**7.6** (3.1-18.5)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.9592	0.0001	4,894.7	**10.8** (3.0-38.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	0.9592	0.011	281.6	**0.4** (0.2-0.9)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.5-1.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.9592	0.042	545.9	**0.7** (0.2-2.4)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	0.9592	0.0001	14,500	1.4 (1.2-1.8)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.07	**26.6** (5.3-134.0)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	0.028	95.86	**45.7** (11.2-185.6)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.001	7.075	**73.0** (29.7-179.7)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.028	7.5	25.7 (4.0-164.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.028	9.7	**312.8** (88.4-1,106.8)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**1.0** (0.4-2.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**1.0** (0.4-2.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.9592	0.014	4,894.7	**0.3** (0.1-0.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.4-1.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.4** (0.2-0.9)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.9592	0.009	977.6	**0.4** (0.2-0.7)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.9592	0.009	281.6	**1.8** (0.6-5.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.4-1.7)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	16.24	**22.2** (5.1-95.7)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.9592	0.0001	50	3.3[b] (1.1-10.0)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.9592	0.0001	46,500	4.8[b] (2.5-9.2)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	8,341.5	**34.4** (16.8-70.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-24. Ranked 7:3 FTCA Genus Mean Acute Values.
Values in bold and highlighted are derived from empirical 7:3 FTCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.2519	0.2519	0.02
2	E	Oriental river shrimp	*Macrobrachium nipponense*	0.3806	0.3806	0.04
3	D	Water flea	*Chydorus sphaericus*	0.4278	0.4278	0.06
4	G	Washboard	*Megalonaias nervosa*	0.4393	0.4393	0.08
5	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	0.5324	0.5324	0.10
6	D	Daphnid	*Ceriodaphnia dubia*	0.6004	0.6004	0.12
7	D	Daphnid	*Simocephalus serrulatus*	0.5972	0.6489	0.14
	D	Daphnid	*Simocephalus vetulus*	0.7051		
8	G	Pheasantshell	*Ortmanniana pectorosa*	0.7379	0.7379	0.16
9	G	Western pearlshell	*Margaritifera falcata*	0.7857	0.7857	0.18
10	G	Neosho mucket	*Lampsilis rafinesqueana*	0.7949	0.8254	0.20
	G	Fatmucket	*Lampsilis siliquoidea*	0.8571		
11	G	Paper pondshell	*Utterbackia imbecillis*	0.8426	0.8426	0.22
12	E	Amphipod	*Gammarus fasciatus*	0.7600	0.8483	0.24
	E	Amphipod	*Gammarus pseudolimnaeus*	0.9468		
13	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	0.8488	0.8488	0.27
14	G	Green floater	*Lasmigona subviridis*	0.8497	0.8497	0.29
15	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	0.8773	0.8773	0.31
16	G	Tadpole physa	*Physella gyrina*	0.9531	0.9531	0.33
17	**D**	**Daphnid**	***Daphnia magna***	**0.9592**	**0.9592**	**0.35**
18	G	Swamp lymnaea	*Lymnaea stagnalis*	1.029	1.029	0.37
19	D	Water flea	*Moina macrocopa*	1.760	1.760	0.39
20	F	Midge	*Chironomus tentans*	4.630	4.630	0.41
21	H	Bryozoan	*Pectinatella magnifica*	5.340**	5.340	0.43
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	7.621	7.621	0.45
23	E	Isopod	*Caecidotea brevicauda*	8.173	8.173	0.47
24	E	Amphipod	*Crangonyx pseudogracilis*	9.512	9.512	0.49
25	H	Oligochaete	*Tubifex tubifex*	10.75	10.75	0.51
26	F	Midge	*Paratanytarsus dissimilis*	105.75	17.45	0.53
	F	Midge	*Paratanytarsus parthenogeneticus*	2.880		
27	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.55
28	B	Yellow perch	*Perca flavescens*	22.20**	22.20	0.57
29	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.59

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
	A	Lake trout	*Salvelinus namaycush*	18.01		
30	B	Zebrafish	*Danio rerio*	24.56	24.56	0.61
31	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.63
32	E	Isopod	*Asellus aquaticus*	27.30	27.30	0.65
33	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.67
34	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.69
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	**32.00**	**0.71**
36	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.73
	A	Brown trout	*Salmo trutta*	33.12		
37	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.76
38	B	Green sunfish	*Lepomis cyanellus*	45.79**	38.92	0.78
	B	Bluegill	*Lepomis macrochirus*	33.08		
39	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.80
40	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.82
41	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.84
42	B	Medaka	*Oryzias latipes*	52.12	52.12	0.86
43	B	Goldfish	*Carassius auratus*	62.18	62.18	0.88
44	B	Bonytail	*Gila elegans*	72.68	72.68	0.90
45	B	Silver perch	*Bidyanus bidyanus*	73.03**	73.03	0.92
46	C	Bullfrog	*Lithobates catesbeianus*	58.75	106.0	0.94
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	312.8**		
47	B	Cape Fear shiner	*Notropis mekistocholas*	114.0**	114.0	0.96
48	H	Leech	*Nephelopsis obscura*	131.2	131.2	0.98

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table 5-25. 7:3 FTCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=48 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	0.2519	-1.38	1.90	0.020	0.143
2	*Macrobrachium*	0.3806	-0.97	0.93	0.041	0.202
3	*Chydorus*	0.4278	-0.85	0.72	0.061	0.247
4	*Megalonaias*	0.4393	-0.82	0.68	0.082	0.286
		Σ (Su):	**-4.02**	**4.23**	**0.20**	**0.88**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 17.53 S = slope
L = -1.923 L = X-axis intercept
A = -0.987 A = lnFAV
FAV = 0.3727
FAV/2 = 0.1864 mg/L (Preliminary Value)
Adjustment = 0.1864 / 15.8 = 0.01179 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.012 mg/L 7:3 FTCA** (rounded to two significant figures)

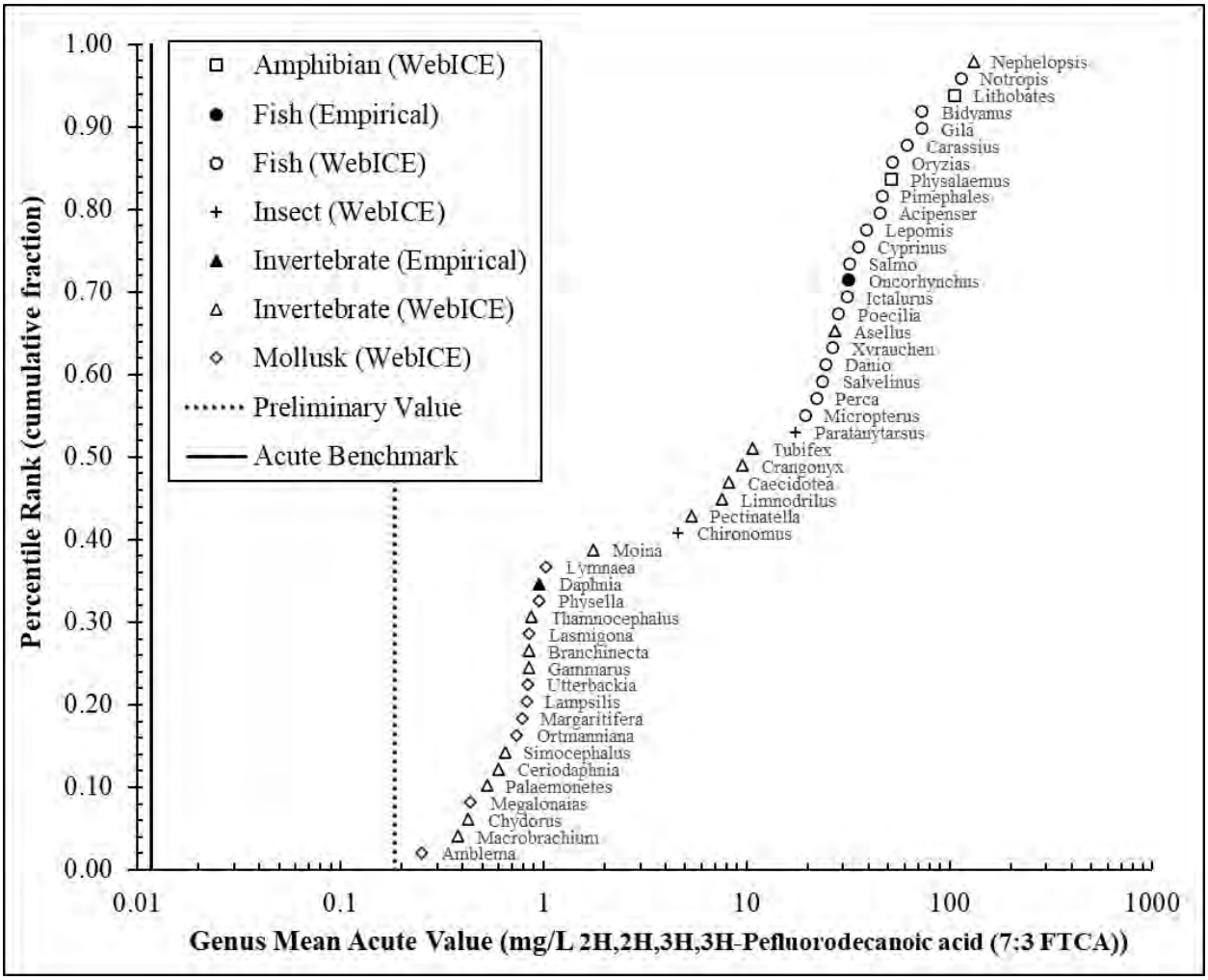


Figure 5-8. Ranked Acute 7:3 FTCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.10 Summary of Benchmarks for Evaluated PFAS Substances and Effects Characterization

5.10.1 Summary and Evaluation of Benchmark Values

Freshwater acute benchmarks for the eight selected PFAS are summarized in **Table 5-26**. These concentrations are expected to be protective of 95% of freshwater genera exposed to these PFAS under short-term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. However, because only limited empirical toxicity test data were available for the eight PFAS and the use of ICE models were required to fill in

data gaps to derive the benchmarks, the recommended aquatic life acute ambient water quality benchmarks are less certain than the standard Aquatic Life AWQC typically developed.

Quantitatively acceptable empirical toxicity data were coupled with ICE-predicted values to try to fulfill the eight MDRs for deriving acute freshwater criteria per the Aquatic Life Criteria Guidelines (U.S. EPA 1985). Using this approach, the eight MDRs were fulfilled for six of the evaluated compounds (PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, and 7:3 FTCA). Seven of the eight MDRs were fulfilled for PFBS while six of the eight MDRs were fulfilled for PFHxS; thus, there is somewhat greater uncertainty associated with these latter benchmarks. It is also noted that ICE models for some chemicals were highly dependent on one or a couple of surrogate species. For example, *Daphnia magna* comprised the majority of surrogate species for PFNA. This heavy reliance on a surrogate potentially increases the uncertainty associated with these ICE models. Conversely, there is somewhat less uncertainty associated with those PFAS benchmarks with empirical test endpoints amongst the most sensitive test species. Notably, empirical test data comprised the mean acute value for the most sensitive test species for PFBA (*Brachionus calyciflorus*), PFHxA (*Brachionus calyciflorus*), and PFHxS (*Danio rerio*), and the second most sensitive species for PFNA (*Chydorus sphaericus*). Mean acute values comprised of empirical test data did not fall amongst the most sensitive test species for the remaining PFAS for which acute benchmark values were derived. The resulting acute water column-based benchmark magnitudes range from 0.012 mg/L for 7:3 FTCA to 5.3 mg/L for PFBA.

Table 5-26. Acute Freshwater Benchmarks for Eight PFAS (mg/L, ppm).

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude	5.3	4.8	0.65	0.50	5.0	0.21	0.037	0.012
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

Table 5-27 compares the model inputs and freshwater acute benchmarks where the ICE input PFAS value (surrogate value) was beyond the model range and the model prediction used for deriving the EPA's acute PFAS benchmark values was based on the extrapolation approach or using an alternative scaled approach, as discussed in **Section 3** (see also **Appendix F)**. The alternative scaled approach adjusts the toxicity values to bring them within the ICE model range, when necessary, by changing the measured toxicity units from mg/L to µg/L so the values fall within the range of ICE toxicity values, rather than allowing regressions to extrapolate beyond the model range. This alternative approach underwent detailed evaluation by Raimondo et al. (2024) and values were calculated using the scaled approach to compare with values calculated using the extrapolation approach.

As shown in **Table 5-27**, the scaled approach allowed for the acceptance of up to two times more models than the extrapolation approach, based primarily on the greater number of scaled inputs and associated output values meeting the confidence interval acceptability parameters presented in **Box 1** (**Section 2**). Although there was a meaningful increase in the number of models accepted with the scaled approach, there was only a slight increase in the number of MDRs fulfilled. Using the scaled approach, MDRs were met for PFBS (an increase of one MDR) and one additional MDR was met for PFHxS (seven versus six MDRs met). The differences between the resulting acute benchmarks, although consistently higher using the scaled approach, were also small, with each of the benchmarks falling within a factor of $\leq$ 2.1 of

one another, indicating close agreement between benchmark values calculated using either approach. Raimondo et al. (2024) compared the extrapolated and scaled approaches and found no significant differences in prediction accuracy between the two approaches. It is likely the small but generally higher benchmark values (values were the same for PFHxS) resulting from the scaled approach result from the increase in the number of GMAVs available using the approach (see in **Table 5-27**), which will tend to mathematically increase the resulting values because of the effect of increasing the number of genera (n) in the Aquatic Life Criteria Guidelines calculation method.

Several investigations have indicated a trend of increasing toxicity with increasing carbon chain length of PFAS (Cousins et al. 2020, Chambers et al. 2021). This pattern was observed for both carboxylic acid and sulfonic acid PFAS in this document, as demonstrated by the decreasing benchmark values with an increasing number of carbons (i.e., increasing chain length) based on both the extrapolation and scaled approaches, as shown in **Table 5-28**. Further, the final acute aquatic life criterion recently derived by the EPA (U.S. EPA 2024b) for the eight carbon carboxylic acid PFOA is 3.1 mg/L, which falls between the benchmark values for PFHxA with six carbons (4.8 mg/L with the extrapolated approach; 6.0 mg/L with the scaled approach) and PFNA with nine carbons (0.65 mg/L with the extrapolated approach; 0.73 mg/L with the scaled approach) (see **Table 5-28**). Similarly, the acute aquatic life criterion recently derived by the EPA (U.S. EPA 2024c) for the eight carbon sulfonic acid PFOS is 0.071 mg/L, which falls below the benchmark values for PFHxS with six carbons (0.21 mg/L for both the extrapolated and scaled approaches). The trend of increasing toxicity with increasing chain length for carboxylic acid and sulfonic acid PFAS thus holds for both the calculated acute benchmark values and with the final acute criteria for PFOA and PFOS.

Table 5-27. Summary of Data Inputs, MDRs Fulfilled and Benchmark Outcomes Using the Extrapolation and Scaled Approach when Web-ICE Input PFAS Values Exceed Model Range.

Chemical	MDRs Met with Empirical Data	Number of Accepted ICE Predictions		MDRs Filled with ICE Models		Total MDRs Met (Number of GMAVs)		Acute Benchmark (mg/L)	
		Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach
Carboxylic Acids									
PFBA	3	22	43	5	5	8(22)	8(33)	5.3	11
PFHxA	3	28	43	5	5	8(24)	8(33)	4.8	6.0
PFNA	2	29	42	6	6	8(25)	8(33)	0.65	0.73
PFDA	3	61	85	5	5	8(42)	8(60)	0.50	0.65
Sulfonic Acids									
PFBS	2	55	119	5	6	7(30)	8(58)	5.0	7.6
PFHxS	2	17	18	4	5	6(12)	7(13)	0.21	0.21
Fluorotelomers									
8:2 FTUCA	2	66	80	6	6	8(45)	8(55)	0.037	0.041
7:3 FTCA	2	70	81	6	6	8(48)	8(59)	0.012	0.015

Table 5-28. Comparison of PFAS Chain Length to Perfluorocarboxylic and Perfluorosulfonic Acid Benchmarks and PFOA and PFOS Acute Criteria Values.

Chemical	Chain Length: Number of Carbons	Acute Benchmark (mg/L)		PFOA and PFOS Aquatic Life Criteria (mg/L) [1]
		Extrapolation Approach	Scaled Approach	
Carboxylic Acids				
PFBA	4	5.3	11	NA
PFHxA	6	4.8	6.0	NA
PFOA	8	NA	NA	3.1
PFNA	9	0.65	0.73	NA
PFDA	10	0.50	0.65	NA
Sulfonic Acids				
PFBS	4	5.0	7.6	NA
PFHxS	6	0.21	0.21	NA
PFOS	8	NA	NA	0.071

NA = Not Applicable

[1] Final aquatic life criteria for PFOA and PFOS are presented in U.S. EPA (2024b) and U.S. EPA (2024c), respectively.

5.10.2 Comparison of Benchmarks to Other Calculation Methods for Data-Limited PFAS

5.10.2.1 Comparison with Giddings et al. (2019) Approach of Combining Data Across Chemical Class

In addition to calculating PFAS benchmarks using a combination of empirical and predicted (ICE model) values for each individual PFAS, the EPA explored the application of an approach used by Giddings et al. (2019) to derive protective values for data-limited synthetic pyrethroid insecticides (detailed in **Appendix G**). In brief, Giddings et al. (2019) combined acute toxicity data across several synthetic pyrethroid insecticide active ingredients (e.g., bifenthrin, cypermethrin, permethrin, etc.) into a single pyrethroid species sensitivity distribution (SSD) to provide a more robust dataset for the derivation of protective aquatic life values. The Giddings et al. (01) approach thus involved combining or "binning" data within a general chemical class, and expected similar mode of action, to develop a larger data set for their analyses. The EPA followed the same binning approach to estimate protective aquatic life values for PFAS, using quantitatively acceptable empirical data (**Appendix A**) for combined carboxylic acid PFAS (PFBA, PFHxA, PFNA, and PFDA) and combined sulfonic acid PFAS (PFBS and PFHxS) datasets. The EPA additionally incorporated quantitatively acceptable empirical data used for derivation of the acute PFOA and PFOS criteria (U.S. EPA 2024b,c) in the carboxylic acid and sulfonic acid data groupings, respectively, to further support derivation of these benchmark values. Data for carboxylic acid and sulfonic acid groupings were each then normalized by a common species and pooled according to PFAS grouping to derive group-specific SSDs. Group-specific SSDs were then generated using both the EPA's SSD Generator (as was done by Giddings et al. 2019) and following procedures described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985). A more detailed description of the process used to derive these benchmark values is presented in **Appendix G**.

Exploratory acute benchmarks derived using the Giddings binning approach and SSD Generator-based values are summarized in **Table 5-29** and compared to those calculated using the Aquatic Life Criteria Guidelines-based values. Exploratory benchmark values for both carboxylic acids (5.6 times) and sulfonic acids (5.0 times) were consistently higher when calculated using the SSD Generator-based values versus the Aquatic Life Criteria Guidelines-based values. However, these benchmarks did not show a consistent pattern in magnitude of differences when compared to the EPA's recommended acute benchmark values (see **Table 5-29**). For carboxylic acid PFAS, for example, exploratory benchmark values calculated using the Aquatic Life Criteria Guidelines-based approach were between 0.5 and 3.7 times different (both higher and lower) compared to the EPA's recommended acute benchmarks. For sulfonic acid PFAS, exploratory benchmark values calculated using the Aquatic Life Criteria Guidelines-based values were between 0.5 and 3.0 times different (both higher and lower) compared to recommended benchmarks. Although the differences between these outcomes cannot be definitively determined, outcomes are likely to reflect inherent differences in the derivation processes being used. For example, use of the EPA's Aquatic Life Criteria Guidelines procedure to derive an SSD and calculate a protective value relies on a single distribution based on averaging the data for the four most sensitive genera around the fifth centile of the distribution, whereas the EPA's SSD Generator (used by Giddings et al. 2019) supports the calculation of four different distributions (normal, logistic, triangular, and Gumbel), with the protective value selected from the best-fit distribution. The limited size of the constituent empirical datasets available for the binning approach used to generate SSDs based on Aquatic Life Criteria Guidelines-based and SSD Generator-based values for the specific PFAS (24 studies total for PFBA, PFNA, PFDA, and PFHxA datasets and seven studies total for PFBS and PFHxS

datasets) and the limited number of datapoints available for the species used for normalization (one to three datapoints for PFBA, PFHxA, PFNA, and PFDA datasets and seven studies total for PFBS and PFHxS datasets) are also likely to influence the individual benchmark outcomes.

Table 5-29. Comparison of Benchmark Outcomes with Acute Benchmarks Calculated Based on Approach by Giddings et al. (2019) (Aquatic Life Criteria Guidelines and SSD Generator-Based Values).

Chemical	EPA Recommended Acute Benchmark (mg/L)	Acute Benchmarks Calculated Using Binning Approaches[1]		Factor Difference Between EPA Acute Benchmark and Binning-based Benchmark Values	
		Aquatic Life Criteria Guidelines-Based Values (mg/L)	SSD Generator-Based Values (mg/L)	Aquatic Life Criteria Guidelines-Based Values	SSD Generator-Based Values
Carboxylic Acids					
PFBA	5.3	19	110	3.6	21
PFHxA	4.8	4.3	24	0.9	5.0
PFNA	0.65	0.35	2.0	0.5	3.0
PFDA	0.50	0.49	2.8	1.0	5.6
Sulfonic Acids					
PFBS	5.0	15	75	3.0	15
PFHxS	0.21	0.11	0.56	0.5	2.7

[1] Acute benchmarks-binning approach is presented in Appendix G.

5.10.2.2 Comparison with Grippo et al. (2021) Approach of Deriving Water Quality-Based Ecological Screening Values

Grippo et al. (2021) calculated aquatic life surface water ecological screening values for each of the carboxylic acid and sulfonic acid PFAS for which the EPA developed benchmarks. Grippo et al. (2021) employed the Great Lakes Initiative (GLI) Tier II water quality value derivation approach (U.S. EPA 1995a,b,c), which uses secondary acute factors to account for the increased uncertainty associated with the reduced number of tested genera, when they fall below the eight MDRs. A comparison of the resulting screening values to the EPA's recommended

benchmark values is presented in **Table 5-30**. The Tier II GLI values calculated by Grippo et al. (2021) using the Tier II water quality value derivation approach were generally in agreement with the EPA benchmarks for carboxylic acid PFAS (within a factor of ≤ 8 of one another), whereas the Tier II GLI value calculated by Grippo et al. (2021) for sulfonic acid PFAS trended towards a greater difference (factor of 8 for PFHxS and 130 for PFBS). This trend towards a larger difference in Tier II GLI value versus the EPA benchmark for sulfonic acid PFAS is likely primarily due to differences between the application factor used by the EPA for calculation of the acute benchmark (42.3) and the application factor used by Grippo et al. (2021) for calculation of the Tier II GLI values (13), and some differences in the data used for the calculation of the Tier II values. If, for example, the Tier II GLI application factor of 13, used by Grippo et al. (2021) for PFBS and PFHxS, were applied to the EPA calculated benchmarks for these compounds (instead of the application factor used by the EPA) the difference between the two values decreases to a factor difference of 9.2 for PFBS and 2.4 for PFHxS). Overall, the EPA's acute benchmark values are lower than the Tier GLI values. This is primarily because the EPA used application factors to account for the high sensitivity of some invertebrate species to PFAS, as represented by the mayfly *Neocloeon triangulifer* (Soucek et al. 2023) and the cladoceran *Moina micrura* (Razak et al. 2023) to PFOA and PFOS. These sensitive species were not represented in the derived Tier II GLI values.

Table 5-30. Comparison of Benchmark Outcomes with Water Quality-Based Ecological Screening Values Calculated by Grippo et al. (2021) Using the Great Lakes Initiative (GLI) Approach.

Chemical	EPA Recommended Acute Benchmark (mg/L)	Grippo et al. (2021) Calculated Tier II GLI Values (mg/L)	Factor Difference Between EPA Acute Benchmark and Calculated Tier II GLI Values
Carboxylic Acids			
PFBA	5.3	13.75	2.6
PFHxA	4.8	17.5	3.6
PFNA	0.65	2.14	3.3
PFDA	0.50	4	8
Sulfonic Acids			
PFBS	5.0	149.1	30
PFHxS	0.21	1.7	8

5.10.3 Summary and Conclusions

The EPA developed recommended aquatic life acute ambient water quality benchmarks for eight PFAS with limited empirical test data in accordance with the provisions of section 304(a) of the CWA. Benchmarks were developed for the following eight chemicals: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), 2H-Perfluoro-2-decenoic acid (8:2 FTUCA), and 2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA).

Because of limitations in the available direct empirical test data, the EPA developed acute protective benchmarks for these PFAS using available freshwater species empirical test data in conjunction with the application of the EPA, f fice of e search and Development's (D 's) peer-reviewed Web-based Interspecies Correlation Estimate tool (Web-ICE; Version 4.0; https://www.epa.gov/webice/) (Raimondo et al. 2024). Two approaches were used for

benchmark development because PFAS acute values can extend beyond the range of values used to calibrate the ICE model. One approach allowed extrapolation of the data beyond the calibrated range of the model, while the other (presented in **Appendix F**) "scaled" the toxicity values to bring them within the ICE model range, when necessary.

Table 5-27 summarizes and compares the model inputs and aquatic life ambient water quality benchmark values between the extrapolation approach and alternative scaled approach. Benchmark values calculated using the scaled approach were consistently higher than those calculated using the extrapolation approach, though the difference was small (within a factor of $\leq$ 2.1 of one another), indicating close agreement between the two approaches. It is likely this small but consistent difference results from an increase in the number of GMAVs available when using the scaled approach, which tends to mathematically increase the resulting values.

Several investigations have indicated a trend of increasing toxicity with increasing carbon chain length of PFAS compounds (Cousins et al. 2020, Chambers et al. 2021). This pattern was also observed for carboxylic acid and sulfonic acid PFAS, as demonstrated by the decreasing benchmark values with an increasing number of carbons (i.e., increasing chain length) for both the extrapolation and scaled approaches (**Table 5-28**).

Table 5-31 compares the EPA benchmark values with those calculated using alternative approaches. The EPA explored the application of an alternative data "binning" approach, developed by Giddings et al. (2019) (see **Table 5-31**), which combines data for multiple related compounds to provide a more robust dataset. This approach supported derivation of protective aquatic life values for grouped perfluoroalkyl carboxylic acids and grouped perfluoroalkyl sulfonic acids. SSDs were derived using both Aquatic Life Criteria Guidelines -based procedures, which derive a single distribution and determine the benchmark value based on

averaging the data for the four most sensitive genera around the fifth centile, and by using the EPA's SSD Generator, which was used by Giddings et al. (2019) and supports the calculation of four different distributions and selects a protective value from the best-fit distribution using all of the genera. Exploratory benchmark values for both carboxylic acids and sulfonic acids were consistently higher (5.6 to 5.8 times and 5.0 times, respectively) when derived using the SSD Generator versus the Aquatic Life Criteria Guidelines procedures but did not show a consistent pattern in magnitude when compared to the EPA's recommended benchmark values (**Tables 5-31** and **5-29**). For carboxylic acid PFAS, for example, exploratory benchmark values calculated using Aquatic Life Criteria Guidelines-based values were between 1.02 and 3.6 times different (both higher and lower) compared to EPA's recommended acute benchmarks. For sulfonic acid PFAS, exploratory benchmark values calculated using Aquatic Life Criteria Guidelines-based values were between 1.9 and 3.0 times different (both higher and lower) compared to recommended benchmarks. These mostly small differences likely reflect inherent differences in the derivation processes being used and the constituent datasets.

Grippo et al. (2021) employed the Great Lakes Initiative (GLI) Tier II water quality value derivation approach (U.S. EPA 1995a,b,c) to derive draft ecological screening values for the same carboxylic acid and sulfonic acid PFAS evaluated in this document. This GLI approach uses secondary acute factors to account for the increased uncertainty associated with the reduced number of tested genera, when the number of genera for which data is available falls below the Aquatic Life Criteria Guidelines eight MDRs. The Grippo et al. (2021) calculated draft Tier II GLI values were in generally close agreement with the EPA's acute benchmarks for both carboxylic acid PFAS (within a factor of ≤ 8 of one another) and sulfonic acid PFAS (30 for PFBS and 8 for PFHxS) (**Table 5-31**). These differences likely reflect the use of different

application factors and some differences in the data used for the calculation of the draft Grippo et al. (2021) Tier II values, which did not include the sensitive mayfly (*N. triangulifer*) data as the Grippo et al. (2021) publication preceded the publication of the mayfly data.

These acute aquatic life benchmarks, developed under Section 304(a)(2) of the CWA, are informational values that the EPA generated because there are limited high quality toxicity data available and data gaps exist for several aquatic organism families, such that 304(a)(1) criteria could not be developed. The EPA developed these aquatic life benchmarks to provide information that states and Tribes may consider in their water quality protection programs, including establishing their water quality criteria. ow ever, the EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's or Tribe's water quality standards.

Table 5-31. Summary of the EPA Acute Benchmark Values and Acute Values Calculated Using Alternative Methods

Chemical	EPA Acute Benchmarks – Extrapolation Approach (mg/L)	Acute Benchmarks Calculated Using Binning Approaches		Grippo et al (2021) Calculated Draft Tier II GLI Values (mg/L)
		Aquatic Life Criteria Guidelines-Based Values (mg/L)	SSD Generator-Based Values (mg/L)	
PFBA	5.3	19	110	13.75
PFHxA	4.8	4.3	24	17.5
PFNA	0.65	0.35	2.0	2.14
PFDA	0.50	0.49	2.8	4
PFBS	5.0	15	75	149.1
PFHxS	0.21	0.11	0.56	1.7
8:2 FTUCA	0.037	-	-	-
7:3 FTCA	0.012	-	-	-

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Appendices

Appendix A Quantitatively Acceptable Freshwater Acute Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Perfluorobutanoic acid (PFBA)									
Rotifer (neonate, <2 h), *Brachionus calyciflorus*	S, U	24 hr	PFBA 98%	7.0	20	LC_{50}	110	110	Wang et al. 2017
Cladoceran (neonate, <24 hours), *Chydorus sphaericus*	S, M	48 hr	PFBA 98%	-	20	EC_{50} (immobilization)	>4,280.8	>4,280.8	Ding et al. 2012
Zebrafish (embryo, 60 mpf), *Danio rerio*	S, U	96 hr	PFBA >97%	7.2-7.5	26	LC_{50}	>3,000[b]	-	Hagenaars et al. 2011
Zebrafish (gastrula, 4.5 hpf), *Danio rerio*	R, U	96 hr	PFBA NR	7.0 - 7.5	28	LC_{50}	13,779	13,779	Godfrey et al. 2017
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, U	48 hr	PFBA >97%	NR	21	EC_{50} (immobilization)	>1,006[b]	-	Boudreau 2002
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBA 98%	8.0	20	EC_{50} (immobilization)	>4,280.8	-	Ding et al. 2012
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBA >98%	7.0 – 7.82	20	EC_{50} (immobilization)	5,251	4,741	Barmentlo et al. 2015
Cladoceran (neonate, <24 hr), *Daphnia pulicaria*	S, U	48 hr	PFBA >97%	-	21	EC_{50} (immobilization)	>1,006	>1,006	Boudreau 2002
Perfluorobutanesulfonic acid (PFBS)									
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBS 97.3-97.9%	8.1 – 8.5	19.8 – 20.1	EC_{50} (immobilization)	2,183	2,183	Wildlife International Ltd. 2001
Fathead minnow (juvenile), *Pimephales promelas*	S, M	96 hr	PFBS 97.3 – 97.9%	8.4 – 8.5	20.7 – 22.8	LC_{50}	1,938	1,938	Wildlife International Ltd. 2001
Bluegill (juvenile), *Lepomis macrochirus*	S, M	96 hr	PFBS 97.3 – 97.9%	8.1 – 8.4	22.1 – 23.9	LC_{50}	6,452	6,452	Drottar and Krueger 2001

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Zebrafish (embryo, 60 mpf), *Danio rerio*	S, U	96 hr	PFBS >97%	7.2 – 7.5	26	LC_{50}	>3,000	>3,000	Hagenaars et al. 2011
Perfluorononanoic acid (PFNA)									
Cladoceran (<24 hours), *Chydorus sphaericus*	S, M	48 hr	PFNA 97%	-	20	EC_{50} (immobilization)	27.84	27.84	Ding et al. 2012
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, U	48 hr	PFNA 98%	7.7 – 8.4	20	EC_{50} (immobilization)	43.42	-	Lu et al. 2016
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, U	48 hr	PFNA >97%	-	21	EC_{50} (immobilization)	91.89	-	Boudreau 2002
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, M	48 hr	PFNA 97%	-	20	EC_{50} (immobilization)	151.29	84.51	Ding et al. 2012
Clawed frog (blastula), *Xenopus sp.*	R, U	96 hr	PFNA NR	-	23	LC_{50}	335.8	335.8	Kim et al. 2013
Perfluorodecanoic acid (PFDA)									
Cladoceran (neonate, <24 hours), *Chydorus sphaericus*	S, U	48 hr	PFDA 98%	-	20	EC_{50} (immobilization)	41.13	41.13	Ding et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA 99.6%	-	20	EC_{50}	>100[b]	-	Hoke et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA >97%	-	21	EC_{50} (immobilization)	129.54	-	Boudreau 2002
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA	-	20	EC_{50} (immobilization)	163.48	145.5	Ding et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia pulicaria*	S, U	48 hr	PFDA, >97%	-	21	EC_{50} (immobilization)	149.59	149.59	Boudreau 2002
Clawed frog (blastula), *Xenopus sp.*	R, U	96 hr	PFDA NR	-	23	LC_{50}	76.5	76.5	Kim et al. 2013

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	PFDA 99.6%	-	12	LC_{50}	32	32	Hoke et al. 2012
Perfluorohexanoic acid (PFHxA)									
Rotifer (neonate, <2 h), *Brachionus calyciflorus*	S, U	24 hr	PFHxA 98%	7.0	20	LC_{50}	140	140	Wang et al. 2017
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, M	48 hr	PFHxA >97%	7.0 – 7.82	20	EC_{50} (immobilization)	1,048	1,048	Barmentlo et al. 2015
American bullfrog (larva, 26 go), *Lithobates catesbeianus* (formerly, *Rana catesbeiana*)	S, U	96 hr	PFHxA >98%	-	21	LC_{50}	1,105	1,105	Tornabene et al. 2021
Green frog (larva, 26 gosner stage), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hr	PFHxA NR	-	21	LC_{50}	758	758	Tornabene et al. 2021
Perfluorohexanesulfonic acid (PFHxS)									
Zebrafish (embryo, 3 hpf), *Danio rerio*	R, U	96 hr	PFHxS NR	7.2	28	NOEC (growth, length)	22.5	22.5	Annunziato et al. 2020
American bullfrog (larva, 26 gosner stage), *Lithobates catesbeianus* (formerly, *Rana catesbeiana*)	S, U	96 hr	PFHxS NR	-	21	LC_{50}	1,105	1,105	Tornabene et al. 2021
Green frog (larva, 26 gosner stage), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hr	PFHxS NR	-	21	LC_{50}	758	758	Tornabene et al. 2021
H-Perfluoro-2-decenoic acid (8:2 FTUCA)									
Cladoceran (neonate, 24 hr), *Daphnia magna*	S, U	48 hr	8:2 FTUCA 99.8%	-	20	EC_{50} (immobilization)	3.2	3.2	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	8:2 FTUCA 99.8%	-	12	LC_{50}	81	81	Hoke et al. 2012
2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)									
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	7:3 FTCA >99.%	-	20	EC_{50} (immobilization)	0.4	-	Hoke et al. 2012
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, U	48 hr	7:3 FTCA >99%	-		EC_{50} (immobilization)	2.3	0.9592	Hoke et al. 2012
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	7:3 FTCA >99%	-	12	LC_{50}	32	32	Hoke et al. 2012

a S=static, R=renewal, F=flow-through, U=unmeasured, M=measured.
b Not used in species mean calculation because the value is greater than low value.

A.1 References

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Appendix B Summary of Lowest Quantitatively Acceptable Freshwater Acute Toxicity Studies

B.1 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies Used to Derive Aquatic Life Benchmarks

Quantitatively acceptable empirical acute toxicity data were available for 27 freshwater species across the 8 PFAS: PFBA (5 species), PFBS (4 species), PFNA (3 species), PFDA (4 species), PFHxA (4 species), PFHxS (3 species), 8:2 FTUCA (2 species), and 7:3 FTCA (2 species) (**Table 4-2**; **Appendix A**). The following presents summaries of the freshwater toxicity studies with empirical effect values that were used quantitatively to derive acute benchmarks, which consist of toxicity values falling amongst the four most sensitive values. These values are used quantitatively to derive the benchmarks consistent with procedures presented in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) designed to protect 95% of aquatic taxa. These "most sensitive" empirical studies consist of one study each for PFBA, PFNA, PFHxA, and PFHxS. Study summaries for the most sensitive taxa are presented below for each of these substances. Acute values are presented as reported by the study authors, unless indicated otherwise.

B.2 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorobutanoic acid (PFBA)

Wang et al. (2014) exposed the rotifer, *Brachionus calyciflorus*, to perfluorobutanoic acid (PFBA, CAS # 375-22-4, 98% purity) in a 24-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <2-hour old neonates. Exposures consisted of 10 test organisms per 15 mL well in 6-well cell culture plates containing 10 mL of test medium. The control and each of nine PFBA test concentrations were replicated three times for a total of 30 neonates per treatment. Nominal test concentrations were 0.0 mg/L for the control and treatments ranged from 40-140 mg/L PFBA. Neonates were exposed at pH 7.0 and 0 ± 1°C under darkness. After 4 h of exposure, the number of dead and live rotifers was

enumerated under a microscope at 10× magnification. Rotifers were deemed inactive if they did not move in a period of 30s. The author-reported acute LC_{50} was 110 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFBA benchmark.

B.3 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorohexanoic acid (PFHxA)

Wang et al. (2014) exposed the rotifer, *Brachionus calyciflorus*, to perfluorohexanoic acid (PFHxA, CAS # 307-24-4, 98% purity) in a 24-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <2-hour old neonates. Exposures consisted of 10 test organisms per 15 mL well in 6-well cell culture plates containing 10 mL of test medium. The control and each of nine PFHxA test concentrations were replicated three times for a total of 30 neonates per treatment. Nominal test concentrations were 0.0 mg/L for the control and treatments ranged from 40-140 mg/L PFHxA. Neonates were exposed at pH 7.0 and 0 ± 1°C under darkness. After 4 h of exposure, the number of dead and live rotifers was enumerated under a microscope at 10× magnification. Rotifers were deemed inactive if they did not move in a period of 30s. The author-reported acute LC_{50} was 140 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFHxA benchmark.

B.4 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorononanoic acid (PFNA)

Ding et al. (2012) exposed the cladoceran, *Chydorus sphaericus*, to perfluorononanoic acid (PFNA, CAS # 375-95-1, 97% purity) in a 48-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <24-hour old neonates. Exposures consisted of five test organisms per 2 ml vial to which 250 µl test solution was added. Because the water solubility of PFNA was low, dimethylsulfoxide (DMSO) was used for preparing the stock solution. The concentration of DMSO in test solutions did not exceed 0.2%.

A solvent control was tested simultaneously with a negative (dilution water) control. The two controls and each of six PFNA test concentrations were replicated four times for a total of 20 neonates per treatment. Nominal test concentrations were 0.0 (controls), 0.05, 0.07, 0.1, 0.15, 0.2 and 0.25 mM PFNA, or 0, 23.20, 32.49, 69.61, 92.82 and 116.0 mg/L after conversion using a molecular weight for PFNA of 464.08 mg/mmol. Samples containing the highest and the lowest test concentrations were analyzed to confirm the nominal concentrations. Although partial chemical analysis was conducted, only nominal concentrations were reported. To assess the test concentration variability, all samples at a selected nominal concentration were analyzed, with a relative standard deviation of 6.5%. The measured concentrations were between 82 and 91% of the nominal concentration. Neonates were exposed at 0 ± 1°C unde r a 1 -hour light:dark cycle. The animals were not fed during the test period and were checked under a reverse dissecting microscope at 24 and 48 h. Immobilization was determined based on inactivation of the animals after slightly tapping with a finger to the vial and monitoring them for 30s. The author-reported acute immobilization LC_{50} was 0.060 mM (or 27.84 mg/L). This value was acceptable for quantitative use in deriving the recommended acute freshwater PFNA benchmark.

B.5 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorohexanesulfonic acid (PFHxS)

Annunziato et al. (2020) exposed the wildtype AB strain of zebrafish, *Danio rerio*, to perfluorohexanesulfonic acid (PFHxS, CAS # 355-46-4, purity not reported) in a 93-hour acute toxicity test following OECD Test No. 236 (OECD 2013). The test was performed under static-renewal conditions beginning with 3-hour post fertilization (hpf) embryos. Fifteen embryos were assigned at random to each treatment or control group, with each embryo placed into an individual glass vial containing 1 mL of solution. Test solutions were prepared and refreshed daily. Because of the low water solubility of PFHxS, dimethylsulfoxide (DMSO) was used for

preparing the stock solution. The concentration of DMSO in test solutions did not exceed 0.01%. A solvent control and each of seven PFHxS test concentrations were replicated three times for a total of 45 organisms per treatment. Nominal test concentrations were 0.0 (control), 0.07, 1.41, . 1, . 3, 11. , . 0, a nd 4 .00 mg/ L PF xS. Em bryos were exposed at p . , ± 1°C under a 14:10-hour light:dark cycle and monitored daily through 96 hpf. There was >90% health and survival in the control group. Test concentrations of PFHxS were too low to derive an LC_{50} value. Larval growth (length) was assessed at test termination. The author-reported a growth (weight) NOEC of 22.5 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFHxS benchmark.

B.6 References

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Appendix C Qualitative Freshwater Acute Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Perfluorobutanoic acid (PFBA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorobutanoic acid / 98%	NR	NR	EC_{50} (population photosynthesis)	262.2[b]	Test duration too short	Ding et al. 2012
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorobutyric acid / ≥ %	NR	25	IC_{50} (population/ weight)	>1,006[b]	Test duration too long	Boudreau 2002
Japanese medaka (embryo, <6 hpf), *Oryzias latipes*	R, U	48 hr post hatch	Perfluorobutyric acid / NR	7.0-7.5	25	LOEC (velocity)	137	Only one concentration tested; atypical endpoint	Godfrey 2017
Japanese medaka, *Oryzias latipes*	S, U	96 hr	Perfluorobutyric acid / NR	7.0-7.5	25	NOEC (mortality)	>13,795	Only one concentration tested	Godfrey et al. 2019
Japanese medaka (embryo, <6 hpf), *Oryzias latipes*	R, U	10 d	Perfluorobutyric acid / NR	7.0-7.5	25	NOEC (growth - length)	137	Only one concentration tested; duration too short for a chronic test	Godfrey et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	144 hr post fert.	Perfluorobutyric acid / NR	7.2-7.6	26	EC_{50} (heart rate, hatching time)	2,200	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Perfluorobutyric acid / >99%	7	28	LC_{50}	9,703	Test duration too short	Wasel et al. 2020

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-butanoic acid / >98%	NR	28	NOEC (mortality)	0.2504[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Perfluorobutanesulfonic acid (PFBS)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, M	96 hr	Perfluorobutane sulfonate potassium salt / 97.3%	6.9-8.7	23.8-24.5	EC_{50} (population abundance)	2,146		Wildlife International 2001
Green alga, *Raphidocelis subcapitata*	S, U	48 hr	1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-Nonafluoro-, Potassium salt / ≥ %	7.2	20	EC_{10} (population growth rate)	299	Test duration too short	Kusk et al. 2018
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorobutane sulfonic acid / NR	7.2-7.6	26	LC_{50}	1,500	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium nonafluoro-1-butanesulfonate / NR	NR	28	NOEC (growth stunting)	20.84[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo), *Danio rerio*	R, U	168 hr post fert.	Nonafluorobutane-1-sulfonic acid / NR	7.5	28-28.5	LC_{50}	921.9	Duration too long for an acute test and too short for a chronic test	Stinckens et al. 2018
Zebrafish (embryo, 3 hpf), *Danio rerio*	R, U	5 d post fert.	Nonafluorobutane-1-sulfonic acid / 97%	NR	NR	LC_{50}	406.2[b]	Test duration too long for an acute test and too short for a chronic test	Sant et al. 2019

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium nonafluoro-1-butanesulfonate / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	27.75[b]	Test duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Potassium perfluorobutane-sulfonate / >99%	NR	28	LC_{50}	1,394	Test duration too short	Wasel et al. 2020
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Potassium perfluoro-1-butanesulfonate / >98%	NR	28	NOEC (mortality)	0.2295[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Zebrafish (3-yr old adults), *Danio rerio*	R, U	14 d	Perfluorobutanesulfonate / >98%	NR	NR	LOEC (multiple genetic and biochemistry endpoints)	0.100	Only non-apical endpoints evaluated	Liu et al. 2022
Perfluorohexanoic acid (PFHxA)									
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hr	Perfluorohexanoic acid / >97%	8.5	6	NOEC (accumulation residue)	10.83	Test duration too short	Raine et al. 2021
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorohexanoic acid / NR	NR	28	NOEC (growth stunting)	20.10[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorohexanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	22.18[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Perfluorohexanoic acid / >99%	7	28	LC_{50}	6,486	Test duration too short	Wasel et al. 2020
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	120 hr	Perfluoro-n-hexanoic acid / >98%	NR	28	MATC (mortality)	5.367	Duration too long for an acute test and too short for a chronic test	Guo et al. 2021

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-hexanoic acid / >98%	NR	28	NOEC (mortality)	0.2512[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Zebrafish (embryo, 0.5-2 hpf), *Danio rerio*	R, U	94-95.5 hr	Perfluorohexanoic acid / >99%	NR	28	MATC (mortality)	5.367	Test duration too short	Zhang et al. 2022
Perfluorohexanesulfonic acid (PFHxS)									
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium perfluorohexanesulfonate / NR	NR	28	MATC (mortality)	8.889[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium perfluorohexanesulfonate / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	1.968[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Sodium perfluoro-1-hexanesulfonate / >98%	NR	28	NOEC (mortality)	0.2503[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Perfluorononanoic acid (PFNA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorononanoic acid / 97%	NR	NR	EC_{50} (population photosynthesis)	481.7[b]	Test duration too short	Ding et al. 2012
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorononanoic acid / ≥ %	NR	25	IC_{50} (population/ weight)	89.10[b]	Test duration too long	Boudreau 2002
Cladoceran (<24 h old), *Daphnia pulicaria*	R, U	21 d	Perfluorononanoic acid / ≥ %	NR	21	MATC (reproduction: young/brood)	8.694[b]	Lack of procedural details cannot judge against data quality objectives	Boudreau 2002
Zebrafish (embryo, 0 hpf), *Danio rerio*	S, U	72 hr post fert.	Perfluorononanoic acid / 97%	8.3	28.5	LC_{50}	84	Test duration too short	Zheng et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorononanoic acid / NR	7.2-7.6	26	LC_{50}	>10	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorononanoic acid / NR	NR	28	MATC (mortality)	0.9392[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, U	23 hr	Perfluorononanoic acid / 97%	7.2-8.0	28	LC_{50}	140.2[b]	Test duration too short	Liu et al. 2015
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	117 hr	Perfluorononanoic acid / NR	7.2-7.7	26-28	MATC (growth - length)	0.2935[b]	Duration too long for an acute test and too short for a chronic test	Jantzen et al. 2016
Zebrafish (embryo, 72 hpf), *Danio rerio*	S, M	48 hr	Perfluorononanoic acid / NR	NR	27	LC_{50}	108.6	Test duration too short	Rainieri et al. 2017
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorononanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	17.10[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-nonanoic acid / 97%	NR	28	LOEC (development deformation)	45.02[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
African clawed frog (embryo, 8-11 stage), *Xenopus laevis*	R, U	96 hr	Perfluorononanoic acid / NR	6.5-7.5	24	NOEC (mortality, growth)	3.5	Only one concentration evaluated	Mitchell 2010
Perfluorodecanoic acid (PFDA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorodecanoic acid / 98%	NR	NR	EC_{50} (population photosynthesis)	437.5[b]	Test duration too short	Ding et al. 2012
Green alga, *Raphidocelis subcapitata*	S, U	72 hr	n-Perfluorodecanoic acid / 99.6%	~7.5	24	EC_{50} (population biomass)	10.6	Test duration too short	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorodecanoic acid / ≥ %	NR	25	IC_{50} (population/ weight)	99.22[b]	Test duration too long	Boudreau 2002
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorodecanoic acid / NR	7.2-7.6	26	LC_{50}	8.4	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorodecanoic acid / NR	NR	28	NOEC (growth stunting)	32.90[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorodecanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	5,141[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-decanoic acid / 98%	NR	28	NOEC (mortality)	50.38[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
African clawed frog (embryo, 8-11 stage), *Xenopus laevis*	R, U	96 hr	Perfluorodecanoic acid / NR	6.5-7.5	24	NOEC (mortality, growth)	3.5	Only one concentration evaluated	Mitchell 2010
Hexadecafluoro-2-decenoic acid (8:2 FTUCA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, M	72 hr	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	24	EC_{10} (population abundance)	2.3	Test duration too short	Mitchell et al. 2011
Green alga, *Raphidocelis subcapitata*	S, U	72 hr	8:2 FTUCA / 99.8%	~7.5	24	EC_{50} (population biomass)	7.5	Test duration too short	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Green alga (exponential growth phase (log), 3-7 d), *Chlorella vulgaris*	S, M	72 hr	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	24	EC_{10} (population abundance)	2.2	Test duration too short	Mitchell et al. 2011
Amphipod, *Hyalella azteca*	R, M	10 d	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	23	NOEC (mortality and growth)	6.1	Duration too long for an acute test and too short for a chronic test	Mitchell et al. 2011
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	2H-Perfluoro-2-decenoic acid / >98%	NR	28	NOEC (mortality)	0.2520[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Pentadecafluorodecanoic acid (7:3 FTCA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	72 hr	4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Pentadecafluorodecanoic acid / >99%	~7.5	24	EC_{50} (population biomass)	2.1	Test duration too short	Hoke et al. 2012
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	3-Perfluoroheptyl propanoic acid / >98%	NR	28	NOEC (mortality)	0.2520[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured.
[b] Converted to mg/L based on molecular weight.

C.1 References

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Appendix D Unused Freshwater Acute Toxicity Studies

Author	Citation	Reason Unused
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Blanc, M., J. Ruegg, N. Scherbak and S.H. Keiter	2019. Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (ZF-L) cell line and in zebrafish embryos. Aquat. Toxicol. 215: 105272-9999.	No control information
Perfluorononanoic acid (PFNA)		
Dale, K., F. Yadetie, T. Horvli, X. Zhang, H.G. Froysa, O.A. Karlsen and A. Goksoyr	2022. Single PFAS and PFAS mixtures affect nuclear receptor- and oxidative stress-related pathways in precision-cut liver slices of Atlantic cod (*Gadus morhua*). Sci. Total Environ. 814: 1-12.	*In vitro* exposure
Garoche, C., A. Boulahtouf, M. Grimaldi, B. Chiavarina, L. Toporova, M.J. Den Broeder, J. Legler, W. Bourguet and P. Ba	2021. Interspecies differences in activation of peroxisome proliferator-activated receptor gamma by pharmaceutical and environmental chemicals. Environ. Sci. Technol. 55(24): 16489-16501.	*In vitro* exposure

Appendix E ICE Models Available in Web-ICE v4.0 for Freshwater Predicted Species Based on Surrogates with Measured Toxicities

Table E-1. All ICE Models Selected for the Derivation of PFAS Benchmarks.

Model parameters are used to evaluate prediction robustness. Cross-validation success is the percentage of all model data that were predicted within five-fold of the measured value through leave-one-out cross-validation (Raimondo et al. 2024). Taxonomic distance describes the relationship between surrogate and predicted species (e.g., 1 = shared genus, 2 = shared family, 3 = shared order, 4 = shared class, 5 = shared phylum, 6 = shared kingdom).

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
African clawed frog (Xenopus laevis)	Fathead minnow (Pimephales promelas)	0.76	0.77	4	0.91	0.00	0.14	18.0	140,225.3	100	5	PFBS
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.91	1.26	12	0.75	0.00	0.76	0.8	166,819.4	50	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.82	0.44	51	0.75	0.00	0.76	0.2	5,000,000.0	49	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus fasciatus)	Mysid (Americamysis bahia)	0.91	0.58	26	0.70	0.00	0.81	0.0	4,400.0	57	4	PFBS
Amphipod (Gammarus minus)	Fathead minnow (Pimephales promelas)	0.73	1.50	2	0.96	0.02	0.05	239.0	107,584.8	75	6	PFBS
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.92	0.25	30	0.75	0.00	0.75	0.1	68,300.0	63	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus pseudolimnaeus)	Mysid (Americamysis bahia)	0.82	0.22	18	0.80	0.00	0.56	0.0	7,170.0	55	4	PFBS
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	1.03	-0.10	30	0.88	0.00	0.68	0.0	193,390.8	63	4	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Apache trout (Oncorhynchus gilae)	Rainbow trout (Oncorhynchus mykiss)	1.10	-0.35	3	0.99	0.00	0.02	4.1	1,624.7	100	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Arctic grayling (Thymallus arcticus)	Bluegill (Lepomis macrochirus)	1.14	-0.59	1	1.00	0.02	0.01	37.9	150,750.0	N/A	4	PFBS
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	1.15	-0.64	11	0.95	0.00	0.13	4.3	123,267.3	92	4	PFBS
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	1.01	-0.07	12	0.96	0.00	0.11	0.2	95,857.7	86	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Atlantic salmon (Salmo salar)	Fathead minnow (Pimephales promelas)	1.28	-1.51	6	0.68	0.01	0.84	44.4	140,225.3	63	4	PFBS
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.91	0.22	18	0.97	0.00	0.12	0.3	8,694,505.9	95	4	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	0.74	1.21	12	0.60	0.00	0.95	0.0	9,809.6	43	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Black bullhead (Ameiurus melas)	Fathead minnow (Pimephales promelas)	1.07	-0.10	3	0.97	0.00	0.17	1.2	20,922.0	80	4	PFBS
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.71	1.18	270	0.65	0.00	0.84	0.1	46,278,306.5	57	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Bluegill (Lepomis macrochirus)	Mysid (Americamysis bahia)	0.71	1.38	150	0.71	0.00	0.55	0.0	79,000.0	72	6	PFBS
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.30	314	0.89	0.00	0.22	0.1	8,341,462.7	90	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Bluegill (Lepomis macrochirus)	Fathead minnow (Pimephales promelas)	0.94	-0.02	108	0.81	0.00	0.43	0.2	50,154,461.4	82	4	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1.10	-0.48	5	0.98	0.00	0.10	2.5	233,000.0	100	5	PFHxS, PFHxA
Bonytail (Gila elegans)	Bluegill (Lepomis macrochirus)	0.96	-0.37	6	0.69	0.01	0.62	53.9	150,750.0	50	4	PFBS
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	0.92	0.70	6	0.89	0.00	0.22	3.3	43,073.0	75	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Bonytail (Gila elegans)	Fathead minnow (Pimephales promelas)	1.01	-0.39	5	0.62	0.04	0.50	58.3	8,320.3	43	2	PFBS
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	0.97	-0.06	21	0.86	0.00	0.27	0.4	123,267.3	83	4	PFBS
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	1.00	0.01	25	0.95	0.00	0.11	0.6	96,692.7	93	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Brook trout (Salvelinus fontinalis)	Fathead minnow (Pimephales promelas)	0.95	-0.43	17	0.70	0.00	0.75	1.2	140,225.3	68	4	PFBS
Brown trout (Salmo trutta)	Bluegill (Lepomis macrochirus)	0.91	-0.10	16	0.73	0.00	0.68	2.3	123,267.3	78	4	PFBS
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	1.00	0.02	20	0.97	0.00	0.07	0.2	95,857.7	100	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	0.87	1.14	2	0.98	0.01	0.01	14.1	231.7	100	6	PFNA, PFDA, 7:3 FTCA, 8:2 FTUCA
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	0.89	0.48	5	0.98	0.00	0.08	0.4	201,000.0	100	5	PFBS
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	0.88	0.82	9	0.95	0.00	0.31	0.6	13,400,000.0	73	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	0.97	0.15	7	0.98	0.00	0.10	1.2	26,500,000.0	100	5	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	1.17	-0.23	3	0.99	0.00	0.01	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Catla (Gibelion catla)	Fathead minnow (Pimephales promelas)	1.09	-0.36	2	0.97	0.02	0.02	333.0	8,320.3	100	2	PFBS
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	0.77	0.95	5	0.91	0.00	0.07	144.8	304,541.0	86	4	PFHxS, PFBA
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	0.81	0.74	77	0.77	0.00	0.43	0.4	517,824.6	82	4	PFBS
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	0.82	0.82	98	0.76	0.00	0.50	0.0	13,400,000.0	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	0.98	0.06	52	0.87	0.00	0.30	1.2	26,500,000.0	85	4	PFBS
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1.02	-0.28	7	0.99	0.00	0.08	2.5	20,900,000.0	100	5	PFHxS, PFHxA
Channel catfish (Ictalurus punctatus)	African clawed frog (Xenopus laevis)	1.00	0.47	3	0.93	0.01	0.20	18.4	122,473.4	100	5	PFNA
Chinook salmon (Oncorhynchus tshawytscha)	Bluegill (Lepomis macrochirus)	0.91	-0.10	13	0.65	0.00	0.95	4.1	150,750.0	67	4	PFBS
Chinook salmon (Oncorhynchus tshawytscha)	Rainbow trout (Oncorhynchus mykiss)	1.02	0.02	18	0.96	0.00	0.11	3.3	724,430.8	95	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Chinook salmon (Oncorhynchus tshawytscha)	Fathead minnow (Pimephales promelas)	1.16	-1.22	9	0.71	0.00	0.82	10.5	140,225.3	45	4	PFBS
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	0.91	0.01	25	0.79	0.00	0.47	0.4	150,750.0	78	4	PFBS
Coho salmon (Oncorhynchus kisutch)	Rainbow trout (Oncorhynchus mykiss)	1.03	0.06	36	0.99	0.00	0.04	0.0	43,073.0	100	1	PFDA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Coho salmon (Oncorhynchus kisutch)	Fathead minnow (Pimephales promelas)	0.91	-0.23	19	0.65	0.00	0.69	1.2	20,922.0	67	4	PFBS
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	0.91	0.92	7	0.73	0.00	0.56	3.3	43,073.0	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Colorado squawfish (Ptychocheilus lucius)	Fathead minnow (Pimephales promelas)	1.07	-0.58	6	0.68	0.01	0.49	58.3	20,922.0	63	2	PFBS
Common carp (Cyprinus carpio)	Zebrafish-embryo (Danio rerio-embryo)	0.83	0.45	5	0.66	0.03	0.37	86.0	34,190.0	71	2	PFBA
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	0.86	0.60	30	0.79	0.00	0.47	0.9	180,000.0	81	4	PFBS
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	0.81	0.92	47	0.74	0.00	0.52	0.2	198,443.9	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	0.99	0.06	25	0.84	0.00	0.21	4.7	133,000.0	89	2	PFBS
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	0.88	0.42	32	0.79	0.00	0.36	0.4	180,000.0	71	4	PFBS
Cutthroat trout (Oncorhynchus clarkii)	Rainbow trout (Oncorhynchus mykiss)	0.95	0.15	36	0.96	0.00	0.08	0.0	198,443.9	97	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	0.99	-0.45	22	0.82	0.00	0.35	1.2	140,225.3	79	4	PFBS
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	0.61	1.97	2	0.99	0.01	0.01	709.9	155,541.7	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1.03	-0.28	40	0.95	0.00	0.24	0.3	46,278,306.5	83	2	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	0.98	-0.21	25	0.84	0.00	0.77	0.0	4,100,000.0	52	5	PFBS
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	0.87	-0.03	7	0.70	0.01	0.24	0.8	231.7	89	2	PFNA, PFDA, 8:2 FTUCA
Daphnid (Daphnia galeata)	Daphnid (Daphnia magna)	0.92	0.27	4	0.96	0.00	0.08	0.1	646.2	83	1	PFNA, PFDA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia longispina)	Daphnid (Daphnia magna)	1.22	-0.57	2	0.99	0.01	0.06	8.9	10,359.2	100	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia magna)	Water flea (Chydorus sphaericus)	1.04	0.27	7	0.98	0.00	0.06	7.1	462,000.0	100	3	PFNA, PFDA
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	0.92	-0.31	11	0.71	0.00	0.74	3.1	36,910.0	54	6	PFHxS, PFBA
Daphnid (Daphnia magna)	Daphnid (Daphnia pulicaria)	0.88	0.51	4	0.94	0.00	0.19	9.3	237,367.4	83	1	PFDA
Daphnid (Daphnia magna)	Mysid (Americamysis bahia)	0.82	0.83	246	0.73	0.00	0.76	0.0	11,000,000.0	70	5	PFBS
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	0.82	0.68	9	0.84	0.00	0.79	2.5	20,900,000.0	73	6	PFHxS, PFHxA
Daphnid (Daphnia pulex)	Daphnid (Daphnia magna)	1.01	-0.17	40	0.96	0.00	0.15	0.2	4,894,739.3	95	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia pulicaria)	Daphnid (Daphnia magna)	1.07	-0.32	4	0.94	0.00	0.23	14.1	281,641.4	83	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.94	-0.02	21	0.86	0.00	0.28	0.2	7,200.0	87	2	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.75	0.61	5	0.88	0.00	0.35	0.1	166,819.4	57	2	PFNA, PFHxA, PFDA, PFBS, 7:3 FTCA, 8:2 FTUCA
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	0.60	2.45	2	0.95	0.03	0.06	4622.4	5,100,709.2	75	6	PFHxS
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	1.07	-0.25	4	0.97	0.00	0.05	18.0	25,076.0	100	2	PFHxS, PFBA
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	1.00	-0.11	41	0.93	0.00	0.22	23.0	54,578,562.0	84	2	PFHxS, PFBA
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.69	1.42	236	0.64	0.00	0.76	0.2	46,500,000.0	66	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Fathead minnow (Pimephales promelas)	Bluegill (Lepomis macrochirus)	0.87	0.62	108	0.81	0.00	0.40	0.1	27,540,000.0	81	4	PFBS
Fathead minnow (Pimephales promelas)	Mysid (Americamysis bahia)	0.62	1.74	104	0.62	0.00	0.75	0.0	11,000,000.0	60	6	PFBS
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.62	146	0.87	0.00	0.27	0.2	13,400,000.0	86	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1.01	-0.10	7	0.98	0.00	0.10	2.5	20,900,000.0	100	5	PFHxS, PFHxA
Fathead minnow (Pimephales promelas)	African clawed frog (Xenopus laevis)	1.20	-0.62	4	0.91	0.00	0.22	44.7	122,473.4	67	5	PFNA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.90	0.24	17	0.94	0.00	0.19	14.1	8,694,505.9	79	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Fatmucket (Lampsilis siliquoidea)	Fathead minnow (Pimephales promelas)	0.94	-0.40	13	0.65	0.00	0.93	13.4	10,830,770.1	47	6	PFBS
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	1.22	-0.78	2	0.99	0.00	0.01	7797.4	649,307.3	100	4	PFHxS, PFBA
Flagfish (Jordanella floridae)	Bluegill (Lepomis macrochirus)	0.86	0.47	7	0.89	0.00	0.30	0.4	113,000.0	78	4	PFBS
Flagfish (Jordanella floridae)	Fathead minnow (Pimephales promelas)	0.91	0.11	8	0.84	0.00	0.46	1.2	32,537.5	80	4	PFBS
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	0.97	0.33	7	0.96	0.00	0.06	144.8	304,541.0	100	2	PFHxS, PFBA
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	0.78	0.98	27	0.71	0.00	0.51	0.4	201,000.0	72	4	PFBS
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	0.82	1.12	35	0.83	0.00	0.49	0.0	13,400,000.0	81	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	0.98	0.19	26	0.94	0.00	0.14	1.2	26,500,000.0	93	2	PFBS
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1.04	-0.30	7	0.98	0.00	0.14	2.5	20,900,000.0	89	5	PFHxS, PFHxA
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.67	0.94	2	0.97	0.02	0.08	14.1	166,819.4	75	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Green floater (Lasmigona subviridis)	Rainbow trout (Oncorhynchus mykiss)	0.60	1.44	4	0.73	0.03	0.36	3.3	95,857.7	67	6	PFDA
Green sunfish (Lepomis cyanellus)	Bluegill (Lepomis macrochirus)	0.85	0.63	13	0.92	0.00	0.14	4.1	113,000.0	93	1	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.60	13	0.95	0.00	0.13	0.0	19,926.0	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Green sunfish (Lepomis cyanellus)	Fathead minnow (Pimephales promelas)	0.90	-0.09	6	0.64	0.02	0.89	10.5	26,685.4	50	4	PFBS
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	1.05	-0.04	3	0.93	0.01	0.11	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Greenthroat darter (Etheostoma lepidum)	Fathead minnow (Pimephales promelas)	0.85	-0.09	3	0.92	0.01	0.13	5.7	8,320.3	100	4	PFBS
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	0.70	1.04	22	0.75	0.00	0.39	0.4	180,000.0	67	4	PFBS
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.24	24	0.63	0.00	0.68	0.6	198,443.9	69	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	0.85	0.35	27	0.78	0.00	0.39	1.2	140,225.3	66	4	PFBS
Indian bullfrog (Euphlyctis hexadactylus)	Bluegill (Lepomis macrochirus)	0.88	0.02	4	0.86	0.01	0.29	37.9	113,000.0	67	5	PFBS
Indian bullfrog (Euphlyctis hexadactylus)	Rainbow trout (Oncorhynchus mykiss)	1.25	-0.12	5	0.78	0.01	0.59	28.3	19,926.0	57	5	8:2 FTUCA
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.86	1.88	7	0.91	0.00	0.31	0.3	166,819.4	67	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Isopod (Asellus aquaticus)	Bluegill (Lepomis macrochirus)	1.09	-0.19	5	0.91	0.00	0.37	2.8	123,267.3	43	6	PFBS
Isopod (Asellus aquaticus)	Mysid (Americamysis bahia)	0.79	1.69	4	0.89	0.00	0.31	0.0	6,982.6	83	4	PFBS
Isopod (Caecidotea brevicauda)	Bluegill (Lepomis macrochirus)	0.86	0.28	17	0.74	0.00	0.48	0.4	26,700.0	68	6	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	0.78	0.41	19	0.70	0.00	0.52	0.6	82,000.0	57	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Isopod (Caecidotea brevicauda)	Fathead minnow (Pimephales promelas)	0.76	-0.05	8	0.73	0.00	0.49	1.2	20,922.0	60	6	PFBS
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	0.69	0.80	20	0.72	0.00	0.27	2.8	180,000.0	77	4	PFBS
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	0.89	0.23	26	0.94	0.00	0.07	0.2	198,443.9	96	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Lake trout (Salvelinus namaycush)	Fathead minnow (Pimephales promelas)	0.77	0.04	12	0.66	0.00	0.34	44.4	133,000.0	79	4	PFBS
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	1.03	-0.10	33	0.93	0.00	0.12	0.4	123,267.3	94	2	PFBS
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.04	32	0.89	0.00	0.21	0.0	95,857.7	91	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	1.16	1.67	2	0.98	0.01	0.13	0.8	4,369.3	75	6	PFNA, PFDA, 7:3 FTCA
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	1.01	0.64	3	0.98	0.00	0.13	0.6	95,857.7	100	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	0.91	0.61	3	0.95	0.01	0.16	6.6	54,399.6	80	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	0.86	0.60	6	0.98	0.00	0.05	2.7	1,420,000.0	88	4	PFBS
Midge (Chironomus plumosus)	Mysid (Americamysis bahia)	0.74	0.93	13	0.78	0.00	0.66	0.0	87,600.0	67	5	PFBS

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.97	0.76	6	0.89	0.00	0.82	0.3	472,000.0	50	5	PFNA, PFDA, 7:3 FTCA
Midge (Chironomus tentans)	Bluegill (Lepomis macrochirus)	1.03	0.11	5	0.94	0.00	0.39	0.1	517,824.6	71	6	PFBS
Midge (Paratanytarsus dissimilis)	Bluegill (Lepomis macrochirus)	0.85	1.02	5	0.89	0.00	0.49	0.4	201,000.0	57	6	PFBS
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	0.89	1.03	8	0.90	0.00	0.33	0.6	1,330,000.0	80	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Midge (Paratanytarsus dissimilis)	Fathead minnow (Pimephales promelas)	0.86	0.97	8	0.85	0.00	0.52	1.2	1,430,000.0	80	6	PFBS
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1.09	-0.27	3	0.97	0.00	0.25	2.5	3,019,983.4	40	6	PFHxS, PFHxA
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.98	0.55	3	0.99	0.00	0.06	370.0	14,500,000.0	100	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Midge (Paratanytarsus parthenogeneticus)	Fathead minnow (Pimephales promelas)	0.99	0.57	2	0.98	0.01	0.09	280.0	10,600,000.0	100	6	PFBS
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.76	0.47	20	0.63	0.00	0.72	0.3	58,000.0	64	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Mosquitofish (Gambusia affinis)	Fathead minnow (Pimephales promelas)	0.95	0.01	2	0.99	0.01	0.12	5.7	26,500,000.0	100	4	PFBS
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	0.97	0.07	1	1.00	0.01	0.00	18600.0	20,900,000.0	N/A	5	PFHxS, PFHxA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Mozambique tilapia (Oreochromis mossambicus)	Fathead minnow (Pimephales promelas)	0.86	0.36	9	0.73	0.00	0.33	44.4	140,225.3	64	4	PFBS
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	0.76	1.03	4	0.71	0.04	0.43	0.6	1,624.7	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Mrigal carp (Cirrhinus mrigala)	Fathead minnow (Pimephales promelas)	1.11	-0.59	3	0.98	0.00	0.01	196.9	8,320.3	100	2	PFBS
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.89	0.24	2	0.99	0.00	0.02	42.0	166,819.4	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	1.04	-0.04	1	1.00	0.00	0.00	2.3	6,969.1	N/A	3	PFBS
Northern leopard frog (Lithobates pipiens)	Bluegill (Lepomis macrochirus)	0.80	1.13	1	1.00	0.02	0.01	7.9	180,000.0	N/A	5	PFBS
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.60	1	1.00	0.01	0.00	2.3	198,443.9	N/A	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	1.04	-0.30	6	0.80	0.00	0.27	4.7	1,822.3	88	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	1.17	-0.31	3	0.92	0.01	0.24	144.8	239,250.6	60	6	PFHxS, PFBA
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.71	1.77	10	0.81	0.00	0.36	3.2	281,641.4	67	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Limnodrilus hoffmeisteri)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.60	6	0.67	0.01	0.76	3.3	707,000.0	50	6	8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Oligochaete (Lumbriculus variegatus)	Fathead minnow (Pimephales promelas)	0.94	0.28	14	0.82	0.00	0.43	1.2	140,225.3	75	6	PFBS
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.89	1.37	13	0.80	0.00	0.78	0.1	4,894,739.3	47	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Tubifex tubifex)	Bluegill (Lepomis macrochirus)	0.72	0.67	7	0.72	0.00	0.84	0.1	113,000.0	56	6	PFBS
Oligochaete (Tubifex tubifex)	Fathead minnow (Pimephales promelas)	1.04	-0.10	8	0.87	0.00	0.53	15.3	10,830,770.1	50	6	PFBS
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1.15	-0.84	3	0.99	0.00	0.06	11.0	281,641.4	100	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.89	0.28	11	0.98	0.00	0.08	14.1	8,694,505.9	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Paper pondshell (Utterbackia imbecillis)	Fathead minnow (Pimephales promelas)	0.86	-0.16	9	0.65	0.00	0.85	134.2	10,830,770.1	18	6	PFBS
Peppered loach (Lepidocephalichthys guntea)	Fathead minnow (Pimephales promelas)	0.78	0.72	1	1.00	0.03	0.00	196.9	43,785.6	N/A	3	PFBS
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.96	0.00	3	0.97	0.00	0.11	42.0	545,927.5	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	1.31	-1.12	5	0.96	0.00	0.24	3.1	26,390.0	71	4	PFHxS, PFBA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	1.16	-1.06	27	0.80	0.00	0.61	23.0	8,843,890.0	66	4	PFHxS, PFBA
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	0.71	1.03	377	0.63	0.00	0.84	0.1	14,500,000.0	60	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	0.95	0.03	314	0.89	0.00	0.22	0.1	7,100,000.0	91	4	PFBS
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	0.66	1.35	201	0.62	0.00	0.72	0.0	113,000.0	68	6	PFBS
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	0.97	-0.20	146	0.87	0.00	0.29	0.2	26,500,000.0	87	4	PFBS
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1.09	-0.71	9	0.95	0.00	0.38	2.5	20,900,000.0	73	5	PFHxS, PFHxA
Razorback sucker (Xyrauchen texanus)	Bluegill (Lepomis macrochirus)	0.81	0.01	6	0.67	0.01	0.48	53.9	150,750.0	75	4	PFBS
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	0.78	0.91	6	0.87	0.00	0.19	3.3	43,073.0	88	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Razorback sucker (Xyrauchen texanus)	Fathead minnow (Pimephales promelas)	0.98	-0.36	5	0.66	0.03	0.40	58.3	8,320.3	71	3	PFBS
Redear sunfish (Lepomis microlophus)	Bluegill (Lepomis macrochirus)	1.03	-0.03	2	1.00	0.00	0.00	17.0	4,500.0	100	1	PFBS
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	0.85	0.97	2	0.95	0.03	0.09	3.3	2,040.4	100	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Rohu (Labeo rohita)	Bluegill (Lepomis macrochirus)	0.94	0.77	4	0.94	0.00	0.21	0.8	7,326.2	83	4	PFBS
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	1.07	0.86	4	0.89	0.00	0.37	0.6	1,624.7	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Rohu (Labeo rohita)	Fathead minnow (Pimephales promelas)	0.98	0.32	4	0.87	0.01	0.30	2.3	8,320.3	83	2	PFBS
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	1.16	-0.58	3	0.98	0.00	0.06	28.3	95,857.7	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	1.09	-0.03	1	1.00	0.01	0.00	1.4	7,075.2	N/A	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.88	1	1.00	0.02	0.00	0.6	68.4	N/A	6	PFDA, 7:3 FTCA
Sockeye salmon (Oncorhynchus nerka)	Rainbow trout (Oncorhynchus mykiss)	0.85	0.60	5	0.85	0.00	0.14	28.3	7,500.0	71	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	1.28	-0.26	4	0.98	0.00	0.04	28.3	9,700.0	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	1.20	-0.41	3	0.97	0.00	0.06	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.94	0.20	6	0.96	0.00	0.23	14.1	8,694,505.9	88	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.96	0.11	8	0.96	0.00	0.20	14.1	8,694,505.9	80	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Tadpole physa (Physella gyrina)	Fathead minnow (Pimephales promelas)	1.22	-1.60	6	0.84	0.00	0.58	813.9	10,830,770.1	25	6	PFBS
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.95	-0.43	4	0.97	0.00	0.13	14.1	4,894,739.3	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1.30	-2.81	3	0.98	0.00	0.13	813.9	10,830,770.1	100	6	PFBS
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.94	0.12	5	0.98	0.00	0.07	14.1	8,694,505.9	100	4	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Walleye (Sander vitreus)	Bluegill (Lepomis macrochirus)	0.74	0.54	7	0.78	0.00	0.22	4.3	6,722.1	67	3	PFBS
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	0.68	0.60	7	0.69	0.01	0.28	4.7	16,235.6	56	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.96	-0.23	9	0.97	0.00	0.13	14.1	8,694,505.9	91	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.95	-0.20	7	0.98	0.00	0.06	8.9	977,589.9	100	3	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.72	1.10	3	0.97	0.00	0.11	8.9	281,641.4	100	3	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1.18	-1.28	1	0.99	0.05	0.01	166819.4	4,894,739.3	N/A	6	PFHxA
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.88	0.27	8	0.96	0.00	0.16	14.1	8,694,505.9	80	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	1.33	-0.51	3	0.90	0.01	0.15	28.3	1,624.7	80	5	PFDA, 7:3 FTCA, 8:2 FTUCA
White sucker (Catostomus commersonii)	Bluegill (Lepomis macrochirus)	1.05	-0.32	7	0.92	0.00	0.18	35.0	123,267.3	89	4	PFBS
White sucker (Catostomus commersonii)	Fathead minnow (Pimephales promelas)	1.15	-0.37	5	0.93	0.00	0.21	43.9	140,225.3	86	3	PFBS
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	0.98	-0.11	17	0.92	0.00	0.15	0.4	9,409.7	89	3	PFBS
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.29	23	0.77	0.00	0.58	0.0	16,235.6	84	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Yellow perch (Perca flavescens)	Fathead minnow (Pimephales promelas)	0.94	-0.38	12	0.74	0.00	0.54	1.2	20,922.0	64	4	PFBS
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.77	1.22	11	0.71	0.00	0.62	0.1	50,000.0	62	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Zebrafish (Danio rerio)	Mysid (Americamysis bahia)	0.73	1.91	7	0.68	0.01	0.83	0.0	5,666.0	33	6	PFBS
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	0.73	0.96	5	0.96	0.00	0.14	0.2	229,100.0	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Zebrafish (Danio rerio)	Fathead minnow (Pimephales promelas)	0.91	0.31	4	0.97	0.00	0.04	13.4	26,685.4	100	2	PFBS
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.66	1.72	43	0.67	0.00	0.87	0.1	46,500,000.0	56	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	0.69	1.43	27	0.80	0.00	0.37	0.2	8,341,462.7	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R^2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µ)	Surrogate model maximum valu (µ)	Cross-validation Success (%)	Taxonomic Distance	PFAS Compound
Zebrafish-embryo (Danio rerio-embryo)	Fathead minnow (Pimephales promelas)	0.93	0.38	41	0.93	0.00	0.20	4.0	70,200,000.0	81	2	PFBS

Appendix F Derivation of PFAS Benchmarks using Scaled Data

As discussed in **Section 3.0**, PFAS acute values (typically reported as mg/L) can be greater than those used to develop an ICE model (ICE database toxicity range $1x10^{-4}$ and $1x10^{8}$ µg/L) such that the input PFAS value of the surrogate would be outside the model domain. In these situations, a user can either enter the measured toxicity value (LC_{50}/EC_{50}) into the ICE model as µg/L and allow the regression to extrapolate beyond the range of the model or enter a "scaled" toxicity value (i.e., enter the measured LC_{50} value as mg/L) (Raimondo et al. 2024). **i u r 3-1** provides an example data plots and calculation of an EC_0 value for *ph* for PF S for a value that falls beyond the model range, when calculated using an "extrapolation" and "scaled" approach. Calculations presented in **Section 5** of this document allowed extrapolation beyond the range of the regression model, recognizing that such an extrapolation has inherent uncertainties and can result in larger confidence intervals around the prediction than would typically occur using the scaled approach. Within this section, we apply the alternative scaled approach to explore calculating benchmark values for the eight PFAS using this alternative method (Raimondo et al. 2024).

For the scaled approach, values are directly entered as µg/L in situations when the surrogate species measured PFAS value falls within the range of the ICE model. In cases where the surrogate species measured value is beyond the range of the ICE model, the value is then entered as mg/L, so that it falls within the range of the model data. For example, if a hypothetical concentration of 15 µg/L is beyond the range of the ICE model data for a chemical, it is then entered as 15 mg/L to bring it within range of the model data. Values were also scaled in some situations where the measured value falls within the model range but where the confidence interval was large (>50 fold), and use of scaling resulted in acceptable confidence intervals.

The use of scaled values has been validated through a comprehensive analysis of ICE data, where it has been demonstrated that 3,104 values predicted in this manner had the same level of accuracy as values entered into ICE models as µg/L within and beyond the model domain for models with slopes ranging from 0.66 - 1.33 (Raimondo et al. 2024). Raimondo et al. (2024) also demonstrated that using ICE models in this manner does not violate any of the model assumptions, which are: 1) they represent the relationship of inherent sensitivity between two species, which is conserved across chemicals, mechanisms of action, and ranges of toxicity and 2) the nature of a contaminant that was tested on the surrogate reflects the nature of the contaminant in the predicted species (e.g., effect concentration [EC_{50}] or lethal concentration [LC_{50}]), percentage of active ingredient, technical grade, toxicity unit). Additionally, there are no statistical assumptions of linear regressions (linear relationship, normality, homoscedasticity, no auto-correlation, no or little multicollinearity; Dowdy et al. 2011) violated by this application. The following sections present the calculations and resulting alternative benchmark values for each of eight selected PFAS using the scaled approach. ICE models selected for use for the derivation of recommended PFAS benchmarks are presented in **Table E-1** and the empirical data used for this evaluation are presented in **Appendix A**.

F.1 Derivation of Acute Water Benchmark for Perfluorobutanoic Acid (PFBA)

Quantitatively acceptable empirical data for PFBA were available for five species comprising four genera and fulfilling three MDR groups (**Table F-2**). A total of 43 ICE models were accepted for use. Of these, seven ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Chydorus sphaericus* and *Daphnia magna*). Three other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Pimephales promelas*). One additional model (*Danio rerio* predicting for *P e ph le pro e l*) was not used because the measured

EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 32 ICE models were selected for use, resulting in ICE models predicting 31 SMAVs, representing 29 genera (**Tables F-1** and **F-2**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-2.** GMAVs for the four most sensitive genera were within a factor of 13.9 of each other (**Table F-3**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFBA is 347.1 mg/L (**Table F-3**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus*, GMAV=110 mg/L). The FAV was divided by two to obtain a preliminary value of 173.5 mg/L PFBA and then adjusted by the carboxylic acid application factor (15.8) to obtain the alternative freshwater acute water column benchmark magnitude of 11 mg/L PFBA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-1**).

Table F-1. Acceptable models for ICE-estimated Species Sensitivity to PFBA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0008	166.8	**44,908.2** (10,018-201,313)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	5,000	**3,060.4** (1,474.4–6,352.5)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	68.3	**4,490.4** (1,523.0–13,240)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0003	8,694.5	**2,248.7** (1,108-4,563)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	46,278.3	**959.02** (507.98-1,810.6)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	304.5	**13,361.7** (6,856.1-26,037)
Common carp (Cyprinus carpio)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.086	34.19	**7,908.4** (1,225.4–51,039)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0003	46,278.3	**4,100.3** (2,125.4-7,909.9)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	10.36	8,995.3 (1,641.2-49,301)
Daphnid (Daphnia magna)*	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.003	36.91	3,291.4 (831.19-13,034)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	4,894.7	3,957.7 (2,788.6-5,616.9)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	281.6	4,361.5 (1,223.6-15,546)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	7.2	**2,939.0** (964.77–8,953.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0002	46,500	1,174.4[b] (655.01–2,105.8)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.018	25.08	15,343.0[c] (7,383.2-31,884)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	13,779	13,779,000 (µg/L)	0.023	54,579	**11,532.0** (6,189.6-21,485)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**2,013.5** (809.37-5,008.9)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	7.797	649.3	**18,640.2** (10,316-33,681)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	304.5	**23,117.1** (14,833-36,028)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	166.8	**2,645.9** (481.11-14,552)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0003	166.8	**116,979.2** (32,795-417,264)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.37	14,500	**12,921.0** (3,394.0-49,189)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0003	58	**1,932.2** (372.75-10,016)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.042	166.8	**3,613.7** (1,572.1-8,306.4)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.003	281.6	**25,412.0** (9,374.1-68,889)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	239.3	35,536.4[b] (6,336.9-199,284)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	4,894.7	**49,159.1** (10,555-228,948)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.011	281.6	**2,680.1** (1,224.4-5,866.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,748.6** (862.33-3,545.6)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.042	545.9	**3,775.7** (1,269.6-11,229)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	14,500	677.8[b] (396.18–1,159.4)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.003	26.39	**20,148.4** (5,451.2-74,471)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.023	8,843.9	**5,447.6** (2,518.2- 11,785)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**3,490.7** (670.81-18,165)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**3,740.1** (1,061.7-13,176)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	4,894.7	**1,262.4** (480.17-3,318.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**2,852.6** (949.6-8,569.8)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,759.2** (742.37-4,168.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	977.6	2,151.3 (1,364.7- 3391.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	281.6	**5,989.5** (1,810.5-19,815)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,528.3** (497.59-4,694.2)
Zebrafish (Danio rerio)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	50	12,209.2 (3,373.7-44,185)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	46,500	1,391.4 (413.39-4,683.2)

*Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.
[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-2. Ranked PFBA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**110**	110	0.03
2	B	Bluegill	*Lepomis macrochirus*	959	959	0.06
3	G	Threeridge	*Amblema plicata*	1,262**	1,262	0.09
4	G	Western pearlshell	*Margaritifera falcata*	1,528	1,528	0.12
5	G	Paper pondshell	*Utterbackia imbecillis*	1,749	1,749	0.15
6	G	Washboard	*Megalonaias nervosa*	1,759	1,759	0.18
7	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1,932**	1,932	0.21
8	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2,249	2,249	0.24
9	G	Green floater	*Lasmigona subviridis*	2,646**	2,646	0.26
10	E	Oriental river shrimp	*Macrobrachium nipponense*	2,680**	2,680	0.29
11	G	Neosho mucket	*Lampsilis rafinesqueana*	3,614**	2,697	0.32
	G	Fatmucket	*Lampsilis siliquoidea*	2,013		
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2,853	2,853	0.35
13	D	Daphnid	*Simocephalus serrulatus*	2,939**	2,939	0.38
14	G	Swamp lymnaea	*Lymnaea stagnalis*	3,491	3,491	0.41
15	E	Amphipod	*Gammarus fasciatus*	3,060**	3,707	0.44
	E	Amphipod	*Gammarus pseudolimnaeus*	4,490**		
16	G	Tadpole physa	*Physella gyrina*	3,740	3,740	0.47
17	G	Pheasantshell	*Ortmanniana pectorosa*	3,776**	3,776	0.50
18	D	Daphnid	*Ceriodaphnia dubia*	4,100	4,100	0.53
19	**D**	**Daphnid**	***Chydorus sphaericus***	**>4,280.8**	>4,280.8	0.56
20	**D**	**Daphnid**	***Daphnia magna***	**4,741**	4,741	0.59
	D	**Daphnid**	***Daphnia pulicaria***	**>1,006[a]**		
21	D	Water flea	*Moina macrocopa*	5,989**	5,989	0.62
22	B	Common carp	*Cyprinus carpio*	7,908**	7,908	0.65
23	A	Rainbow trout	*Oncorhynchus mykiss*	10,477**	10,477	0.68
24	B	Fathead minnow	*Pimephales promelas*	11,532	11,532	0.71
25	F	Midge	*Paratanytarsus parthenogeneticus*	12,921	12,921	0.74
26	C	Channel catfish	*Ictalurus punctatus*	13,361**	13,361	0.76
27	**B**	**Zebrafish**	***Danio rerio***	**13,779**	13,779	0.79
28	C	Flagfish	*Jordanella floridae*	18,640**	18,640	0.82
29	B	Goldfish	*Carassius auratus*	23,117**	23,117	0.85
30	H	Oligochaete	*Limnodrilus hoffmeisteri*	25,412**	25,412	0.88
31	E	Amphipod	*Crangonyx pseudogracilis*	44,908**	44,908	0.91
32	H	Oligochaete	*Tubifex tubifex*	49,159**	49,159	0.94
33	E	Isopod	*Asellus aquaticus*	116,979**	116,979	0.97

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

a Not used in GMAV calculation, because value represents a greater than low value

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes

B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-3. PFBA Protective Aquatic Life Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**110.0**	4.70	22.09	0.029	0.171
2	*Lepomis*	959.0	6.87	47.14	0.059	0.243
3	*Amblema*	1262.4	7.14	50.99	0.088	0.297
4	*Margaritifera*	1528.3	7.33	53.76	0.118	0.343
		Σ (Su):	**26.04**	**173.98**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 273.69 S = slope
L = 2.150 L = X-axis intercept
A = 5.849 A = lnFAV
FAV = 347.1
FAV/2 = 173.5 mg/L (Preliminary Value)
Adjustment = 173.5 / 15.8 = 10.98 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **11 mg/L PFBA** (rounded to two significant figures)

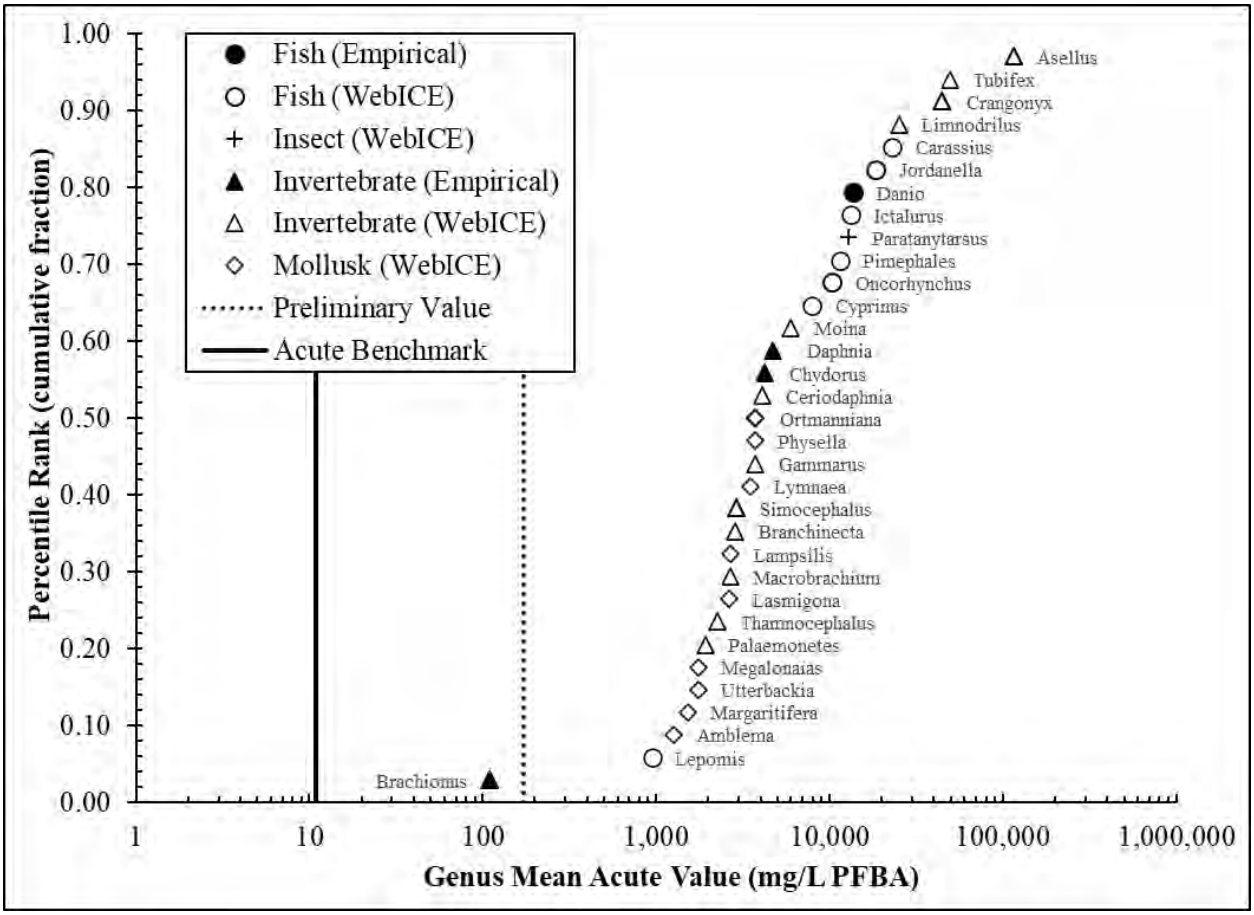


Figure F-1. Ranked Acute PFBA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.2 Derivation of Acute Water Benchmark for Perfluorohexanoic Acid (PFHxA)

Quantitatively acceptable empirical data for PFHxA were available for four species comprising three genera and fulfilling three MDR groups (**Table F-5**). A total of 43 ICE models were accepted for use. Of these, four ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Daphnia longispina* and *Daphnia magna*). Three other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus*). One additional model (*Daphnia magna* predicting for *o re r o*) was not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related

species with measured EC_{50} values within the ICE model range were used. A total of 35 ICE models were selected for use, resulting in ICE models predicting 35 SMAVs representing 30 genera (**Tables F-4** and **F-5**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-5**. GMAVs for the four most sensitive genera were within a factor of 2.7 of each other (**Table F-6**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxA is 189.1 mg/L (**Table F-6**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus*, GMAV=140 mg/L). The FAV was divided by two to obtain a preliminary value of 94.57 mg/L PFHxA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 6.0 mg/L PFHxA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-2**).

Table F-4. Acceptable models for ICE-estimated Species Sensitivity to PFHxA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0008	166.8	**10,313.0** (3,000.6-35,445)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	5,000	**234.7** (64.5-853.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	68.3	**1,026.8** (431.3-2,444.8)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0003	8,694.5	**517.3** (280.8-953.1)
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	1,105 (mg/L)	0.003	233	**766.4** (378-1,553.6)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	46,278	303.5[b] (176-523.2)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**719.8** (362.4-1,429.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0003	46,278	**785.2** (446.2-1,381.6)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	10.36	1,267.8 (334.3-4,808.1)
Daphnid (Daphnia magna)*	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	424.1 (68.5-2,627.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	4,894.7	841.4 (444.8-1,591.6)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.014	281.6	783.4 (181.5-3,381.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0002	7.2	**648.9** (269.3-1,563.9)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	166.8	**753.7** (136-4,175.6)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**1,083.6** (468.5-2,506.4)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	46,500	384.7[b] (235.8-627.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**470.8** (220.2-1,006.8)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**1,027.7** (405.9-2,602.1)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.014	166.8	**901.5** (216-3,761.6)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0003	166.8	**29,446.1** (9,967-86,994)
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1,105^	1,105 (mg/L)	0.003	3,020.0	**1,069.7** (176.6-6,480.9)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.37	14,500	**2,676.6** (917.7-7,806.3)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0003	58	**569.4** (155.9-2,080.1)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	18.6	20,900	**805.1** (394.5-1,643.4)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.042	166.8	**860.1** (421.3-1,755.9)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.003	281.6	**8,114.2** (3,337.6-19,727)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	1,048^	1,048 (mg/L)	0.0001	4,894.7	**11,637.0** (3,223.3-42,013)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.011	281.6	**421.3** (176.7-1004.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**418.5** (231.7-755.9)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.042	545.9	**803.4** (252.6-2,555.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**713.5** (200.3-2,541.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	14,500	214.2[b] (135.8-337.8)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**762.0** (188.8-3,075.8)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**794.5** (277.3-2,276.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	4,894.7	**191.3** (48-761.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**623.8** (247-1,575.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**372.3** (181.1-765.2)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	977.6	**465.4** (271.8-796.9)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	281.6	**1,876.3** (633.2-5,560.2)
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	166.8	4,894.7	**686.2** (127.8-3,684.7)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**370.2** (145.8-940.1)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	50	3,520.9[c] (1,155.2-10,731)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	46,500	**481.7** (172.4-1,346.1)

*****Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.
^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.
[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-5. Ranked PFHxA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**140.0**	140.0	0.03
2	G	Threeridge	*Amblema plicata*	191.3	191.3	0.06
3	G	Western pearlshell	*Margaritifera falcata*	370.2	370.2	0.09
4	G	Washboard	*Megalonaias nervosa*	372.3	372.3	0.12
5	G	Paper pondshell	*Utterbackia imbecillis*	418.5	418.5	0.15
6	E	Oriental river shrimp	*Macrobrachium nipponense*	421.3**	421.3	0.18
7	D	Water flea	*Chydorus sphaericus*	465.4**	465.4	0.21
8	B	Zebrafish	*Danio rerio*	481.7	481.7	0.24
9	E	Amphipod	*Gammarus fasciatus*	234.7	490.9	0.26
	E	Amphipod	*Gammarus pseudolimnaeus*	1,027**		
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	517.3	517.3	0.29
11	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	569.4**	569.4	0.32
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	623.8	623.8	0.35
13	G	Wavyrayed lampmussel	*Lampsilis fasciola*	686.2	652.6	0.38
	G	Neosho mucket	*Lampsilis rafinesqueana*	860.1**		
	G	Fatmucket	*Lampsilis siliquoidea*	470.8		
14	D	Daphnid	*Simocephalus serrulatus*	648.9**	699.3	0.41
	D	Daphnid	*Simocephalus vetulus*	753.7**		
15	A	Rainbow trout	*Oncorhynchus mykiss*	713.5	713.5	0.44
16	B	Channel catfish	*Ictalurus punctatus*	719.8	719.8	0.47
17	G	Swamp lymnaea	*Lymnaea stagnalis*	762.0	762.0	0.50
18	B	Bluegill	*Lepomis macrochirus*	766.4**	766.4	0.53
19	D	Daphnid	*Ceriodaphnia dubia*	785.2	785.2	0.56
20	G	Tadpole physa	*Physella gyrina*	794.5	794.5	0.59
21	G	Pheasantshell	*Ortmanniana pectorosa*	803.4**	803.4	0.62
22	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.65
23	G	Green floater	*Lasmigona subviridis*	901.5**	901.5	0.68
24	**C**	**Bullfrog**	***Lithobates catesbeianus***	**1,105**	915.2	0.71
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
25	B	Goldfish	*Carassius auratus*	1,028	1,028	0.74
26	**D**	**Daphnia**	***Daphnia magna***	**1,048**	1,048	0.76
27	B	Fathead minnow	*Pimephales promelas*	1,084	1,084	0.79
28	F	Midge	*Paratanytarsus dissimilis*	1,070	1,692	0.82
	F	Midge	*Paratanytarsus parthenogeneticus*	2,677		
29	D	Water flea	*Moina macrocopa*	1,876**	1,876	0.85
30	H	Oligochaete	*Limnodrilus hoffmeisteri*	8,114**	8,114	0.88
31	E	Amphipod	*Crangonyx pseudogracilis*	10,313**	10,313	0.91
32	H	Oligochaete	*Tubifex tubifex*	11,637	11,637	0.94
33	E	Isopod	*Asellus aquaticus*	29,446**	29,446	0.97

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes

B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-6. PFHxA Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**140.0**	4.94	24.42	0.029	0.171
2	*Amblema*	191.3	5.25	27.60	0.059	0.243
3	*Margaritifera*	370.2	5.91	34.98	0.088	0.297
4	*Megalonaias*	372.3	5.92	35.04	0.118	0.343
		Σ (Su):	**22.03**	**122.04**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 44.02 S = slope
L = 3.759 L = X-axis intercept
A = 5.242 A = lnFAV
FAV = 189.1
FAV/2 = 94.57 mg/L (Preliminary Value)
Adjustment = 94.57 / 15.8 = 5.985 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **6.0 mg/L PFHxA** (rounded to two significant figures)

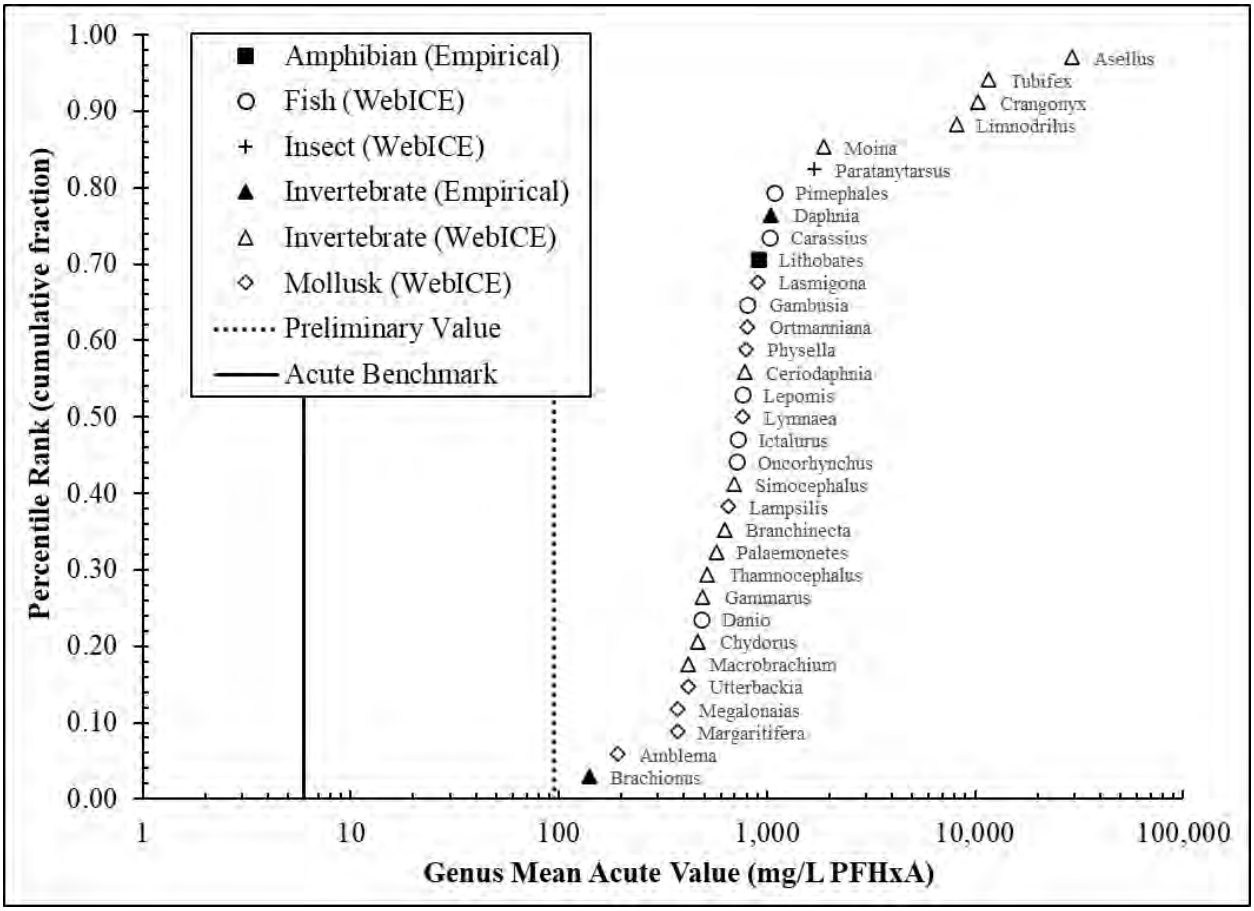


Figure F-2. Ranked Acute PFHxA GMAVs Used for the Aquatic Life Acute Benchmark Calculations.

F.3 Derivation of Acute Water Benchmark for Perfluorononanoic Acid (PFNA)

Quantitatively acceptable empirical data for PFNA were available for three species comprising three genera and fulfilling two MDR groups (**Table F-8**). A total of 42 ICE models were accepted for use. Of these, six ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Daphnia longispina* and *Daphnia magna*). One other model was not used because there was an acceptable model for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Pimephales promelas*). One additional model (i.e., *Daphnia magna* predicting for *o rer o*) was not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related

species with measured EC_{50} values within the ICE model range were used. A total of 34 ICE models were selected for use, resulting in ICE models predicting 34 SMAVs representing 30 genera (**Tables F-7** and **F-8**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-8**. GMAVs for the four most sensitive genera were within a factor of 2.0 of each other (**Table F-9**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFNA is 23.18 mg/L (**Table F-9**), which is lower than all of the GMAVs except for the one ICE-derived species value (the threeridge mussel, *Amblema plicata*, GMAV=17.58 mg/L). The FAV was divided by two to obtain a preliminary value of 11.59 mg/L PFNA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.73 mg/L PFNA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFNA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-3**).

Table F-7. Acceptable models for ICE-estimated Species Sensitivity to PFNA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0008	166.8	**1,035.5** (296.9-3,611.9)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	5,000	**29.8** (10.9-81.6)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	68.3	**102.4** (51.2-204.7)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	8,694.5	**52.1** (32.2-84.2)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	46,278	**50.3** (33.3-75.9)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.014	0.232	**648.8** (426-988.3)
Channel catfish (Ictalurus punctatus)	African clawed frog (Xenopus laevis)	335.8**	335.8 (mg/L)	0.018	122.5	**977.6** (162.9-5,867.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	46,278	**59.4** (38.2-92.1)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0008	0.232	**44.5** (16.1-123.4)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	0.646	109.2 (49.2-242.4)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.009	10.36	59.4 (16.2-217.7)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	27.84	27,840 (µg/L)	0.007	462	76.4 (48-121.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	4,894.7	65.8 (40.3-107.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	281.6	84.2 (18-392.8)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0002	7.2	**61.3** (33.8-111)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0001	166.8	**113.7** (29.4-439.6)
Fathead minnow (Pimephales promelas)	African clawed frog (Xenopus laevis)	335.8**	335.8 (mg/L)	0.045	122.5	**260.9** (48.9-1,393.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	46,500	67.3[b] (47.1-96.2)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**48.6** (27.6-85.6)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.014	166.8	**167.6** (38.9-721.5)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	166.8	**1,261.8** (217.7-7,314.9)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0008	4.369	**7,904.0** (1,279-48,846)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0003	472	**435.6** (71.7-2,647.8)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.37	14,500	**228.8** (101.9-513.5)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0003	58	**84.4** (33.9-210)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.042	166.8	**42.9** (12.1-152.7)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.003	281.6	**181.9** (43.1-768.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0001	4,894.7	**1,225.2** (393.2-3,817.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.011	281.6	**65.1** (24.3-174.3)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**44.8** (28.6-70.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.042	545.9	**54.4** (13.3-222.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	14,500	**35.4** (25.2-49.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**70.7** (24-208)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**70.6** (31.3-159.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	4,894.7	**17.6** (6.1-50.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**58.0** (28.6-117.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**32.9** (18.5-58.5)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.009	977.6	30.1 (19.3-47.1)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.009	281.6	**44.3** (8.8-223.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**40.4** (19.8-82.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	50	504.6[c] (137.4-1,853.4)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	46,500	**91.9** (42.1-200.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-8. Ranked PFNA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFNA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	17.58	17.58	0.03
2	**D**	**Daphnid**	***Chydorus sphaericus***	**27.84**	27.84	0.06
3	G	Washboard	*Megalonaias nervosa*	32.90	32.90	0.09
4	A	Rainbow trout	*Oncorhynchus mykiss*	35.40	35.40	0.12
5	G	Western pearlshell	*Margaritifera falcata*	40.41	40.41	0.15
6	D	Water flea	*Moina macrocopa*	44.31	44.31	0.18
7	G	Paper pondshell	*Utterbackia imbecillis*	44.82	44.82	0.21
8	G	Neosho mucket	*Lampsilis rafinesqueana*	42.93	45.69	0.24
	G	Fatmucket	*Lampsilis siliquoidea*	48.63		
9	B	Bluegill	*Lepomis macrochirus*	50.29	50.29	0.26
10	D	Daphnid	*Ceriodaphnia dubia*	59.35	51.41	0.29
	D	Daphnid	*Ceriodaphnia reticulata*	44.54**		
11	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	52.08	52.08	0.32
12	G	Pheasantshell	*Ortmanniana pectorosa*	54.41	54.41	0.35
13	E	Amphipod	*Gammarus fasciatus*	29.82	55.26	0.38
	E	Amphipod	*Gammarus pseudolimnaeus*	102.4**		
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	58.02	58.02	0.41
15	E	Oriental river shrimp	*Macrobrachium nipponense*	65.1	65.10	0.44
16	G	Tadpole physa	*Physella gyrina*	70.62	70.62	0.47
17	G	Swamp lymnaea	*Lymnaea stagnalis*	70.68	70.68	0.50
18	D	Daphnid	*Simocephalus serrulatus*	61.28**	83.48	0.53
	D	Daphnid	*Simocephalus vetulus*	113.7		
19	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	84.41**	84.41	0.56
20	**D**	**Daphnid**	***Daphnia magna***	**84.51**	84.51	0.59
21	B	Zebrafish	*Danio rerio*	91.85	91.85	0.62
22	G	Green floater	*Lasmigona subviridis*	167.6	167.6	0.65
23	H	Oligochaete	*Limnodrilus hoffmeisteri*	181.9	181.9	0.68
24	F	Midge	*Paratanytarsus parthenogeneticus*	228.8	228.8	0.71
25	B	Fathead minnow	*Pimephales promelas*	260.9**	260.9	0.74
26	**C**	**Clawed frog**	***Xenopus sp.***	**335.8**	335.8	0.76
27	F	Midge	*Chironomus tentans*	435.6	435.6	0.79
28	H	Bryozoan	*Pectinatella magnifica*	648.8**	648.8	0.82
29	B	Channel catfish	*Ictalurus punctatus*	977.6**	977.6	0.85
30	E	Amphipod	*Crangonyx pseudogracilis*	1,035	1,035	0.88
31	H	Oligochaete	*Tubifex tubifex*	1,225	1,225	0.91
32	E	Isopod	*Asellus aquaticus*	1,262	1,262	0.94
33	H	Leech	*Nephelopsis obscura*	7,904**	7,904	0.97

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes

B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-9. PFNA Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	17.58	2.87	8.22	0.029	0.171
2	***Chydorus***	**27.84**	3.33	11.07	0.059	0.243
3	*Megalonaias*	32.90	3.49	12.20	0.088	0.297
4	*Oncorhynchus*	35.40	3.57	12.72	0.118	0.343
		Σ (Su):	**13.25**	**44.21**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 18.12 S = slope
L = 2.192 L = X-axis intercept
A = 3.143 A = lnFAV
FAV = 23.18
FAV/2 = 11.59 mg/L (Preliminary Value)
Adjustment = 11.59 / 15.8 = 0.7337 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.73 mg/L PFNA** (rounded to two significant figures)

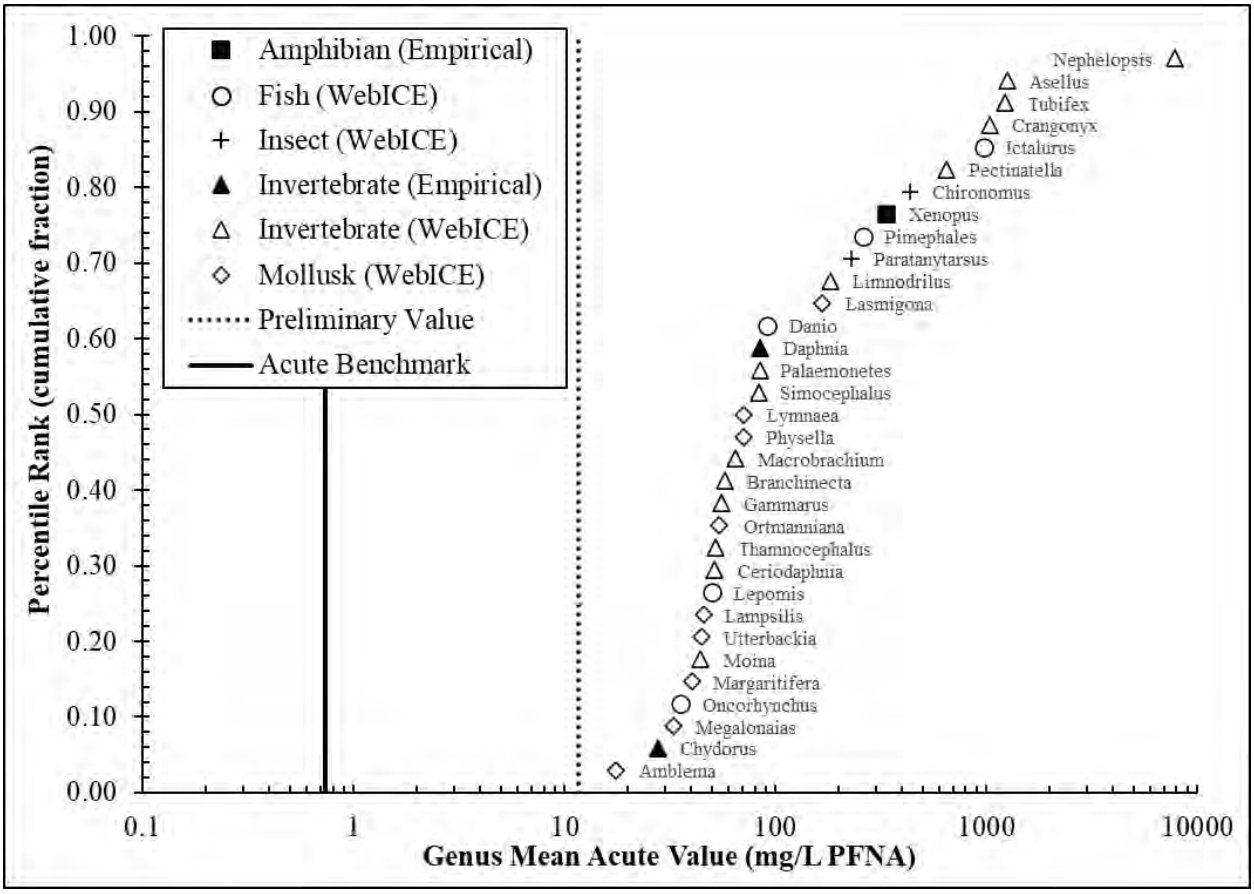


Figure F-3. Ranked Acute PFNA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.4 Derivation of Acute Water Benchmark for Perfluorodecanoic Acid (PFDA)

Quantitatively acceptable empirical data for PFDA were available for five species comprising four genera and fulfilling three MDR groups (**Table F-11**). A total of 85 ICE models were accepted for use. Of these, thirteen ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Chydorus sphaericus* and *Daphnia magna*). Four other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Pimephales promelas*). A total of 68 ICE models were selected for use, resulting in ICE models predicting 67 SMAVs representing 56 genera (**Table F-10** and **F-11**).

The combined empirical and ICE data resulted in 60 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-11**. GMAVs for the four genera used in the FAV calculation were within a factor of 1.3 of each other (**Table F-12**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFDA is 20.53 mg/L (**Table F-12**), which is lower than all of the GMAVs except for three of the ICE-derived species values (the isopod, *Caecidotea brevicauda*, GMAV= 8.170 mg/L the northern pike, *Esox lucius*, GMAV=18.36 mg/L, and the largemouth bass, *Micropterus salmoides*, GMAV=19.52 mg/L). The FAV was divided by two to obtain a preliminary value of 10.27 mg/L PFDA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.65 mg/L PFDA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFDA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-4**).

Table F-10. Acceptable models for ICE-estimated Species Sensitivity to PFDA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0008	166.8	**1,422.9** (423.1-4,784.7)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	5,000	**39.7** (14-112.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0001	68.3	**140.9** (70-283.6)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	20.0 (12.3-32.6)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**31.0** (15.7-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	8,694.5	**71.5** (43.6-117.5)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	9.810	**207.8** (56.1-770)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	46,278	64.5[b] (42-99)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**33.1** (19.8-55.3)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.014	0.232	**877.8** (553.4-1,392.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**34.4** (22.8-51.9)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	724.4	40.0 (24-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	43.07	49.4 (38.4-63.6)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0033	43.07	**195.1** (34.5-1,103.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	46,278	**84.8** (53.8-133.7)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0008	0.232	**60.4** (19.8-184.4)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0001	0.646	150.2 (65.6-344.1)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.009	10.36	90.7 (25.8-318.7)
Daphnid (Daphnia magna)*	Daphnid (Daphnia pulicaria)	149.6	149,600 (µg/L)	0.009	237.4	122.1 (27.4-544.5)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	41.13	41,130 (µg/L)	0.007	462	114.5 (70.9-184.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	4,894.7	93.7 (56.2-156)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	281.6	122.0 (24.2-614.7)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0002	7.2	**84.9** (45.5-158.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0001	166.8	**147.7** (37.1-587.8)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	46,500	85.6[b] (58.9-124.4)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	13,400	**46.7** (36-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**66.6** (37-119.8)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.014	166.8	**211.5** (51.2-874.2)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	19.93	**90** (54.2-149.3)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**34.9** (11-110.9)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	166.8	**1,699.9** (273.7-10,559)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	82	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**18.0** (11.4-28.4)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0008	4.369	**11,822** (1,982-70,511)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0006	95.86	**143.5** (43.1-477.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.007	54.4	**52.1** (10.9-248.7)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0003	472	**611.2** (100.7-3,709.3)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.37	14,500	**321.5** (140.5-735.3)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0003	58	**109.9** (42.7-282.7)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**147.7** (24.4-896.1)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.042	166.8	**58.5** (15.4-222.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.002	198.4	**64.7** (33.7-124.2)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	1.822	**18.4** (6.5-52)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.003	281.6	**232.8** (51.5-1,052.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0001	4,894.7	**1,672.6** (538.3-5,196.9)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.011	281.6	**97.1** (34.6-272.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**61.0** (38.4-97.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.042	545.9	**76.0** (17.4-332.2)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	14,500	45.4 (31.9-64.7)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0033	43.07	**26.6** (5.3-134)
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.003	2.04	**177.6** (34.2-922.7)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**302** (61.5-1,484.6)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.028	95.86	**45.7** (11.2-185.7)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0014	7.075	**40.3** (25.8-62.8)
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	0.068	**194.9** (89.6-424)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	7.5	74.6 (17.1-325.1)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	9.7	**46.3** (19.9-107.9)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**24.5** (10.4-57.6)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**98.2** (32.2-299.3)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**98.7** (42.7-227.9)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	4,894.7	**24.5** (8.2-73.1)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.506	**80.6** (38.8-167.4)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	16.24	**41.8** (12.1-144.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**46.0** (25.6-82.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.009	977.6	41.9 (26.5-66.4)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.009	281.6	**56.9** (10.6-305.9)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**54.9** (26.3-114.5)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	1.625	**31.3** (4.6-211.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	16.24	**44.5** (21-94.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	119.7**	1,19.7(mg/L)	0.0001	50	660.1[b] (190-2,293.1)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	46,500	115.5[b] (51.4-259.4)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	8,341.5	**34.4** (16.8-70.4)

*Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

Table F-11. Ranked PFDA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFDA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	E	Isopod	*Caecidotea brevicauda*	8.170	8.170	0.02
2	B	Northern pike	*Esox lucius*	18.36**	18.36	0.03
3	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.05
4	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.07
	A	Lake trout	*Salvelinus namaycush*	18.01		
5	G	Threeridge	*Amblema plicata*	24.46	24.46	0.08
6	B	Spotfin chub	*Erimonax monachus*	24.47**	24.47	0.10
7	B	Zebrafish	*Danio rerio*	24.57	24.57	0.11
8	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.13
9	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.15
10	C	Western toad	*Anaxyrus boreas*	31.29**	31.29	0.16
11	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.18
12	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	32.00	0.20
13	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.21
	A	Brown trout	*Salmo trutta*	33.12		
14	B	Cape Fear shiner	*Notropis mekistocholas*	34.42**	34.42	0.23
15	B	Greenthroat darter	*Etheostoma lepidum*	34.87**	34.87	0.25
16	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.26
17	B	Silver perch	*Bidyanus bidyanus*	40.28**	40.28	0.28
18	**D**	**Daphnid**	***Chydorus sphaericus***	**41.13**	41.13	0.30
19	B	Walleye	*Sander vitreus*	41.80**	41.80	0.31
20	B	Yellow perch	*Perca flavescens*	44.48**	44.48	0.33
21	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.34
22	G	Washboard	*Megalonaias nervosa*	46.01	46.01	0.36
23	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.38
24	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.39
25	B	Medaka	*Oryzias latipes*	52.12	52.12	0.41
26	B	Green sunfish	*Lepomis cyanellus*	89.98**	54.56	0.43
	B	Bluegill	*Lepomis macrochirus*	33.08		
27	G	Western pearlshell	*Margaritifera falcata*	54.89	54.89	0.44
28	C	Bullfrog	*Lithobates catesbeianus*	58.75	56.04	0.46
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	46.28**		
29	D	Water flea	*Moina macrocopa*	56.93	56.93	0.48
30	G	Paper pondshell	*Utterbackia imbecillis*	61.04	61.04	0.49
31	B	Goldfish	*Carassius auratus*	62.18	62.18	0.51
32	G	Neosho mucket	*Lampsilis rafinesqueana*	58.54	62.42	0.52
	G	Fatmucket	*Lampsilis siliquoidea*	66.56		
33	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	71.54	71.54	0.54
34	D	Daphnid	*Ceriodaphnia dubia*	84.82	71.55	0.56
	D	Daphnid	*Ceriodaphnia reticulata*	60.35**		
35	B	Bonytail	*Gila elegans*	72.68	72.68	0.57
36	E	Amphipod	*Gammarus fasciatus*	39.66	74.75	0.59
	E	Amphipod	*Gammarus pseudolimnaeus*	140.9**		

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
37	G	Pheasantshell	*Ortmanniana pectorosa*	76.02	76.02	0.61
38	**C**	**Clawed frog**	***Xenopus sp.***	**76.50**	76.50	0.62
39	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	80.58	80.58	0.64
40	G	Swamp lymnaea	*Lymnaea stagnalis*	98.19	98.19	0.66
41	G	Tadpole physa	*Physella gyrina*	98.69	98.69	0.67
42	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	109.9**	109.9	0.69
43	D	Daphnid	*Simocephalus serrulatus*	84.92**	112.0	0.70
	D	Daphnid	*Simocephalus vetulus*	147.7		
44	E	Riceland prawn	*Macrobrachium lanchesteri*	177.6**	131.3	0.72
	E	Oriental river shrimp	*Macrobrachium nipponense*	97.09		
45	F	Mayfly	*Drunella grandis*	143.5	143.5	0.74
46	**D**	**Daphnid**	***Daphnia magna***	**145.5**	147.5	0.75
	D	**Daphnid**	***Daphnia pulicaria***	**149.6**		
47	B	Mrigal carp	*Cirrhinus mrigala*	147.7**	147.7	0.77
48	F	Midge	*Paratanytarsus dissimilis*	105.8	184.4	0.79
	F	Midge	*Paratanytarsus parthenogeneticus*	321.5		
49	F	Snipefly	*Atherix variegata*	194.9**	194.9	0.80
50	B	Colorado squawfish	*Ptychocheilus lucius*	195.1	195.1	0.82
51	B	Black bullhead	*Ameiurus melas*	207.8**	207.8	0.84
52	G	Green floater	*Lasmigona subviridis*	211.5	211.5	0.85
53	H	Oligochaete	*Limnodrilus hoffmeisteri*	232.8	232.8	0.87
54	B	Rohu	*Labeo rohita*	302.0**	302.0	0.89
55	F	Midge	*Chironomus tentans*	611.2	611.2	0.90
56	H	Bryozoan	*Pectinatella magnifica*	877.8**	877.8	0.92
57	E	Amphipod	*Crangonyx pseudogracilis*	1,423	1,423	0.93
58	H	Oligochaete	*Tubifex tubifex*	1,673	1,673	0.95
59	E	Isopod	*Asellus aquaticus*	1,700	1,700	0.97
60	H	Leech	*Nephelopsis obscura*	11,822**	11,822	0.98

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-12. PFDA Final Acute Value and Protective Aquatic Acute Benchmark

Calculated Freshwater FAV based on 4 values closest to 0.05 percentile; N=60 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
2	*Esox*	18.36	2.91	8.47	0.033	0.181
3	*Micropterus*	19.52	2.97	8.83	0.049	0.222
4	*Salvelinus*	23.80	3.17	10.05	0.066	0.256
5	*Amblema*	24.46	3.20	10.22	0.082	0.286
		Σ (Su):	**12.25**	**37.57**	**0.23**	**0.95**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 =	9.92	S = slope
L =	2.318	L = X-axis intercept
A =	3.022	A = lnFAV
FAV =	20.53	
FAV/2 =	10.27 mg/L (Preliminary Value)	
Adjustment =	10.27 / 15.8 = 0.6498 (Preliminary Value / Carboxylic Acid Application Factor)	
Benchmark =	**0.65 mg/L PFDA** (rounded to two significant figures)	

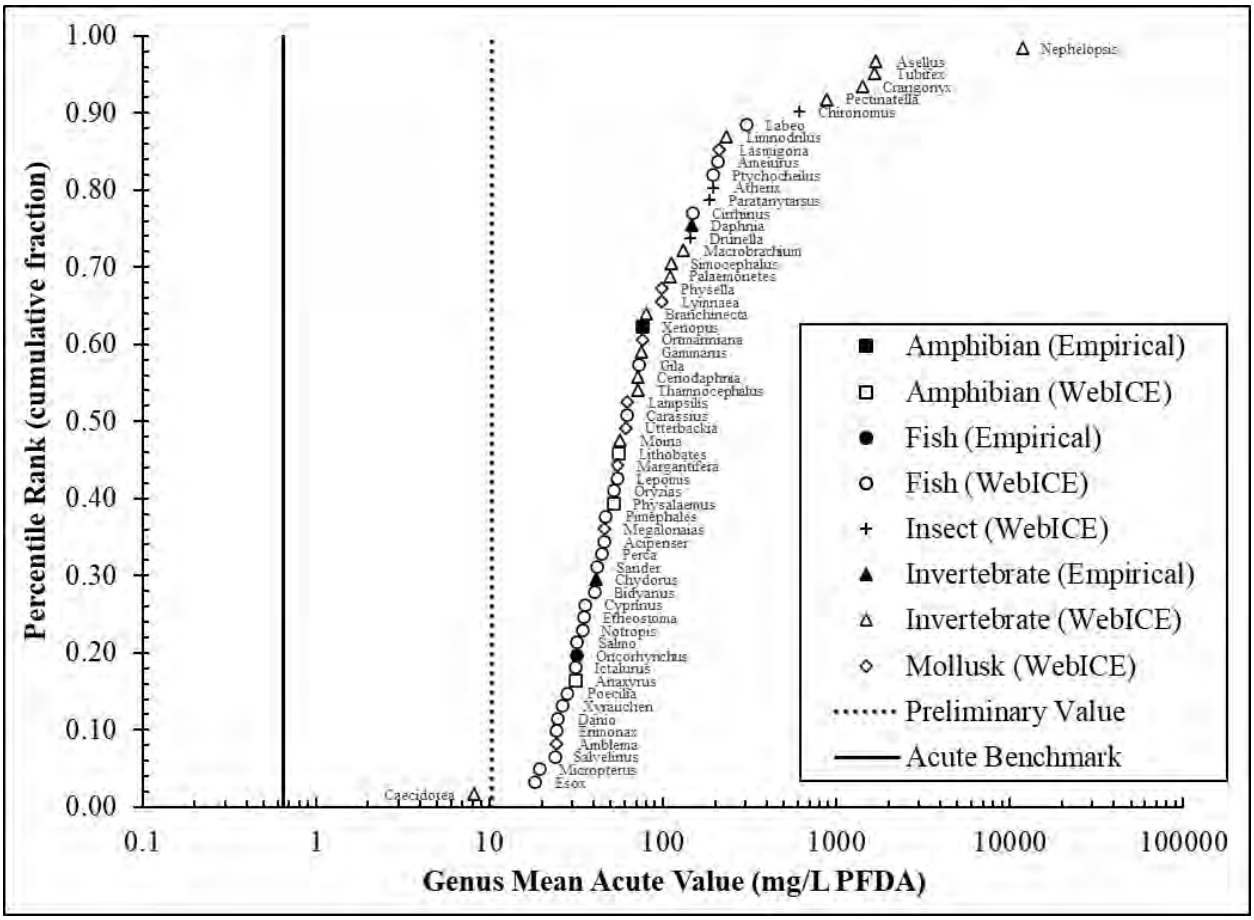


Figure F-4. Ranked Acute PFDA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.5 Derivation of Acute Water Benchmark for Perfluorobutanesulfonic Acid (PFBS)

Quantitatively acceptable empirical data for PFBS were available for four species comprising four genera and fulfilling two MDR groups (**Table F-14**). A total of 119 ICE models were accepted for use. Of these, seventeen ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Lepomis macrochirus* and *Daphnia magna*). Seven ICE models were not selected for use because they were derived based on saltwater species surrogates (e.g., *Americamysis bahia* predicting for *Gammarus fasciatus*). Sixteen other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Lepomis macrochirus* predicting for *Cyprinus carpio*). Two

additional models (e.g., *Ictalurus punctatus* predicting for *Lithobates catesbeianus*) were not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 77 ICE models were selected for use, resulting in ICE models predicting 66 SMAVs, representing 54 genera (**Tables F-13** and **F-14**).

The combined empirical and ICE data resulted in 58 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-14**. GMAVs for the four most sensitive genera were within a factor of 1.9 of each other (**Table F-15**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFBS is 646.3 mg/L (**Table F-15**), which is lower than all of the GMAVs except for two of the ICE-derived species values (the threeridge mussel, *Amblema plicata*, GMAV=383.6 mg/L and the greenthroat darter, *Etheostoma lepidum*, GMAV=518.0 mg/L). The FAV was divided by two to obtain a preliminary benchmark value of 323.1 mg/L PFBS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 7.6 mg/L PFBS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-5**).

Table F-13. Acceptable models for ICE-estimated Species Sensitivity to PFBS using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
African clawed frog (Xenopus laevis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.018	140.2	**1,868.8** (685.1-5,097.5)
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0008	166.8	**20,152** (5,302.8-76,585)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	5,000	**428.1** (107.9-1,697.9)
Amphipod (Gammarus fasciatus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	4.4	826.2[a] (280.5-2,433.7)
Amphipod (Gammarus minus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.239	107.6	**7,872.6** (1,934.1-32,046)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	68.3	**2,010.4** (770.9-5,242.9)
Amphipod (Gammarus pseudolimnaeus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	7.17	220.9[a] (87-560.8)
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	193.4	357.7[a] (156.5-817.5)
Arctic grayling (Thymallus arcticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.038	150.8	**5,722.4** (958.9-34,148)
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	123.3	**5,330.1** (2,929.8-9,696.8)
Atlantic salmon (Salmo salar)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**493.4** (76.8-3,169.5)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0003	8,694.5	**1,010.1** (525.1-1,942.9)
Black bullhead (Ameiurus melas)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**2,628.4** (591-11,689)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	46,278	512.5 (285.3-920.4)
Bluegill (Lepomis macrochirus)*	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.0002	50,154	751.0 (390-1,446)
Bluegill (Lepomis macrochirus)*	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	79	1,574.6 (1,174.5-2,111)
Bonytail (Gila elegans)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.054	150.8	1,895.4[b] (374.7-9,588.1)
Bonytail (Gila elegans)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	8.32	**880.6** (168.2-4,610.9)
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	123.3	**4,301.9** (2,141.8-8,640.5)
Brook trout (Salvelinus fontinalis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**506.7** (192.5-1,334)
Brown trout (Salmo trutta)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.002	123.3	**2,390.2** (732.9-7,795.5)
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	7,340[c] (3,632.2-14,833)
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**1,791.4** (756.1-4,244.6)
Catla (Gibelion catla)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.333	8.32	**1,718.3** (810.4-3,643.3)
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	517.8	6,851.9[c] (4,607-10,191)
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**1,617.3** (755.1-3,464.4)
Chinook salmon (Oncorhynchus tshawytscha)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	150.8	**2,417.4** (631.8-9,249.7)
Chinook salmon (Oncorhynchus tshawytscha)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.011	140.2	**397.2** (94.8-1,664.3)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	150.8	**3,010.5** (1,399.5-6,475.7)
Coho salmon (Oncorhynchus kisutch)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**589.1** (232.4-1,493.1)
Colorado squawfish (Ptychocheilus lucius)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	20.92	**868.0** (215.4-3,498.7)
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0009	180	7,708.8[b] (3,816-15,573)
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.005	133	**2,074.7** (1,359.3-3,166.5)
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	180	**5,874.5** (3,202.5-10,776)
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**645.0** (354.7-1,172.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0003	46,278	**1,666.6** (909-3,055.6)
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	4,100	171.7[a] (36.6-804.9)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	10.36	3,094.2 (706.1-13,559)
Daphnid (Daphnia magna)*	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	11,000	243.9 (143.9-413.4)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	4,894.7	1,768.1 (893.9-3,497.1)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.014	281.6	1,712.1 (445.5-6,580.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0002	7.2	**1,291.0** (482.8-3,451.9)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	166.8	**1,307.9** (202.3-8,457.1)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)*	Bluegill (Lepomis macrochirus)	6,452	6,452,000 (µg/L)	0.0001	27,540	3,399.2 (1,631.9-7,080.3)
Fathead minnow (Pimephales promelas)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	46,500	639.5 (375.7-1,088.5)
Fathead minnow (Pimephales promelas)*	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	11,000	158.6 (74.6-337.5)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**912.5** (399-2,086.8)
Fatmucket (Lampsilis siliquoidea)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.013	10,831	487.4[b] (125.7-1,889.9)
Flagfish (Jordanella floridae)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	113	**5,648.7** (1,648.7-19,354)
Flagfish (Jordanella floridae)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	32.54	**1,232.7** (388.5-3,911.1)
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	8,832.2[b] (4,286.5-18,199)
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**2,152.4** (1,075.2-4,309)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.014	166.8	**1,471.9** (318.2-6,807.8)
Green sunfish (Lepomis cyanellus)*	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	113	7,710.8 (3,925.7-15,146)
Green sunfish (Lepomis cyanellus)*	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.011	26.69	720.9 (107.3-4,841.9)
Greenthroat darter (Etheostoma lepidum)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.006	8.32	**518.0** (105.9-2,533.1)
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	180	**5,295.7** (2,358.2-11,892)
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**1,407.4** (816.4-2,426.3)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Indian bullfrog (Euphlyctis hexadactylus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.038	113	**2,330.8** (410.6-13,232)
Isopod (Asellus aquaticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.003	123.3	9,389.6[b] (2,238.7-39,383)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0003	166.8	**55,185** (17,354-175,483)
Isopod (Asellus aquaticus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	6.983	5,240.6[a] (964.7-28,468.1)
Isopod (Caecidotea brevicauda)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	26.7	**3,753.7** (1,068.9-13,183)
Isopod (Caecidotea brevicauda)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**286.2** (83.9-976.8)
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.003	180	**2,719.2** (1,374-5,381.5)
Lake trout (Salvelinus namaycush)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	133	**374.7** (171.7-817.4)
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	123.3	**6,684.3** (4,375-10,213)
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.003	1,420	**2,670.2** (1,679.9-4,244.3)
Midge (Chironomus plumosus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	87.6	672.1[a] (228.5-1,977.3)
Midge (Chironomus tentans)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0001	517.8	**10,675** (1,696.1-67,189)
Midge (Paratanytarsus dissimilis)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	**18,234** (3,711.6-89,583)
Midge (Paratanytarsus dissimilis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	1,430	**6,295.4** (1,795.6-22,072)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.37	14,500	**5,481.6** (1,674.6-17,944)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Paratanytarsus parthenogeneticus)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.28	10,600	6,562.8[b] (1,034.9-41,616)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0003	58	**993.2** (233.2-4,230.7)
Mosquitofish (Gambusia affinis)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.006	26,500	**1,349.6** (216.3-8,421.7)
Mozambique tilapia (Oreochromis mossambicus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**1,578** (636.6-3,911.6)
Mrigal carp (Cirrhinus mrigala)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.1969	8.32	**1,132.5** (742.8-1,726.6)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.042	166.8	**1,653.6** (781.8-3,497.4)
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.002	6.969	**8,705.2** (7,662.2-9,890.2)
Northern leopard frog (Lithobates pipiens)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.008	180	**14,976.0** (2,672.6-83,915)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.003	281.6	**13,646.0** (5,444.3-34,203)
Oligochaete (Lumbriculus variegatus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**2,368.1** (1,042.6-5,378.4)
Oligochaete (Tubifex tubifex)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0001	113	2,696.5[b] (423.5-17,169)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	2,183^	2,183 (mg/L)	0.0001	4,894.7	**22,428.0** (5,594.7-89,907)
Oligochaete (Tubifex tubifex)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.015	10,831	2,039.0[b] (591.9-7,024)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.011	281.6	**978.3** (434.4-2,203.5)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**802.5** (422.2-1,525.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.134	10,831	478.6[b] (99.6-2,299.1)
Peppered loach (Lepidocephalichthys guntea)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.1969	43.79	**1,937.8** (699.6-5,367.4)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.042	545.9	**1,625.4** (540-4,892.6)
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	6,452**	6,452,000 (µg/L)	0.0001	7,100	**3,208.6** (2,275.7-4,524)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	14,500	361.9[b] (221.2-592.1)
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.0002	26,500	**788.9** (505.7-1,230.8)
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	113	1,127.7[a] (854.1-1,489)
Razorback sucker (Xyrauchen texanus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.054	150.8	1,223[b] (295.2-5,067.6)
Razorback sucker (Xyrauchen texanus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	8.32	**742.7** (170.7-3,231.1)
Rohu (Labeo rohita)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0008	7.326	22,073[b] (3,315.4-146,959)
Rohu (Labeo rohita)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.002	8.32	**3,518.1** (750.6-16,489)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,523.7** (337.4-6,881.2)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,608.6** (512.8-5,046.1)
Tadpole physa (Physella gyrina)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.814	10,831	263.4[b] (41-1,694.5)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	4,894.7	**383.6** (85.7-1,717.6)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.814	10,831	250.3[b] (39.4-1,592.2)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,246.4** (457-3,399.4)
Walleye (Sander vitreus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	6.722	**2,272.6** (535.8-9,639.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**755.1** (345.4-1,650.5)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	977.6	**934.5** (569.8-1,532.6)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	281.6	**3,183.1** (1,038.3-9,758.3)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**706.1** (255.8-1,948.9)
White sucker (Catostomus commersonii)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.035	123.3	4,920.4[b] (2,000.4-12,103)
White sucker (Catostomus commersonii)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**2,552.9** (896.1-7,273)
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	9.41	**4,050.0** (2,122-7,729.6)
Yellow perch (Perca flavescens)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	504.1[b] (168.3-1,509.3)
Zebrafish (Danio rerio)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	50	6,202.4 (1,930.8-19,924)
Zebrafish (Danio rerio)*	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.013	26.69	2,005.9 (1,169.6-3,440.3)
Zebrafish (Danio rerio)*	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	5.666	6,109.6 (1,057.2-35,308)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	46,500	780.8 (257.2-2,370.6)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Zebrafish-embryo (Danio rerio-embryo)*	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.004	70,200	1,707.9 (1,061.5-2,748)

*Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-14. Ranked PFBS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	383.6	383.6	0.02
2	B	Greenthroat darter	*Etheostoma lepidum*	518.0**	518.0	0.03
3	G	Western pearlshell	*Margaritifera falcata*	706.1	706.1	0.05
4	B	Razorback sucker	*Xyrauchen texanus*	742.7**	742.7	0.07
5	G	Washboard	*Megalonaias nervosa*	755.1	755.1	0.08
6	G	Paper pondshell	*Utterbackia imbecillis*	802.5	802.5	0.10
7	B	Colorado squawfish	*Ptychocheilus lucius*	868.0**	868.0	0.12
8	B	Bonytail	*Gila elegans*	880.6**	880.6	0.14
9	D	Water flea	*Chydorus sphaericus*	934.5**	934.5	0.15
10	E	Oriental river shrimp	*Macrobrachium nipponense*	978.3**	978.3	0.17
11	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	993.2**	993.2	0.19
12	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	1,010	1,010	0.20
13	E	Isopod	*Caecidotea brevicauda*	1,036**	1,036	0.22
14	B	Mrigal carp	*Cirrhinus mrigala*	1,132**	1,132	0.24
15	A	Brook trout	*Salvelinus fontinalis*	1,476**	1,221	0.25
	A	Lake trout	*Salvelinus namaycush*	1,009**		
16	G	Neosho mucket	*Lampsilis rafinesqueana*	1,654**	1,228	0.27
	G	Fatmucket	*Lampsilis siliquoidea*	912.5		
17	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	1,246	1,246	0.29
18	D	Daphnid	*Simocephalus serrulatus*	1,291**	1,299	0.31
	D	Daphnid	*Simocephalus vetulus*	1,308**		
19	B	Mosquitofish	*Gambusia affinis*	1,350	1,350	0.32
20	A	Cutthroat trout	*Oncorhynchus clarkii*	1,946**	1,418	0.34
	A	Coho salmon	*Oncorhynchus kisutch*	1,332**		
	A	Rainbow trout	*Oncorhynchus mykiss*	1,591		
	A	Chinook salmon	*Oncorhynchus tshawytscha*	979.9**		
21	G	Green floater	*Lasmigona subviridis*	1,472**	1,472	0.36
22	G	Swamp lymnaea	*Lymnaea stagnalis*	1,524	1,524	0.37
23	G	Tadpole physa	*Physella gyrina*	1,609	1,609	0.39
24	B	Channel catfish	*Ictalurus punctatus*	1,617	1,617	0.41
25	G	Pheasantshell	*Ortmanniana pectorosa*	1,625**	1,625	0.42
26	D	Daphnid	*Ceriodaphnia dubia*	1,667	1,667	0.44
27	B	Catla	*Gibelion catla*	1,718**	1,718	0.46
28	C	African clawed frog	*Xenopus laevis*	1,869**	1,869	0.47
29	E	Amphipod	*Gammarus fasciatus*	428.1	1,892	0.49
	E	Amphipod	*Gammarus minus*	7,873**		
	E	Amphipod	*Gammarus pseudolimnaeus*	2,010**		
30	B	Peppered loach	*Lepidocephalichthys guntea*	1,938**	1,938	0.51
31	**B**	**Fathead minnow**	***Pimephales promelas***	**1,938**	1,938	0.53
32	A	Atlantic salmon	*Salmo salar*	1,622**	1,969	0.54
	A	Brown trout	*Salmo trutta*	2,390**		
33	B	Common carp	*Cyprinus carpio*	2,075**	2,075	0.56

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
34	B	Goldfish	*Carassius auratus*	2,152	2,152	0.58
35	**D**	**Daphnid**	***Daphnia magna***	**2,183**	2,183	0.59
36	B	Walleye	*Sander vitreus*	2,273**	2,273	0.61
37	C	Indian bullfrog	*Euphlyctis hexadactylus*	2,331**	2,331	0.63
38	H	Oligochaete	*Lumbriculus variegatus*	2,368**	2,368	0.64
39	B	White sucker	*Catostomus commersonii*	2,553**	2,553	0.66
40	B	Black bullhead	*Ameiurus melas*	2,628**	2,628	0.68
41	B	Flagfish	*Jordanella floridae*	2,639**	2,639	0.69
42	B	Medaka	*Oryzias latipes*	2,670**	2,670	0.71
43	B	Guppy	*Poecilia reticulata*	2,730**	2,730	0.73
44	**B**	**Zebrafish**	***Danio rerio***	**>3,000**	>3,000	0.75
45	D	Water flea	*Moina macrocopa*	3,183**	3,183	0.76
46	B	Rohu	*Labeo rohita*	3,518**	3,518	0.78
47	B	Mozambique tilapia	*Oreochromis mossambicus*	1,578**	3,706	0.80
	B	Nile tilapia	*Oreochromis niloticus*	8,705**		
48	B	Yellow perch	*Perca flavescens*	4,050**	4,050	0.81
49	C	Bullfrog	*Lithobates catesbeianus*	1,791	5,180	0.83
	C	Northern leopard frog	*Lithobates pipiens*	14,976**		
50	A	Arctic grayling	*Thymallus arcticus*	5,722**	5,722	0.85
51	**B**	**Bluegill**	***Lepomis macrochirus***	**6,452**	6,452	0.86
52	B	Largemouth bass	*Micropterus salmoides*	6,684**	6,684	0.88
53	F	Midge	*Paratanytarsus dissimilis*	10,714**	7,664	0.90
	F	Midge	*Paratanytarsus parthenogeneticus*	5,482		
54	F	Midge	*Chironomus tentans*	10,675**	10,675	0.92
55	H	Oligochaete	*Limnodrilus hoffmeisteri*	13,646**	13,646	0.93
56	E	Amphipod	*Crangonyx pseudogracilis*	20,152**	20,152	0.95
57	H	Oligochaete	*Tubifex tubifex*	22,428**	22,428	0.97
58	E	Isopod	*Asellus aquaticus*	55,185**	55,185	0.98

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-15. PFBS Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=58 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	383.6	5.95	35.40	0.017	0.130
2	*Etheostoma*	518.0	6.25	39.06	0.034	0.184
3	*Margaritifera*	706.1	6.56	43.03	0.051	0.225
4	*Xyrauchen*	742.7	6.61	43.70	0.068	0.260
		Σ (Su):	**25.37**	**161.18**	**0.17**	**0.80**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 29.92 S = slope
L = 5.248 L = X-axis intercept
A = 6.471 A = lnFAV
FAV = 646.3
FAV/2 = 323.1 mg/L (Preliminary Value)
Adjustment = 323.1 / 42.3 = 7.639 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **7.6 mg/L PFBS** (rounded to two significant figures)

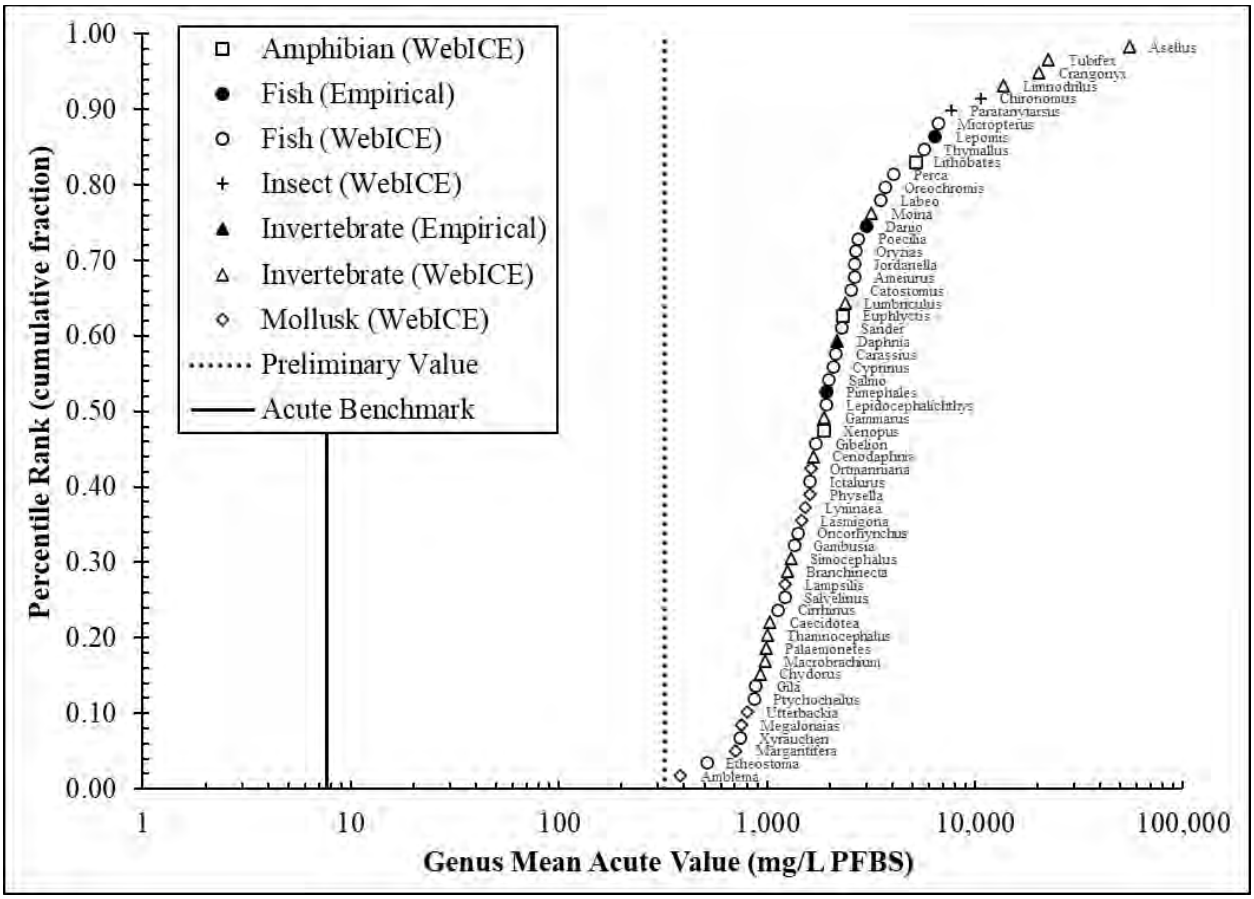


Figure F-5. Ranked Acute PFBS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.6 Derivation of Acute Water Benchmark for Perfluorohexanesulfonic Acid (PFHxS)

Quantitatively acceptable empirical data PFHxS were available for three species comprising two genera and fulfilling two MDR groups (**Table F-17**). A total of 18 ICE models were accepted for use. Of these, four other models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lithobates catesbeianus* predicting for *Ictalurus punctatus*). A total of 14 ICE models were selected for use, resulting in ICE models predicting 11 SMAVs representing 11 genera (**Tables F-16** and **F-17**).

The combined empirical and ICE data resulted in 13 GMAVs that collectively fulfill seven of the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life

Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-17**. GMAVs for the four most sensitive genera were within a factor of 1.2 of each other (**Table F-18**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxS is 18.17 mg/L (**Table F-18**), which is lower than all of the GMAVs. The FAV was divided by two to obtain a preliminary value of 9.084 mg/L PFHxS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 0.21 mg/L PFHxS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-6**).

Table F-16. Acceptable models for ICE-estimated Species Sensitivity to PFHxS using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	1,105 (mg/L)	0.003	233	**766.4** (378-1,553.6)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	719.8[b] (362.4-1,429.7)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	304.5	**19.5** (9.3-41)
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**424.1** (68.5-2,627.9)
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.003	36.91	**5.2** (1.2-22.7)
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	4.622	5,100.7	**114.2** (33.4-390.3)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	1,083.6[b] (468.5-2,506.4)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.018	25.08	**26.0** (11.6-58.2)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.023	54,579	**18.3** (13.3-25.2)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	7.797	649.3	**33.9** (19.7-58.4)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	1,027.7[b] (405.9-2,602.1)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	304.5	**37.3** (23.2-59.9)
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	3,020.0	**1,069.7** (176.6-6,480.9)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	18.6	20,900	**805.1** (394.5-1,643.4)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	239.3	**63.2** (9.9-403.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	713.5[b] (200.3-2,541.4)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.003	26.39	**38.3** (9.5-155.1)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.023	8,843.9	**9.6** (4.2-22)

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-17. Ranked PFHxS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	A	Rainbow trout	*Oncorhynchus mykiss*	19.19	19.19	0.07
2	B	Channel catfish	*Ictalurus punctatus*	19.46	19.46	0.14
3	B	Fathead minnow	*Pimephales promelas*	21.80	21.80	0.21
4	**B**	**Zebrafish**	***Danio rerio***	**22.50**	22.50	0.29
5	B	Flagfish	*Jordanella floridae*	33.90	33.90	0.36
6	B	Goldfish	*Carassius auratus*	37.28	37.28	0.43
7	D	Daphnid	*Daphnia magna*	46.87	46.87	0.50
8	H	Oligochaete	*Limnodrilus hoffmeisteri*	63.23	63.23	0.57
9	E	Fairy shrimp	*Streptocephalus proboscideus*	114.2	114.2	0.64
10	B	Bluegill	*Lepomis macrochirus*	766.4**	766.4	0.71
11	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.79
12	**C**	**Bullfrog**	***Lithobates catesbeiana***	**1,105**	915.2	0.86
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
13	F	Midge	*Paratanytarsus dissimilis*	1,070	1,070	0.93

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-18. PFHxS Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.
Note: Missing MDR Group: G

Calculated Freshwater FAV based on 4 lowest values; N=13 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Oncorhynchus*	19.19	2.95	8.73	0.071	0.267
2	*Ictalurus*	19.46	2.97	8.81	0.143	0.378
3	*Pimephales*	21.80	3.08	9.50	0.214	0.463
4	***Danio***	**22.50**	3.11	9.69	0.286	0.535
		Σ (Su):	**12.12**	**36.73**	**0.71**	**1.64**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 0.48 S = slope
L = 2.744 L = X-axis intercept
A = 2.900 A = lnFAV
FAV = 18.17
FAV/2 = 9.084 mg/L (Preliminary Value)
Adjustment = 9.084 / 42.3 = 0.2147 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **0.21 mg/L PFHxS** (rounded to two significant figures)

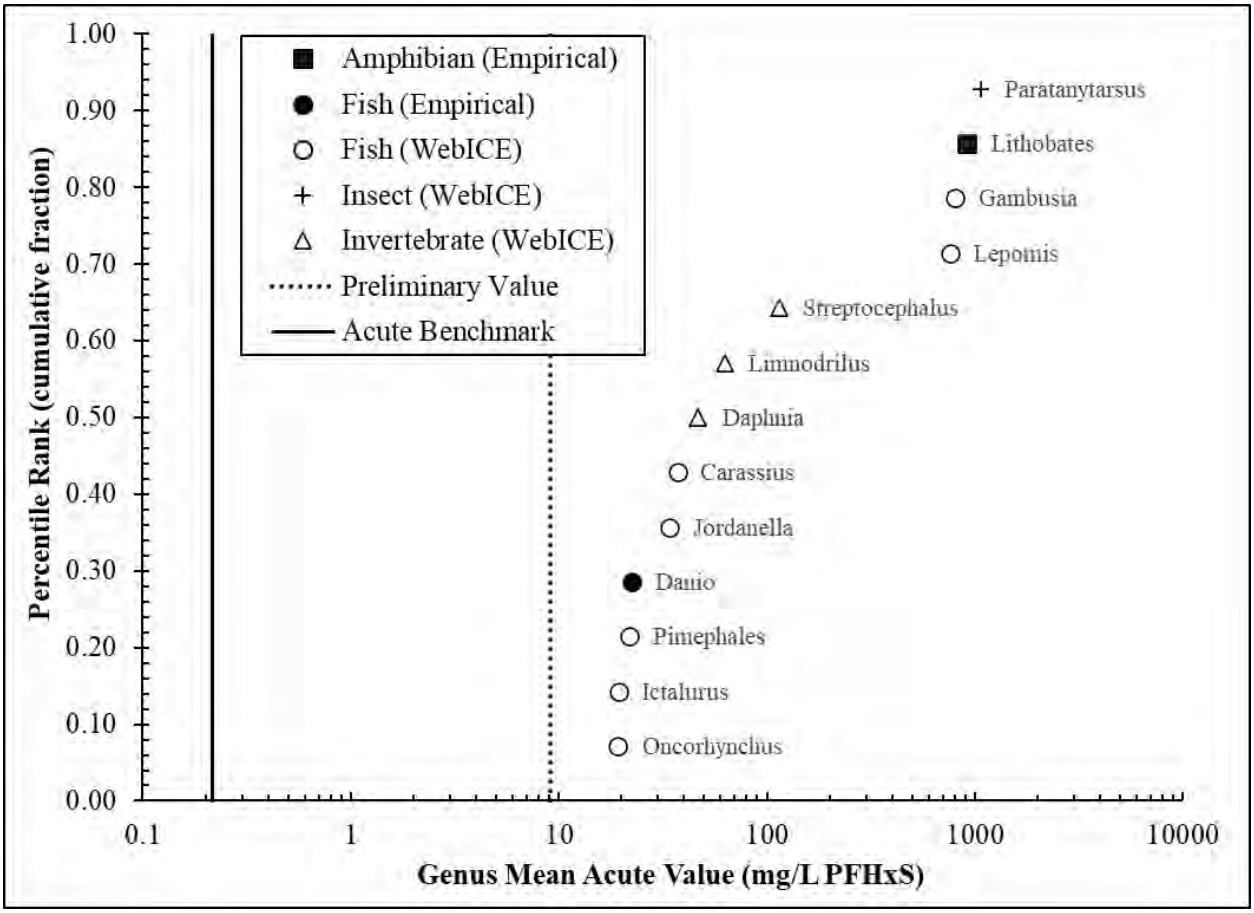


Figure F-6. Ranked Acute PFHxS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.7 Derivation of Acute Water Benchmark for Hexadecafluoro-2-decenoic Acid (8:2 FTUCA)

Quantitatively acceptable empirical data for HEXA were available for two species comprising two genera and fulfilling two MDR groups (**Table F-20**). A total of 80 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Oncorhynchus tshawytscha* and *Daphnia magna*). Five other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus* predicting for *Cyprinus carpio*). A total of 65 ICE models were selected for use, resulting in ICE models predicting 64 SMAVs representing 53 genera (**Tables F-19** and **F-20**).

The combined empirical and ICE data resulted in 55 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV is summarized in **Table F-20**. GMAVs for the four most sensitive genera were within a factor of 1.8 of each other (**Table F-21**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 8:2 FTUCA is 1.292 mg/L (**Table F-21**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata*, GMAV = 0.79 mg/L. The FAV was divided by two to obtain a preliminary value of 0.6458 mg/L 8:2 FTUCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.041 mg/L 8:2 FTUCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 8:2 FTUCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-7**).

Table F-19. Acceptable models for ICE-estimated Species Sensitivity to 8:2 FTUCA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0008	166.8	**28.6** (7-116.1)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	5,000	**2.0** (1-4.1)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	68.3	**2.9** (1-7.8)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	55.5 (35-87.9)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	95.86	**79.3** (36.6-171.6)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	8,694.5	**2.6** (1.8-3.9)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	9.81	**411.3** (105.2-1,607.8)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	46,278	4.9[b] (3.7-6.4)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0001	8,341.5	**79.0** (64.3-97.1)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**288.8** (102.7-811.8)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	96.69	**79.4** (44.1-142.9)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	95.86	**83.8** (47-149.4)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	13,400	**132.5** (51-344.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**102.3** (69.6-150.5)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	13,400	**67.4** (39.8-114)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.003	724.4	102.9 (57.1-185.4)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	43.07	106.1 (91.6-122.9)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**453.5** (101.9-2,018.3)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	198.4	**75.2** (31-182.2)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	198.4	65.5 (42.9-100)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.7099	155.5	**91.1** (48.4-171.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	46,278	**2.1** (1.5-2.9)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	3.2**	3.2 (mg/L)	0.0008	0.232	**2.6** (0.6-11.3)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	3.2**	3.2 (mg/L)	0.0001	0.646	5.4 (2.1-14.2)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	10.36	4.9 (1-23.7)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	4,894.7	2.4 (1.7-3.3)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	281.6	2.6 (0.7-9.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	7.2	**1.9** (0.7-5.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	166.8	**1.7** (0.3-12.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	46,500	7.0[b] (5.4-9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	13,400	**107.7** (79.8-145.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.5** (1.6-4.1)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	13,400	**132.7** (62.2-283.3)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	166.8	**1.9** (0.4-9.4)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	19.93	**208** (129.4-334.4)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**92.6** (31.2-274.7)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	198.4	**54.9** (12.3-243.7)
Indian bullfrog (Euphlyctis hexadactylus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	19.93	**184.0** (26-1,301.7)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	166.8	**76.6** (23-255.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	82	**16.8** (3.2-89.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	198.4	**41.3** (24.5-69.8)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	95.86	**46.8** (20.7-106.2)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	81^	81 (mg/L)	0.0006	95.86	**365.0** (106.1-1,255.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	1,330	**241.0** (72.2-804.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.37	14,500	**9.3** (3.5-24.7)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	58	**1.3** (0.3-6.2)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.0006	1.625	**298.8** (53.6-1,666.3)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.042	166.8	**2.3** (1.1-5.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.002	198.4	**125.5** (62.3-252.8)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.005	1.822	**48.1** (16.4-141.3)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.003	281.6	**17.9** (6.9-46.2)
Oligochaete (Limnodrilus hoffmeisteri)	Rainbow trout (Oncorhynchus mykiss)	81^	81 (mg/L)	0.003	707	889.5[b] (128.1-6,176.8)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	4,894.7	**31.6** (7.4-134.7)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.011	281.6	**1.5** (0.7-3.4)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.5** (1.6-3.7)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.042	545.9	**2.4** (0.8-7)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	3.2**	3200 (µg/L)	0.0001	14,500	3.4 (2.7-4.3)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**250.4** (96.5-649.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	2.04	**392.0** (87.1-1,764.3)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.0006	1.625	**818.9** (162.1-4,136.2)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.028	95.86	**134.5** (26.7-677.1)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.001	7.075	**110.5** (70.9-172.1)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	7.5	163.6 (50.5-529.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	9.7	**151.5** (76.9-298.6)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**74.3** (33.2-166.2)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**3.2** (1.2-8.4)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**3.0** (1.4-6.7)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	4,894.7	**0.8** (0.3-2.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.6** (1.4-4.9)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	81*	81 (mg/L)	0.005	16.24	**78.4** (27.6-222.5)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**1.4** (0.8-2.6)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	977.6	**1.3** (0.8-2.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	281.6	**4.2** (1.3-13.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.3** (1.1-4.6)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	1.625	**107.9** (25.9-450.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	16.24	**102.6** (49.6-212)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	50	8.3[b] (2.5-28)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	229.1	**34.6** (11.3-106)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	46,500	10.7[b] (5.7-19.9)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	8,341.5	**65.2** (29-146.3)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC 0 falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-20. Ranked 8:2 FTUCA Genus Mean Acute Values.
Values in bold and highlighted are derived from empirical 8:2 FTUCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.7900	0.7900	0.02
2	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1.330	1.330	0.04
3	D	Daphnid	*Chydorus sphaericus*	1.340	1.340	0.05
4	G	Washboard	*Megalonaias nervosa*	1.400	1.400	0.07
5	D	Daphnid	*Simocephalus serrulatus*	1.850	1.794	0.09
	D	Daphnid	*Simocephalus vetulus*	1.740		
6	G	Green floater	*Lasmigona subviridis*	1.900	1.900	0.11
7	G	Western pearlshell	*Margaritifera falcata*	2.270	2.270	0.13
8	D	Daphnid	*Ceriodaphnia reticulata*	2.560**	2.302	0.14
	D	Daphnid	*Ceriodaphnia dubia*	2.070		
9	G	Pheasantshell	*Ortmanniana pectorosa*	2.350	2.350	0.16
10	E	Amphipod	*Gammarus fasciatus*	2.040	2.411	0.18
	E	Amphipod	*Gammarus pseudolimnaeus*	2.850		
11	G	Neosho mucket	*Lampsilis rafinesqueana*	2.320	2.428	0.20
	G	Fatmucket	*Lampsilis siliquoidea*	2.540		
12	G	Paper pondshell	*Utterbackia imbecillis*	2.450	2.450	0.21
13	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2.630	2.630	0.23
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2.640	2.640	0.25
15	G	Tadpole physa	*Physella gyrina*	3.030	3.030	0.27
16	**D**	**Daphnid**	***Daphnia magna***	**3.200**	3.200	0.29
17	G	Swamp lymnaea	*Lymnaea stagnalis*	3.210	3.210	0.30
18	D	Water flea	*Moina macrocopa*	4.190	4.190	0.32
19	E	Isopod	*Caecidotea brevicauda*	16.82	16.82	0.34
20	H	Oligochaete	*Limnodrilus hoffmeisteri*	17.89	17.89	0.36
21	E	Riceland prawn	*Macrobrachium lanchesteri*	392.0**	24.41	0.375
	E	Oriental river shrimp	*Macrobrachium nipponense*	1.520		
22	E	Amphipod	*Crangonyx pseudogracilis*	28.57	28.57	0.39
23	H	Oligochaete	*Tubifex tubifex*	31.57	31.57	0.41
24	B	Largemouth bass	*Micropterus salmoides*	46.83	46.83	0.43
25	F	Midge	*Paratanytarsus dissimilis*	241.0	47.44	0.45
	F	Midge	*Paratanytarsus parthenogeneticus*	9.340		
26	B	Zebrafish	*Danio rerio*	47.48	47.48	0.46
27	B	Northern pike	*Esox lucius*	48.13**	48.13	0.48
28	B	Guppy	*Poecilia reticulata*	54.85	54.85	0.50
29	A	Brook trout	*Salvelinus fontinalis*	79.36	57.25	0.52
	A	Lake trout	*Salvelinus namaycush*	41.30		
30	B	Channel catfish	*Ictalurus punctatus*	67.38	67.38	0.54
31	B	Spotfin chub	*Erimonax monachus*	74.30**	74.30	0.55
32	B	Common carp	*Cyprinus carpio*	75.18	75.18	0.57
33	E	Isopod	*Asellus aquaticus*	76.56	76.56	0.59
34	B	Walleye	*Sander vitreus*	78.36**	78.36	0.61

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**81.00**	81.00	0.63
36	A	Atlantic salmon	*Salmo salar*	79.29	81.49	0.64
	A	Brown trout	*Salmo trutta*	83.76		
37	C	Cuvier's foam froglet	*Physalaemus cuvieri*	91.14	91.14	0.66
38	B	Greenthroat darter	*Etheostoma lepidum*	92.55**	92.55	0.68
39	B	Cape Fear shiner	*Notropis mekistocholas*	102.3**	102.3	0.70
40	B	Yellow perch	*Perca flavescens*	102.6**	102.6	0.71
41	B	Fathead minnow	*Pimephales promelas*	107.7	107.7	0.73
42	C	Western toad	*Anaxyrus boreas*	107.9**	107.9	0.75
43	B	Silver perch	*Bidyanus bidyanus*	110.5**	110.5	0.77
44	B	Green sunfish	*Lepomis cyanellus*	208.0**	128.2	0.79
	B	Bluegill	*Lepomis macrochirus*	78.99		
45	B	Goldfish	*Carassius auratus*	132.7	132.7	0.80
46	B	Shortnose sturgeon	*Acipenser brevirostrum*	134.5	134.5	0.82
47	C	Bullfrog	*Lithobates catesbeianus*	132.5	136.0	0.84
	C	Northern leopard frog	*Lithobates pipiens*	125.5		
	C	Southern leopard frog	*Lithobates sphenocephalus*	151.5**		
48	C	Indian bullfrog	*Euphlyctis hexadactylus*	184.0**	184.0	0.86
49	B	Razorback sucker	*Xyrauchen texanus*	250.4**	250.4	0.88
50	B	Bonytail	*Gila elegans*	288.8**	288.8	0.89
51	B	Mrigal carp	*Cirrhinus mrigala*	298.8**	298.8	0.91
52	F	Mayfly	*Drunella grandis*	365.0	365.0	0.93
53	B	Black bullhead	*Ameiurus melas*	411.3**	411.3	0.95
54	B	Colorado squawfish	*Ptychocheilus lucius*	453.5**	453.5	0.96
55	B	Rohu	*Labeo rohita*	818.9**	818.9	0.98

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-21. 8:2 FTUCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=55 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	0.79	-0.24	0.06	0.018	0.134
2	*Palaemonetes*	1.33	0.29	0.08	0.036	0.189
3	*Chydorus*	1.34	0.29	0.09	0.054	0.231
4	*Megalonaias*	1.40	0.34	0.11	0.071	0.267
		Σ (Su):	**0.68**	**0.34**	**0.18**	**0.82**

P = cumulative probability
R = rank
N = number of GMAVs
S = slope
L = X-axis intercept
A = lnFAV

S^2 = 22.23
L = -0.798
A = 0.256
FAV = 1.292
FAV/2 = 0.6458 mg/L (Preliminary Value)
Adjustment = 0.6458 / 15.8 = 0.04087 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.041 mg/L 8:2 FTUCA** (rounded to two significant figures)

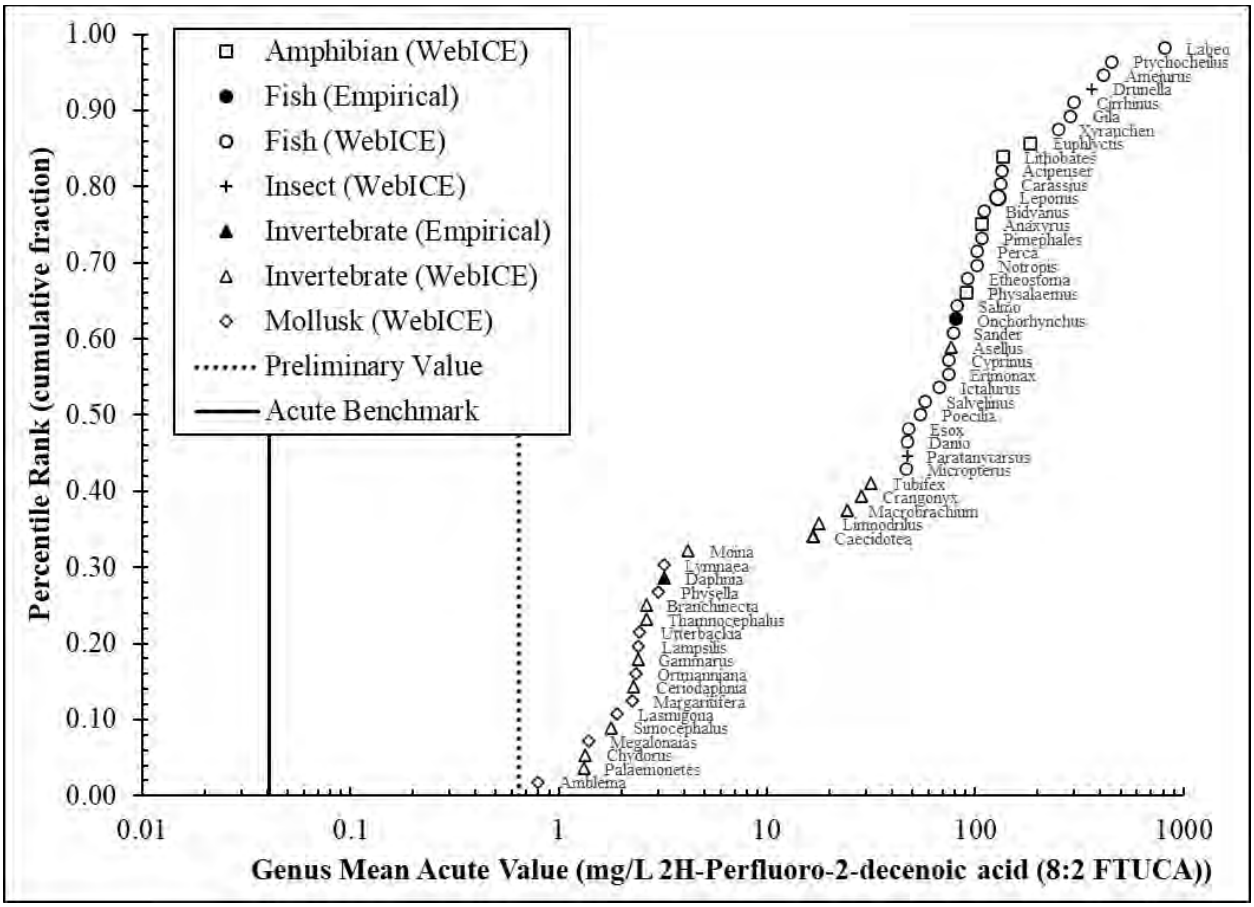


Figure F-7. Ranked Acute 8:2 FTUCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.8 Derivation of Acute Water Benchmark for Pentadecafluorodecanoic Acid (7:3 FTCA)

Quantitatively acceptable empirical data for 7:3 FTCA were available for two species comprising two genera and fulfilling two MDR groups (**Table F-23**). A total of 81 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Oncorhynchus tshawytscha* and *Daphnia pulex*). Four other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus* predicting for *Cyprinus carpio*). A total of 67 ICE models were selected for use, resulting in ICE models predicting 66 SMAVs representing 57 genera (**Tables F-22** and **F-23**).

The combined empirical and ICE data resulted in 59 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-23**. GMAVs for the four most sensitive genera were within a factor of 2.1 of each other (**Table F-24**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 7:3 FTCA is 0.4609 mg/L (**Table F-24**), which is lower than all of the GMAVs except for three of the ICE-derived species values (the threeridge mussel, *Amblema plicata*, GMAV=0.25 mg/L, the water flea *Chydorus sphaericus*, GMAV=0.43 mg/L, and the washboard, *Megalonaias nervosa,* GMAV=0.44 mg/L). The FAV was divided by two to obtain a preliminary value of 0.2305 mg/L 7:3 FTCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.015 mg/L 7:3 FTCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 7:3 FTCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-8**).

Table F-22. Acceptable models for ICE-estimated Species Sensitivity to 7:3 FTCA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0008	166.8	**9.5** (2.8-32.4)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	5,000	**0.8** (0.4-1.4)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	68.3	**1.0** (0.4-2.2)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	20.0 (12.3-32.6)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**31.0** (15.7-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	8,694.5	**0.9** (0.6-1.3)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	9.81	**207.8** (56.1-770)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	46,278	2.1[b] (1.6-2.7)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**33.1** (19.8-55.3)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	13,400	**58.8** (23.7-145.4)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**34.4** (22.8-51.9)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	724.4	40.0 (24-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	43.07	49.4 (38.4-63.6)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.003	43.07	**195.1** (34.5-1,103.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	46,278	**0.6** (0.4-0.9)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	0.9592**	0.9592 (mg/L)	0.0001	0.646	1.8 (0.6-5.8)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	10.36	1.1 (0.3-4.3)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	4,894.7	0.7 (0.5-1)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	281.6	0.7 (0.2-3.1)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	7.2	**0.6** (0.3-1.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	166.8	**0.7** (0.1-3.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	46,500	3.0[b] (2.3-3.9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	13,400	**46.7** (36-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.9** (0.5-1.5)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	166.8	**0.9** (0.2-3.5)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	19.93	**90.0** (54.2-149.3)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**34.9** (11-110.9)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	198.444	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	166.8	**27.3** (9.3-80.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	82	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**18.0** (11.4-28.4)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0008	4.369	**131.2** (18.7-920.2)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0006	95.86	**143.5** (43.1-477.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.007	54.400	**52.1** (10.9-248.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	472	**4.6** (0.7-32.5)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.37	14,500	**2.9** (0.9-9.1)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	58	**0.5** (0.2-1.9)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**147.7** (24.4-896.1)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.042	166.8	**0.8** (0.4-1.6)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.002	198.4	**64.7** (33.7-124.2)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	1.822	**18.4** (6.5-52)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.003	281.6	**7.6** (3.1-18.5)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	4,894.7	**10.8** (3-38.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.011	281.6	**0.4** (0.2-0.9)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.8** (0.5-1.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.042	545.9	**0.7** (0.2-2.4)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	14,500	1.4 (1.2-1.8)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**26.6** (5.3-134)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.003	2.04	**177.6** (34.2-922.7)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**302.0** (61.5-1,484.6)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.028	95.86	**45.7** (11.2-185.7)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.001	7.075	**40.3** (25.8-62.8)
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	0.068	**194.9** (89.6-424)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	7.5	74.6 (17.1-325.1)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	9.7	**46.3** (19.9-107.9)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**24.5** (10.4-57.6)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**1.0** (0.4-2.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**1.0** (0.4-2.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.9592	0.9592 (mg/L)	0.014	4,894.7	**0.3** (0.1-0.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.9** (0.4-1.6)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	16.24	**41.8** (12.1-144.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.4** (0.2-0.9)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	977.6	**0.4** (0.3-0.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	281.6	**1.8** (0.6-5.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.8** (0.4-1.7)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	1.625	**31.3** (4.6-211.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	16.24	**44.5** (21-94.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	50.000	3.3[b] (1.1-10)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	46,500	4.8[b] (2.5-9.2)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	8,341.5	**34.4** (16.8-70.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-23. Ranked 7:3 FTCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 7:3 FTCA toxicity tests with the species.

Rank	MDR Group[1]	Common Name	Species	SMAV (mg/L)	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.2500	0.2500	0.02
2	D	Daphnid	*Chydorus sphaericus*	0.4300	0.4300	0.03
3	G	Washboard	*Megalonaias nervosa*	0.4400	0.4400	0.05
4	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	0.5300	0.5300	0.07
5	D	Daphnid	*Ceriodaphnia dubia*	0.6000	0.6000	0.08
6	D	Daphnid	*Simocephalus serrulatus*	0.6000	0.6527	0.10
	D	Daphnid	*Simocephalus vetulus*	0.7100		
7	G	Pheasantshell	*Ortmanniana pectorosa*	0.7400	0.7400	0.12
8	G	Western pearlshell	*Margaritifera falcata*	0.7900	0.7900	0.13
9	G	Neosho mucket	*Lampsilis rafinesqueana*	0.7900	0.8243	0.15
	G	Fatmucket	*Lampsilis siliquoidea*	0.8600		
10	G	Paper pondshell	*Utterbackia imbecillis*	0.8400	0.8400	0.17
11	E	Amphipod	*Gammarus fasciatus*	0.7600	0.8497	0.18
	E	Amphipod	*Gammarus pseudolimnaeus*	0.9500		
12	G	Green floater	*Lasmigona subviridis*	0.8500	0.8500	0.20
13	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	0.8500	0.8500	0.22
14	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	0.8800	0.8800	0.23
15	G	Tadpole physa	*Physella gyrina*	0.9500	0.9500	0.25
16	**D**	**Daphnid**	***Daphnia magna***	**0.9592**	0.9592	0.27
17	G	Swamp lymnaea	*Lymnaea stagnalis*	1.030	1.030	0.28
18	D	Water flea	*Moina macrocopa*	1.760	1.760	0.30
19	F	Midge	*Paratanytarsus parthenogeneticus*	2.880	2.880	0.32
20	F	Midge	*Chironomus tentans*	4.630	4.630	0.33
21	H	Oligochaete	*Limnodrilus hoffmeisteri*	7.620	7.620	0.35
22	E	Isopod	*Caecidotea brevicauda*	8.170	8.170	0.37
23	E	Riceland prawn	*Macrobrachium lanchesteri*	177.6**	8.214	0.38
	E	Oriental river shrimp	*Macrobrachium nipponense*	0.3800		
24	E	Amphipod	*Crangonyx pseudogracilis*	9.510	9.510	0.40
25	H	Oligochaete	*Tubifex tubifex*	10.75	10.75	0.42
26	B	Northern pike	*Esox lucius*	18.36**	18.36	0.43
27	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.45
28	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.47
	A	Lake trout	*Salvelinus namaycush*	18.01		
29	B	Spotfin chub	*Erimonax monachus*	24.47**	24.47	0.48
30	B	Zebrafish	*Danio rerio*	24.57	24.57	0.50
31	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.52
32	E	Isopod	*Asellus aquaticus*	27.30	27.30	0.53
33	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.55
34	C	Western toad	*Anaxyrus boreas*	31.29**	31.29	0.57
35	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.58
36	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	32.00	0.60

Rank	MDR Group[1]	Common Name	Species	SMAV (mg/L)	GMAV	Percentile Rank
37	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.62
	A	Brown trout	*Salmo trutta*	33.12		
38	B	Cape Fear shiner	*Notropis mekistocholas*	34.42**	34.42	0.63
39	B	Greenthroat darter	*Etheostoma lepidum*	34.87**	34.87	0.65
40	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.67
41	B	Silver perch	*Bidyanus bidyanus*	40.28**	40.28	0.68
42	B	Walleye	*Sander vitreus*	41.80**	41.80	0.70
43	B	Yellow perch	*Perca flavescens*	44.48**	44.48	0.72
44	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.73
45	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.75
46	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.77
47	B	Medaka	*Oryzias latipes*	52.12	52.12	0.78
48	B	Green sunfish	*Lepomis cyanellus*	89.98**	54.56	0.80
	B	Bluegill	*Lepomis macrochirus*	33.08		
49	C	Bullfrog	*Lithobates catesbeianus*	58.75	56.04	0.82
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	46.28**		
50	B	Goldfish	*Carassius auratus*	62.18	62.18	0.83
51	B	Bonytail	*Gila elegans*	72.68	72.68	0.85
52	F	Midge	*Paratanytarsus dissimilis*	105.8	105.8	0.87
53	H	Leech	*Nephelopsis obscura*	131.2	131.2	0.88
54	F	Mayfly	*Drunella grandis*	143.5	143.5	0.90
55	B	Mrigal carp	*Cirrhinus mrigala*	147.7**	147.7	0.92
56	F	Snipefly	*Atherix variegata*	194.9**	194.9	0.93
57	B	Colorado squawfish	*Ptychocheilus lucius*	195.1	195.1	0.95
58	B	Black bullhead	*Ameiurus melas*	207.8**	207.8	0.97
59	B	Rohu	*Labeo rohita*	302.0**	302.0	0.98

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR Groups

A) The family Salmonidae in the class Osteichthyes
B) A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
C) A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
D) A planktonic crustacean (e.g., cladoceran, copepod, etc.)
E) A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
F) An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
G) A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
H) A family in any order of insect or any phylum not already represented.

Table F-24. 7:3 FTCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=59 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	0.25	-1.39	1.92	0.017	0.129
2	*Chydorus*	0.43	-0.84	0.71	0.033	0.183
3	*Megalonaias*	0.44	-0.82	0.67	0.050	0.224
4	*Palaemonetes*	0.53	-0.63	0.40	0.067	0.258
		Σ (Su):	**-3.69**	**3.71**	**0.17**	**0.79**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 33.93 S = slope
L = -2.077 L = X-axis intercept
A = -0.775 A = lnFAV
FAV = 0.4609
FAV/2 = 0.2305 mg/L (Preliminary Value)
Adjustment = 0.2305 / 15.8 = 0.01459 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.015 mg/L 7:3 FTCA** (rounded to two significant figures)

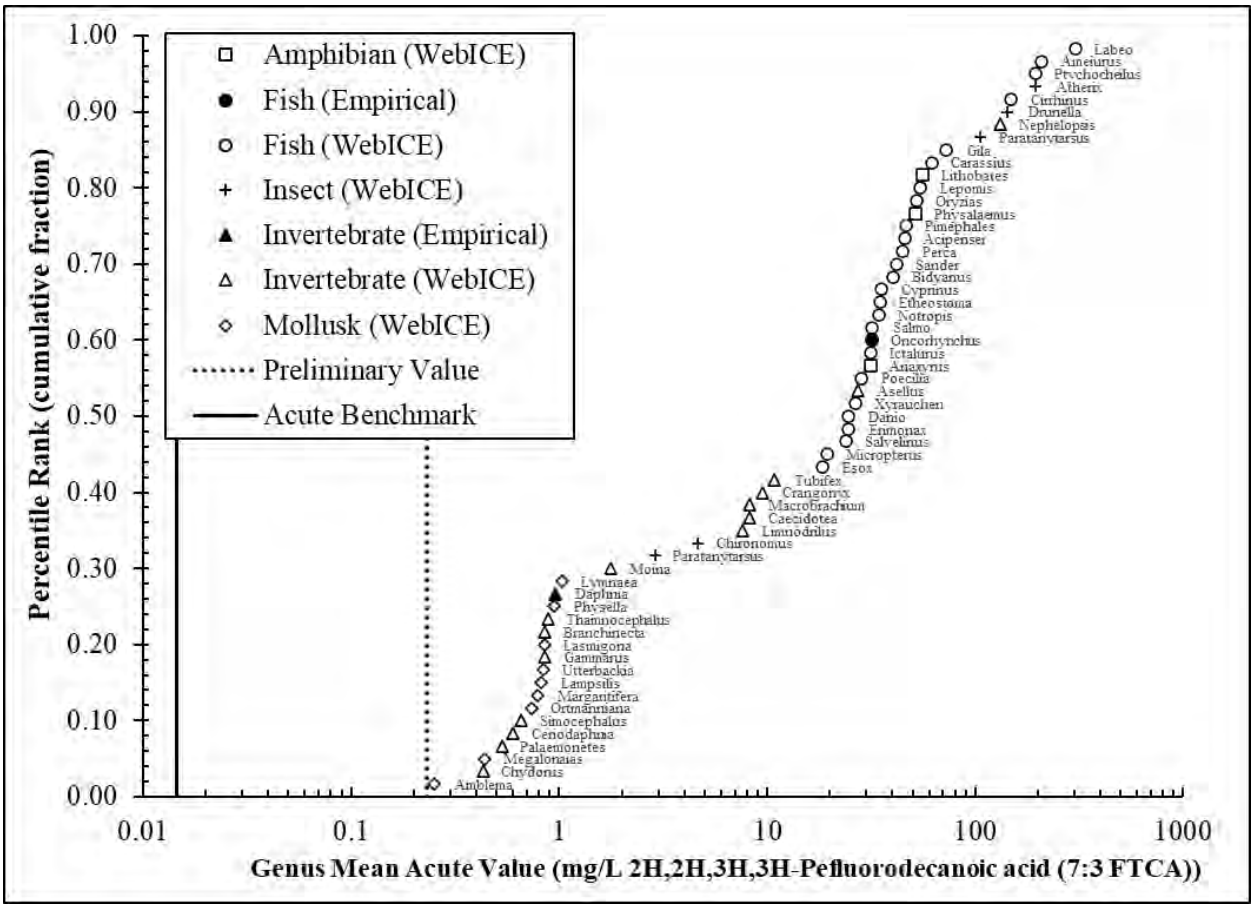


Figure F-8. Ranked Acute 7:3 FTCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.9 Summary of Benchmarks for Evaluated PFAS Substances

Acute freshwater Aquatic Life Ambient Water Quality Benchmarks for the eight selected PFAS are summarized in **Table F-25**. These concentrations are expected to be protective of 95% of freshwater genera exposed to the listed PFAS under short term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. Quantitatively-acceptable empirical toxicity data were coupled with ICE predicted values to fulfill the eight MDRs for deriving acute freshwater criteria per the Aquatic Life Criteria Guidelines (U.S. EPA 1985). Using this approach, the eight MDRs were fulfilled for seven of the evaluated compounds (PFBA, PFHxA, PFNA, PFDA, PFBS, 8:2 FTUCA, and 7:3 FTCA).

Seven of the eight MDRs were fulfilled for PFHxS and there is considered to be greater uncertainty associated with this benchmark value compared to benchmarks developed with all MDRs met. The resulting acute water column-based benchmark magnitudes range from 0.015 mg/L for 7:3 FTCA to 11 mg/L for PFBA.

Table F-25. Acute Freshwater Benchmarks for Eight PFAS.

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude[1]	11	6.0	0.73	0.65	7.6	0.21	0.041	0.015
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

[1] Values expressed as mg/L, or ppm.

F.1 References

Dowdy, S., S. Wearden and D. Chilko. 2011. Statistics for research. John Wiley & Sons.

Raimondo, S., C. Lilavois and S.A. Nelson. 2024. Uncertainty analysis and updated user guidance for interspecies correlation estimation (ICE) models and low toxicity compounds. Integr. Environ. Assess. Manag. Accepted Author Manuscript. https://doi.org/10.1002/ieam.4884

U.S. EPA. (United States Environmental Protection Agency). 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. EPA Report 822/R-85-100 (NTIS Report PB85-227049).

Appendix G PFAS Acute Benchmark Calculations using a Data Binning Approach

The EPA investigated the utility of applying an approach described in Giddings et al. (2019) for synthetic pyrethroid insecticides as another alternative for deriving benchmark values for data-limited PFAS. For this approach, Giddings et al. (2019) combined acute toxicity data available for multiple data-limited synthetic pyrethroid insecticide active ingredients (e.g., bifenthrin, cypermethrin, permethrin, etc.) to create a more robust combined species sensitivity distribution (SSD) with the grouped pyrethroids. Before combining, the available toxicity data were normalized across the individual pyrethroid active ingredients. This was done by normalizing the data across the individual pyrethroid active ingredients based on the relative sensitivity of a single selected "key" species, that has data for each constituent active ingredient. Giddings et al. (2019) then combined the normalized species data (i.e., key species equivalents) for the individual pyrethroids and calculated an HC_5 (hazard concentration at the 5% level) based on the combined pyrethroid SSD composed of normalized acute toxicity values. Estimated HC_5s were then back-calculated for individual constituent pyrethroids based on the relative sensitivity of the species used for the normalization.

The EPA followed the same methodology to calculate predicted HC_5 values for selected PFAS combined into two groups: carboxylic acids and sulfonic acids. Constituent PFAS for these groups are as follows:

- Carboxylic acid PFAS substances for which acceptable acute toxicity data were available:
    - PFOA (perfluorooctanoic acid or perfluorooctanoate)
    - PFBA (perfluorobutanoic acid or perfluorobutanoate)
    - PFHxA (perfluorohexanoic acid or perfluorohexanoate)
    - PFNA (perfluorononanoic acid or perfluorononanoate)
    - PFDA (perfluorodecanoic acid or perfluorodecanoate)
- Sulfonic acid PFAS substances for which acceptable acute toxicity data were available:
    - PFOS (perfluorooctane sulfonate or perfluorooctane sulfonate acid)
    - PFBS (perfluorobutane sulfonic acid or perfluorobutane sulfonate)
    - PFHxS (perfluorohexane sulfonic acid or perfluorohexane sulfonate)

The EPA conducted the following steps to complete this evaluation:

1. High quality acute toxicity data for each PFAS were compiled. These toxicity data consist of the same quantitatively acceptable empirical data used for derivation of the ICE-based values (see **Appendix A**) for PFBA, PFBS, PFHxA, PFHxS, PFNA, PFDA, and the same quantitatively acceptable data used for derivation of the PFOA and PFOS criteria (U.S. EPA 2024a,b). All data were converted into mg/L.
2. Species mean acute values (SMAVs) were identified. If multiple acute toxicity values were available for a single species and PFAS substance, then the geometric mean of those values was calculated.
3. "Key" species to be used for normalizing the data across the constituent substances within each PFAS grouping (sulfonic acid PFAS and carboxylic acid PFAS grouping) were identified and species equivalent values were calculated. Zebrafish (*Danio rerio*) was identified as the key species for sulfonic acid PFAS, and the planktonic crustacean (*Daphnia magna*) was identified as the key species for carboxylic acid

PFAS. The SMAV for each constituent substance within each PFAS grouping was then divided by the corresponding normalization value for the key species to calculate species equivalent values. The following examples illustrate the procedure for calculating key species equivalents for daphnids for PFOA within the carboxylic acid PFAS group:

- Equivalent for *Daphnia magna*:
    - *D. magna* PFOA SMAV of 213.9 mg/L ÷ *D. magna* PFOA SMAV of 213.9 mg/L PF A = 1.00 *D. magna* equivalent
- Equivalent for *Daphnia pulicaria*:
    - *D. pulicaria* PFOA SMAV of 203.7 mg/L PFOA ÷ *D. magna* PFOA SMAV of 213.9 mg/L PF A = 0.9523 *D. magna* equivalent

The species equivalent values were then ranked. The key species equivalent acute toxicity values and their rankings are shown in **Table G-1** for sulfonic acids and **Table G-2** for carboxylic acids.

4. Species equivalent values for constituent substances were pooled for each PFAS grouping to derive SSDs for sulfonic acid PFAS and for carboxylic acid PFAS. If a key species equivalent value was available for a species having two or more constituent PFAS within a group, then the geometric mean of those values was calculated as the SMAV equivalent for that species. These SMAVs representing key species equivalent values were then ranked to create SSDs for their respective PFAS group (sulfonic acid and carboxylic acid PFAS). SSDs were derived for sulfonic acid PFAS and carboxylic acid PFAS using both the EPA SSD Generator[1], as was done by Giddings et al. (2019), and procedures described in the Aquatic Life Criteria

[1] Available online at: Download Software | US EPA. Use of this tool results in the estimation of an HC_5.

Guidelines (U.S. EPA 1985), as is typically done for the derivation of aquatic life criteria. The SSDs generated by the EPA's SSD Generator are shown in **Figure G-1a** for sulfonic acid PFAS and **Figure G-2a** for carboxylic acid PFAS. The SSDs generated using procedures described in the Aquatic Life Criteria Guidelines are shown in **Figure G-1b** for sulfonic acid PFAS and **Figure G-2b** for carboxylic acid PFAS.

5. HC_5 values were calculated for sulfonic acid PFAS and carboxylic acid PFAS from the SSDs using both the EPA SSD Generator[2] and procedures described in the Aquatic Life Criteria Guidelines (termed Final Acute Value [FAV] for the Aquatic Life Criteria Guidelines calculations [U.S. EPA 1985]). For any given species within the PFAS group-specific SSD, a key species equivalent greater than 1.0 indicated it was less sensitive than the key species, and a value less than 1.0 indicated it was more sensitive than the key species. Once the key species equivalent HC_5 (or FAV) was calculated, the HC_5 (or FAV) species equivalent value calculated from the SSD was multiplied by the key species toxicity value (i.e., SMAV) for the PFAS of interest within that group (sulfonic or carboxylic PFAS) to generate a substance-specific HC_5 (or FAV). For example:

   - FAV for PFHxS:
     - FAV for Sulfonic Acid PFAS of 0.01004 *x D. rerio* PFHxS SMAV of . mg/ L = PF xS F A of 0.2260 mg/L
   - FAV for PFBA:
     - FAV for Carboxylic Acid PFAS of 0.008213 *x D. magna* PFBA SMAV of 4, 41 mg/ L = PF A FA of 38.94 mg/L

The calculations and values are summarized in **Tables G-3** through **G-6**.

[2] Available online at: Download Software | US EPA. Use of this tool results in the estimation of an HC_5.

Table G-1. Acute LC_{50}s, SMAVs, normalized *Danio rerio* equivalent values and rank of SMAV *D. rerio* equivalent values for the sulfonic acid PFAS.

Bolding indicates key species used for normalizing data across constituent substances.

PFAS	Species	Species Common Name	SMAV[a] (mg/L)	*Danio rerio* equivalent[b]	SMAV *D. rerio* equivalent[c]	Rank of SMAV *D. rerio* equivalent[d]
PFOS	*Ambystoma jeffersonianum*	Jefferson salamander	51.71	1.86	1.86	17
PFOS	*Ambystoma texanum*	Small-mouthed salamander	30.00	1.08	1.08	14
PFOS	*Ambystoma tigrinum*	Eastern tiger salamander	68.63	2.46	2.46	23
PFOS	*Anaxyrus americanus*	American toad	56.49	2.03	2.03	18
PFOS	*Brachionus calyciflorus*	Rotifer	61.80	2.22	2.22	21
PFBS	*Danio rerio*	**Zebrafish**	**>3,000**	**1.00**	**1.00**	**13**
PFHxS	*Danio rerio*	**Zebrafish**	**22.50**	**1.00**		
PFOS	*Danio rerio*	**Zebrafish**	**27.86**	**1.00**		
PFOS	*Daphnia carinata*	Daphnid	11.56	0.41	0.41	5
PFBS	*Daphnia magna*	Daphnid	2,183	0.73	1.16	15
PFOS	*Daphnia magna*	Daphnid	51.86	1.86		
PFOS	*Daphnia pulicaria*	Daphnid	134.0	4.81	4.81	27
PFOS	*Dugesia japonica*	Planaria	22.48	0.81	0.81	12
PFOS	*Elliptio complanata*	Eastern elliptio	64.35	2.31	2.31	22
PFOS	*Hyla versicolor*	Gray treefrog	19.88	0.71	0.71	11
PFOS	*Lampsilis siliquoidea*	Fatmucket	16.50	0.59	0.59	9
PFBS	*Lepomis macrochirus*	Bluegill	6,452	2.15	2.15	20
PFOS	*Ligumia recta*	Black sandshell	13.50	0.48	0.48	6
PFHxS	*Lithobates catesbeianus*	Bullfrog	1,105	49.11	15.33	30
PFOS	*Lithobates catesbeianus*	American bullfrog	133.3	4.78		
PFOS	*Lithobates clamitans*	Green frog	113.0	4.06	4.06	25
PFOS	*Lithobates pipiens*	Northern leopard frog	72.72	2.61	2.61	24
PFOS	*Lithobates sylvatica*	Wood frog	130.0	4.67	4.67	26

PFAS	Species	Species Common Name	SMAV[a] (mg/L)	*Danio rerio* equivalent[b]	SMAV *D. rerio* equivalent[c]	Rank of SMAV *D. rerio* equivalent[d]
PFOS	*Moina macrocopa*	Daphnid	17.20	0.62	0.62	10
PFOS	*Moina micrura*	Daphnid	0.5496	0.02	0.02	2
PFOS	*Neocaridina denticulata*	Japanese swamp shrimp	15.61	0.56	0.56	7
PFOS	*Neocleon triangulifer*	Mayfly	0.07617	0.003	0.003	1
PFOS	*Oncorhynchus mykiss*	Rainbow trout	7.515	0.27	0.27	3
PFOS	*Physella acuta*	Bladder snail	183.0	6.57	6.57	29
PFOS	*Physella heterostropha pomilia*	Snail	161.8	5.81	5.81	28
PFBS	*Pimephales promelas*	Fathead minnow	1,938	0.65	0.40	4
PFOS	*Pimephales promelas*	Fathead minnow	6.950	0.25		
PFOS	*Pontastacus leptodactylus*	Crayfish	48.81	1.75	1.75	16
PFOS	*Procambarus fallax f. virginalis*	Crayfish	59.87	2.15	2.15	19
PFOS	*Xenopus laevis*	African clawed frog	15.99	0.57	0.57	8

[a] Data used for PFBS and PFHxS are presented in **Appendix A**; data used for PFOS are the same as the quantitatively acceptable data used for derivation of the PFOS criteria (US EPA 2024b).

[b] *Danio rerio* equivalents determined according to the following equation: Species *x* SMAV for PFAS ÷ *D. rerio* SMAV for PFAS *x* = *D. rerio* equivalent for species.

[c] SMAV *Danio rerio* equivalents are the geometric means of the *D. rerio* equivalents for that species.

[d] Rank order of the *D. rerio* equivalents.

Table G-2. Acute LC_{50}s, SMAVs, normalized *Daphnia magna* equivalent values and rank of SMAV *D. magna* equivalent values for carboxylic acid PFAS.

Bolding indicates key species used for normalizing data across constituent substances.

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFOA	*Ambystoma jeffersonianum*	Jefferson salamander		1,070	5.00	5.00	27
PFOA	*Ambystoma texanum*	Small-mouthed salamander		407.3	1.90	1.90	14
PFOA	*Ambystoma tigrinum*	Eastern tiger salamander		752.0	3.52	3.52	24
PFOA	*Anaxyrus americanus*	American toad		793.9	3.71	3.71	25
PFBA	*Brachionus calyciflorus*	Rotifer	110	110.0	0.02	0.13	3
PFHxA	*Brachionus calyciflorus*	Rotifer	140	140.0	0.13		
PFOA	*Brachionus calyciflorus*	Rotifer		150.0	0.70		
PFBA	*Chydorus sphaericus*	Daphnid	>4,280.8	>4,280.8	0.90	0.46	5
PFDA	*Chydorus sphaericus*	Daphnid	41.13	41.13	0.34		
PFNA	*Chydorus sphaericus*	Daphnid	27.84	27.84	0.33		
PFOA	*Chydorus sphaericus*	Daphnid		93.17	0.44		
PFBA	*Danio rerio*	Zebrafish	>3,000[e]	13,779	2.91	2.47	18
PFBA	*Danio rerio*	Zebrafish	13,779				
PFOA	*Danio rerio*	Zebrafish		450.4	2.11		
PFOA	*Daphnia carinata*	Daphnid		66.80	0.31	0.31	4
PFBA	***Daphnia magna***	**Daphnid**	**>1,006**	**4,741**	**1.00**	**1.00**	**10**
PFBA	***Daphnia magna***	**Daphnid**	**>4,280.8**				
PFBA	***Daphnia magna***	**Daphnid**	**5,251**				
PFDA	***Daphnia magna***	**Daphnid**	**81**	**119.7**	**1.00**		
PFDA	***Daphnia magna***	**Daphnid**	**129.54**				
PFDA	***Daphnia magna***	**Daphnid**	**163.48**				
PFHxA	***Daphnia magna***	**Daphnid**	**1048**	**1,048**	**1.00**		
PFNA	***Daphnia magna***	**Daphnid**	**43.42**	**84.51**	**1.00**		
PFNA	***Daphnia magna***	**Daphnid**	**91.89**				

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFNA	***Daphnia magna***	**Daphnid**	**151.29**				
PFOA	***Daphnia magna***	**Daphnid**		**213.9**	**1.00**		
PFBA	*Daphnia pulicaria*	Daphnid	>1,006	>1,006	0.21	0.63	6
PFDA	*Daphnia pulicaria*	Daphnid	149.59	149.6	1.25		
PFOA	*Daphnia pulicaria*	Daphnid		203.7	0.95		
PFOA	*Dugesia japonica*	Planaria		383.6	1.79	1.79	12
PFOA	*Hyla versicolor*	Gray treefrog		646.2	3.02	3.02	20
PFOA	*Lampsilis siliquoidea*	Fatmucket		164.4	0.77	0.77	8
PFOA	*Lepomis macrochirus*	Bluegill		664.0	3.10	3.10	21
PFOA	*Ligumia recta*	Black sandshell		161.0	0.75	0.75	7
PFHxA	*Lithobates catesbeiana*	Bullfrog	1,105	1,105	1.05	2.24	17
PFOA	*Lithobates catesbeiana*	American bullfrog		1,020	4.77		
PFHxA	*Lithobates clamitans*	Green frog	758	758.0	0.72	1.90	13
PFOA	*Lithobates clamitans*	Green frog		1,070	5.00		
PFOA	*Lithobates pipiens*	Northern leopard frog		751.7	3.51	3.51	23
PFOA	*Lithobates sylvatica*	Wood frog		999.0	4.67	4.67	26
PFOA	*Moina macrocopa*	Daphnid		166.3	0.78	0.78	9
PFOA	*Moina micrura*	Daphnid		0.4747	0.0022	0.0022	1
PFOA	*Neocaridina denticulata*	Green neon shrimp		431.5	2.02	2.02	15
PFOA	*Neocloeon triangulifer*	Mayfly		13.05	0.061	0.061	2
PFDA	*Oncorhynchus mykiss*	Rainbow trout	32	32.00	0.27	2.24	16
PFOA	*Oncorhynchus mykiss*	Rainbow trout		4,001	18.71		
PFOA	*Physella acuta*	Bladder snail		681.1	3.18	3.18	22
PFOA	*Pimephales promelas*	Fathead minnow		593.6	2.78	2.78	19
PFDA	*Xenopus sp.*	Clawed frog	76.5	76.50	0.64	1.65	11
PFNA	*Xenopus sp.*	Clawed frog	335.8	335.8	3.97		

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFOA	*Xenopus sp.*	Frog		377.0	1.76		

[a] Data used for PFBA, PFNA, PFDA, and PFHxA are presented in **Appendix A**; data used for PFOA are the same as the quantitatively acceptable data used for derivation of the PFOA criteria (US EPA 2024a).

[b] *Daphnia magna* equivalents determined according to the following equation: Species x SMAV for PFAS ÷ *D. magna* SMAV for PFAS x = *D. magna* equivalent for species.

[c] SMAV *Daphnia magna* equivalents are the geometric means of the *D. magna* equivalents for that species.

[d] Rank order of the *D. magna* equivalents.

[e] Non-definitive value, not used in SMAV calculation.

Table G-3. Results of lognormal regression analysis of combined PFAS group SSDs based on key species equivalents and using the 's SSD G n rator

HC5 value expressed as mg/L.

PFAS Group	N[a]	Intercept	Slope	R^2	HC_5 (95% prediction interval)
Sulfonic acid[b]	30	4.970	1.241	0.827	0.0500 (0.0120 – 0.2079)
Carboxylic acid[c]	27	4.946	1.193	0.732	0.04636 (0.00705 – 0.30478)

[a] Number of species in SSD.
[b] *Danio rerio* equivalents.
[c] *Daphnia magna* equivalents.

Table G-4. Calculated freshwater FAV based on four lowest SMAV key species equivalents for sulfonic acid PFAS.

Rank	Species	SMAV (mg/L)	ln(SMAV)	$ln(SMAV)^2$	P=R/(N+1)	sqrt(P)
1	*Neocloeon triangulifer*	0.003	-5.90	34.83	0.032	0.180
2	*Moina micrura*	0.02	-3.93	15.41	0.065	0.254
3	*Oncorhynchus mykiss*	0.27	-1.31	1.72	0.097	0.311
4	*Pimephales promelas*	0.40	-0.91	0.83	0.129	0.359
		Σ(Su):	-12.05	52.79	0.32	1.10
	N	30				
	S^2 =	919.63		**S = slope**		
	L =	-11.382		**L = X-axis intercept**		
	A =	-4.601		**A = lnFAV**		
	FAV =	0.01004		**P = cumulative probability**		

Table G-5. Calculated freshwater FAV based on four lowest SMAV key species equivalents for carboxylic acid PFAS.

Rank	Species	SMAV (mg/L)	ln(SMAV)	$ln(SMAV)^2$	P=R/(N+1)	sqrt(P)
1	*Moina micrura*	0.0022	-6.11	37.34	0.036	0.189
2	*Neocloeon triangulifer*	0.061	-2.80	7.82	0.071	0.267
3	*Brachionus calyciflorus*	0.13	-2.04	4.18	0.107	0.327
4	*Daphnia carinata*	0.31	-1.16	1.35	0.143	0.378
		Σ(Su):	-12.11	50.69	0.36	1.16
	N	27				
	S^2 =	705.20		**S = slope**		
	L =	-10.740		**L = X-axis intercept**		
	A =	-4.802		**A = lnFAV**		
	FAV =	0.008213		**P = cumulative probability**		

Table G-6. PFAS acute benchmarks for individual PFAS within sulfonic and carboxylic acid groups calculated using approach presented by Giddings et al. (2019): Summary of values determined from SSDs derived using Aquatic Life Criteria Guidelines-based and SSD Generator-based values.

PFAS Substance Group	Aquatic Life Criteria Guidelines-Based Values, mg/L		SSD Generator-Based Values, mg/L	
	FAV	Benchmark[a]	HC_5,	Benchmark[a]
PFBS FAV	30.13	15.07	150.0	74.98
PFHxS FAV	0.2260	0.1130	1.125	0.5623
PFOS FAV	0.2798	0.1399	1.393	0.6963
PFBA FAV	38.94	19.47	219.8	109.9
PFNA FAV	0.6941	0.3471	3.918	1.959
PFDA FAV	0.983	0.4916	5.550	2.775
PFHxA FAV	8.607	4.304	48.59	24.29
PFOA FAV	1.757	0.8784	9.917	4.959

[a] Benchmarks by dividing the FAV or HC_5 by two, consistent with approach described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) for derivation of acute aquatic life criteria.

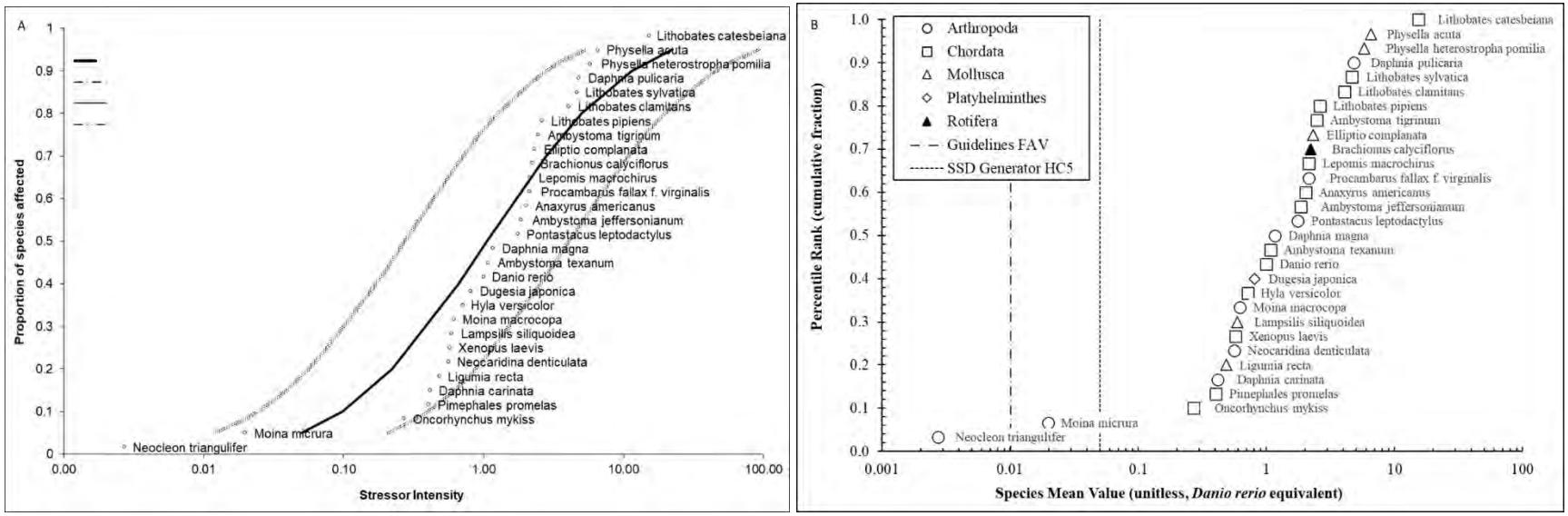


Figure G-1. Species sensitivity distributions for sulfonic acid PFAS based on *Danio rerio* equivalents, using the 's SSD Generator (left panel - A) or the Aquatic Life Criteria Guidelines procedure (right panel - B).

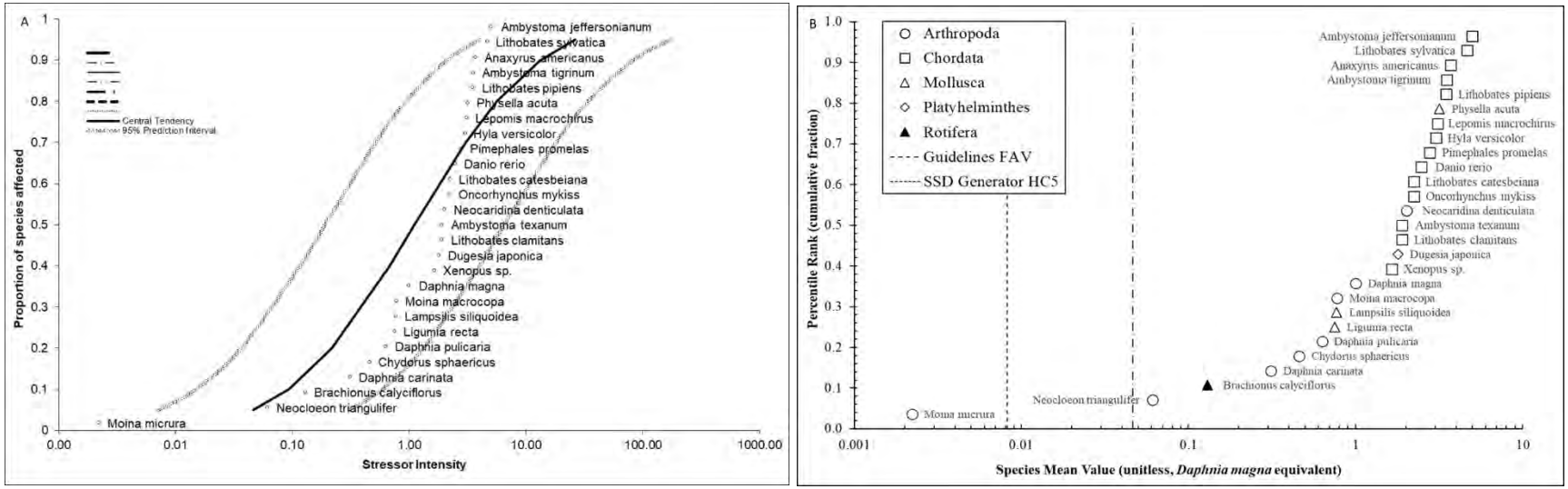


Figure G-2. Species sensitivity distributions for carboxylic acid PFAS based on *Daphnia magna* equivalents, using the 's SSD Generator (left panel - A) or the Aquatic Life Criteria Guidelines procedure (right panel - B)

G.1 References

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United States Environmental Protection Agency

EPA-815-R-24-003

FINAL

Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

AB Ex. 8

Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Prepared by:

U.S. Environmental Protection Agency
Office of Water & Office of Research and Development
Washington, DC

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Notices

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

This document provides a framework for estimating noncancer human health risks associated with mixtures of per- and polyfluoroalkyl substances (PFAS), based on longstanding EPA mixtures guidelines. This document is not a regulation and does not impose legally binding requirements on the EPA, states, Tribes, or the regulated community, and might not apply to a particular situation based on the circumstances. The extent of the utility of this document for a particular programmatic application will need to be assessed on a case-by-case basis within each specific decision context under applicable statutory and regulatory authority. The framework included in this document does not supersede previously published EPA guidelines on mixtures (e.g., USEPA, 1986, 2000b) or EPA approaches used to assess cumulative risks of contaminants including chemical mixtures under various environmental statutes (e.g., Federal Insecticide, Fungicide, and Rodenticide Act; Food Quality Protection Act; Comprehensive Environmental Response, Compensation, and Liability Act). The EPA may change certain aspects of this document in the future based on evolving availability of information relevant to human health risk assessment and increasing confidence in New Approach Methods.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Dedication

This document is dedicated to the memory of Dr. Jane Ellen Simmons and Mr. Jeffrey Swartout. Jane Ellen and Jeff were both dedicated civil servants in the EPA's Office of Research and Development for more than 30 years where they conducted rigorous chemical mixtures research and championed cumulative risk assessment approaches for exposure to multiple stressors. Their contributions to the field live on in this framework document.

Authors, Contributors, and Reviewers

Prepared by

Justin Conley, PhD – EPA Office of Research and Development (ORD)
Colleen Flaherty, MS (co-lead) – EPA Office of Water (OW)
Earl Gray, PhD – ORD
Brittany Jacobs, PhD – OW
Jason C. Lambert, PhD, DABT (co-lead) – ORD
Alex Lan, MPH – OW
Casey Lindberg, PhD – OW
Kathleen Raffaele, PhD (Retired) – EPA Office of Land and Emergency Management

Contributors

Carlye Austin, PhD, DABT – OW
Kelly Cunningham, MS – OW
Hannah Holsinger, MPH – OW
Amanda Jarvis, MS – OW
James R. Justice, MS – OW

Internal Technical Reviewers

Andrew Kraft, PhD – ORD
Allison Phillips, PhD – ORD
Glenn Rice, ScD – ORD
Jane Ellen Simmons, PhD – ORD

Executive Direction

Elizabeth Behl (Retired) – OW
Eric Burneson, P.E. – OW
Santhini Ramasamy, PhD – ORD
Jamie Strong, PhD – ORD
Russell Thomas, PhD – ORD
Tim Watkins, PhD – ORD

Formatting By

Tetra Tech, Inc.

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Abbreviations and Acronyms

6:2 FTS	6:2 fluorotelomer sulfonic acid
ADONA	4,8-dioxa-3H-perfluorononanoic acid
AED	administered equivalent dose
AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criteria
AOF	adsorbable organofluorine
AOP	adverse outcome pathway
AR	androgen receptor
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the concentration vs. time curve
BBP	butyl benzyl phthalate
BMD	benchmark dose
BMDL	lower statistical bound on a BMD
BMR	benchmark response
CF	carbon-fluorine
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CCL 5	fifth Contaminant Candidate List
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CPSC CHAP	Consumer Product Safety Commission Chronic Hazard Advisory Panel
DA	dose additivity
DAF	dosimetric adjustment factor
DBP	di-n-butyl phthalate
DEHP	di(2-ethylhexyl)phthalate
DIBP	diisobutyl phthalate
DLC	dioxin-like chemical
DWI	drinking water intake
DWI-BW	body weight-based drinking water intake
E	duration-relevant exposure
EC_X	effect concentration
ED_X	effective dose in x percent of test animals
EOF	extractable organofluorine
EPA	U.S. Environmental Protection Agency
EU	European Union
FT4	free serum thyroxine
FQPA	Food Quality Protection Act
GenX chemicals	hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt
GD	gestational day

HBWC	health-based water concentration
HED	human equivalent dose
HepaRG	epoxide hydrolase endpoint in liver
HFPO	hexafluoropropylene oxide
HFPO-DA	hexafluoropropylene oxide dimer acid
HI	hazard index
HQ	hazard quotient
HQ-115	lithium bis[(trifluoro-methyl)sulfonyl]azanide
IA	integrated addition
IC	index chemical
ICEC	index chemical equivalent concentration
$ICEC_{MIX}$	total mixture index chemical equivalent concentration
$ICEC_{NAM}$	new approach methodology (NAM)-based ICEC
ICED	index chemical equivalent dose
IRIS	Integrated Risk Information System
IVIVE	*in vitro*-to-*in vivo* extrapolation
k_e	elimination rate constant
KE	key event
L	liter
LOAEL	lowest-observed-adverse-effect level
M-BMD	mixture benchmark dose
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg/kg/day	milligrams per kilogram per day
MIE	molecular initiating event
MOA	mode of action
MRL	minimal risk level
NAM	new approach methodology(ies)
NAS	National Academy of Sciences
NBP2	Nafion byproduct 2
ng/g	nanograms per gram
ng/kg/week	nanograms per kilogram per week
ng/L	nanograms per liter
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OP	organophosphate
ORD	Office of Research and Development
osRfV	organ-specific reference value

PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PECO	Population, Exposure, Comparator, and Outcome
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonic acid
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFDS	perfluorodecanesulfonate
PFECHS	perfluoroethylcyclohexane sulfonate
PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptanesulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFNS	perfluorononanesulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFOSA	perfluorooctane sulfonamide
PFPA	perfluoroalkyl phosphonic acids
PFPeA	perfluoropentanoic acid
PFPeS	perfluoropentanesulfonic acid
PFPIA	perfluoroalkyl phosphinic acid
PFPrA	perfluoropropanoic acid
PFPS	perfluoropropane sulfonic acid
PFSA	perfluoroalkane sulfonic acid
PFSIA	perfluoroalkane sulfinic acid
PFTA	perfluorotetradecanoic acid
PFTrDA	perfluorotridecanoic acid
PFUnA	perfluoroundecanoic acid
PND	postnatal day
POD	point of departure
POD_{HED}	human-equivalent point of departure
PPARα	peroxisome proliferator-activated receptor alpha
PPARγ	peroxisome proliferator-activated receptor gamma
PPRTV	Provisional Peer-Reviewed Toxicity Value
ppt	parts per trillion
PWS	public water system

RA	response addition
RfD	reference dose
RfV	reference value
RPF	relative potency factor
RPF_{NAM}	new approach methodology (NAM)-based RPF
RSC	relative source contribution
rTK	reverse toxicokinetic
SAB	Science Advisory Board
SD	standard deviation
T3	triiodothyronine
T4	serum thyroxine
TT4	total serum thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TD	toxicodynamic
TEF	toxicity equivalence factor
TEQ	toxic equivalent
TK	toxicokinetic
TOSHI	target-organ-specific hazard index
$TOSHI_{DEV}$	target-organ-specific hazard index for developmental effects
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
TTD	target-organ toxicity dose
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
UF_A	interspecies UF
UF_C	composite UF
UF_D	database UF
UF_H	human interindividual variability UF
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	extrapolation from subchronic to a chronic exposure duration UF
Vd	volume of distribution

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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency is releasing the final *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* ("PFAS Mixtures Framework" or "framework"). This document is designed to communicate and illustrate the practical application of existing EPA chemical mixtures assessment approaches and methods to assess noncancer human health hazards and risks associated with exposure to two or more PFAS co-occurring in environmental media, using hypothetical drinking water examples. In November 2021, the EPA released a draft version of this document for Science Advisory Board (SAB) review, and in March 2023, this document underwent public comment as part of the proposed National Primary Drinking Water Regulation for PFAS (USEPA, 2023b). The EPA has considered the SAB and public comments and revised the document accordingly.

In a chemical mixtures risk assessment context, while it would be optimal to leverage whole-mixture hazard and dose-response data, such data are extremely rare, particularly at component-chemical proportions and concentrations consistent with environmentally occurring mixtures. As such, mixtures risk assessment commonly relies upon the integration of available toxicity information for the individual component chemicals that co-occur in environmental media.

This PFAS Mixtures Framework describes flexible, data-driven approaches that facilitate practical component chemical-based mixtures evaluation of two or more PFAS based on dose additivity. Dose additivity (described in detail in Section 3.0) means that the combined effect of the component chemicals in the mixture is equal to the sum of the individual doses or concentrations scaled for potency. Several perfluoroalkyl acid species (PFAAs) of PFAS tested to date have been shown to elicit the same or similar profiles of adverse effects in several organs and systems (ATSDR, 2021; EFSA, 2018, 2020; USEPA, 2021a, 2021b). Studies with PFAS and other classes of chemicals (e.g., phthalates, polycyclic aromatic hydrocarbons, etc.) support the EPA's health-protective conclusion that chemicals that elicit similar adverse health effects following individual exposure will act in a dose-additive manner when present in a mixture (unless data demonstrate otherwise). Although similarities among some PFAS have been shown at the level of molecular and cellular perturbations, no conserved modes of action (MOAs) have been identified across PFAS for noncancer health effects assessed thus far. As such, in this framework, the evaluation of toxicological similarity among component PFAS in a mixture is proposed at the level of adverse health outcome. This concept and proposed application of dose additivity for PFAS mixtures assessment are consistent with the EPA's mixtures guidelines (USEPA, 1986, 2000b) and the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023h).

Descriptions of dose additivity-based approaches such as the hazard index (HI), relative potency factor (RPF), and mixture benchmark dose (M-BMD) are presented here to demonstrate potential application to PFAS mixtures, but they are not intended to provide a comprehensive treatise on the methods themselves; EPA chemical mixtures guidelines (USEPA, 1986, 2000b) and the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023h) exist for such a purpose. The EPA's mixture assessment concepts and associated illustrative examples presented in this framework may inform PFAS evaluation(s) by

federal, state, and Tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders.

PFAS are a large and structurally diverse family of compounds used in myriad commercial applications due to their unique physicochemical properties. Although PFAS have been manufactured and used broadly in commerce since the 1940s, particular concern over potential adverse effects on human health grew in the early 2000s with the discovery of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in human blood. Since then, hundreds of PFAS have been identified in environmental media, including water, soil, and air.

Many PFAS and/or their precursors or degradants are environmentally persistent, bioaccumulative, and have long half-lives in humans, particularly the longer-chain perfluoroalkyl carboxylic acid (PFCA) and perfluoroalkane sulfonic acid (PFSA) species such as PFOA and PFOS, respectively. PFCAs/PFSAs with shorter carbon chain lengths, such as perfluorobutanesulfonic acid (PFBS) and hexafluoropropylene oxide dimer acid (HFPO-DA) (also known as GenX Chemicals[1]), were developed and integrated into various consumer products and industrial applications because they have the desired performance properties and characteristics associated with this class of compounds but are more rapidly eliminated from the human body than PFOA and PFOS. The range of PFAS encountered in environmental media is often a diverse milieu of linear, branched, cyclic, and/or aromatic parent species, metabolites, and/or abiotic degradants, leading to significant potential for PFAS mixture exposures in aquatic, terrestrial, and human populations.

As of April 2024, final EPA human health assessments are available for PFBS (USEPA, 2021a), HFPO-DA (USEPA, 2021b), perfluorobutanoic acid (PFBA; USEPA, 2022e), perfluorohexanoic acid (PFHxA; USEPA, 2023c), PFOA (USEPA, 2024a), PFOS (USEPA, 2024b), perfluoropropanoic acid (PFPrA; USEPA, 2023d), and lithium bis[(trifluoromethyl)sulfonyl]azanide (HQ-115) (USEPA, 2023e). In addition, the EPA's Integrated Risk Information System (IRIS) program is developing PFAS human health assessments for perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA), which are expected to be completed in 2024. In May 2021, the Agency for Toxic Substances and Disease Registry (ATSDR) published a *Toxicological Profile for Perfluoroalkyls* that included quantitative minimal risk levels (MRLs) for PFAS, including PFOA, PFOS, PFHxS, and PFNA (ATSDR, 2021).

A significant challenge in evaluating PFAS is the lack of hazard and dose-response data suitable for human health risk assessment for the large majority of individual PFAS. In response to the critical need, the EPA and the National Institute of Environmental Health Sciences are actively engaged in research and testing to help address data gaps for a broad landscape of PFAS (approximately 150 structures at the time of the drafting of this document). Examples of this coordination include publishing systematic evidence maps for hundreds of PFAS (e.g., Carlson et al., 2022), generating new hazard and dose-response data (e.g., new approach methodologies or NAMs), applying read-across tools, and developing the EPA Transcriptomic Assessment Product, which entails the derivation of toxicity reference values using transcriptomic pathway-

[1] The EPA notes that the chemical HFPO-DA is used in a processing aid technology developed by DuPont to make fluoropolymers without using PFOA. The chemicals associated with this process are commonly known as GenX Chemicals, and the term is often used interchangeably for HFPO-DA along with its salts.

based data from 5-day *in vivo* rat studies (see: https://www.epa.gov/chemical-research/epa-transcriptomic-assessment-product-etap-and-value-information-voi-case-study). Until results from ongoing research and testing efforts are available, the evaluation of potential toxicity/risk associated with PFAS mixtures is primarily limited to existing hazard and dose-response data under the purview of human health assessments by federal, state, and/or international entities.

This framework describes component-based mixture assessment methods that can be used to assess noncancer human health hazards and risks associated with exposure to PFAS mixtures. It is not the intent of the framework to ignore potential carcinogenic effects associated with PFAS exposure(s); however, at present, few PFAS have information available to evaluate potential carcinogenic effects via any route of exposure. Should such information become available for an increasing number of PFAS in the future, the EPA would consider approaches for addressing joint carcinogenic effects. The EPA's *National PFAS Testing Strategy: Identification of Candidate Per- and Polyfluoroalkyl Substances (PFAS) for Testing* (USEPA, 2021e) is underway to develop and issue test orders on data-poor[2] PFAS. Testing requirements encompass physicochemical properties, environmental fate and transport, and human health hazards, including mechanistic information (e.g., genotoxicity).

It is anticipated that real-world practical application of the approaches communicated and demonstrated in this framework may entail collecting, evaluating, and integrating diverse hazard and dose-response information. For example, only a small fraction of the thousands of PFAS have existent human health noncancer toxicity reference values, dozens more PFAS have gradations of traditional *in vivo* bioassay data available, and dozens more have data only from NAM assays/platforms (e.g., *in vitro* cell bioactivity). As such, to facilitate the use of potentially disparate sources of PFAS toxicity information in a mixtures assessment context, the application of the component-based methods presented in this framework is demonstrated using a hypothetical example mixture of five PFAS:

> **PFAS 1** = comprehensively studied, most potent for effect(s), and has formal noncancer human health assessment value(s) (i.e., reference dose [RfD]) and a health-based water concentration (HBWC) available;
>
> **PFAS 2** = well-studied, second-most potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;
>
> **PFAS 3** = studied, least potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;
>
> **PFAS 4** = *in vivo* animal toxicity data available but no formal human health assessment and no HBWC; and
>
> **PFAS 5** = data-poor; no *in vivo* animal toxicity data or human data available.

The hypothetical PFAS mixture is purposefully designed to demonstrate how this framework allows for flexible integration of information derived from health assessment data sources (e.g., federal, state, international), available human and/or experimental animal hazard and dose-

[2] In this framework document, "data-poor" refers to the lack or absence of hazard and dose-response data traditionally used to support noncancer and/or cancer human health assessment (e.g., chronic oral exposure studies in humans and/or animals).

response data (that have not yet been formally evaluated in an assessment product), and information from NAMs. Opportunities for integrating additional PFAS into the context of a mixture assessment are expected to evolve over time and will depend on the decision context and availability of hazard and dose-response data from traditional and/or NAM-based assays and *in silico* platforms.

1.0 Introduction and Background

1.1 Purpose

Per- and polyfluoroalkyl substances (PFAS) are an urgent public health and environmental issue facing communities across the United States. In April 2021, Administrator Michael Regan established the Environmental Protection Agency's Council on PFAS and charged the Council to develop a whole-of-EPA strategy to protect public health and the environment from the impacts of PFAS. In October 2021, the EPA released the PFAS Strategic Roadmap[3] (the Roadmap), which lays out the EPA's approach to tackling PFAS and sets timelines by which the agency plans to take concrete actions to deliver results for the American people. The Roadmap is built on a number of key principles, including considering the lifecycle of PFAS, getting upstream of the problem, holding polluters accountable, ensuring science-based decision-making, and prioritizing the protection of disadvantaged communities. In November 2022, the EPA released *EPA's PFAS Strategic Roadmap: A Year of Progress*, which underscores key actions taken by the agency during the first year of implementing the Roadmap (USEPA, 2022f).

Recognizing that PFAS tend to occur in mixtures in environmental media (see Section 1.5), the EPA has developed this data-driven framework for assessing the noncancer human health risks associated with oral exposures to mixtures of PFAS. The approaches presented in this document are based on longstanding EPA guidelines related to human health risk assessment for mixtures (USEPA, 1986, 1991, 2000b). Although the framework and illustrative hypothetical examples contained within focus on PFAS in drinking water, the framework itself is not limited to specific media and may be useful for understanding the potential noncancer health effects of PFAS mixtures under various authorities or decision contexts.

The approaches presented here are not intended to be used to assign groups or subclasses or otherwise classify PFAS (instead, see the EPA *National PFAS Testing Strategy: Identification of Candidate Per- and Polyfluoroalkyl Substances (PFAS) for Testing* for categorization efforts; USEPA, 2021e). Rather, the framework is designed for the practical application of the EPA's mixtures assessment approaches and methods to gain insight into the potential joint toxicity associated with mixtures of PFAS. The mixtures assessment concepts and associated illustrative examples presented in this framework may inform PFAS evaluation(s) by federal, state, and Tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential noncancer human health risks associated with exposure to PFAS mixtures.

The framework and hypothetical examples presented here are intended to demonstrate data-driven application of EPA component-based mixture assessment methods based on gradations of data availability and completeness anticipated to occur in real-world scenarios for PFAS. Although the examples provided are focused on drinking water, the approaches described in this framework could also be applied to other environmental media with oral[4] exposure routes (e.g., soil, fish/shellfish, food). Due to the constantly evolving science related to PFAS, the approaches

[3] https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

[4] In general, the component-based approaches presented in this document may also be applicable in assessing health risks associated with inhalation exposures to PFAS mixtures. However, the dosimetry differences across categories of (volatile/semi-volatile) PFAS gases/vapors would need to be considered in such an assessment. Data regarding the volatilization and toxicity of inhaled PFAS are generally limited.

presented herein have the flexibility to consider information as it becomes available, including forthcoming EPA human health assessments, assessments from other sources (e.g., federal, state, international), available hazard and dose-response data in the public domain, and information from high(er)-throughput bioassays and other new approach methodologies (NAMs), including data submitted to the agency under the Toxic Substances Control Act (TSCA).

Experimental evidence supports dose-additive effects from combined exposure to multiple PFAS. Dose additivity, described in detail in Section 3.0, means that the combined effect of the component chemicals in the mixture is equal to the sum of the individual doses or concentrations scaled for potency. Several perfluoroalkyl acid species (PFAAs) of PFAS tested to date, including perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), hexafluoropropylene oxide dimer acid (HFPO-DA), and perfluorobutanesulfonic acid (PFBS), have been shown to elicit the same or similar profiles of adverse effects in mammalian biological systems including effects on thyroid hormone levels, lipid synthesis and metabolism, development, immune system function, and liver function (ATSDR, 2021; EFSA, 2018, 2020; USEPA, 2021a, 2021b). An increasing body of evidence also shows similarities in molecular and cellular perturbations (e.g., common receptor binding/activation) across some PFAS; however, no conserved noncancer or cancer mode(s) of action (MOA(s)) have been identified to date.

The framework is not a regulation and does not impose legally binding requirements on the EPA, states, Tribes, or the regulated community, and might not apply to a particular situation based on the circumstances.

1.2 The EPA Science Advisory Board Review

In November 2021, the EPA released the *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS* ("draft framework;" USEPA, 2021d) for the Science Advisory Board (SAB) review. The SAB held public meetings on December 16, 2021; January 4, 6, and 7, 2022; and July 20, 2022, to discuss the draft framework and three other technical documents supporting the EPA's development of a National Primary Drinking Water Regulation for PFAS under the Safe Drinking Water Act. The EPA sought SAB comment on whether the draft framework and illustrative examples provided within were scientifically supported, clearly described, and informative for assessing potential health risk(s) associated with exposure to mixtures of PFAS. The EPA asked specific charge questions on PFAS dose additivity and three component-based approaches: hazard index (HI), relative potency factor (RPF), and mixture benchmark dose (M-BMD). A draft of the written SAB recommendations was published on April 1, 2022, and the EPA received the final report from the SAB on August 22, 2022 (SAB, 2022).

The EPA received a generally favorable review from SAB (SAB, 2022) for its development of component-based mixture assessment approaches that rely on a health-protective conclusion of dose additivity based on the same or similar adverse health outcome(s) instead of a shared MOA to evaluate risks from exposure to PFAS mixtures in drinking water and other environmental media. The EPA responded to the SAB's consensus advice in the development of this final *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)*. The SAB's overarching consensus recommendations and the

EPA's responses are summarized below. To view the EPA's complete responses to SAB comments on the draft framework, please see USEPA (2023a).

- "The SAB supports dose additivity based on a common outcome, instead of a common mode of action as a health protective default assumption and does not propose another default approach. However, EPA should more thoroughly and clearly present the uncertainties associated with this approach along with information supporting this approach" (SAB, 2022).
    - The EPA has added text in Section 3.0 (Dose Additivity for PFAS) to address the SAB's comments related to uncertainties associated with dose additivity as the default assumption for assessment of PFAS mixtures. The EPA has added further discussion on deviations from dose additivity, such as synergy or antagonism, but available evidence suggests that dose additivity should be considered the default model.
- "The SAB expressed concern regarding the requirement for "external peer review" of toxicity values developed by states and recommends that this phrase in the draft framework be broadened to recommend the need for scientific input and review in general" (SAB, 2022).
    - In response to this point of clarification, the EPA has removed the text related to external peer review. The text now reads, "If de novo derivation of toxicity values is necessary, it is recommended that experts in hazard identification and dose-response assessment be consulted for scientific input and review, and the associated uncertainties (e.g., data gaps) be transparently characterized."
- "EPA should consider using a menu-based framework to support selection of fit-for-purpose approaches, rather than a tiered approach as described in the draft Mixtures document. Tiered approaches that require increasingly complex information before reaching a final decision point can be extremely challenging for data-poor chemicals such as PFAS" (SAB, 2022).
    - In response to this and other SAB comments, the EPA has eliminated the tiered approach and restructured the framework as a data-driven, flexible approach to facilitate PFAS mixtures assessment in various decision contexts (e.g., at a contaminated site, water system, etc.) (see Section 4.2 and Figure 4-1). With "fit-for-purpose assessment" in mind, the EPA has included a discussion of key steps in the framework, including problem formulation and scoping, assembling information, evaluating data objectives, considering the data landscape to select component-based mixture assessment approach(es), and implementing component-based mixture assessment approach(es) (see Section 4.2.1).
- "EPA should provide clarification regarding the conceptual similarities and differences between the target-organ-specific hazard index (TOSHI) approach, the relative potency factor (RPF) approach, and the mixture benchmark dose (BMD) approach, since all are based on health effect-specific values (i.e., Reference Values [RfVs] or RPFs) for the individual PFAS in the PFAS mixture. More discussion and comparison of approaches, as well as when they converge, is needed. For instance, given the mathematical correspondence between the RPF and mixture BMD approaches, EPA should consider revising the discussion of these two approaches to present them as essentially the same (or highlighting any essential differences), and perhaps also merging them into a single section" (SAB, 2022).

- The EPA has added a section (Section 8.0) that describes similarities and differences among the different component-based mixtures assessment approaches. In addition, the EPA has revised the framework to use the same hypothetical example mixture of five PFAS (ranging from data-poor to well-studied) for all the illustrative examples so that the user can better understand similarities/differences among the approaches.

- "For both the RPF and mixture BMD approach, EPA's approach would be strengthened by using PODs from animal studies that are based on human equivalent doses (HEDs) rather than administered doses. The SAB found it difficult to envision situations in which the mixture BMD was advantageous; therefore, EPA should provide additional information on how the proposed Mixtures BMD approach will be applied in practice" (SAB, 2022).
  - Text has been added in several places to indicate that it is optimal to calculate and use HEDs rather than oral-administered doses in test animals when possible. This includes additional text that walks the reader through the EPA's logic flow for cross-species scaling (see new Subsection 5.2.1). Regarding the M-BMD approach, text has been added to better articulate when this specific approach is more appropriate (e.g., component chemical data that indicate common health outcome but with non-similarly shaped dose-response functions). Further, Subsection 7.3 has been revised to reiterate the conditions that warrant consideration of this specific component-based mixtures approach (as opposed to the RPF approach).

1.3. Public Review

On March 14, 2023, the EPA released the draft framework for public comment (revised in response to the SAB review, as summarized in Section 1.2) as part of the proposed National Primary Drinking Water Regulation for six PFAS (USEPA, 2023b). The public comment period ended on May 30, 2023. The public docket can be accessed at www.regulations.gov under Docket ID: EPA-HQ-OW-2022-0114. The EPA has developed responses to public comments to support the final National Primary Drinking Water Regulation, including responses to comments on PFAS dose additivity and regulation of PFAS mixtures in drinking water using an HI approach (USEPA, 2024d).

1.4 Background on PFAS

PFAS are a large group of structurally diverse anthropogenic chemicals that include perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and thousands of other fully or partially fluorinated chemicals. There is no consensus definition of PFAS as a class of chemicals (OSTP, 2023). Based on three related structural definitions associated with the EPA's identification of PFAS to be included in the fifth Contaminant Candidate List (CCL 5; see below), the universe of environmentally relevant PFAS, including parent chemicals, metabolites, and degradants, is approximately 15,000 compounds.[5] The Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)* includes over 4,700 PFAS (OECD, 2018). Comparatively,

[5] See the EPA List of PFAS Structures: https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCT

the EPA has identified more than 1,300 PFAS on the TSCA Inventory, of which more than 600 are considered "active" in U.S. commerce.

PFAS have been manufactured and used in a wide variety of industries worldwide, including in the United States, since the 1940s. The chemical structures and physicochemical properties of some PFAS enable them to repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties; these properties confer utility in commercial and industrial applications but are also, in part, what make some PFAS persistent in the human body and the environment (Calafat et al., 2007, 2019). In general, PFAAs studied to date have strong, stable carbon-fluorine (CF) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Beach et al., 2006; Buck et al., 2011; Evich et al., 2022). Conversely, the larger PFAS universe is more structurally and physicochemically diverse and includes categories of substances that may be more or less stable, persistent, and/or bioaccumulative compared to PFAAs studied thus far (see the EPA's *National PFAS Testing Strategy: Identification of Candidate Per- and Polyfluoroalkyl Substances (PFAS) for Testing*; USEPA, 2021e). Due to their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many PFAS co-occur in exposure media (e.g., indoor air/house dust, water, ice, sediment) and in tissues and blood of aquatic and terrestrial organisms and humans.

There are many families or subclasses of PFAS, and each contains many individual structural homologues and can exist as either branched-chain or straight-chain isomers (Buck et al., 2011; USEPA, 2021c). These PFAS families can be divided into two primary categories: nonpolymers and polymers. Nonpolymer PFAS include perfluoroalkyl and polyfluoroalkyl substances and encompasses parent structures, precursors, and some environmental degradation and transformation products. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-1). Several U.S. federal, state, and industry stakeholders and European entities have posited various definitions of what constitutes a PFAS. The OECD-led "Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance" workgroup provided an updated definition of PFAS (OECD, 2021), originally posited in part by Buck et al. (2011), as follows: "PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–CF3) or a perfluorinated methylene group (–CF2–) is a PFAS." It is not within the scope of this framework to compare and contrast the various definitions or the nuances associated with defining or scoping PFAS; rather, the reader is referred to OECD (2021) for review. However, for the purposes of development of the EPA's CCL 5, the structural definition of PFAS includes chemicals that have <u>at least one of the following three structures</u>:

1. R-(CF2)-CF(R′)R″, where both the CF2 and CF moieties are saturated carbons, and none of the R groups can be hydrogen.
2. R-CF2OCF2-R′, where both the CF2 moieties are saturated carbons, and none of the R groups can be hydrogen.
3. CF3C(CF3)RR′, where all the carbons are saturated, and none of the R groups can be hydrogen.

It should also be noted that what defines or constitutes a PFAS may change or evolve over time and under different purviews (e.g., federal, state, international).

Table 1-1. Two primary categories of PFAS.[a]

PFAS nonpolymers	Structural elements	Example PFAS families
Perfluoroalkyl acids	Compounds in which all carbon-hydrogen bonds, except those on the functional group, are replaced with carbon-fluorine bonds	Perfluoroalkyl carboxylic and sulfonic acids (e.g., PFOA, PFOS), perfluoroalkyl phosphonic and phosphinic acids, perfluoroalkylether carboxylic and sulfonic acids
Polyfluoroalkyl acids	Compounds in which carbon-hydrogen bonds on at least one carbon (but not all) are replaced with carbon-fluorine bonds	polyfluoroalkyl carboxylic acids, polyfluoroalkylether carboxylic and sulfonic acids
PFAS polymers	**Structural elements**	**Example PFAS families**
Fluoropolymers	Carbon-only polymer backbone with fluorines directly attached	polytetrafluoroethylene, polyvinylidene fluoride, fluorinated ethylene propylene, perfluoroalkoxy polymer
Polymeric perfluoropolyethers	Carbon and oxygen polymer backbone with fluorines directly attached to carbon	F-(CmF2mO-)nCF3, where the CmF2mO represents -CF2O, -CF2CF2O, and/or -CF(CF3)CF2O distributed randomly along polymer backbone
Side-chain fluorinated polymers	Nonfluorinated polymer backbone with fluorinated side chains with variable composition	n:1 or n:2 fluorotelomer-based acrylates, urethanes, oxetanes, or silicones; perfluoroalkyl fluorides; perfluoroalkane sulfonyl fluorides

Note:
[a] Amalgamation of information from Figure 9 in OECD (2021) and Buck et al. (2011).

PFOA and PFOS are PFAAs in the nonpolymer PFAS category and are among the most studied PFAS in terms of human health toxicity and biomonitoring (see USEPA, 2024a, 2024b; Podder et al., 2021). The PFAA family includes perfluoroalkyl carboxylic, phosphonic, and phosphinic acids and perfluoroalkane sulfonic and sulfinic acids (Table 1-2). Many PFAA are highly persistent and are frequently found in the environment (Ahrens, 2011; Brendel et al., 2018; Wang et al., 2017). Although the EPA defines, specifically for purposes under the purview of TSCA, long-chain perfluoroalkyl carboxylate substances as having perfluorinated carbon chain lengths equal to or greater than seven carbons and less than or equal to 20 carbons (85 Federal Register [FR] 45109, July 27, 2020), a more comprehensive delineation of what constitutes short-chain vs. long-chain PFAAs is provided by the OECD (OECD, 2021). Specifically, the OECD established long-chain perfluoroalkyl carboxylic acids (PFCAs) as those species with

eight or more carbons (seven or more carbons are perfluorinated) and short-chain PFCAs as those with seven or fewer carbons (six or fewer carbons are perfluorinated). Conversely, long-chain perfluoroalkane sulfonic acids (PFSAs) are identified as those species with six or more carbons (six or more carbons are perfluorinated), and short-chain PFSAs are identified as those with five or fewer carbons (five or fewer carbons are perfluorinated) (see Table 1-3).

Table 1-2. Groups, structural traits, and examples of perfluoroalkyl acids (PFAAs), including perfluoroalkylether acids.[a]

Group	Functional group	Examples
Perfluoroalkyl carboxylic acids (PFCAs)	-COOH	Perfluorooctanoic acid (PFOA), C7F15COOH
Perfluoroalkane sulfonic acids (PFSAs)	-SO3H	Perfluorooctane sulfonic acid (PFOS), C8F17SO3H
Perfluoroalkyl phosphonic acids (PFPAs)	-PO3H2	Perfluorooctyl phosphonic acid (C8-PFPA)
Perfluoroalkyl phosphinic acids (PFPIAs)	-PO2H	Bis(perfluorooctyl) phosphinic acid (C8/C8-PFPIA)
Perfluoroalkylether carboxylates (PFECAs)	-OC2F4OCF2COOH	Perfluoro-2-methyl-3-oxahexanoic acid (GenX chemicals), 4,8-Dioxa-3H-perfluorononanoic acid (ADONA)
Perfluoroalkylether sulfonic acids (PFESAs)	-OCF2CF2SO3H	Nafion byproduct 2 (NBP2)
Perfluoroalkyl dicarboxylic acids (PFdiCAs)	HOOC-CnF2n-COOH	Perfluoro-1,10-decanedicarboxylic acid, Perfluorosebacic acid
Perfluoroalkane disulfonic acids (PfdiSAs)	HO3S-CnF2n-SO3H	
Perfluoroalkane sulfinic acids (PFSIAs)	-SO2H	Perfluorooctane sulfinic acid

Note:
[a] Modified from Figure 9 in OECD (2021).

Table 1-3. Characterization system of short-chain and long-chain PFAAs.[a]

Total # of carbons	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
# of fluorinated carbons	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
PFCAs	**Short-chain PFCAs**					**Long-chain PFCAs**		
	PFPrA	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA
# of fluorinated carbons	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
PFSAs	PFPS	PFBS	PFPeS	PFHxS	PFHpS	PFOS	PFNS	PFDS
	Short-chain PFSAs			**Long-chain PFSAs**				

Notes:
PFPrA = perfluoropropanoic acid; PFBA = perfluorobutanoic acid; PFPeA = perfluoropentanoic acid;
PFHxA = perfluorohexanoic acid; PFHpA = perfluoroheptanoic acid; PFOA = perfluorooctanoic acid;
PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFPS = perfluoropropane sulfonic acid;
PFBS = perfluorobutanesulfonic acid; PFPeS = perfluoropentanesulfonic acid; PFHxS = perfluorohexanesulfonic acid;
PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid; PFNS = perfluorononanesulfonic acid;
PFDS = perfluorodecanesulfonate.
For brevity, Table 1-3 only includes PFAAs of 3–10 carbons; the long-chain class of PFCAs and PFSAs can be expanded considerably.
[a] Modification of Table 2-2 in ITRC (2022).

Although many PFAS are manufactured in various salt forms (e.g., potassium [K+] PFBS), they typically fully dissociate to their protonated acid and/or anionic forms depending on their acid strength (pK_a value) in aqueous environmental media, soils or sediments, and the human body. Importantly, the protonated and anionic forms may have different physicochemical and environmental fate and transport properties. It should also be noted that the structural diversity of PFAS goes beyond the PFCAs and PFSAs indicated in Table 1-3. There are branched, cyclic, aromatic, and multi-component (e.g., polymers) structures that have been or are currently classified as PFAS. However, in general, the linear PFCAs and PFSAs have been the most studied PFAS to date and have been the primary focus of formal human health risk assessment activities in the federal and state sectors.

1.5 Occurrence of PFAS Mixtures

Improved analytical monitoring and detection methods have enabled detection of the co-occurrence of multiple PFAS in drinking water, ambient surface waters, aquatic organisms, biosolids (sewage sludge), and other environmental media.[6] PFOA and PFOS have historically been target analytes, but recent water monitoring studies have begun to focus on additional PFAS via advanced analytical instrumentation/methods and nontargeted analysis (De Silva et al., 2020; McCord and Strynar, 2019; McCord et al., 2020). The proposed framework for estimating the likelihood of noncancer human health risks associated with oral exposure to mixtures of

[6] For a more detailed discussion of the occurrence of PFOA, PFOS, and other PFAS in potential human exposure sources, see the relative source contribution (RSC) sections in USEPA (2024f, 2024g).

PFAS (described in Section 4) is flexible to accommodate information for any PFAS mixture of interest, provided sufficient hazard and dose-response information is available.

The EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect occurrence data nationwide for contaminants suspected to be present in drinking water. Between 2013 and 2015, the EPA's third UCMR (UCMR 3) required all large public water systems (PWSs) (each serving more than 10,000 people) and a statistically selected, representative national sample of 800 small PWSs (each serving 10,000 people or fewer) to monitor for 30 unregulated contaminants in drinking water, including six PFAS: PFOS, PFOA, PFNA, PFHxS, PFBS, and perfluoroheptanoic acid (PFHpA). UCMR 3 data demonstrated that two or more of those six PFAS co-occurred in 48% (285 / 598) of sampling events with PFAS detected, and PFOA and PFOS co-occurred in 27% (164 / 598) of sampling events with two or more PFAS detected (Guelfo and Adamson, 2018; USEPA, 2019b). The EPA found that 4% of PWSs reported results for which one or more of these six PFAS were measured at or above their respective minimum reporting levels (USEPA, 2019b).[7] Under UCMR 5 (2023 to 2025), PWSs will monitor for 29 PFAS. A small subset of UCMR 5 data (24% of the total results that the EPA expects to receive) was released to the public in early 2024. Preliminary sampling results from UCMR 5 are available in the *PFAS Occurrence and Contaminant Background Support Document for the Final PFAS NPDWR* (USEPA, 2024c).

Outside of the UCMR data collection, many states have undertaken individual efforts to monitor for PFAS in both source and finished drinking water. These results show that occurrence in multiple geographic locations is consistent with what was observed during UCMR 3 monitoring, as well as the occurrence and co-occurrence of other PFAS not included in the UCMR 3. Additionally, these results show that PFAS are very likely to co-occur as mixtures in the environment. These data suggest that PWSs with high concentrations of one PFAS are likely to have high concentrations of other PFAS and that there is notable co-occurrence at elevated concentrations (Cadwallader et al., 2022; USEPA, 2024c).

PFAS mixtures have also been reported in U.S. ambient surface waters and aquatic biota (Ahrens, 2011; Benskin et al., 2012; Burkhard, 2021; McCord and Strynar, 2019; Nakayama et al., 2007; Remucal, 2019; Zareitalabad et al., 2013). Most environmental monitoring of PFAS in surface waters has focused on sites of historical manufacturing and known contamination (3M Company, 2000; Boulanger et al., 2004; Cochran, 2015; Hansen et al., 2002; Jarvis et al., 2021; Konwick et al., 2008; Nakayama et al., 2007). Simcik and Dorweiler (2005) consistently detected both PFOA and PFHpA in all 12 surface waters sampled across the U.S. Midwest and PFOS in all but two locations. Sinclair and Kannan (2006) detected PFOA and PFOS in all effluent-dominated samples collected across New York State; Sinclair and Kannan (2006) also detected PFHxS, but PFBS and perfluorooctane sulfonamide (PFOSA) were below detection limits in all samples. De Silva et al. (2011) detected PFOS and additional PFAS (i.e., perfluoropentanoic acid [PFPeA] [C5], perfluorohexanoic acid [PFHxA] [C6], PFHpA [C7], and PFOA [C8]) co-occurring as mixtures in all surface water samples (n = 32) collected across the five Laurentian Great Lakes. Other PFAS, including PFNA (C9), perfluorodecanoic acid (PFDA) (C10), perfluoroundecanoic acid (PFUnA) (C11), PFBS (C4), PFHxS (C6),

[7] The 4% figure is based on 198 PWSs reporting measurable PFAS results for one or more sampling events from one or more of their sampling locations. Those 198 PWSs serve an estimated total population of approximately 16 million (USEPA, 2019b).

perfluoroethylcyclohexane sulfonate (PFECHS) (C8), and perfluoromethylcyclohexane sulfonate (C7), were also quantified in at least 20 of the 32 samples collected from the Great Lakes.

PFAS mixtures in the environment can be linked to the direct application of manufactured products that contain a specific mixture of PFAS. For example, Anderson et al. (2016) quantified PFAS in ambient surface waters across 10 U.S. Air Force bases where there were known historical uses of aqueous film-forming foam, which is used in firefighting and training activities and can contain hundreds of polyfluoroalkyl precursors (Ruyle et al., 2021). PFOA and PFOS largely co-occurred and were detected in 88% and 96% of samples, respectively. Anderson et al. (2016) also detected PFBA, PFBS, PFPeA, PFHxA, PFHxS, and PFHpA in ≥ 80% of samples.

Environmental monitoring of PFAS in aquatic biota has primarily focused on fish. Generally, PFCAs are less bioaccumulative than PFSAs in aquatic systems, with longer-chain PFAS being more bioaccumulative than short-chain PFAS (Burkhard, 2021; Conder et al., 2008; Kannan et al., 2005). Within the United States, PFAS in aquatic biota have been measured in major rivers, in the Laurentian Great Lakes region, in several estuaries, and in targeted studies of sites of known contamination (e.g., industrial). A recent national probabilistic survey by the EPA measured up to 33 PFAS in fish samples collected in 2013–2014 and 2018–2019 from hundreds of river sites across the U.S. One or more PFAS were detected in 99.7% of fish fillet samples collected in 2013–2014 and in 95.2% of samples collected in 2018–2019. For both sampling periods, detection frequency was dominated by PFOS (91%–99%), PFUnA (85%–88%), PFDA (84%–88%), and PFDoA (69%–70%) (Stahl et al., 2023). De Silva et al. (2011) measured PFAS from lake trout (*Salvelinus namaycush*) samples collected in 2001 from each of the Great Lakes. Eight different PFAS (i.e., PFNA, PFDA, PFUnA, PFDoDA, perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTA), PFHxS, and PFOS) were detected in tissues of lake trout from across all the Great Lakes, with PFOA, PFECHS, and perfluorodecanesulfonic acid (PFDS) also being detected in lake trout from Lake Ontario (De Silva et al., 2011). Sedlak et al. (2017) measured PFAS in composite samples containing yellowfin gobies (*Acanthogobius flavimanus*), chameleon/cheekspot gobies (*Tridentiger trigonocephalus/Ilypnus gilberti*), northern anchovy (*Engraulis mordax*), shiner surfperch (*Cymatogaster aggregata*), and staghorn sculpin (*Leptocottus armatus*) that were collected from the San Francisco Bay estuary. PFOS and PFOSA were detected in nearly all composite samples and at relatively high concentrations (geometric mean PFOS concentration of 3.9 nanograms (ng) per gram (g); geometric mean PFOSA concentration of 3.2 nanograms per gram [ng/g]). Other longer-chain PFAS, including PFNA, PFDA, PFUnA, and perfluorododecanoic acid (PFDoDA), were also frequently detected in the fish composite samples but at relatively low concentrations (geometric mean concentrations < 2.4 ng/g). Shorter-chain PFAS, including PFBS, PFBA, PFHxA, and PFHpA, were not detected in any of the fish composite samples. Houde et al. (2006) measured whole-body PFAS in six fish species in Charleston Harbor, South Carolina, and in five fish species in Sarasota Bay, Florida. Charleston Harbor (the more developed of the two sites) had higher overall PFAS concentrations. PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoDA, PFHxS, and PFOSA were all commonly detected in tissues of the six fish species from Charleston Harbor. PFOS and PFDoDA were the only two PFAS detected at elevated concentrations in the fish from Sarasota Bay (Houde et al., 2006). A study in New Jersey found co-occurrence of PFAS in ambient water, sediment, and fish at sites with historical and current industrial activities (Goodrow et al., 2020). Fish tissue concentrations of PFOS were generally higher than other PFAS and high enough in nearly all fish species to trigger fish consumption advisories.

Within the United States, PFAS occurrence in invertebrate tissues, such as shellfish, has not been as extensively monitored as PFAS occurrence in fish. Kannan et al. (2005) measured PFAS in several species, including zebra mussels, from two rivers in southern Michigan (Raisin River, St. Claire River) and one in northern Indiana (Calumet River). Overall, PFAS concentrations in zebra mussels were lower than in fish. Nevertheless, PFOS and PFOSA were both detected in zebra mussels in the Raisin River (PFOS concentration = 3.1 ng/g wet weight; PFOSA concentration = 2.7 ng/g wet weight). Interestingly, PFOA was not detected in zebra mussel tissues even though it was detected in elevated concentrations in the Raisin River water column (PFOA water concentration = 14.7 nanograms/liter [ng/L]), suggesting that chemical-specific considerations (e.g., carbon chain length, functional group differences) affect bioaccumulation dynamics in aquatic organisms and resultant human exposures to PFAS mixtures via ingestion of fish and shellfish (Kannan et al., 2005).

1.6 Evidence of PFAS Exposure in Humans

Humans can be exposed to PFAS through a variety of sources, including food packaged in PFAS-containing materials, processed with equipment that uses PFAS, or grown or raised in PFAS-contaminated soil or water (including livestock and seafood); commercial household products, including stain- and water-repellent fabrics, nonstick products, polishes, waxes, paints, and cleaning products; the fire suppressant, aqueous film-forming foam; production facilities or industries that use PFAS; and drinking water, where these chemicals have contaminated water supplies. Although humans may be exposed to PFAS via dermal and inhalation routes, the primary focus of this document is the oral route of exposure, including via drinking water, food, fish/shellfish, and incidental soil/dust ingestion (Egeghy and Lorber, 2010; Lorber and Egeghy, 2011; Poothong et al., 2020).

The Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999 (CDC, 2023). Results from this nationally representative biomonitoring study in which data were gathered from 1999–2000 through 2017–2018 documented measurable serum levels of PFOS, PFOA, PFHxS, and PFNA in more than 95% of participants, indicating widespread exposure to these PFAS in the U.S. population. PFOA and PFOS have been detected in up to 98% of serum samples collected in biomonitoring studies that are representative of the U.S. general population; however, from 1999 to 2018, blood levels of PFOA and PFOS declined by > 70% and > 85%, respectively, presumably due to restrictions on commercial use of PFOA and PFOS in the United States. Under the EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, including PFNA and longer-chain PFCAs, by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (USEPA, 2021c). However, since the voluntary phase-out of these longer-chain PFAS in the United States, manufacturers have been shifting to shorter-chain and alternative forms of PFAS, such as HFPO-DA. The most recent available NHANES survey (2017–2018) measured ADONA, HFPO-DA, perfluoroheptanesulfonic acid (PFHpS), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate, and PFHxA in blood and found that PFHpS was detected in 78% of samples and 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate was detected in 12% of samples (the others were not detected or found in less than 1% of samples). The 2015–

2016 NHANES survey detected some other PFAS in more than 30% of samples, including PFDA, PFUA, and 2-(N-methylperfluoroctanesulfonamido)acetic acid (Me-PFOSA-AcOH). Studies of residents in locations of suspected PFAS contamination show higher serum levels of PFAS compared to the general U.S. population reported by NHANES (ATSDR, 2022; Table 17-6 in ITRC, 2022; Kotlarz et al., 2020; Yu et al., 2020). Compared to PFOA and PFOS, there is less publicly available information on the occurrence and health effects of replacements for PFOA and PFOS and other members of the carboxylic acid and sulfonate PFAS families.

1.7 Brief Summary of State, National, and International Approaches to Address PFAS Mixtures in Water

In 2016, the EPA finalized drinking water Health Advisories of 70 parts per trillion (ppt or ng/L) for PFOA and PFOS, both individually and when present as a mixture (USEPA, 2016a, 2016b), because the reference doses (RfDs) were based on developmental effects and numerically identical. Subsequently, some states developed state-specific cleanup levels or drinking water or groundwater guidelines, advisories, or standards for PFOA, PFOS, and other PFAS. In some cases, the state values are the same as the EPA's 2016 drinking water Health Advisory; in other cases, states developed different values (see examples in Table 1-4).

In June 2022, the EPA issued interim updated drinking water Health Advisories for PFOA and PFOS and final Health Advisories for HFPO-DA and PFBS (USEPA, 2022a, 2022b, 2022c, 2022d). The EPA's interim updated Health Advisories for PFOA and PFOS are 0.004 ng/L and 0.02 ng/L, respectively, and the final Health Advisories for HFPO-DA and PFBS are 10 ng/L and 2,000 ng/L, respectively. Each of the health advisory documents provides an example of how to use the HI approach (see Section 5.0) to assess the potential noncancer human health risks of exposure to a mixture of PFAS (USEPA, 2022a, 2022b, 2022c, 2022d) consistent with the approach presented in this framework.

In March 2023, the EPA proposed and requested comment on a National Primary Drinking Water Regulation that included an HI public health goal (i.e., Maximum Contaminant Level Goal [MCLG]) and enforceable level (i.e., Maximum Contaminant Level [MCL]) to protect public health from exposure to mixtures of any combination of two or more of PFHxS, PFNA, HFPO-DA, and/or PFBS, four PFAS that individually can affect similar health endpoints/outcomes and co-occur in drinking water (USEPA, 2023b). After consideration of prior peer-review advice and public comment, and consistent with the provisions set forth under the Safe Drinking Water Act, the EPA finalized an HI MCLG and MCL for mixtures of these four PFAS. In consideration of their known toxic effects, dose additivity health concerns, and occurrence and likely co-occurrence in drinking water, the EPA finalized an HI of 1 (unitless) as the MCLG and MCL for any mixture containing two or more of PFHxS, PFNA, HFPO-DA, and PFBS (USEPA, 2024e).

International approaches to addressing multiple PFAS in drinking water have resulted in a range of proposed and promulgated standards and guideline values, as well as a variety of grouping methods (Table 1-4). Canada proposed a drinking water objective of 30 ng/L as a summed total of all PFAS measured in drinking water (using EPA Method 533 or EPA Method 537.1 or both). Australia has established a combined level of 70 ppt for PFOS and PFHxS, based on the assumption that PFHxS is similar in toxicity to PFOS (i.e., PFOS tolerable daily intake also applies to PFHxS). Several countries have expanded the combined toxicity approach to include a

variety of other PFAS. For instance, Denmark has set a limit of 100 ppt to account for any combination of the following: C4–C10 PFCAs, PFBS, PFHxS, PFOS, PFOSA, and 6:2 fluorotelomer sulfonic acid (6:2 FTS). Sweden has adopted the same approach, not including PFOSA, and set a maximum limit of 90 ppt. In both Denmark and Sweden, it is assumed that these PFAS are similar in toxicity to PFOS. Most recently, the European Union (EU) adopted a level of 100 ppt for the sum of 20 PFAS, including C4–C13 PFSAs and C4–C13 PFCAs and a level of 500 ppt for all PFAS, as measured by extractable organofluorine (EOF) or adsorbable organofluorine (AOF) (Cousins et al., 2020; EU, 2020). Further, Sweden and the Netherlands have evaluated the potential human health risk(s) associated with mixtures of PFAS using component-based methods consistent with the HI or RPF approaches presented in this framework (Borg et al., 2013; RIVM, 2018). Although not specifically related to drinking water, the European Food Safety Authority has also taken PFAS mixture toxicity into consideration in its development of a Tolerable Weekly Intake for the sum of PFOA, PFNA, PFHxS, and PFOS (4.4 nanograms per kilogram per week [ng/kg/week]) (EFSA, 2020).

Table 1-4. U.S. and international approaches to addressing the combined toxicity of multiple PFAS in drinking water or groundwater[a,b] (only combined PFAS approaches are presented).

Entity	Date	Conc. (ng/L)	Sum of PFAS	Background
EPA (USEPA, 2024e, 2022a, 2022b, 2022c, 2022, 2016a, 2016b)	2024	10 PFHxS 10 PFNA 10 HFPO-DA 2000 PFBS	HI MCLG and MCL = 1 for any combination of two or more of four PFAS	Final PFAS National Primary Drinking Water Regulation Rulemaking.
	2022	0.004 PFOA 0.02 PFOS 10 HFPO-DA 2000 PFBS	Example HI for four PFAS	Interim Updated Drinking Water Health Advisories (HAs) for PFOA and PFOS; Final HAs for PFBS and HFPO-DA.
	2016	70	PFOA and PFOS	Drinking Water Health Advisory.
Alaska (USA) (Alaska DEC, 2019)	2019	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
Colorado (USA) (CDPHE, 2020)	2020	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
Delaware (USA) (DE DHHS, 2021)	2018	17[c]	PFOA and PFOS	Based on the sum of approximately 50% of each individual MCL[d]
Florida (USA) (Florida Health, 2020)	2019	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.

Entity	Date	Conc. (ng/L)	Sum of PFAS	Background
Maine (USA) (Maine DEP, 2021)	2021	20	PFOA, PFOS, PFNA, PFHxS, PFHpA, and PFDA	Based on similarities in chemical structure and toxicities of four PFAS to PFOS and PFOA. Same approach as the EPA 2016 Health Advisory but includes an additional uncertainty factor.
Massachusetts (USA) (Mass DEP, 2019)	2019	20	PFOA, PFOS, PFNA, PFHxS, PFHpA, and PFDA	Based on similarities in chemical structure and toxicities of four PFAS to PFOS and PFOA. Same approach as the EPA 2016 Health Advisory but includes an additional uncertainty factor.
Montana (USA) (MT DEQ, 2021)	2019	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
Ohio (USA) (Ohio EPA, 2019)	2019	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
Rhode Island (USA) (RIDEM, 2017)	2019	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
Vermont (USA) (Levine, 2018; VT DEC, 2021)	2019	20	PFOA, PFOS, PFNA, PFHxS, and PFHpA	PFHxS, PFHpA, and PFNA are considered sufficiently similar to PFOA and PFOS. Difference from the EPA 2016 Health Advisory is due to Vermont's calculation being based on infant consumption rates.
Wisconsin (USA) (WI DHS, 2022)	2022	70	PFOA and PFOS	Application of the EPA 2016 Health Advisory.
European Union (EU, 2020)	2020	100 500	100 ng/L for sum of 20 PFAS (C4–C13 PFSAs and C4–C13 PFCAs) 500 ng/L for "PFAS Total" – the total of all PFAS	"PFAS Total" proposed to be enforced through measurement of EOF/AOF once validated or 100 ppt for the sum of 20 PFAS considered to be a concern for drinking water (implementation January 12, 2023).
Denmark (Danish Environmental Protection Agency, 2015)	2015	100	C4–C10 PFCAs, PFBS, PFHxS, PFOS, PFOSA, and 6:2 FTS	Assumes all 12 PFAS are similarly toxic as PFOS. Rationale: PFOS is the most toxic and toxicity data on PFAS other than PFOS and PFOA are limited.

Entity	Date	Conc. (ng/L)	Sum of PFAS	Background
Sweden (Swedish Food Agency, 2021)	2014	90	C4–C10 PFCAs, PFBS, PFHxS, PFOS, and 6:2 FTS	Assumes all 11 PFAS are similarly toxic as PFOS. Rationale: PFOS is the most toxic and toxicity data on PFAS other than PFOS and PFOA are limited.
Australia (Australian Government Department of Health, 2019)	2017	70	PFOS and PFHxS combined, if both present	Assumes PFHxS is similarly toxic as PFOS. Rationale: PFOS is the most toxic and toxicity data on PFAS other than PFOS and PFOA are limited.
Canada (Health Canada, 2023)	2023	30	Total all PFAS measured using EPA Method 533, 537.1 or both	Technology-based.

Notes:
[a] Modified from Cousins et al. (2020).
[b] As of July 2021, several states have passed or proposed compound-specific MCLs or Health Advisories (e.g., California, Illinois, Michigan, Minnesota, New Jersey, New York, Pennsylvania, Texas, and Washington). Some states have applied the EPA's Health Advisory to interpret narrative water quality standards under the Clean Water Act (e.g., Colorado, Montana). Only approaches using the sum of PFAS parameters are presented in this table.
[c] Proposed level based on the Delaware PFOA and PFOS MCL Implementation Plan.
[d] Based on a PFOA MCL of 21 ppt and PFOS MCL of 14 ppt.

1.8 Overview of Proposed Framework for Estimating Noncancer Health Risks for PFAS Mixtures

This document describes a framework of component-based options with different levels of data requirements and objectives for estimating the noncancer human health risks associated with exposure to mixtures of PFAS based on longstanding EPA chemical mixtures guidelines. To address concerns over health risks from multichemical exposures, the EPA issued the *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (USEPA, 1986). The 1986 guidelines were followed in 2000 by the *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 2000b). These documents define a chemical mixture as "any combination of two or more chemical substances, regardless of source or of spatial or temporal proximity, that can influence the risk of chemical toxicity in the target population" (USEPA, 1986, 2000b); this definition is used in this framework document.

Several laws direct the EPA to address health risks posed by exposures to chemical mixtures, including the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, the Superfund Amendments and Reauthorization Act of 1986, and amendments in 2002 (CERCLA, 2002; SARA, 2002) (commonly referred to as Superfund); the Clean Air Act Amendments of 1990 (CAA, 1990); the Safe Drinking Water Act Amendments of 1996 (SDWA, 1996); and the Food Quality Protection Act (FQPA) of 1996 (FQPA, 1996). Both the 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (USEPA, 1986) and the 2000 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 2000b) were developed, in part, to be responsive to these laws. When developing assessment information for exposures to chemical mixtures, risk assessors and risk managers in

the EPA's programs currently implement environmental laws through regulations that rely on the methods articulated in these two chemical mixtures guidelines documents. This framework does not supersede previously published EPA guidelines on mixtures or longstanding EPA approaches used to assess health risks of contaminants, including chemical mixtures under various environmental statutes (e.g., Federal Insecticide, Fungicide, and Rodenticide Act; FQPA; CERCLA).

The objective of this document is to provide a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on dose additivity. All approaches presented involve integrating dose-response metrics that have been scaled based on the potency of each PFAS in the mixture. Three approaches are presented:

1) The general HI (based on overall RfDs [or similar noncancer toxicity value such as an ATSDR minimal risk level (MRL)] irrespective of similarity in target organ or system) and TOSHI (based on RfDs in same target organ) for each component chemical provide an indication of noncancer risk associated with exposure to a PFAS mixture of concern (Section 5).

2) The RPF approach provides a mixture toxicity estimate by scaling the potency of component chemicals, for a common health effect, relative to a well-characterized member of the mixture, referred to as the index chemical (IC) (Section 6).

3) The M-BMD approach uses a dose additivity (DA) model-based equation (similar to the Berenbaum equation; Section 4.2.6 in USEPA, 2000b) to calculate a BMD (e.g., $BMD_{X\text{-}HED}$) for the mixture (Section 7).

The HI facilitates the estimation of potential combined toxicity associated with the co-occurrence of chemicals in environmental media (e.g., water, soil) (USEPA, 1991, 2000b). The RPF method is more data-intensive than the HI approach in that the mixture component chemicals typically must meet two requirements: (1) there are data to demonstrate or suggest that component chemicals share either a similar toxicological MOA[8] or have a conserved toxicological target (i.e., share a common apical endpoint/effect); and (2) the dose-response functions for the effect of concern exhibit similar shape and slope over the exposure ranges most relevant to the decision context (USEPA, 2000b). The RPF method is illustrated in Section 6 using the same target organs/systems, including liver, thyroid, and developmental. An MOA for a given toxic effect is a detailed description of the source-to-outcome pathway, including the key molecular/cellular or organellar events, leading to a defined health effect or syndrome of effects (e.g., "developmental" can be a collection of related outcomes). In general, a health effect or outcome is the terminus of one or more operant MOA(s). In addition to the HI and RPF methods, the assumption of similarity in MOA or toxicological target is also inherent when applying the M-BMD approach; however, in contrast to the RPF method there is no necessity or assumption of similar dose-response functions (i.e., same/similar shape or slope) across component chemicals. This approach provides predictions of a mixture effect even if the slopes of the dose-response curves differ among the chemicals (Section 7). Considering that PFAS are an emerging chemical class of interest for toxicological evaluations and human health risk assessment, data pertaining

[8] Mode of action is a sequence of key events and processes, starting with interaction between an agent and a cell, proceeding through operational and anatomical changes, and resulting in a noncancer effect or cancer formation (modification of footnote 2 in USEPA, 2005).

to biological pathway perturbations are limited or not available for many PFAS; so, while there is an evolving landscape of evidence demonstrating shared molecular and cellular effects by some PFAS, no conserved noncancer or cancer MOA(s) have been identified across PFAS to date. As such, this framework focuses the biological level of organization for evaluation of potential dose additivity on ***similarity of toxicological endpoint/effect/adverse outcome*** rather than similarity in MOA, which is consistent with EPA chemical mixtures guidelines (USEPA, 1986, 1991, 2000b), the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023h), and expert opinion from the National Academy of Sciences, National Research Council (NRC, 2008).

Recognizing the evolving and dynamic nature of PFAS science, the component-based mixtures assessment approaches described herein are flexible to allow for consideration of new or evolving dose-response data and toxicity assessments as they become available. Additionally, because publicly available traditional (e.g., *in vivo* mammalian) toxicity studies are limited to only a small fraction of the ~15,000 PFAS estimated at the time of this writing, this framework also provides suggestions for practical integration of validated NAMs such as toxicogenomics (e.g., *in vitro* cell bioactivity) and *in silico* platforms (e.g., structure-activity, read-across) into the HI, RPF, and M-BMD approaches. The illustrative examples in Sections 5, 6, and 7 are intended to demonstrate the application of dose-additivity-based component chemical mixture approaches using hypothetical human health-relevant toxicity and exposure information.

2.0 Background on EPA Mixtures Additivity Guidelines

Exposure to mixtures of environmental chemicals occurs in human populations through ingestion, inhalation, and/or dermal contact with contaminated media (e.g., water, air, food). It should be noted that a "mixture" of chemicals may be a function of both co-occurrence in exposure media and/or internal bioaccumulation and persistence in biological matrices. In recognition of the need for methods and approaches that inform the evaluation of potential health risks associated with chemical mixtures, the EPA developed the 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* and, subsequently, the 2000 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 1986, 2000b). In those guideline documents, the EPA proposed a hierarchy of mixtures approaches where the preferred approach is to evaluate health risk using hazard and dose-response data for a specific whole mixture of concern or, alternatively, a sufficiently similar mixture. However, whole-mixture data are rare; there are often many chemical combinations and proportions in the environment (e.g., parent chemicals, metabolites, and/or abiotic degradants), introducing a level of complexity that is difficult to evaluate and characterize.

Further, most controlled experimental toxicity data derive from single-chemical exposures or, at best, small mixtures (i.e., a limited number of component chemicals at fixed proportions/ratios). As such, the EPA also developed multiple component chemical-based mixtures assessment approaches. Component-based methods are used more frequently than whole-mixture methods. These component-based methods are based on assumptions about how the chemicals behave biologically when co-occurring. Although observed toxicity could be related to direct chemical-to-chemical interaction(s), the manner in which co-occurring chemicals induce toxicity in a coordinated or independent way is the basis for the concept of "additivity." The basic tenets of the EPA mixtures additivity theory and practice are:

- Additivity-based methods are used to estimate the probability or magnitude of a given health outcome (e.g., incidence and/or severity, or change in magnitude, of a noncancer target organ effect) associated with exposure to mixtures of two or more component chemicals. In the 1986 and 2000 EPA mixtures guidelines documents, the development of component-based mixture approaches was informed by two main concepts, simple similar action and simple independent action, as described by Bliss (1939) and Finney (1971).
- *Simple similar action* applies to mixture component chemicals that *cause a common health effect* via *toxicologically similar* pathway(s). Under simple similar action (i.e., DA), the evidence associated with toxic responses to mixture component chemicals demonstrates or suggests coordinated (i.e., same/similar) pathway events. DA is generally applied when mixture chemicals are assumed to act through simple similar action.
- *Simple independent action* applies to mixture component chemicals that cause a *common health effect* via *toxicologically independent* pathways. Under simple independent action (i.e., response addition [RA]), the evidence associated with toxic responses to different mixture component chemicals demonstrates or suggests independent pathway events. RA is generally applied when mixture chemicals are assumed to act through simple independent action.

2.1 Component-Based Mixtures Assessment Methods

Component-based methods that the EPA has developed for evaluating potential additivity of dose, response, or both are shown in Figure 2-1. Based primarily on similarity in toxicity endpoint/health effect of PFAS, this framework document focuses on the use of dose-additive, component-based methods (left side of Figure 2-1; shaded box), specifically the HI (Section 5), RPF (Section 6), and M-BMD (Section 7) approaches. The methods, although all based on dose addition, involve different assumptions, data requirements and objectives for evaluating the joint toxicity of component chemicals in a mixture. Each method is introduced and detailed in Sections 5–7 and includes a demonstration of the application using a hypothetical five-component PFAS mixture. Specifically, to facilitate the use of potentially disparate sources and types of PFAS information in a mixture context, the data-driven application of the component-based methods presented in this framework document is demonstrated using a hypothetical example mixture of five PFAS:

PFAS 1 = comprehensively studied, most potent for effect(s), and has formal noncancer human health assessment value(s) and an HBWC available;

PFAS 2 = well-studied, second-most potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;

PFAS 3 = studied, least potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;

PFAS 4 = *in vivo* animal toxicity data available but no formal human health assessment and no HBWC; and

PFAS 5 = data-poor; no *in vivo* animal toxicity data or human data available.

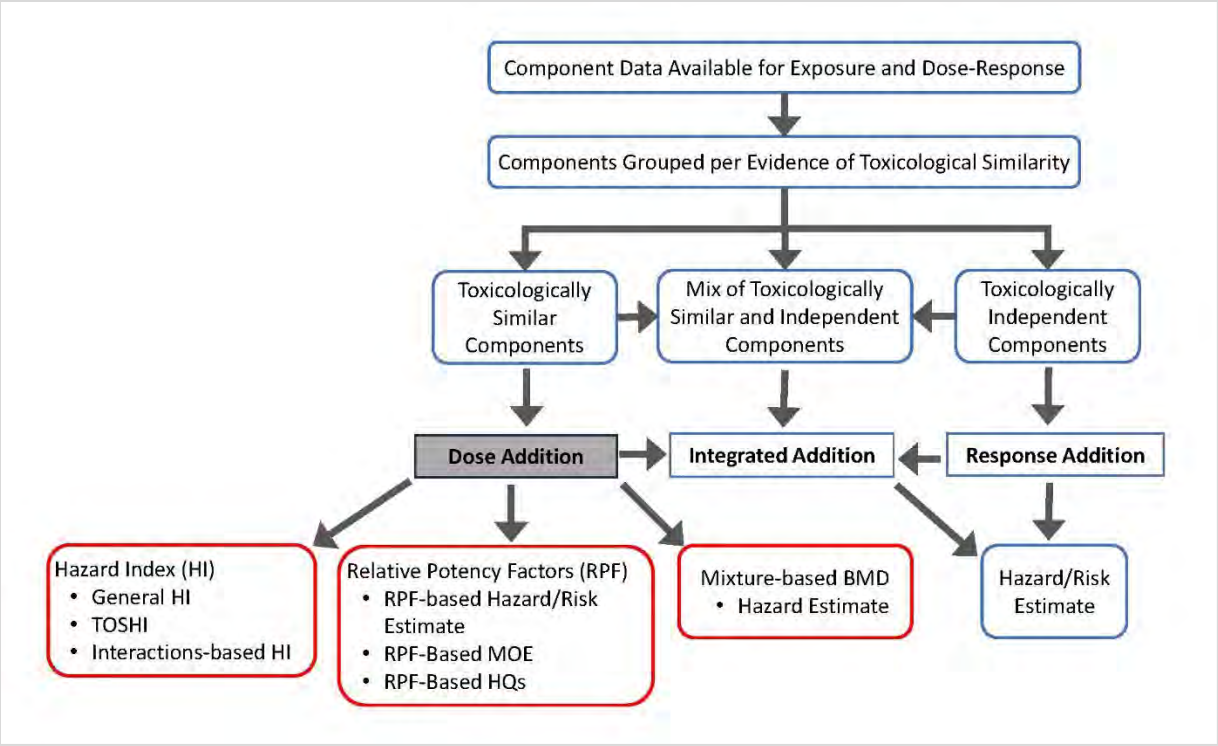
This hypothetical PFAS mixture is purposefully designed to demonstrate how the framework allows for the flexible integration of information derived from diverse data types and sources. Opportunities for integrating PFAS into a mixture assessment are expected to evolve over time and will depend on the decision context and availability of hazard and dose-response data from traditional and/or NAM-based assays and/or *in silico* platforms.

An important property of DA-based methods is that they can aid in the indication or estimation of the effects of a mixture even when all the individual component chemical exposures are at or below their individual no-observed-adverse-effect levels (NOAELs; i.e., "something from nothing"). For example, in the hypothetical PFAS mixture applications (Sections 5–7), the HI in general indicates a point (i.e., 1) above which a hazard might be anticipated for the mixture, or below which adverse effects are not expected (see Section 5). In the RPF approach, the sum of the scaled IC[9] equivalent doses/concentrations across component chemicals is compared to the equivalent threshold dose of the IC (Jonker et al., 1996; Silva et al., 2002). In the context of water-specific application, a mixture IC equivalent dose/concentration may be compared to a health-based water concentration (HBWC) for the IC to indicate if the selected health effect may or may not be expected (see Section 6). Finally, no IC is required in the M-BMD approach, and

[9] An IC is that mixture component that is typically the most toxicologically well-studied. The qualitative and quantitative hazard and dose-response data for an IC serve as an index or anchor against which all other components are compared. IC equivalent doses/concentrations represent scaled dose(s) of mixture components, based on potency for a given toxicity endpoint/health effect, in a corresponding dose of the IC.

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Notes:
Modification of Figure 4-3b (USEPA, 2007).
BMD = benchmark dose; HI = hazard index; HQ = hazard quotient; MOE = margin of exposure; RPF = relative potency factor; TOSHI = target organ-specific hazard index.
Component-based methods selection is based on the relevant evidence supporting toxicological similarity (DA) or toxicological independence (RA or effect summation). Integrated addition methods are reserved for mixtures of component chemicals that demonstrate a profile of both toxicological similarity and independence.

Figure 2-1. Flow chart for evaluating chemical mixtures using component-based additive methods.

dose-response shape(s) and slope(s) do not have to be similar among components. The aggregated M-BMD is converted to a noncancer RfV and corresponding HBWC and then compared directly to the total measured mixture PFAS concentration to indicate if the selected health effect may or may not be expected following exposure to the combinations and proportions for that specific mixture (see Section 7).

2.1.1 Application of Dose Addition as the EPA's Default Approach

Several *in vivo* studies have examined predicted mixture responses based on dose-addition models for specific groups of chemicals (e.g., Altenburger et al., 2000; Crofton et al., 2005; Gennings et al., 2004; Hass et al., 2017; Howdeshell et al., 2015; Kortenkamp and Haas, 2009; Moser et al., 2005, 2012; Mwanza et al., 2012; Rider et al., 2008, 2009, 2010; USEPA, 2007; Walker et al., 2005), focusing primarily on whether experimentally observed toxicity is consistent with modeled predictions of dose additivity. Many of these studies examined groups of chemicals that are thought to target the same biological signal transduction pathways (Moser et al., 2012; Mwanza et al., 2012; Walker et al., 2005), while others have examined chemicals thought to target disparate pathways that lead to the same health outcome (NRC, 2008; Rider et al., 2009; Van Der Ven et al., 2022). In general, the results of such studies listed here, and many

others, support the continued application of DA as the EPA's default component-based mixture assessment approach. Further discussion and examples of the basis for the use of dose additivity for component-based evaluation of PFAS mixtures are provided in Section 3.

3.0 Dose Additivity for PFAS

This section presents a review of *in vivo* chemical mixture studies for different biological pathways that provide information on how mixtures of chemicals with similar and dissimilar molecular and cellular perturbations interact. Section 3.2 discusses the evidence demonstrating that mixtures of chemicals disrupting common pathway events typically produce dose-additive alterations. In *in vivo* studies that rigorously tested accuracy for DA, Integrated Addition (IA), and RA model predictions for mixtures with components that disrupted common pathway events, DA models provided predictions that were better than or equal to IA and RA predictions of the observed mixture effects (Section 3.2). Consistent with the conclusions of the National Academy of Sciences (NAS) (NRC, 2008), Boobis et al. (2011) and Martin et al. (2021) found that published studies in the literature (Section 3.2) support DA as the default model for estimating mixture effects, even when the mixtures included chemicals with diverse biological signal transduction pathways (but common target organs/effects). Further, these two large systematic reviews of the literature on chemical mixtures found little evidence for deviations from dose additivity, such as synergy or antagonism (Boobis et al., 2011; Martin et al., 2021). For example, Martin et al. (2021), following a review of more than 1,200 mixture studies (selected from > 10,000 reports), concluded that there was little evidence for synergy or antagonism among chemicals in mixtures and that DA should be considered as the default model. Taken together, this supports the health-protective conclusion that a mixture of chemicals with similar apical effect profiles should be assumed to also act in a dose-additive manner unless data demonstrate otherwise. Further, experimental data demonstrate that PFOS, PFOA, and other PFAS disrupt signaling in multiple biological pathways, resulting in common adverse effects on several of the same biological systems and functions, including thyroid hormone signaling, lipid synthesis and metabolism, developmental toxicity, and immune and liver function, and are reviewed in Section 3.4. Finally, Section 3.4 summarizes several EPA Office of Research and Development (ORD) PFAS developmental toxicity mixture studies that provide robust evidence that PFAS behave in a dose-additive manner.

3.1 Overview of Assessment Approaches for Chemical Mixtures

Over 30 years ago, scientists developed quantitative dose metrics and methods to assess the joint toxicity of mixtures of large classes of chemicals that disrupt a common biological pathway (NATO, 1988). For example, toxicity equivalence factors (TEFs) were initially developed in the mid-1980s for hundreds of dioxin-like polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs) based on their potency relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Many of the lessons learned from assessing the effects of mixtures of dioxin-like chemicals (DLCs) are also generally applicable to assessing the effects of PFAS mixtures. Since that time, TEF-like approaches (e.g., RPFs) have been used to evaluate mixtures of other chemical classes. However, the evolving picture since early applications of RPFs or TEFs is that some chemicals, regardless of similarities or dissimilarities in molecular initiating events (MIEs) or early key events (KEs), produce mixture effects on common apical endpoints that generally are well predicted using DA models. This has, in part, been explained by the concept of pathway convergence; that is, across mixture component chemicals, some pathway perturbations may qualitatively look dissimilar at the level of MIEs or early KEs but ultimately converge upon shared or common events nearer to the terminus of a pathway leading to health effects (for further details, see the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* [USEPA, 2023h]).

The general applicability of DA models is based on reviews of studies specifically designed to evaluate how well different mixture models predict how chemicals in a mixture interact to produce effects. Studies evaluating mixture models and effects typically include an evaluation of individual chemical dose-response curves and apply this information to different statistical models. In general, systematic reviews have noted that many mixture studies do not include information conducive for evaluating the utility of different mixture models. The data from several studies (reviewed in Section 3.2) indicate that chemicals that produce common adverse effects will typically interact in a dose-additive manner when they occur together in a mixture. Thus, the effects of any combination of co-occurring chemicals can be predicted when sufficient chemical dose-response data are available for all the individual components within an environmentally relevant mixture. For example, the Consumer Product Safety Commission Chronic Hazard Advisory Panel (CPSC CHAP) on Phthalates used DA models to predict the hazard posed by mixtures of phthalates to pregnant women and children. In its assessment, phthalate mixture exposures from NHANES data were used to predict individual hazard scores for each person and then determine the percentage of people who exceeded a point-of-departure (POD) (CHAP, 2014).

In the absence of an adequate *in vivo* database to evaluate mixture models, it should be assumed that any mixture acts in a dose-additive manner if the individual mixture components produce common effects. This approach was fully endorsed by NRC (2008) and Martin et al. (2021).

3.2 Examples of Chemical Classes and Toxicological Pathways Utilizing Mixture Assessment Approaches

3.2.1 Dioxin-Like Chemicals and Aryl Hydrocarbon Receptor Pathway Toxicity Equivalence Factors

In 2010, the EPA published guidelines for using TEFs for human health risk assessment of DLCs, which produce many of their adverse effects by acting as aryl hydrocarbon receptor (AhR) agonists (USEPA, 2010). It should be noted that the TEF approach is a specialized application under the RPF umbrella that is only applicable when all mixture components induce an effect via an identical MIE/MOA (e.g., AhR agonism). DLCs such as PCBs, PCDFs, and PCDDs have been identified as AhR agonists. As such, the EPA and the World Health Organization have recommended the use of the TEF methodology to evaluate risks associated with exposure to mixtures of TCDD and DLCs for human health (USEPA, 1987, 1989, 2003) and ecological risk assessments (USEPA, 2008). TEFs can be calculated for each DLC based on dietary dose or internal whole-body toxic equivalent concentrations.

The joint toxicity of a DLC mixture is based on toxic equivalents (TEQs), which are toxicity-weighted masses of mixtures of PCDDs, PCDFs, and PCBs. The TEQ for each chemical in the mixture is calculated by multiplying each TEF by the corresponding chemical concentration in the mixture. The individual TEQs are then summed to calculate the TEQ of the mixture. The TEQ provides toxicity information about the mixture of chemicals and is more meaningful than reporting the total mass of DLCs in grams.

This approach assumes:

- Chemical mixture components interact in a dose-additive manner;
- They all act via a common AhR-mediated pathway, among other pathways;

- Synergistic and antagonistic interactions are uncommon within the group (Safe, 1994); and
- TEFs and TEQs based on AhR agonism are not necessarily predictive of chemical potency for effects mediated by other receptors or pathways.

The EPA's TEFs have undergone several revisions (Van den Berg et al., 2006). In 2010, the EPA published guidelines for the use of TEFs for human health risk assessment of DLCs (USEPA, 2010). Although the AhR is present in all classes of vertebrates, vertebrate species vary greatly in their sensitivity to environmental TEQ levels. Sensitive species include terns and cormorants (bill deformities), herons (embryo mortality), and mink (lethality and reproductive failure) (Beckett et al., 2008; Restum et al., 1998), for example. Adverse effects also occur in frogs (amphibians) (Gutleb et al., 2000), fish (Monosson, 2000), and snapping turtles (reptiles) (Bishop et al., 1998; Gale et al., 2002). The EPA stated that the TEQ methodology was appropriate for evaluating risks to fish, birds, and mammals associated with AhR agonists (USEPA, 2008).

Studies of AhR agonists in various species indicate:

- Species and tissues differ in sensitivity to the effects of a DLC mixture; and
- Even though the AhR pathway is conserved, the adverse outcomes can vary greatly from species to species.

One common effect of DLCs is a reduction in serum thyroxine (T4). Crofton et al. (2005) conducted a mixture study of 18 thyroid-disrupting DLCs consisting of 12 PCBs, 4 PCDFs, and 2 PCDDs at 6 dilutions of the highest dose, which contained effective dose (ED_{30}) concentrations of each chemical in the high dose. This mixture reduced serum T4 in a dose-related manner. The reduction in T4 was dose additive in the low dose range of interest, but the observed reduction in T4 at the high dose (46% reduced) exceeded DA predictions (28% reduced) by about 18%. In a review of the literature on the effects of mixtures on the thyroid axis, Crofton (2008) concluded, "To date, the limited data from thyroid disrupting chemical mixture studies suggest that DA is reasonably accurate in predicting the effects on serum T4 concentrations."

3.2.2 Pyrethroids/Pyrethrins – Central Nervous System and Behavior

Pyrethrins and pyrethroids share the ability to interact with voltage-gated sodium channels, ultimately leading to neurotoxicity. Wolansky et al. (2009) administered a single oral gavage dose of a mixture of 11 pyrethroid pesticides to adult male rats using a fixed-ratio dilution design at eight dose levels and measured locomotor activity on the day of dosing. The reduction in exploratory activity by the mixture was accurately predicted by DA modeling.

The EPA has determined that naturally occurring pyrethrins and synthetic pyrethroid pesticides form a common mechanism group. This common mechanism grouping is based on (1) shared structural characteristics; (2) a shared ability to interact with voltage-gated sodium channels, resulting in disruption of membrane excitability in the nervous system; and (3) neurotoxicity characterized by two different toxicity syndromes. In 2011, after establishing a common mechanism grouping for the pyrethroids and pyrethrins, the EPA conducted a cumulative risk assessment using an RPF approach (USEPA, 2011a).

3.2.3 Organophosphates – Lethality, Central Nervous System, and Behavior

In the late 1950s, Murphy and Dubois (1957) reported that O-ethyl O-p-nitrophenyl phenylphosphonothioate potentiated the lethality of malathion when the two chemicals were administered simultaneously. Subsequently, all organophosphate (OP) pesticides in use were evaluated in binary mixture studies to determine if deviation from additivity (e.g., synergism or antagonism) was a common outcome among this class of insecticides (reviewed by Moser et al., 2005; Padilla, 2006). An examination of the interactions of 43 pairs of OP insecticides revealed that four pairs showed greater-than-additive effects on lethality (Dubois, 1961). Moser et al. (2005, 2006) reported a range of responses with mixtures of four or five OPs. The ratios of the predicted-to-observed ED_{20}s and ED_{50}s of the mixtures indicated that several effects displayed greater-than-additive effects (ratios = 1.2 to 2.6), a few were less than additive (ratio = 0.5 to 0.9), and most were dose additive (ratio = 1).

In 1999, the EPA determined that the OPs form a common mechanism group based on their shared ability to bind to and phosphorylate the enzyme acetylcholinesterase (AchE), leading to the accumulation of acetylcholine and, ultimately, cholinergic neurotoxicity (USEPA, 1999). As such, the cumulative risk to OPs has been assessed using AchE inhibition as the effect on which dose-response data are integrated under the assumption of dose addition. The most recent OP cumulative assessment was conducted in 2006 and employed an RPF approach (USEPA, 2006).

Further, in 2018, ATSDR concluded that the "default assumption of dose-additive joint action at shared targets of toxicity (i.e., effects on neurological endpoints) be used for screening level assessments of the potential adverse health outcome from concurrent oral exposure to mixtures of pyrethroids, organophosphorus, and carbamate insecticides" (ATSDR, 2018).

3.2.4 Estrogen Agonists – Mixture Effects on the Female Reproductive Tract

Scientists have examined the effects of mixtures of estrogenic chemicals in the female rat using a uterotrophic assay, an EPA Endocrine Disruptor Screening Program Test Guideline that is a sensitive *in vivo* test for estrogenicity (USEPA, 2009). In this assay, immature or adult ovariectomized female rats are typically exposed to test chemicals for 3–4 days, after which uterine weights are measured. Exposures can be oral or through subcutaneous injection. Tinwell and Ashby (2004) exposed immature female rats for 3 days to several known xenoestrogens, either individually or as mixtures. In a reanalysis of the data, predictions of a DA model for a binary mixture of bisphenol A and genistein were consistent with the observed effects of the mixture, with an average deviation of observed results vs. the DA model of 4%. Similarly, Conley et al. (2016) found that the effects of mixtures of bisphenol S + methoxychlor, bisphenol AF + methoxychlor, and bisphenol F + bisphenol S + methoxychlor + bisphenol C + ethinyl estradiol, administered orally to female rats, produced effects that were comparable to predictions using DA models. Because the chemicals all stimulate uterine growth via a common estrogen receptor alpha pathway and produce a common effect, Conley et al. (2016) determined that DA was the most appropriate model for mixtures of these estrogenic compounds.

3.2.5 *Phthalates* in utero – *Mixture Effects on the Female Reproductive Tract*

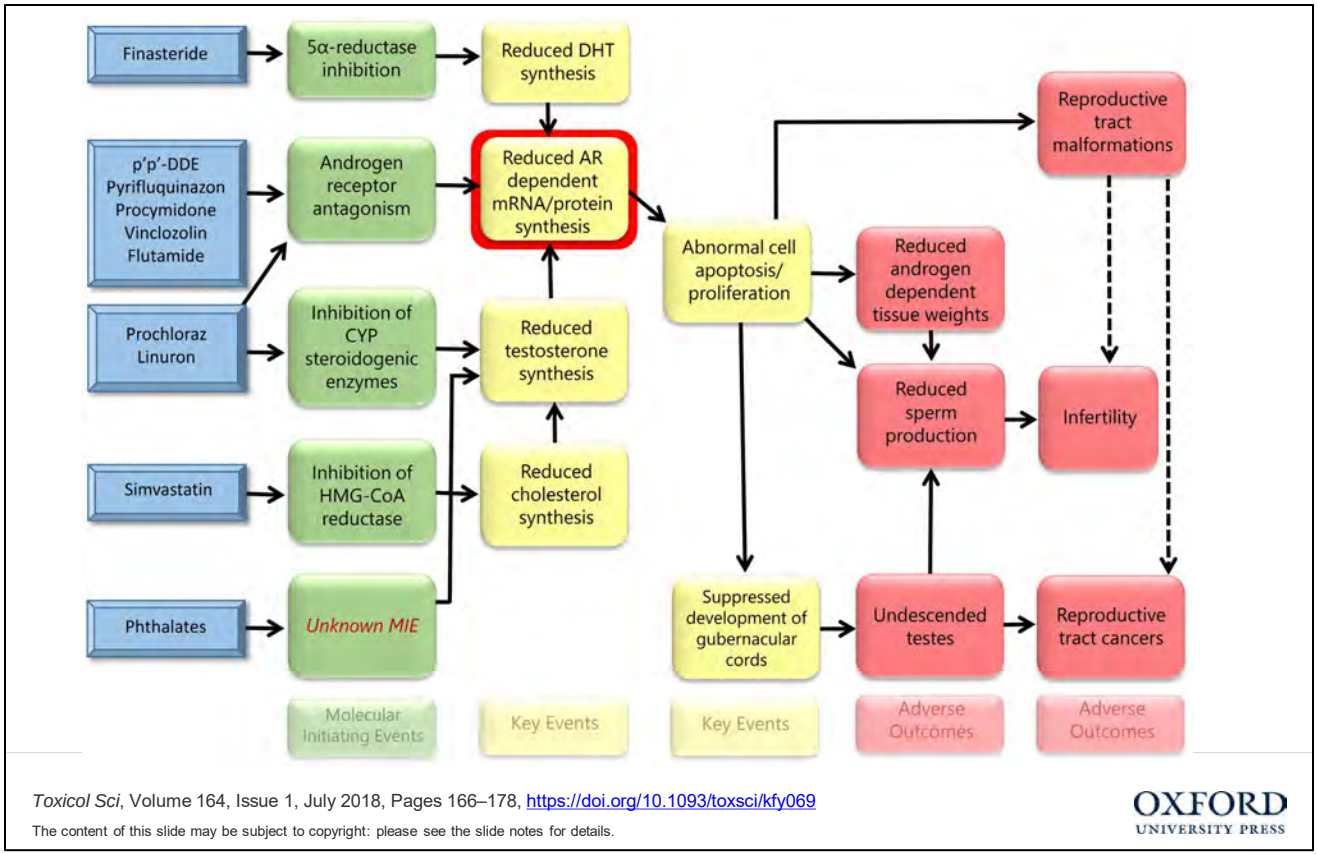
Hannas et al. (2013) reported that administration of a mixture of five phthalates (> 520 mg total phthalate) to pregnant rats from gestational days (GDs) 8 to 13 induced reproductive tract malformations in female rat offspring. These malformations included complete to partial uterine

agenesis and agenesis of the lower vagina, an effect similar to a human congenital condition known as the Mayer-Rokitansky-Küster-Hauser syndrome that occurs in about 1 in 4,500 female newborns. The phthalate mixture was a fixed-ratio dilution and contained five phthalates that do not produce malformations in either female or male offspring when administered individually at the doses used in the mixture. These malformations have been seen in dibutyl (500 milligrams per kilogram per day [mg/kg/day]) and diethylhexyl (750 mg/kg/day) phthalate studies at a low incidence and at high doses but were not seen in similar studies with the other three phthalates. Although there was not enough individual phthalate data to compare DA and RA prediction models, it is clear these effects exceed RA (i.e., 0 + 0 + 0 + 0 + 0 = 75% for uterine agenesis) and is an example of "something from nothing" (Silva et al., 2002).

3.2.6 Antiandrogens – Male Reproductive Tract Development

Historically, it has been hypothesized that mixtures of chemicals with dissimilar MIEs would interact in an RA or IA manner. However, this conclusion is not currently supported by a large body of literature on the effects of chemical mixtures and was rejected by the NRC (2008). Studies on the effects of mixture exposures on male reproductive development provide one of the larger databases supporting the use of DA as the default model. These studies include chemical mixtures with common MIEs and those with multiple MIEs that converge on a common KE in multiple adverse outcome pathways (AOPs) in an AOP network. These studies focus on chemicals that disrupt androgen signaling *in utero* during the critical period of mammalian sexual differentiation. For over 20 years, scientists have examined the *in utero* effects of mixtures of chemicals that disrupt androgen signaling on the male reproductive tract (e.g., Gray et al, 2001; reviewed by Haas et al., 2007; Howdeshell et al., 2017; Metzdorff et al., 2007). These studies include defined binary or multi-chemical fixed-ratio dilution mixtures and were designed to compare the observed effects to DA, RA, and IA model predictions. The number of chemicals used in these studies range from 2 to 18, administered at a range of doses, enabling one to discriminate additive from antagonistic or synergistic interactions. In all these studies, the DA model predicted the effects of the mixture on the male reproductive tract more accurately than IA or RA. Likewise, Metzdorff et al. (2007) concluded that the "Effects of a mixture of similarly acting anti-androgens can be predicted fairly accurately based on the potency of the individual mixture components by using the DA concept. Exposure to anti-androgens, which individually appear to exert only small effects, may induce marked responses in concert with, possibly unrecognized, similarly acting chemicals."

In addition, two recent studies were designed to specifically address a gap in the literature identified by the CPSC CHAP (Lioy et al., 2015). At the time of its review, no published studies addressed whether or not phthalate mixtures exhibited mixture effects when administered at levels below the lowest-observed-adverse-effect levels (LOAELs) of each individual chemical. In the first study, a mixture of 18 administered chemicals induced effects at dose levels about 80-fold below each chemical's individual LOAEL (Conley et al., 2018). These 18 chemicals disrupt androgen signaling via five different MIEs (Figure 3-1) and multiple AOPs that converge on common KEs, resulting in common adverse reproductive effects in male rat offspring.



Notes:
Adapted from Conley et al. (2018).
The bold outlined KE indicates the critical node that links the various MIEs to the downstream adverse outcomes.
DHT = dihydrotestosterone; AR = androgen receptor; CYP = cytochrome P450; HMG-CoA = 3-hydroxy-3-methyl-glytaryl coenzyme A.

Figure 3-1. AOP network for chemicals that disrupt AR-mediated cellular signaling leading to adverse effects on the development of male reproductive tract resulting from *in utero* exposure.

In the second study (Conley et al., 2021a), 15 chemicals (acting via at least three MIEs) demasculinized male rat offspring at dose levels 2- to 4-fold lower than the individual no-observed-effect levels for each chemical, and the DA models were always as good or better than RA or IA models in estimating mixture effect. For example, 60% of male offspring were found to have penile malformations that resulted in infertility; DA accurately predicted this effect, whereas IA and RA predicted that none of the males would be malformed. This is not a unique observation; rather, it is a typical finding with male reproductive tract malformations.

Recently, Gray et al. (2022) demonstrated that the *in utero* effects of a PFAS pesticide, pyrifluquinazon (contains a heptafluoroisopropyl side chain; see https://comptox.epa.gov/dashboard/chemical/details/DTXSID6058057) combined with the di-ortho phthalate ester dibutyl phthalate produced dose-additive effects that were more accurately predicted with DA models that did not require parallel slopes than with RA models for multiple male reproductive abnormalities.

All these endocrine-active chemicals act via AOPs that converge on a common KE in an AOP network (Figure 3-1) that regulates the sequence of molecular events in cells involved in the development of androgen-dependent tissues. Each of the identified chemicals/classes reduces the number of androgen receptor (AR) dimers, AR/AR, activated by an androgen agonist. AR antagonists, like vinclozolin or procymidone, accomplish abrogation of androgen-dependent signaling by blocking androgens from binding to Ars, and the PFAS pesticide pyrifluquinazon has been hypothesized to act by enhancing AR degradation (Gray et al., 2019; Yasunaga et al., 2013). Chemicals like the phthalates di-n-butyl phthalate (DBP), di(2-ethylhexyl)phthalate (DEHP), dipentyl phthalate (DpeP), butyl benzyl phthalate (BBP), and diisobutyl phthalate (DIBP) reduce the levels of androgens available to respondent cell populations (Hannas et al., 2011; Howdeshell et al., 2008; Furr et al., 2014). In contrast, chemicals like finasteride inhibit the enzyme in tissues that converts testosterone to dihydrotestosterone (a more active androgen with a higher affinity for the AR) (Clark et al., 1990).

The result of these related androgen-disrupting AOPs is that fewer activated AR/AR heterodimers bind the promoter region on the DNA of androgen-regulated genes, androgen-dependent mRNA and protein synthesis levels are reduced, and growth and differentiation of androgen-dependent tissues in the fetus is inhibited. As a result, male offspring display agenesis or hypoplasia or malformations in androgen-dependent tissues. In summary, an examination of KEs disrupted in androgen signaling pathways by chemicals such as those identified in Figure 3-1, at the cellular-molecular level, explains why one should expect the mixtures to behave in a dose-additive manner.

In summary, an examination of the literature on the effects of mixtures on male reproductive tract development demonstrates that common effects are most accurately modeled by DA; the chemicals discussed above acted in a dose-additive manner even when including chemicals with different MIEs, and IA and RA models consistently underestimated the hazard of a mixture of chemicals acting on common molecular or cellular (more downstream) events resulting in a common profile of apical effects.

3.3 Systematic Reviews of Mixtures Toxicity: Quantification of Deviations from Dose Additivity

Boobis et al. (2011) examined the literature from 1990 to 2008 that discussed synergy in mammalian test systems with an emphasis on “low dose” studies. Of the 90 papers identified, 43 papers had original data from which synergy could be examined, and only 11 studies reported the magnitude of the difference between the dose-additive estimates of toxicity with the observed results. Of these 11 studies, six reported magnitudes of synergy that were generally less than 2-fold with a maximum value of 3.5-fold. As a result, the authors concluded that deviations from DA at low doses were not common.

The issue of the occurrence of greater-than-DA (sometimes referred to as synergistic) vs. DA or less-than-DA (sometimes referred to as antagonistic) interactions was recently reassessed by Martin et al. (2021). The authors conducted a systematic literature review and quantitative reappraisal of 10 years of a broad range of mixture studies published between 2007 and 2017. Martin et al. (2021) identified 1,220 mixture studies, ~65% of which did not incorporate more than 2 component chemicals. They reported that “relatively few claims of synergistic or antagonist effects stood up to scrutiny in terms of deviations from expected additivity that exceed

the boundaries of acceptable between-study variability" and that the observed effects were not more than 2-fold greater than the predicted effects of the mixture based on an assumption of DA.

3.3.1 Deviation from Additivity

Although the literature indicates that significant deviations from dose additivity are not common among mixtures containing chemicals that disrupt common targets via common AOPs or AOP networks, greater-than-additive (i.e., synergism) and less-than-additive (i.e., antagonism) interactions may occur with co-exposure to chemicals that affect different target organs or different, unrelated AOPs. There are several examples of chemical interactions that deviate from DA in which one chemical has the capability to alter the metabolism of the other chemical(s). For example, 20 years of research have identified at least 85 drugs whose metabolism is inhibited by a chemical in grapefruit, potentially resulting in serious side effects (Bailey, 2013). Furanocoumarins in grapefruit bind to the active site on the CYP3A4 enzyme, causing irreversible inactivation that prolongs the half-life and AUC (the area under the concentration vs. time curve) of some drugs, like some statins, for example.

The effects of metabolic alterations of chemical toxicity are not limited to drug-drug interactions. Hodgson (2012) published a comprehensive review of the effects of metabolism on the toxicity of a large number of pesticides and also described the metabolic mechanisms of chemical activation and/or inactivation.

In addition to alterations in metabolic activity leading to synergistic or antagonistic dose-response among chemicals in mixture, there are other examples of deviations from DA that do not include interactions across different levels of biological organization but instead entail physical-chemical interactions. For example, although melamine and its derivatives, including cyanuric acid, individually present low toxicity, together the compounds can physically interact to lead to the formation of cyanurate crystals in nephrons, causing kidney effects and kidney failure in mammals. Such impacts have been observed in cats and dogs from adulteration of pet food (Jacob et al., 2011), as well as in infants and young children in China from contaminated infant formula and related dairy products (WHO, 2008).

3.4 PFAS Dose Additivity

PFAA, such as PFOA and PFOS, with linear or branched alkyl or alkyl ether chains and sulfonic or carboxylic acid functional groups, as well as PFAA precursors, while not necessarily toxicologically identical, do elicit similar toxicological effects across different levels of biological organization, tissues/organs, life stages, and species (ATSDR, 2021; EFSA et al., 2018, 2020). As described above (Section 3.2), precedents of prior research conducted on mixtures of various chemical classes with common pathway perturbations or KEs (for those chemicals with established MOAs) and adverse outcomes, support the use of dose-additive models for estimating mixture-based risks, even in instances where chemicals with disparate MIEs were included. Thus, in the absence of identification and characterization of MOA(s) for most PFAS, it is considered a health-protective conclusion that PFAS that can be demonstrated to share one or more molecular/cellular pathway events and/or adverse health outcomes will produce dose-additive effects from co-exposure. The EPA's SAB supported this approach in its review of a draft version of this document (SAB, 2022). PFOA and PFOS have historically been the most studied and well-characterized PFAS, but recent work has also provided supportive evidence of similar effects of other PFAAs, including ether-linked structures. Below is a brief

overview of similarities and differences in MIEs, intermediate pathway events, and adverse outcomes that have been reported for those PFAS studied to date and experimental evidence that supports dose-additive effects from combined exposure to multiple PFAS. This overview highlights study results from, among others, the National Institute of Environmental Health Sciences' National Toxicology Program (NTP) 28-day repeat dose guideline toxicity studies of perfluoroalkyl carboxylates (PFHxA, PFOA, PFNA, and PFDA) (NTP, 2019a) and perfluoroalkyl sulfonates (PFBS, PFHxS, and PFOS) (NTP, 2019b). The NTP studies provide high-quality side-by-side comparisons of multiple PFAS from experiments conducted by a single lab with rigorous exposure characterization and multiple effects/endpoints spanning MIEs, intermediate pathway events, and adverse outcomes. More comprehensive reviews of PFAS toxicity endpoints in experimental animal studies and observational human studies can be found elsewhere (e.g., ATSDR, 2021; EFSA et al., 2018, 2020).

Mechanistically, *in vitro* and *in vivo* studies have demonstrated the activation of multiple nuclear receptors associated with exposure to many structurally diverse PFAS, indicating several potential MIEs for PFAS-relevant toxicity pathways. The most commonly reported MIE associated with many PFAS is the activation of peroxisome proliferator-activated receptor alpha (PPARα) based on *in vitro* binding and transcriptional activation assays (Behr et al., 2020; Evans et al., 2022; Ishibashi et al., 2019; Nielsen et al., 2022; Takacs and Abbott, 2007; Vanden Heuvel et al., 2006; Wolf et al., 2012), *in vitro* upregulation of PPARα target genes (Bjork et al., 2011), and *in vivo* tissue-specific upregulation of PPARα target genes (Bjork et al., 2008; Rosen et al., 2007). All PFAA carboxylates and sulfonates included in the NTP 28-day studies displayed upregulation of the PPARα target genes Acox1 and Cyp4a1 in male and female rat livers (NTP, 2019a, 2019b). PPARα is a highly conserved transcription factor that regulates pleiotropic effects on mammalian energy homeostasis and lipid metabolism, among others. Similarly, multiple PFAS have been shown to activate peroxisome proliferator-activated receptor gamma (PPARγ) *in vitro* (Evans et al., 2022; Houck et al., 2021; Vanden Heuvel et al., 2006) and upregulate PPARγ target genes *in vitro* (Marques et al., 2022) and *in vivo* (Rosen et al., 2017). PPARγ is also a highly conserved transcription factor that regulates multiple physiological processes, including adipogenesis and glucose metabolism. Further, *in vivo* studies of tissue-specific gene expression patterns have also demonstrated the activation of the constitutive androstane receptor (CAR) for both PFOA and PFOS, among other PFAS, due to the upregulation of CAR-dependent genes (Rosen et al., 2017). All PFAA carboxylates and sulfonates that were tested in the NTP 28-day studies and displayed upregulation of the PPARα target genes also displayed upregulation of the CAR-inducible genes Cyp2b1 and Cyp2b2 in adult male and female rat livers (NTP, 2019a, 2019b). Additional *in vitro* data indicate potential involvement from several other nuclear receptors following PFAS exposures including estrogen receptor alpha (Evans et al., 2022; Houck et al., 2021, Kjeldsen and Bonefeld-Jorgensen, 2013), pregnane X receptor (Bjork et al., 2011; Houck et al., 2021), farnesoid X receptor (Bjork et al., 2011), and liver X receptor (Bjork et al., 2011, Houck et al., 2021). Multiple PFAAs, including PFOA and PFOS, activate multiple nuclear receptors and gene transcription pathways, which is a data-driven basis for positing shared or overlapping pathway perturbations across PFAAs.

In addition to the cell- and/or tissue-specific gene expression changes described above, multiple pathway events or markers of toxicity downstream of the above-mentioned potential MIEs are also shared between PFOA, PFOS, and other PFAAs. In both rodent and nonhuman primate studies, serum lipids (cholesterol, triglycerides) are altered, and markers of liver injury or

dysfunction (ALT, AST, and/or ALP) are consistently elevated in a dose-responsive manner (ATSDR, 2021; EFSA et al., 2018, 2020). Specifically, the NTP 28-day studies reported reduced serum cholesterol, triglycerides, and globulin and elevated serum ALT, AST (males only), ALP, and bile acids in rats following exposure to PFHxA, PFOA, PFNA, PFDA, PFBS, or PFOS (NTP, 2019a, 2019b). Further, circulating thyroid hormone concentrations were reduced following oral PFAA exposure, with all compounds tested in the NTP 28-day studies being associated with decreased serum total and free thyroxine (T4) (NTP, 2019a, 2019b). Ether-linked PFAAs have also been shown to reduce circulating thyroid hormone concentrations (Conley et al., 2019, 2022a). In combination with a common profile of nuclear receptor activity and gene expression, there is a pronounced similarity in the landscape of serum clinical chemistry and thyroid hormone-based markers of altered physiology for PFOA, PFOS, and several other studied PFAS. Identification and characterization of PFAS-relevant pathway events as formal Kes in established MOA(s) is currently an area of high research activity, as additional associations across different levels of biological organization with the reported MIEs continue to be investigated across various life stages and species.

Similar adverse health outcomes at the organ and whole animal levels have been described for PFAAs, including PFOA and PFOS. Developmental exposure studies with PFOA, PFOS, PFNA, HFPO-DA and Nafion byproduct 2 (NBP2; an emerging polyfluoroethersulfonic acid compound recently detected in human serum) in rats and/or mice have reported consistent effects on pups including reduced offspring survival/viability and reduced offspring body weight (Abbott et al., 2007, 2009; Blake et al., 2020; Butenhoff et al., 2004; Conley et al., 2021b, 2022a; Das et al., 2015; Lau et al., 2003, 2006; Luebker et al., 2005a, 2005b; Thibodeaux et al., 2003). PFAS studied by NTP (2019a, 2019b) have been observed to increase rat liver weights and produce hepatocyte hypertrophy. PFOA and PFOS, and potentially other PFAS, have also been shown to produce functional immunotoxicity (i.e., reduced antibody response) in animal studies (NTP, 2016). Taken together, there are numerous adverse effects that occur in laboratory animals that are shared across PFAS such as PFOA, PFOS and other PFAAs. These adverse effects are consistent with the molecular and cellular pathway perturbations highlighted above. However, it is important to recognize that while there are the same/similar qualitative effect profiles across many PFAS (e.g., liver injury, decreased thyroid hormones), there are quantitative oral potency differences in reported effects, and not all effects appear to be shared across all PFAS, or even across all PFAAs, at the dose levels reported in published studies. For example, PFHxS exposure did not result in changes in serum ALT in male or female rats at any dose in the NTP 28-day studies, while all other PFAAs tested increased serum ALT levels, indicative of hepatocellular injury.

The specific molecular mechanisms or precise MOAs for a given adverse health outcome may be disparate across some PFAS. For example, studies utilizing transgenic mice with PPARα deletion have demonstrated that some effects, such as survival of neonates following *in utero* PFOA exposure, were dependent on PPARα involvement, while this effect appeared independent of PPARα for PFOS (Abbott et al., 2007, 2009). It is important to note in those studies that fetal mortality (as opposed to neonatal mortality) was independent of the PPARα genotype for PFOA. The relevance of rodent PPARα-based effects has been debated in the toxicology literature for decades, largely as it relates to hepatocarcinogenesis, yet the pharmacological utility of PPARα modulation is widely accepted and exploited in the development of therapeutics. Further, studies of PPARα knockout mice have also demonstrated that many liver effects are PPARα

independent for PFOA and PFOS (Abbott et al., 2007, 2009). Although there is potential for disparate MIEs, and more importantly the lack of formal MOA(s), in PFAS-related adverse health outcomes, it is a reasonable health-protective conclusion that effects shared across PFAS in a given mixture will be dose additive.

Some studies have evaluated other models for quantifying potential joint toxicity (e.g., dose addition, synergism, antagonism) associated with combined exposure to PFAS in experimental systems either *in vitro* or *in vivo*. *In vitro* studies have directly assessed the mixture-based effects of combined PFAS exposures by comparing observed experimental data with model-based predictions. For example, Wolf et al. (2014) evaluated *in vitro* PPARα activation and observed joint effects of combined exposure to binary combinations of PFOA and PFOS, PFNA, PFHxA, and PFHxS that were consistent with dose additivity in the lower dose ranges, but the authors reported slightly greater than additive effects at higher mixture doses. In contrast, Carr et al. (2013) reported slightly less than additive responses for *in vitro* PPARα activation of binary mixtures of PFAAs, including PFOA, PFNA, PFOS, and PFHxS. Further, Ojo et al. (2022) reported both synergistic and antagonistic effects compared to a dose-addition model for binary, ternary, and multicomponent mixtures of PFAAs for cytotoxicity in HepG2 cells. Nielsen et al. (2022) demonstrated the utility of generalized dose addition for PPARα activation of PFAA mixtures in an *in vitro* system with variable efficacy across compounds; however, there are no studies indicating this model variation on simple dose addition is applicable for prediction of *in vivo* mixture effects. Most recently, Addicks et al. (2023) conducted *in vitro* exposures of primary human liver spheroids to seven different PFAS mixtures, including combinations of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFBS, PFHxS, PFOS, PFOSA, 6-2 FtS, and 8-2 FtS. Liver spheroids were evaluated for mRNA transcriptomic points of departure, and all mixtures produced effects that were within 2- to 3-fold of mixture effects predicted using dose addition. Similarly, as described above, systematic reviews of chemical mixture studies across various compound classes indicate that departures from dose additivity are uncommon and rarely exceed minor deviations (~2-fold) from predictions based on dose additivity (Martin et al., 2021). Recent PFAS mixture studies in zebrafish reported interactions for combinations of PFOA and PFOS, but departures from additive models were also minor (Ding et al., 2013). Menger et al. (2020) reported zebrafish behavioral effects from a PFAS mixture that were less than individual PFAS; however, an evaluation of chemical dose-response and comparison to mixture models was not conducted. Regarding zebrafish PFAS effects, it is notable that fish PPARγ has a relatively low sequence homology to that of mammalian PPARγ (Zhao et al., 2015), and the potent PPARγ agonist rosiglitazone activates this rat, mouse, and human receptor *in vitro* but not in three species of fish or the clawed frog (Medvedev et al., 2020). The interactions described in the literature thus far for combined *in vitro* exposure to PFAAs demonstrate results that are either consistent with or have relatively minor deviations from predictions based on dose-additive models.

Mammalian *in vivo* toxicity studies evaluating exposure to multiple PFAS are more limited, but recent studies indicate that exposure to a mixture of PFOA, PFOS, and PFHxS in mice (Marques et al., 2021), a mixture of PFOA, PFOS, PFNA, PFHxS, and HFPO-DA in mice (Roth et al., 2021), or a mixture of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS in rabbits (Crute et al., 2022) produced numerous significant health effects compared to control animals, which were consistent with the spectrum of individual PFAS effects described above (e.g., liver injury; thyroid hormone alterations). However, these studies did not

include individual PFAS dose-response data or conduct any mixture model-based analyses, so it is impossible to ascertain if the mixtures behaved in a DA or RA manner or if interactions occurred (i.e., deviations from DA).

Recent and ongoing work at the EPA includes developmental toxicity studies of PFAS mixtures in rats. One study recently investigated *in vivo* effects in maternal rats and offspring from combined exposure to PFOA and PFOS during gestation and early lactation (Conley et al., 2022b). The study included a series of experiments designed to characterize dose-response curves across multiple endpoints for PFOA and PFOS individually, followed by a mixture study of the two chemicals combined. The mixture experiment was designed to test for shifts in the PFOA dose-response curves from combined exposure to a fixed dose of PFOS, compare DA and RA model predictions, and conduct an RPF analysis to evaluate mixture effects. Exposure to binary combinations of PFOA and PFOS significantly shifted the PFOA dose-response curves left towards elicitation of effects at lower doses compared to PFOA-only exposure. This clearly indicated mixture effects for a range of endpoints, including decreased pup survival, maternal and pup body weight, pup serum triiodothyronine (T3) and glucose, and increased maternal kidney weight, maternal and pup liver weight, and pup bile acids, BUN, and bilirubin. Maternal kidneys and maternal and pup livers in the mixture study also displayed a range of treatment-related histopathological lesions. For nearly all endpoints amenable to mixture model analyses, the DA equation produced equivalent or better estimates of observed data than RA. Similarly, for nearly all maternal and neonatal endpoints modeled, the RPF approach produced accurate estimates of dose-additive mixture effects. Only maternal body weight at term and gestational weight gain demonstrated departures from dose additivity, and these effects were slightly less than additive. This work is ongoing, with multiple KE analyses still to be conducted on samples collected during the studies. However, results thus far support the hypothesis of joint toxicity on shared endpoints from PFOA and PFOS co-exposure and dose additivity as a reasonable conclusion for predicting mixture effects of co-occurring PFAS.

A second PFAS mixture study by Conley et al. (2023) reported data on a mixture of PFOS, HFPO-DA, and NBP2 (an emerging polyfluoroethersulfonic acid compound recently detected in human serum [Kotlarz et al., 2020]). Multiple endpoints, including maternal serum cholesterol, triglyceride, and thyroid hormone concentrations (total T3 and T4), pup birthweight and body weight at 2 days of age, and pup mortality, all conformed to dose additivity. Similar to the PFOA + PFOS mixture above, effects on maternal weight gain were slightly less than additive, and no endpoints demonstrated synergy. Further, the mixture shifted the dose-response curves for increased maternal and pup liver weights towards effects at lower doses when comparing the HFPO-DA responses in the mixture to the dose responses from HFPO-DA exposure alone. Finally, preliminary data from a third *in vivo* study of a mixture of six PFAS (PFOA, HFPO-DA, PFMOAA, PFOS, PFHxS, and NBP2) further indicate dose-additive effects on maternal and neonatal endpoints including pup body weight and pup survival. The published and preliminary results discussed above provide robust evidence of combined toxicity of PFOA, PFOS, and other PFAS on multiple maternal and developmental endpoints and the greater accuracy of DA for predicting mixture effects *in vivo* than RA. Similarly, the joint toxicity of a mixture of PFOS and PFOA on Japanese quail chick 10-day survival is accurately predicted by DA but not RA (Gray et al., 2023).

In summary, PFAS data reported in the literature support an assumption of similarity in toxicity profiles for several health effect domains (for review, see Carlson et al., 2022). Importantly, study results reported in this section across multiple chemical classes, biological effects, and study designs clearly support a dose-additive mixture assessment approach. Most notably, recent efforts to characterize *in vivo* mixture effects from combined exposure to multiple PFAS provide key supportive evidence that co-exposure produces dose-additive effects on several endpoints within the range of "same/similar" endpoints shared across the spectrum of PFAS effects. Further, the National Academies of Sciences, Engineering, and Medicine (NASEM, 2022) recently recommended clinicians apply an additive approach for evaluating patient levels of PFAS currently measured in NHANES. The EPA will continue to review how mixtures of PFAS and other chemicals interact. Dose additivity is proposed as the "default" model for PFAS mixtures assessment, and other models will be evaluated when data empirically support or demonstrate significant deviations from dose additivity.

4.0 Introduction to Estimating Noncancer PFAS Mixture Hazard or Risk

4.1 Whole Mixtures Approach

The preferred hazard and dose-response knowledge base for any mixture of environmental chemicals would be derived from exposure to a whole mixture of concern. However, the exponential diversity of chemicals such as PFAS co-occurring in different component combinations and proportions makes whole mixture evaluations difficult and complex. That is, in the environment, due to differing fate and transport properties of chemicals, biotic (metabolism) and abiotic (degradation) processes, pH, ultraviolet radiation, media temperature, and so on, components commonly co-occur in an array of parent species, metabolites, and/or degradants, making characterization of any given mixture complicated. In controlled experimental study designs, whole mixtures can be assembled with defined component membership and proportions. However, the relevance of toxicity associated with exposure to a defined mixture in a laboratory setting may not be translatable to environmental mixtures of different component combinations and proportions across time and space in environmental media. In the context of PFAS, increasing environmental evidence (e.g., ambient and drinking water, fish, air, and soil sampling results) suggests that the complexities briefly summarized above with regard to the diversity of chemicals co-occurring in different component proportions make evaluating each unique whole mixture of PFAS intractable, which is why component-based mixture approaches are considered particularly useful and appropriate for addressing human exposure(s) to mixtures of PFAS (see Sections 5–7).

The EPA's *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 2000b) indicates that there may be opportunities to infer hazard and dose-response for a mixture of concern from a "sufficiently similar mixture." A mixture is considered sufficiently similar to a mixture of concern when the components and respective proportions exist in approximately the same pattern. There are clearly gradations of expert judgment involved in determining what constitutes a sufficiently similar mixture, but determinations should be based on a comparison of similarities or differences in the components' chemical fate and transport in the environment, persistence, bioaccumulative potential, kinetics, and toxicity profiles. If no significant qualitative differences are identified in a systematic comparison of mixtures of chemicals, the hazard and dose-response information associated with the sufficiently similar mixture could be used as a surrogate for the mixture of concern. However, as with a whole mixture of concern, information pertaining to a sufficiently similar mixture is rare. The whole mixture options are most appropriate for localized, site-specific assessments with stable mixtures (i.e., low/no temporal and spatial variability) and should be considered before moving to a component-based mixtures approach.

4.2 Data-Driven Component-Based Mixtures Approaches for PFAS

As a result of both the complexities associated with the characterization and evaluation of whole mixtures (see Section 4.1 above) and the reality that most toxicological information derives from exposure-response studies of individual chemicals, component-based mixtures risk assessment is particularly relevant (Figure 4-1). In addition, although the methodological approaches and associated illustrative examples in this framework are targeted at application to water, the concepts may facilitate the evaluation of PFAS mixtures in other exposure media as well (e.g.,

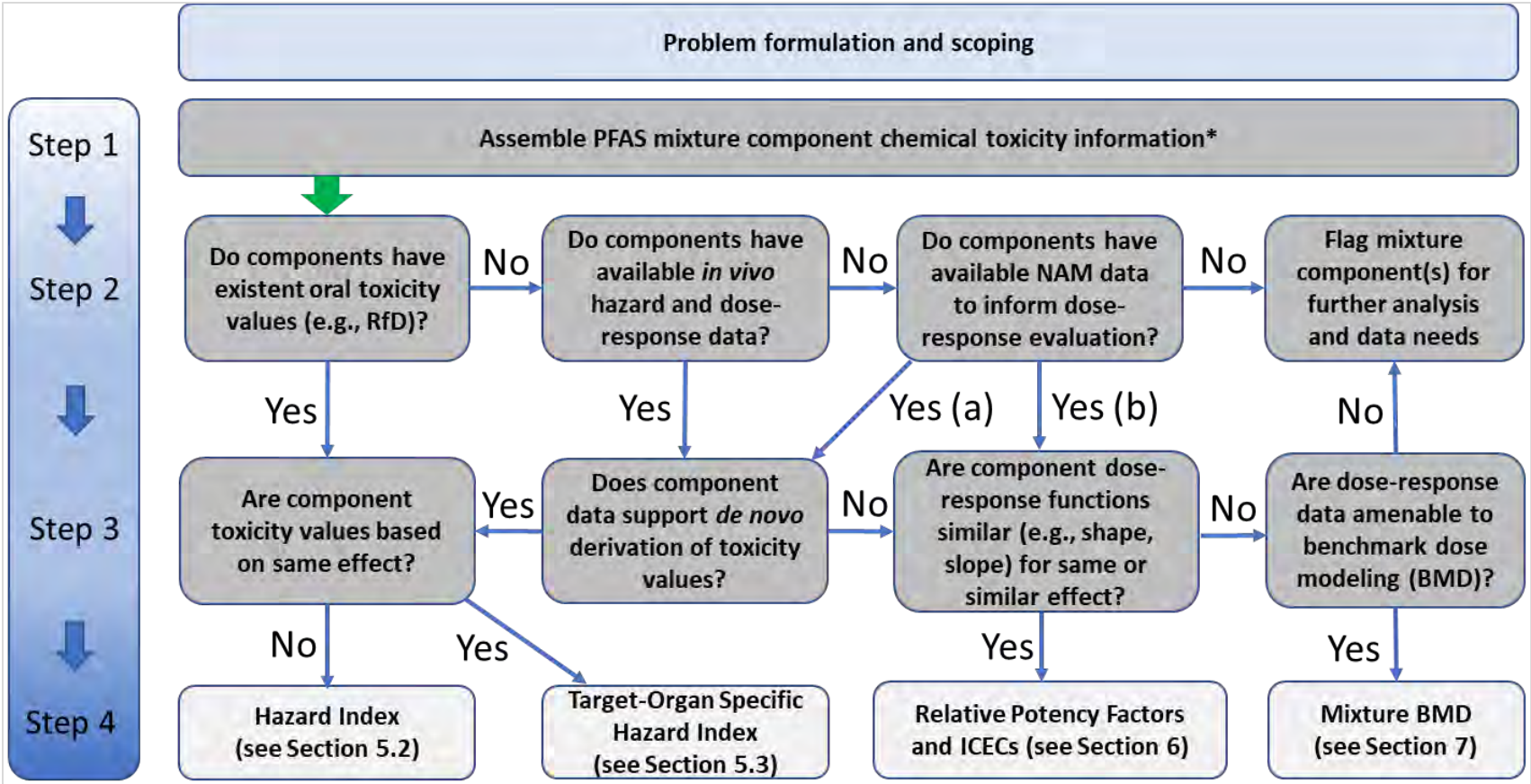


Figure 4-1. Framework for data-driven application of component-based assessment approaches for mixtures of PFAS based on dose additivity.

soil, air). As outlined in earlier sections of this framework, while EPA component-based methods and approaches are available for an evaluation of mixtures of chemicals under different assumptions of additivity (USEPA, 2000b), the currently available hazard evidence on PFAS, and several other classes of environmental chemicals, support an assumption of dose additivity (see Section 3). The HI and RPF are two component-based mixture approaches based on dose additivity that are well validated, supported by peer-reviewed guidelines, and actively used by the EPA. These two approaches are discussed below and include illustrative examples based on a hypothetical five-component mixture of PFAS (see Sections 5 and 6). An alternative M-BMD approach, generally based on the Berenbaum equation (see Section 4.2.6 in the EPA mixtures guidelines [USEPA, 2000b]), is also a dose-additive approach that is described and illustrated (see Section 7). The primary difference between the RPF and M-BMD approaches is that RPF assumes component chemical dose-response curves are similarly shaped, while the M-BMD approach is more applicable for mixture component chemicals with dissimilar dose-response curve shapes/slopes. It should be noted that others have recently demonstrated the application of the HI and RPF approaches in the evaluation of PFAS (Bil et al., 2021, 2022; Mumtaz et al., 2021), lending confidence to the use of this framework document in guiding formal component-based assessment of PFAS mixtures. The M-BMD approach was indirectly described within the context of a mixture RfD in the EPA's mixtures guidelines (USEPA, 2000b) and by the National Research Council (NRC) (NRC, 2008); further, laboratory studies have provided empirical evidence in support of this approach (Gray et al., 2022; see example in Section 7).

4.2.1 Conceptual Framework of the Approach

A pragmatic data-driven approach to the application of component-based evaluation of mixtures of PFAS with variable hazard and dose-response databases is presented in Figure 4-1. The general steps of the component-based approach, as shown in Figure 4-1, are:

Problem formulation and scoping. Problem formulation is the part of the risk assessment framework that articulates the purpose of the assessment and defines the problem (e.g., PFAS source and occurrence, fate and transport, populations/subpopulations potentially at risk, health endpoints). Problem formulation also typically includes developing a conceptual model and analysis plan and engaging with potentially affected stakeholders (e.g., states and Tribes, risk managers, affected community) to discuss foreseeable science and implementation issues.

Step 1: Assemble information.

Step 1 of the data-driven mixture assessment approach is to identify the available hazard and dose-response information for: (1) a whole mixture of the PFAS of potential concern at component proportions consistent with the environmental sampling data, (2) a sufficiently similar mixture, and/or (3) data for the individual component PFAS. If toxicity data for the whole mixture itself or a sufficiently similar mixture are not available or are insufficient, then a structured search, collection, and assembly of all available toxicity data for mixture component PFAS is conducted. Although the optimal approach would be to use formal systematic literature search and review principles as set forth by the EPA (please see the Integrated Risk Information System [IRIS] systematic review protocol for PFBA, PFHxA, PFHxS, PFNA, and PFDA as an example[10]), the user of this framework may employ a structured literature search approach of their choosing so long as the underpinning decisions resulting in the literature inventory and data

[10] https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065

landscape used in steps 2–4 of the framework approach are transparent. It should be noted that while this step is primarily intended for the identification and collation of human epidemiological and/or traditional experimental animal toxicity data, it is ideal to also assemble information such as toxicokinetic (TK) parameters (e.g., clearance, plasma/serum half-life, volume of distribution), mechanistic pathway data, empirical (or predicted) physicochemical properties and, if available, validated NAM data such as cell bioactivity, high(er)-throughput transcriptomics, and/or structure-activity/read-across.

Step 2: Evaluate data objectives.

Once collected, curated, and arrayed, the user should be able to evaluate the following primary data objectives: (a) Existence of human health risk assessment values (e.g., EPA RfD; ATSDR MRL); duration-specific values (e.g., subchronic [note: ATSDR refers to this duration as "intermediate"], or chronic RfVs) may be available from various sources and should be assembled and incorporated into the data-driven mixture assessment approach(es) as deemed appropriate by the user; it should be noted that human health assessment values may be available from different sources utilizing different levels of analytical rigor and/or peer-review. The practitioner is advised to consider the confidence associated with noncancer assessment values located across mixture components and integrate accordingly into subsequent steps of this workflow; and (b) Development of health effect domain profiles (see example literature inventory in Figure 4-2 excerpted from the IRIS systematic review protocol for PFBA, PFHxA, PFHxS, PFNA, and PFDA[10]), and associated dose-response information sorted based on exposure duration (e.g., acute or short[er]-term, longer-term [i.e., subchronic, chronic]; developmental/reproductive), across component PFAS supported by the assembled (i.e., human epidemiological and/or experimental animal) toxicity data from Step 1.

Users of this framework may find that many component PFAS of interest are data-poor (i.e., no traditional human health assessment relevant epidemiological or experimental animal study data are available). In such cases, NAM platforms or assays might provide opportunities to inform dose response for PFAS mixture components. For example, read-across is a NAM approach that could potentially be leveraged to identify surrogate dose-response metrics (e.g., POD, EC_X, IC_X) for integration into the component-based mixtures assessment approaches presented in the subsections below. Analog-based read-across, in general, is a process in which chemicals (i.e., analogs) with relatively replete toxicity databases are compared to a data-poor target chemical across similarity domains including structural, physicochemical, TK, and/or toxicodynamic (TD) similarity (Lizarraga et al., 2023; Wang et al., 2012; Wu et al., 2010). Based on weight-of-evidence for similarity between a data-poor target chemical and candidate analogs, hazard and dose-response data (e.g., PODs) are then adopted from a selected (single-best) analog as a surrogate for the target chemical. This read-across approach might facilitate incorporation of data-poor PFAS into the component-based methods presented in this framework, as surrogate PFAS data that inform similarity of toxic endpoint/health effect and dose-response could potentially: (a) be used in the derivation of a noncancer RfD (using uncertainty factors appropriate for the data-poor target chemical) and subsequent calculation of a hazard quotient (HQ); (b) be used in the calculation of RPF(s); or (c) the surrogate health-effect dose-response data could undergo BMD modeling and be included in the calculation of an overall M-BMD. The EPA's ORD has been employing expert-driven analog-based read-across in the evaluation of data-poor chemicals for over a decade; for an illustrative example human health assessment application of the approach, please see Appendix A of the Provisional Peer-Reviewed Toxicity

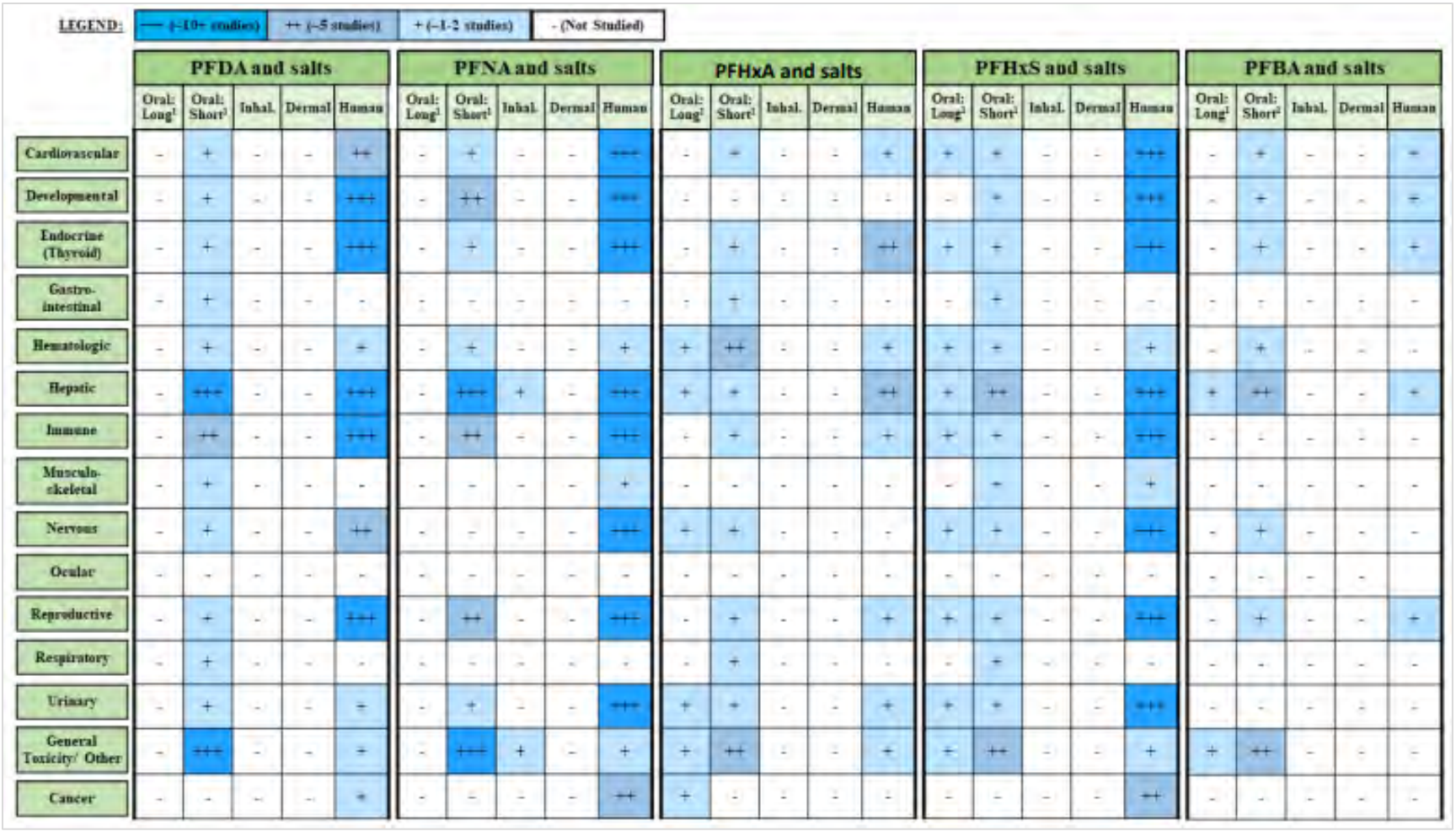


Figure 4-2. Example literature inventory heatmap for epidemiological or traditional experimental animal studies for five PFAS currently under development/review in the EPA/ORD's IRIS program (heat map circa 2018). Health effects are based on groupings from the IRIS website (https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=542033).

Value (PPRTV) document for 2,3-toluenediamine at https://cfpub.epa.gov/ncea/pprtv/recordisplay.cfm?deid=352932 and/or for perylene at https://cfpub.epa.gov/ncea/pprtv/recordisplay.cfm?deid=357451.

Another opportunity for integration of NAMs into the proposed mixture approaches involves cell bioactivity (e.g., ToxCast/Tox21), including pathway-based transcriptomic and metabolomic data from experimental animals and/or *in vitro* cell cultures. For over a decade, the EPA and the National Institute of Environmental Health Sciences have invested significant resources into high(er)-throughput assay development and application to hundreds of chemicals (Richard et al., 2016; Kavlock et al., 2012; Dix et al., 2007). The cell bioactivity assays are primarily targeted at nuclear receptor activity but also include several other assays that inform cell viability, enzyme activity, DNA reactivity, cell transport, and macromolecular/cellular dysfunction. The bioactivity information is quality assured, assembled, curated, and presented in a manner that is intended to facilitate incorporation into risk-based decision contexts such as human health assessment (see example bioactivity plot in Figure 4-3). However, inherent complexities and challenges are associated with study designs and data interpretation using NAM assays/platforms, such as *in vitro* cell culture. For example, there is no *a priori* assumption that molecular/cellular perturbations observed in cells *in vitro* have any direct qualitative relationship to phenotypic health effect(s). Further, considerations such as metabolic capacity, shorter-term exposure durations (e.g., hours to days), and specificity or sensitivity of *in vitro* effect(s), and how these factors influence precision, accuracy and/or reproducibility of quantitative concentration-response relationship within an assay, within a lab, across labs, etc., continue to present challenges for integration into decision-making foci.

Recent investigation has demonstrated that quantitative data (e.g., PODs) from *in vitro* cell bioactivity and corresponding traditional *in vivo* toxicity assay-based PODs differ by approximately 100-fold (median of range); however, the NAM-based PODs were found to be lower than *in vivo* PODs for 89% of chemicals evaluated (Paul-Friedman et al., 2020). Likewise, over the past decade, systematic comparisons of pathway-based (e.g., Gene Ontology or "GO"[11]) transcriptomic PODs to phenotypic health outcome PODs has illustrated that for most chemicals evaluated to date, the dose-response relationship between genotype and phenotype for toxic effects is typically within an order of magnitude (Johnson et al., 2020; Thomas et al., 2011, 2013). For many chemicals, bioactivity assays may also provide information on the potential to disrupt specific MIEs and KEs of known or postulated MOAs or AOPs and could potentially inform the relevance of specific pathways to humans. While *in vitro* assays can be informative, they are not without limitations. For example, to use the cell bioactivity data in the component-based mixture approaches discussed in this framework, the *in vitro* concentration-based metrics should be first converted to administered equivalent doses (AEDs) in humans. Converting *in vitro* bioactivity concentrations to estimated human *in vivo* doses (i.e., AEDs) requires the application of *in vitro*-to-*in vivo* extrapolation (IVIVE) and reverse toxicokinetics (rTK), which introduces additional uncertainty and might not be possible for many data-poor PFAS. In addition, several *in vitro* cell-based assays to date employ truncated nuclear receptors with only the ligand binding domain, and, as a consequence, the transcriptional events that follow binding may not be fully representative of quantitative chemical potencies compared to that seen with

[11] http://geneontology.org/docs/ontology-documentation/

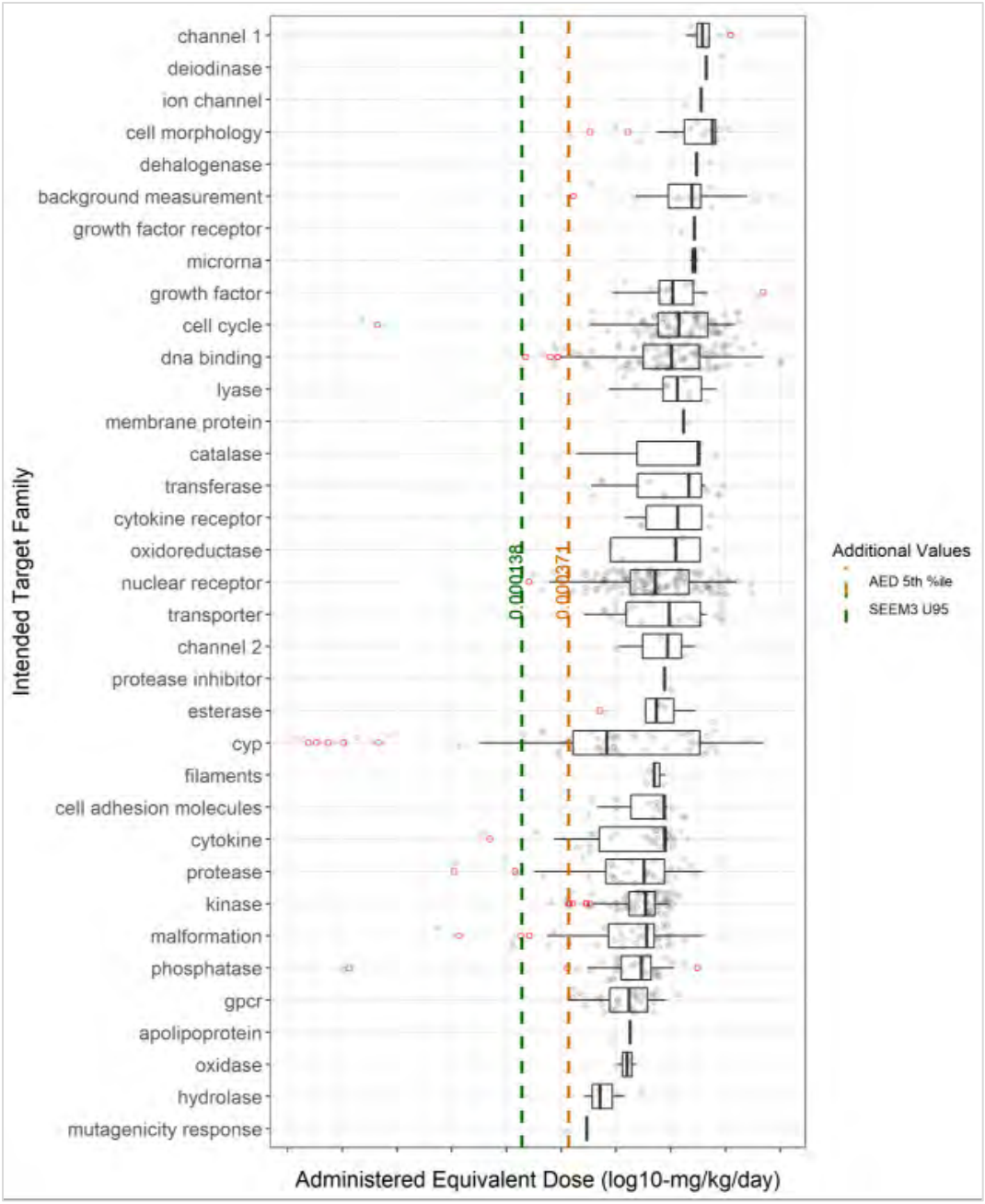


Figure 4-3. Example plot illustrating *in vitro* cell bioactivity expressed in AEDs. An AED is an estimated oral exposure dose that results in an internal steady-state concentration consistent with the *in vitro* concentration associated with a biological perturbation or activity. The example shows the distribution of AEDs; the vertical orange dashed line indicates the 5th percentile on the bioactivity landscape. The green dashed line corresponds to an upper bound on the estimated general population-based median exposure (generated from the EPA's Systematic Empirical Evaluation of Models (SEEM3): https://www.epa.gov/chemical-research/computational-toxicology-communities-practice-systematic-empirical-evaluation).

full-length receptors in native *in vivo* systems. Further, for such approaches to gain widespread regulatory acceptance, it will be important to demonstrate that the NAMs under consideration are reproducible, robust, and can be transferred to other laboratories and produce results that are relevant to *in vivo* adverse effects.

Step 3: Consider data landscape to select component-based approach(es).

Considering the data landscape across mixture component PFAS in Step 2 will help the user ascertain which option(s) in Step 4 is the most supportable. For example, dose-response data for PFAS mixture components may apply to more than one option in Step 4; however, characteristics of the data (e.g., shape or slope of dose-response between components) may not be similar, leading the user to select a M-BMD approach over the RPF approach. Another example entails dose-response data for components that do not indicate "same" health outcomes or MOA; in this case, it may be more practicable to use the available data in a general HI approach (assuming human health assessment values already exist or dose-response data support *de novo* derivation of assessment values). When using any of the component-based mixture approaches, it is optimal to calculate and use HEDs (for PODs, Edx, etc.) rather than orally administered doses in test animals where and when possible. The user is not precluded from applying available hazard and dose-response data across the entirety of the options in Step 4 when/where supported; however, study data types, design (e.g., exposure duration, route), and confidence will likely dictate the optimal selection of component-based approach for PFAS on a case-by-case basis. In most cases, a user would likely select only one of the approaches based on the data available for the PFAS components in a mixture of concern (e.g., if toxicity values and HBWCs are available or can be calculated, then the HI approach is appropriate). If, instead, toxicity values are not available or cannot be readily derived, then the RPF or M-BMD approach may be more suitable depending on characteristics and confidence in available dose-response data across mixture components.

Step 4: Perform component-based approach(es).

a. HI and TOSHI. For component PFAS that have human health risk assessment value(s), the user in Step 3 should have determined if the critical effect(s) for two or more PFAS fit into the same health effect domain (e.g., liver, thyroid, developmental). If not, then those PFAS are entered into a general HI approach (Section 5). For many PFAS, no formal health assessment exists; however, human epidemiological and/or experimental animal hazard and dose-response data may be available in the public domain. Should such data be available, the user has the option of performing *de novo* derivation of duration-specific noncancer toxicity values using assessment guidance and practices accepted under their specific purview. Again, if the *de novo*-derived values are not based on the same/similar effect, then the general HI approach is recommended. In brief, the HI approach entails the use of duration-relevant exposure (E) and toxicity values (e.g., RfV) for each component PFAS in a simple ratio (E/RfV) to calculate an HQ. The general HI involves the use of RfVs for each PFAS mixture component irrespective of the health outcome domain. Because each mixture component HQ is calculated using a corresponding RfV (protective of all effects), the mixture HI may represent a health-protective indicator of potential mixture risk. The component PFAS HQs are then summed to generate a mixture HI (see Equation 5-1). A mixture HI exceeding one (1) indicates potential concern for health risk(s) associated with a given environmental media

or site. The HI provides an indication of: (1) risk associated with the overall PFAS mixture; and (2) potential driver PFAS (i.e., those PFAS with high(er) HQs). Conversely, those PFAS with low(er) HQs (e.g., ≤ 0.0X) might be deprioritized as they may not have a significant impact(s) on overall mixture risk at the specific media concentrations identified. It should be noted that a user of this approach should consider the potential exposure (e.g., water concentration), the potency for toxic effect (e.g., low(er) or high(er) RfVs, PODs), the duration associated with exposure and toxicity, and qualitative and quantitative uncertainty (i.e., totality of UF application) for each PFAS mixture component.

If RfVs across PFAS mixture components are based on the same effect, then a TOSHI is recommended (see Section 5.3). The TOSHI approach is exactly as the name suggests; that is, it entails calculating component chemical HQs and corresponding mixture His for specific target-organ effects/endpoints *using only those mixture components with a reference value for the specified effect*. As such, while more consistent with the concept of toxicological similarity, a potential limitation of the TOSHI approach might entail the loss or exclusion of one or more mixture components for which a toxicity value for the specific health effect does not exist. A further advancement under the TOSHI is the development of target-organ toxicity doses (TTDs) (note: some TTDs could also be the overall RfD for a given PFAS). In practice, it is recommended to calculate TTDs for all health outcomes associated with component PFAS, where/when hazard and dose-response data support. This may facilitate the calculation of TOSHIs for more mixture component PFAS across more health outcomes, thus enriching the evaluation of PFAS co-occurring in a given environmental medium.

If data are available that indicate and support deviations from dose additivity (e.g., synergy or antagonism), an interactions-based HI may be employed (see USEPA, 2000b). However, in this framework, based on dose additivity, only the HI and TOSHI are included. Specifically, data to inform deviations from dose additivity (e.g., interactions such as synergism or antagonism) are virtually nonexistent for PFAS co-occurring in the mixture; as such, an interactions-based HI is not feasible at present.

b. RPF. In contrast to the HI, the RPF approach provides a PFAS mixture risk estimate for a selected health effect when specific media concentrations of the component PFAS are available (see Section 6). In the RPF approach, potency for an effect across each component PFAS is scaled to a selected IC for critical health effect domains of concern. In the illustrative example in Section 6.2, application of the RPF method is demonstrated for liver, thyroid, and developmental effects associated with the hypothetical five-component PFAS mixture. These three health effect domains were selected primarily because: (1) the effects are common across several PFAS assessed by the EPA (and ATSDR) thus far; and (2) each hypothetical example PFAS has differing levels of hazard and dose-response data available across the three health effect domains, to best illustrate demonstration of the RPF methodology. In practice, for application in a water context, each respective PFAS RPF is multiplied by its corresponding specific media concentration (e.g., water concentration), resulting in an Index Chemical Equivalent Concentration (ICEC). The ICECs across PFAS mixture components are summed to generate an overall mixture ICEC (see Equation 6-2), which is effectively a total

concentration of the IC, for each health effect domain. In traditional EPA mixtures risk assessment practice, the mixture ICEC is then mapped to the dose-response function of the IC to arrive at a "mixture response." In this framework, in the context of water, the mixture ICEC (i.e., the total dose of IC) is compared directly to an HBWC (e.g., Health Advisory, MCLG) based on the relevant health effect domain (e.g., liver, thyroid, developmental) for the IC. If the mixture ICEC for one or more of the selected effect domains exceeds the corresponding IC HBWC then there may be cause for concern for the mixture at the reported/measured component water concentrations. Conversely, health risk is not anticipated if the mixture ICECs for all effect domains are below the corresponding IC HBWC. Additionally, component PFAS with large(r) RPFs and corresponding ICECs should be flagged regardless of whether the total mixture ICEC is above or below an IC HBWC.

c. M-BMD. An additional option entails the calculation of an M-BMD (see Section 7) and is applicable even when PFAS in the mixture have dissimilarly shaped dose-response curves for the same or similar effect. In contrast to the RPF approach, there is no need for identification of mixture ICs, calculation of RPFs or ICECs, or existence of HBWCs. The final determination of risk is based on a comparison of the observed total mixture water concentration with an HBWC derived from the most sensitive effect-based M-BMD. Similar to the RPF approach, dose-response data across one or more health effect domains for each PFAS in the mixture are needed to determine the corresponding dose at the benchmark response (BMR) for each PFAS component (i.e., each component PFAS BMD). Then, the individual component chemical BMDs are scaled based on their proportion in the mixture and added using a simple dose-addition-based equation to arrive at a total M-BMD. The M-BMD approach does not require that component chemicals meet an assumption of similarly shaped dose-response functions (i.e., slopes). The resulting M-BMD (i.e., mixture POD) could be converted into a mixture RfD, using expert judgment in UF application, and subsequently incorporated into the calculation of a corresponding mixture-specific HBWC (e.g., Health Advisory or MCLG). However, it is cautioned that such values would be specific to a given mixture of PFAS at defined component proportions (e.g., individual PFAS water concentrations). Consistent with the RPF approach, Section 7.2 presents an illustrative example for liver, thyroid, and developmental effects associated with the hypothetical five component PFAS mixture. If the total measured PFAS mixture water concentration exceeds the mixture-specific HBWC, derived using the mixture toxicity value based on the M-BMD (and appropriate composite UF application), for one or more effect domains, then there may be cause for concern for the mixture at the reported/measured component water concentrations. If the total mixture concentration is below all mixture-specific HBWCs calculated, then a health risk is not anticipated.

4.2.2 Introduction to a Hypothetical Example with Five PFAS

In the following sections, the HI (Section 5), RPF (Section 6), and M-BMD (Section 7) approaches will be detailed and accompanied by a demonstration of practical application to a hypothetical five-component mixture of PFAS. As a reminder, PFAS 1–5 are:

PFAS 1 = comprehensively studied, most potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and an HBWC available;

PFAS 2 = well-studied, second most potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;

PFAS 3 = studied, least potent for effect(s) among PFAS 1–3, and has formal noncancer human health assessment value(s) and HBWC available;

PFAS 4 = *in vivo* animal toxicity data available but no formal human health assessment and no HBWC; and

PFAS 5 = data-poor; no *in vivo* animal toxicity data or human available.

To help introduce the illustrative case study, the hypothetical drinking water scenario is as follows: Periodic analysis of drinking water samples obtained at the tap across a community revealed the presence of five PFAS, referred to as PFAS 1–5 (median concentrations shown in Table 4-1), above the hypothetical analytical quantitation limits[12] (Table 4-2).

Problem formulation and scoping. For simplicity, the problem formulation is scoped to "What are the potential (noncancer) public health risks associated with exposure to the mixture of PFAS 1–5 in drinking water for the community?" A formal problem formulation and scoping exercise might include identifying population/community exposure details (e.g., distribution of sexes, ages, exposure frequency, exposure duration), seasonal variations in PFAS 1–5 levels in the drinking water, groundwater/surface water PFAS 1–5 concentrations, density of wells in the community, and other modifying circumstances or factors.

Table 4-1. Hypothetical drinking water concentrations for five hypothetical PFAS.

PFAS exposure estimates (measured in drinking water) (ng/L)					
	PFAS 1	**PFAS 2**	**PFAS 3**	**PFAS 4**	**PFAS 5**
Median	4.8	55	172	58	120

Note:
The values represent the median of a distribution of sampling data collected across a community over time.

Table 4-2. Hypothetical analytical quantitation limits for drinking water for five hypothetical PFAS.

PFAS analytical quantitation limits (ng/L)					
	PFAS 1	**PFAS 2**	**PFAS 3**	**PFAS 4**	**PFAS 5**
Analytical limit	3.0	5.0	5.0	4.0	5.0

Step 1: Assemble information.

The structured literature search for the hypothetical mixture of PFAS 1–5 included comprehensive Boolean search strings applied across information databases such as PubMed,

[12] Analytical quantitation limits for chemicals are generally defined as the lowest detectable concentration of an analyte where the accuracy achieves the objectives of the intended purpose. For example, the EPA's UCMR Program, the agency establishes "minimum reporting levels" to ensure consistency in the quality of the information reported to the agency. Under UCMR 5, an analytical quantitation limit is the minimum quantitation level that, with 95% confidence, can be achieved by capable analysts at 75% or more of the laboratories using a specified analytical method.

Web of Science, Toxline, and the Toxic Substances Control Act Test Submissions (TSCATS) (Figure 4-4). Please note that the specific PFAS names, synonyms, and Chemical Abstracts Service (CAS) registry numbers in Figure 4-4 are for illustrative purposes only. In an application, the search string(s) would need to be scoped and developed to optimize the literature search for component PFAS on a case-by-case basis.

The assembled literature inventory was then screened at the title and abstract level to determine preliminary relevance to informing human health risk assessment using defined Population, Exposure, Comparator, and Outcome (PECO) elements, as illustrated in Figure 4-5. Again, specific details provided in Figure 4-5 are for illustrative purposes only; mention of PFAS other than the hypothetical PFAS 1–5 should not be construed as the basis of the illustrative PFAS mixture example in subsequent sections.

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- Toxline (National Library of Medicine)

Search	Search strategy	Dates of search
PubMed		
Search terms	375-22-4[rn] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Kyselina heptafluormaselna"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "2,2,3,3,4,4,4-heptafluoro-Butanoic acid"[tw] OR "Butanoic acid, 2,2,3,3,4,4,4-heptafluoro-"[tw] OR "Butanoic acid, heptafluoro-"[tw] OR "Perfluoro-n-butanoic acid"[tw] OR "Perfluorobutanoate"[tw] OR "2,2,3,3,4,4,4-Heptafluorobutanoic acid"[tw] OR "Butyric acid, heptafluoro-"[tw] OR "Fluorad FC 23"[tw] OR "H 0024"[tw] OR "NSC 820"[tw] OR ((PFBA[tw] OR "FC 23"[tw] OR HFBA[tw]) AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw]))	No date limit

Figure 4-4. Hypothetical PFAS-specific literature search string applied to toxicity information databases such as PubMed, Web of Science, Toxline, and TSCATS.

PECO element	Description
Population	Human: Any population and lifestage (occupational or general population, including children and other sensitive populations). Animal: Nonhuman mammalian animal species (whole organism) of any lifestage (including fetal, early postnatal, adolescents and adults).
Exposures	Relevant forms: [chemical X] (CAS number) Other forms of [chemical X] that readily dissociate (e.g., list any salts, etc.) Metabolites of interest, including metabolites used to estimate exposures to [chemical X] Occupations that may be considered surrogates of exposure Human: Any exposure to [chemical X] [via [oral or inhalation] route[s] if applicable]. Studies will also be included if biomarkers of exposure are evaluated (e.g., measured chemical or metabolite levels in tissues or bodily fluids) but the exposure route is unclear or likely from multiple routes. Other exposure routes, such as those that are clearly dermal, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental material." Animal: Any exposure to [chemical X] via [oral or inhalation] route[s] of >1 day duration, or any duration assessing exposure during reproduction or development. Studies involving exposures to mixtures will be included only if they include an experimental arm with exposure to [chemical X] alone. Other exposure routes, including [dermal or injection], will be tracked during title and abstract as "potentially relevant supplemental material."
Comparators	Human: A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits), or exposure for shorter periods of time, or cases versus controls, or a repeated measures design. However, worker surveillance studies are considered to meet PECO criteria even if no statistical analyses using a referent group is presented. Case reports or case series of > 3 people will be considered to meet PECO criteria, while case reports describing findings in 1–3 people will be tracked as "potentially relevant supplemental material." Animal: A concurrent control group exposed to vehicle-only treatment and/or untreated control (control could be a baseline measurement, e.g., acute toxicity studies of mortality, or a repeated measure design).
Outcomes	All health outcomes (cancer and noncancer). In general, endpoints related to clinical diagnostic criteria, disease outcomes, biochemical, histopathological examination, or other apical/phenotypic outcomes are considered to meet PECO criteria.

Figure 4-5. Hypothetical PECO criteria and considerations used to determine study relevance in the systematic review and evaluation of a literature inventory for chemicals such as PFAS.

Following the removal of duplicate references and systematic screening of the initial inventory using the defined PECO, nonrelevant studies/reports were excluded, and the remaining references were full-text screened. The full-text screening resulted in three buckets of references: (1) studies or reports meeting PECO; (2) studies or reports tagged as supplemental (i.e., do not meet PECO criteria but are potentially relevant to the specific aims of the assessment); and (3) studies or reports that upon further review were excluded as not PECO-relevant (bottom of Figure 4-6).

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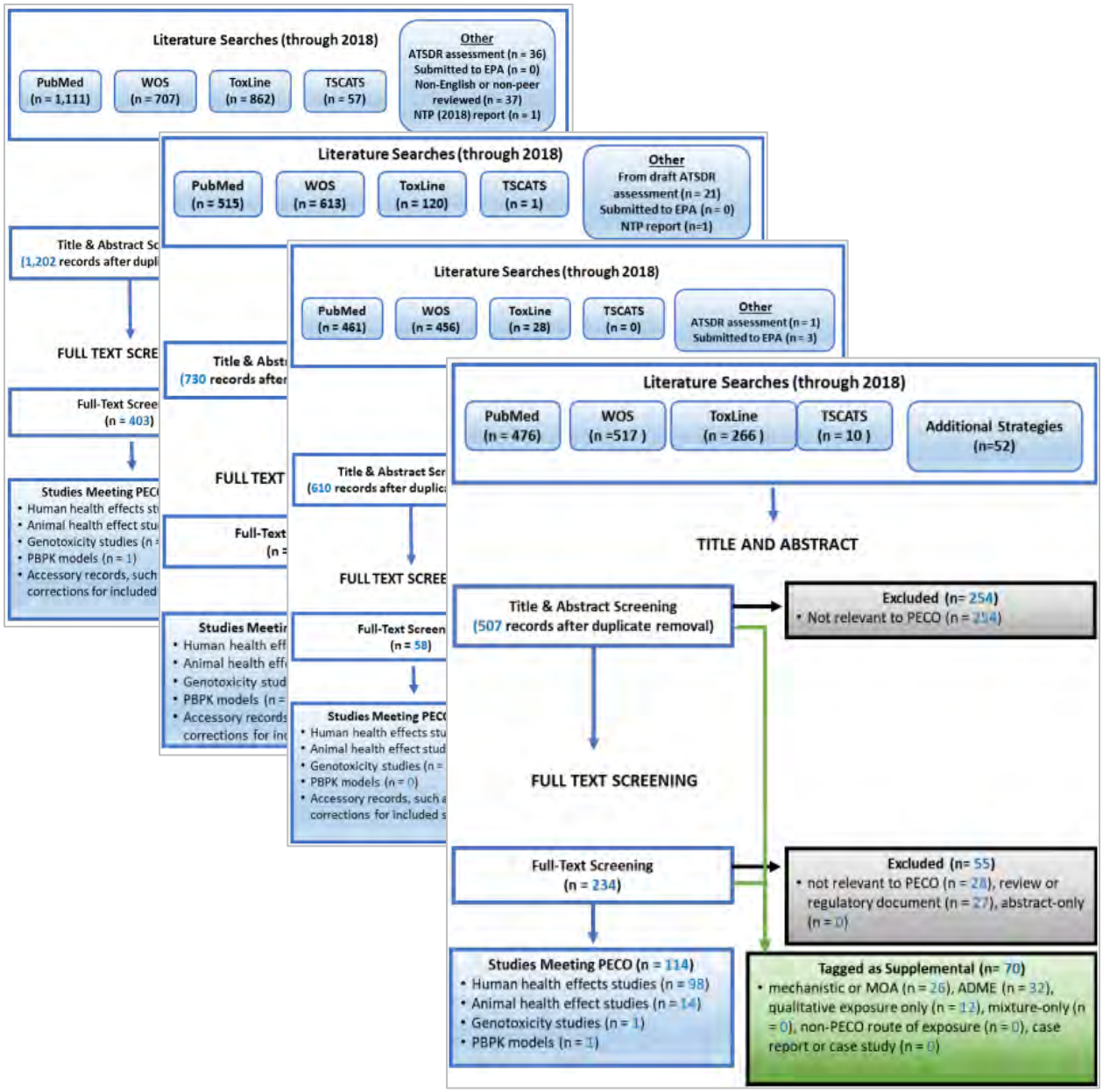


Figure 4-6. Example literature screening logic flow for hypothetical PFAS using an EPA systematic review approach. The figure depicts example PECO-dependent development of evidence bases to support human health assessment application(s). Note: Only four hypothetical PFAS (i.e., PFAS 1–4) are represented, as PFAS 5 is data-poor.

Step 2: Evaluate data objectives.

For the hypothetical example, the systematic literature search and screening resulted in studies meeting the PECO criteria for only PFAS 1–4. PFAS 5 was identified as data-poor (no *in vivo* animal toxicity data or human data available) and further interrogated in the EPA's Computational Toxicology Dashboard (https://comptox.epa.gov/dashboard/) for the presence of alternative toxicity testing data (e.g., *in vitro* cell bioactivity assay data). At the conclusion of the data gathering exercise for the five PFAS in Step 1 of the framework approach, and once all the

hazard and dose-response studies and data were assembled and evaluated, it was determined under Step 2 that: (1) there are no whole mixture/sufficiently similar mixture studies available for the combination of PFAS of concern; (2) PFAS 1–3 have existing human health assessment values for the oral route of exposure (Figure 4-7); (3) PFAS 4 has existing hazard and potentially useful dose-response data but no known health assessment value(s); and (4) PFAS 5, although data-poor, has predicted physicochemical property and empirical *in vitro* cell bioactivity data (identified from the systematic literature search/publications tagged as supplemental and/or searching of the EPA's Computational Toxicology Dashboard). In addition, across the five hypothetical PFAS, different levels/types of TK data were identified. Specifically, clearance[13] values for experimental rats and humans were located, for example, for PFAS 1–3. No clearance or volume of distribution (Vd)[14] values were identified for PFAS 4; only serum half-life data were harvested where available. For PFAS 5, only rat serum half-life data were located from publications tagged as supplemental during the literature search. This type of data is important because it may inform data-driven cross-species extrapolation of TKs between experimental animals and humans (i.e., kinetic adjustment of PODs and corresponding reduction of the animal-to-human uncertainty factor [UF_A]).

For brevity, in the evaluation of the hypothetical five-component PFAS mixture, the health effect domains are truncated to three targets: liver, thyroid, and developmental. Across these three target health effect domains, the studies meeting the PECO criteria were subjected to systematic review principles and practice (e.g., risk of bias analysis; evidence integration) across the available data sources/streams (Figure 4-8).

Step 3: Consider data landscape to select a component-based approach(es).

As illustrated in the hypothetical evidence heat map in Figure 4-8, PFAS 1, 2 and 4 have traditional experimental animal assay hazard and dose-response data available for component-based mixture assessment of liver effect(s); for thyroid effect(s), PFAS 1–3; and for developmental effect(s), PFAS 1–4. The landscape of duration-relevant dose-response data (e.g., chronic) is then interrogated for identification of the single best study/dataset for a critical effect to represent the PFAS in a given effect domain (Table 4-3). "Single best" study/dataset will be subjective and user-dependent; however, considerations such as the methodological strengths of the study(ies) (e.g., power of study/high N per treatment group, comprehensiveness and transparency of toxicity evaluation; statistics), effect level identification (e.g., are both a LOAEL and NOAEL identifiable?), and amenability to BMD modeling are just a few factors for which a study might be selected. For PFAS with an existing health assessment (e.g., in the hypothetical example, PFAS 1–3), the publishing authors have already made such decisions. In practice, if the user of this framework deviates from use of existing health assessment dose-response metrics, a clear rationale must be provided in the mixtures assessment. The dose-response data/metrics (e.g., PODs, dose-response curves) selected across component PFAS should be clearly presented. It should be noted that for NAM-based data (such as *in vitro* cell bioactivity in the hypothetical

[13] Clearance represents the combined intrinsic ability of organs and tissues to remove chemical(s) from the plasma and is commonly expressed in units of volume/time-body mass (e.g., L/day-kg body weight); it is typically calculated as the product of the elimination rate constant (k_e) × volume of distribution (Vd). An elimination rate constant represents the fraction of chemical eliminated from the body per unit of time, commonly expressed in units of hour(s) or day(s).

[14] The Vd represents the degree to which a chemical is distributed in body tissues. For example, chemicals highly bound to plasma proteins and not broadly distributed in tissues have a low Vd; conversely, chemicals with low affinity for plasma proteins typically have a high Vd and distribute broadly across tissues/compartments. Vd is commonly expressed in units of volume/body mass (e.g., L/kg).

PFAS mixture example), the "POD" should be clearly described consistent with the datastream from which it was derived and expressed in terms of an HED. This facilitates comparisons to other PODs (i.e., human epidemiological or experimental animal-based) for other mixture components.

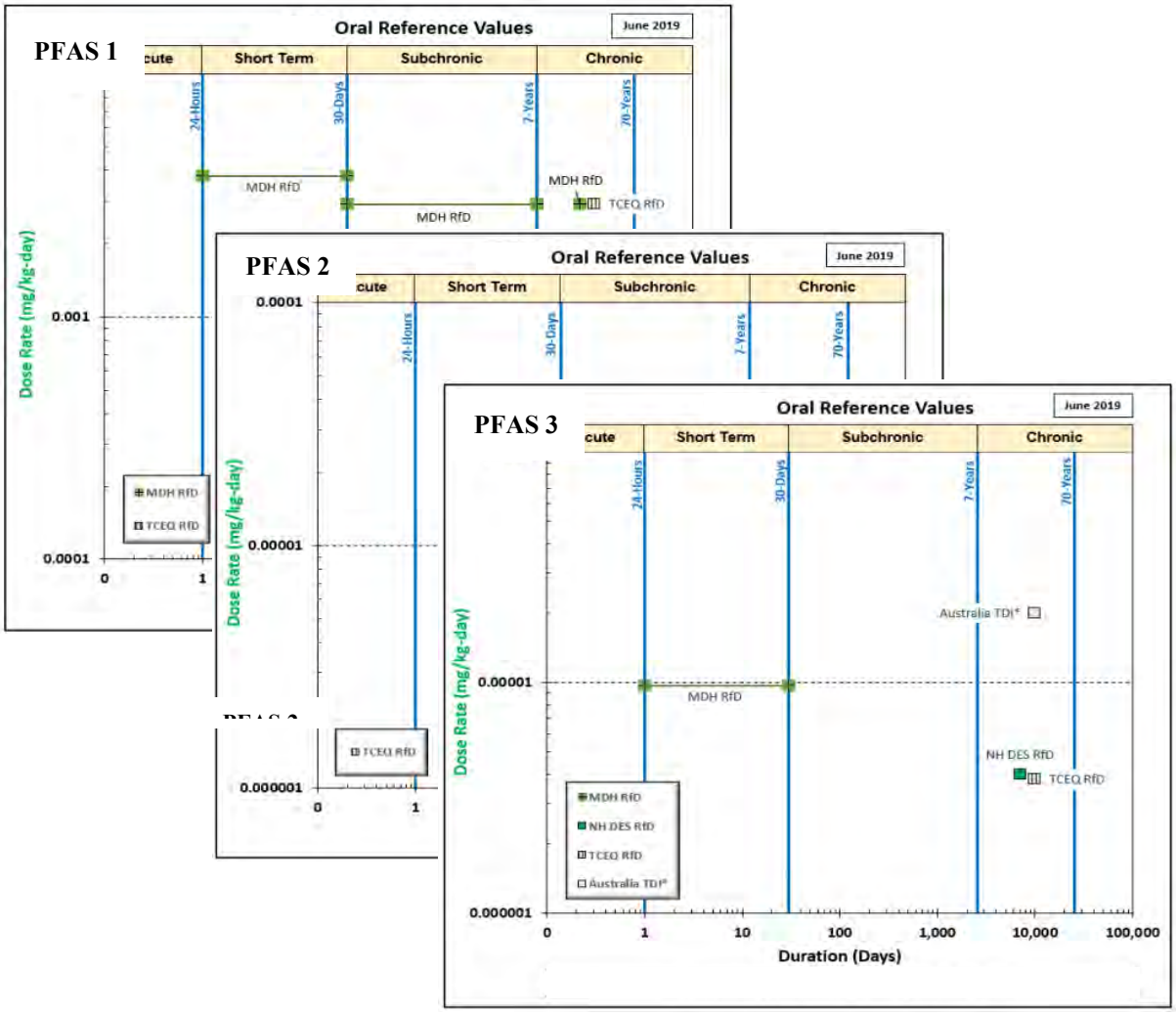


Figure 4-7. Example exposure-response arrays for the hypothetical example PFAS 1–3 identified as having existing human health risk assessment values for one or more exposure durations.

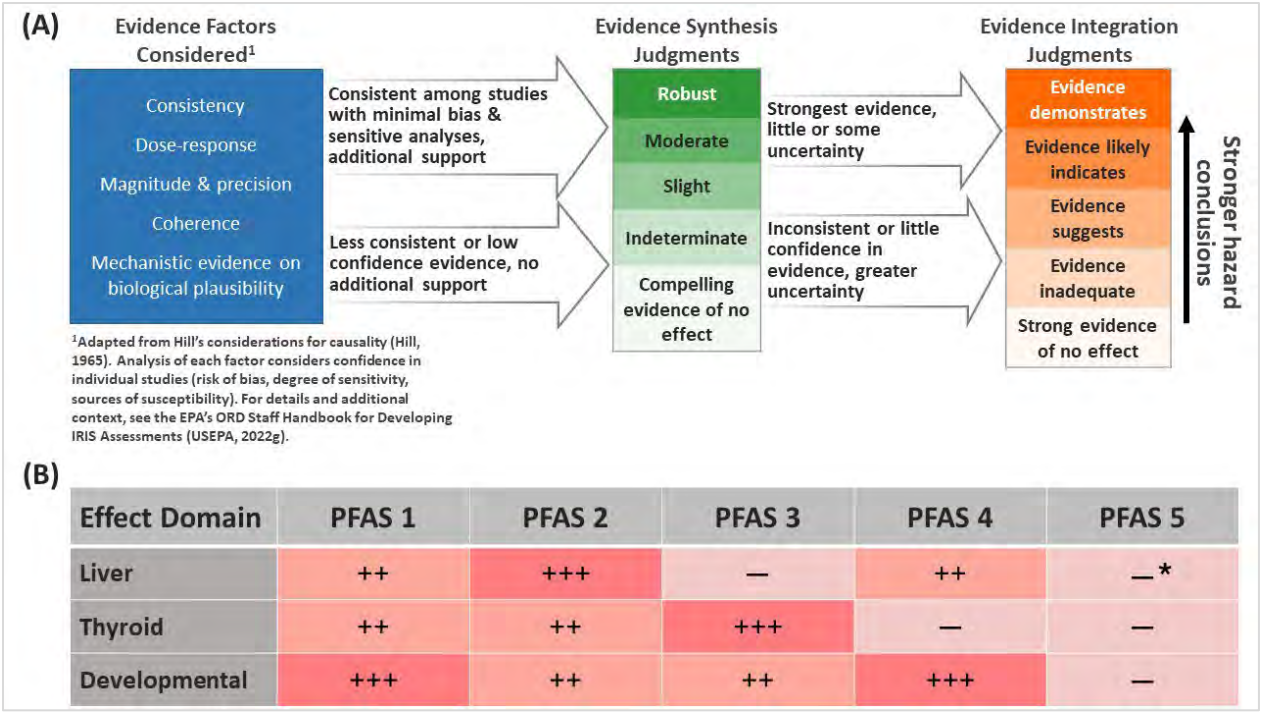
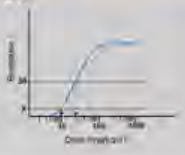
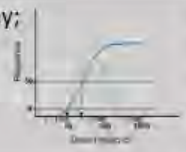
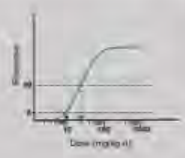
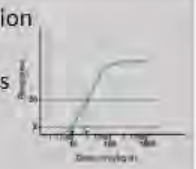


Figure 4-8. Evidence synthesis and integration across three target health effects domains for a mixture of five hypothetical PFAS. (A) Figure depicting evidence synthesis and integration considerations and judgments, based on USEPA (2022g). (B) The heat map indicates the strength of evidence supporting an effect of each hypothetical PFAS in each of three target health effects domains. (+++) evidence likely indicates an effect; (++) evidence suggests an effect; (—) evidence is inadequate to determine an effect. *Although PFAS 5 has no applicable human epidemiological or traditional experimental animal assay data available, *in vitro* cell bioactivity data are available from assays performed predominately in hepatocyte cell lines.

Table 4-3. Example data array to inform decisions in Steps 2 and 3 of the framework approach for component-based mixtures assessment of PFAS.

	Existent HHRA value (mg/kg-day)	Existent dose-response data for critical effect from principal study	Existent NAM data
PFAS 1	RfD: 3 E-8 POD_{HED}: 0.00001 UF_C: 300 Critical effect: Delayed growth and development in offspring (ABC et al. 2022)	S-D rat; single generation repro/dev study; Daily gavage GD 1-20 (ABC et al. 2022) 	–
PFAS 2	RfD: 1 E-5 POD_{HED}: 0.0013 UF_C: 100 Critical effect: Liver necrosis (DEF et al. 2022)	S-D rat; 2-year bioassay; DW ad libitum (DEF et al. 2022) 	Bioactivity profile in ToxCast; Biological perturbation at AED (2.8 E-4 mg/kg-day) = ↓ epoxide hydrolase in HepaRG cells; ↑ oxidative stress
PFAS 3	RfD: 7 E-4 POD_{HED}: 0.21 UF_C: 300 Critical effect: Decreased thyroid hormones (T4/T3) (GHI et al. 2022)	C57BL6 mouse; 90-day gavage; (GHI et al. 2022) 	–
PFAS 4	–	F344 Rat; two generation repro/dev study; Multiple dev outcomes in offspring; Feed ad libitum (JKL et al. 2022) 	–
PFAS 5	–	–	Bioactivity profile in ToxCast; Biological perturbation at AED (8 E-5 mg/kg-day) = ↓ epoxide hydrolase in HepaRG cells; ↑ oxidative stress

Notes:
AED = administered equivalent dose[15]; GD = gestational day; HHRA = human health risk assessment; POD_{HED} = human equivalent point of departure; RfD = oral reference dose; UF_C = composite uncertainty factor.

Step 4: Perform a component-based mixture assessment approach(es).

At this juncture in the data-driven framework approach, information has been assembled to facilitate application in Step 4. In practice, the user may choose to select one component-based mixture approach over others based on data evaluation/interpretation or apply data where appropriate to more than one of the approaches. For the purposes of demonstrating practical application using the hypothetical PFAS 1–5 mixture, all approaches (i.e., Step 4/bottom row of Figure 4-1) will be selected and demonstrated in the following sections.

[15] An AED is an estimated oral exposure dose that results in an internal steady-state concentration in humans consistent with the *in vitro* concentration associated with a biological perturbation or activity.

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5.0 Hazard Index Approach

5.1 Background on the HI Approach

The HI is the EPA's most commonly used component-based mixture risk assessment method. Because the HI employs a population-level human exposure and human health assessment value, such as an oral RfD, this ratio indicates potential health risk(s). The HI is based on an assumption of DA among the mixture components (USEPA, 2000b; Svendsgaard and Hertzberg, 1994). In the HI approach, an HQ is calculated as the ratio of human exposure I to a health-based RfV for each mixture component chemical (USEPA, 1986). The HI is unitless, so in the HI formula, E and the RfV must be in the same units (Equation 5-1). For example, if E is the oral intake rate (mg/kg-day), the RfV could be the RfD, which has the same units. Alternatively, the exposure metric can be a media-specific metric such as water concentration, and the toxicity value is best represented as a duration-specific HBWC such as an EPA lifetime drinking water Health Advisory (e.g., USEPA, 2022a, 2022b) or MCLG, or a similar value (e.g., developed by a state). In this case, the HQ is calculated as the ratio of water concentration (in mass/volume) to an HBWC (also in mass/volume). The component chemical HQs are then summed across the mixture to yield the HI, as illustrated in Equation 5-1.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i} \qquad \text{(Eqn. 5-1)}$$

Where:

- HI = Hazard Index
- HQ_i = Hazard Quotient for chemical i
- E_i = Exposure, i.e., dose (mg/kg-day) or media concentration, such as in drinking water (ng/L), for chemical i
- RfV_i = Reference value (e.g., oral RfD or MRL (mg/kg-day), or corresponding health-based, media-specific value; e.g., such as an HBWC, for example, a drinking water Health Advisory or MCLG for chemical i (ng/L)

Because the numerator of each component chemical HQ is the estimated population-level human exposure, the noncancer health RfVs used in the denominator must be based on human toxicity. These RfVs are derived either directly from human study-based PODs (or measured or modeled ED_X from exposure-response data in a cohort or population) or as human-equivalent PODs converted from experimental animal studies (e.g., conversion of a rodent POD to an HED (POD_{HED}) using cross-species TK-based modeling or allometric body-weight scaling).

The HI approach in practical application may be subdivided into a "general" HI and a "target-organ-specific" HI (i.e., TOSHI). In either case, following the logic flow in Figure 4-1 to the general HI or the TOSHI, they are both applied under an assumption of dose additivity. In the general HI, the RfV for each mixture component chemical is used in the calculation of an HQ, irrespective of the effect on which each component RfV is based (e.g., RfD for mixture chemical 1 may be based on liver effect, for chemical 2 thyroid effect, and chemical 3 developmental effect). The resultant HI is generally a health-protective indicator because the most sensitive health effects are often used as the basis for each respective chemical RfV and

corresponding HQ. Conversely, the TOSHI entails derivation of HQs for each mixture component chemical based on a toxicity value for the "same" effect, which may or may not be the most sensitive or potent effect across the landscape of identified hazards. For example, in the case of a liver-specific HI, for some mixture components, liver effect(s) may indeed be the basis for the RfD, whereas for other components, the liver might be among the least sensitive of effects.

In some cases, the liver may not be identified as a hazard for a given component chemical; for example, the available toxicity data may be insufficient or lacking to support the derivation of a toxicity value. To use this TOSHI approach more fully, organ-specific reference values (osRfVs) or target-organ toxicity doses (TTDs) are needed (note: these are the same type of noncancer values, just with different naming conventions) for each mixture component of potential concern. Under the TOSHI approach, for chemicals lacking hazard and dose-response data from traditional or NAM-based data streams for the selected health effect, it may not be possible to determine their potential contribution to joint toxicity of the mixture, which might result in an underestimation of the overall mixture risk.

An HI greater than one (1) is generally regarded as an indicator of potential adverse health risks associated with exposure to the mixture. An HI less than or equal to 1 is generally regarded as having no appreciable risk (recall that an RfV, such as an oral RfD, represents an estimate at which no appreciable risk of deleterious effects exists), typically requiring no further analysis (USEPA, 1986, 1991, 2000b). However, in some circumstances, the user may want to consider an HI less than 1, for example, for screening when multiple contaminants of concern are present at a site or one or more are present in multiple exposure media. In the case of PFAS, final peer-reviewed toxicity assessments are only available for a small proportion of the approximately 15,000 environmentally relevant PFAS (e.g., see the summary of the EPA and ATSDR PFAS assessments in Table 5-1). The EPA's primary source of peer-reviewed human health toxicity assessments is its IRIS program, but in some cases (e.g., when no IRIS assessment exists or there is a more current assessment from another authoritative source), the agency relies on assessments from other EPA program offices, and other state, national, and international programs. U.S. federal human health assessments, such as EPA's IRIS[16], PPRTV[17], the EPA Office of Water toxicity assessments[18], TSCA risk evaluations[19], and ATSDR's ToxProfiles[20], undergo rigorous peer and public review processes (note: PPRTV assessments do not include public review); as a result, they are considered to be of high scientific quality. The chronic RfDs for PFOA (USEPA, 2024a), PFOS (USEPA, 2024b), PFHxA (USEPA, 2023c), PFPrA (USEPA, 2023d), HQ-115 (USEPA, 2023e), PFBA (USEPA, 2022e), PFBS (USEPA, 2021a), and HFPO-DA (USEPA, 2021b) represent the only final EPA toxicity values for PFAS available at the time of drafting of this document. Several more PFAS assessments are under development in the EPA/ORD (e.g., PFHxS, PFNA, and PFDA; see Table 5-1 below) that can be considered in the future. Also, the use of this approach could consider other PFAS toxicity values (e.g., ATSDR MRLs).

[16] https://www.epa.gov/iris
[17] https://www.epa.gov/pprtv
[18] e.g., https://www.epa.gov/system/files/documents/2021-10/genx-chemicals-toxicity-assessment_tech-edited_oct-21-508.pdf
[19] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluations-existing-chemicals-under-tsca
[20] https://www.atsdr.cdc.gov/toxprofiledocs/index.html

Table 5-1. EPA and ATSDR peer-reviewed human health assessments containing noncancer toxicity values (RfDs or MRLs) for PFAS that are final or under development.

Chemical	EPA chronic oral RfD	ATSDR intermediate oral MRL[a]
PFOA	**Final 2024 RfD = 3×10^{-8} mg/kg/day** (USEPA, 2024a)	**Final 2021 MRL = 3×10^{-6} mg/kg/day** (ATSDR, 2021)
PFOS	**Final 2024 RfD = 1×10^{-7} mg/kg/day** (USEPA, 2024b)	**Final 2021 MRL = 2×10^{-6} mg/kg/day** (ATSDR, 2021)
PFNA	Under development in the EPA IRIS program	**Final 2021 MRL = 3×10^{-6} mg/kg/day** (ATSDR, 2021)
PFDA	***Draft*** **2023 RfD = 4×10^{-10} mg/kg/day** (USEPA, 2023f)	N/A
PFBA	**Final 2022 RfD = 1×10^{-3} mg/kg/day** (USEPA, 2022e)	N/A
PFBS	**Final 2021 RfD = 3×10^{-4} mg/kg/day** (USEPA, 2021a)	N/A
PFHxA	**Final 2023 RfD = 5×10^{-4} mg/kg/day** (USEPA, 2023c)	N/A
PFHxS	***Draft*** **2023 RfD = 4×10^{-10} mg/kg/day** (USEPA, 2023g)	**Final 2021 MRL = 2×10^{-5} mg/kg/day** (ATSDR, 2021)
HFPO-DA	**Final 2021 RfD = 3×10^{-6} mg/kg/day** (USEPA, 2021b)	N/A
PFPrA	**Final 2023 RfD = 5×10^{-4} mg/kg/day** (USEPA, 2023d)	N/A
HQ-115[b]	**Final 2023 RfD = 3×10^{-4} mg/kg/day** (USEPA, 2023e)	N/A

Notes: N/A = Not available.

[a] Note that MRLs and RfDs are not necessarily equivalent (e.g., intermediate duration MRL vs. chronic duration RfD; the EPA and ATSDR may apply different uncertainty/modifying factors) and are developed for different purposes.

[b] HQ-115 is the trade name for lithium bis[(trifluoromethyl)sulfonyl]azanide (CASRN 90076-65-6).

Some state health agencies publish toxicological assessments for PFAS that could potentially be used in HI calculations. For example, the Minnesota Department of Health publishes Toxicological Summaries that include the assessment of available toxicological information and subsequent development of oral toxicity values if adequate data are available (MN DOH, 2021). It should be noted that state or other (e.g., international) assessments may have varying levels of peer and public review and may reflect different risk assessment practices or policy choices as compared to the EPA or ATSDR assessments.

There may be scenarios where a final peer-reviewed toxicity assessment for one or more component chemicals in a mixture is not available. In these cases, evaluating available hazard and dose-response information for PFAS in the mixture may be necessary under an HI approach. For instance, there may be a need to develop toxicity value(s) to estimate potential risks associated with site-specific/localized contamination from a PFAS mixture with a component(s) that may not be relevant to other areas, sites, or exposure sources, and/or has not been prioritized

for assessment at the federal level. In such cases, the user of this framework might need to develop a targeted, fit-for-purpose assessment, if possible (i.e., based on the availability of hazard and dose-response data, resources, and expertise). Excluding component PFAS that lack off-the-shelf toxicity values from further analysis could result in an underestimation of the potential health risk(s) of the mixture. If *de novo* derivation of toxicity values is necessary, it is recommended that experts in hazard identification and dose-response assessment be consulted for scientific input and review, and the associated uncertainties (e.g., data gaps) be transparently characterized. The EPA has published several peer-reviewed documents that may assist in efforts to derive chronic (or subchronic) oral RfDs for chemicals with no available peer-reviewed toxicological assessment (for more information, see the EPA's Human Health Risk Assessment website at https://www.epa.gov/risk/human-health-risk-assessment).

To date, the majority of environmental chemicals, including PFAS, are data-poor, having no known or available information to inform hazard or dose-response in a screening/prioritization or assessment context. Considering that the number of legacy and new(er) chemicals present in commerce and the environment is in the tens of thousands, the generation of traditional animal toxicity data to support hazard identification and dose-response assessment would take decades and extraordinary numbers of animals and fiscal resources to complete. As human populations and biota are currently exposed to mixtures of chemicals such as PFAS, it is critical to identify methods, approaches, and platforms that can provide some reasonable context for potential human health hazard(s) and associated dose-response/potency for effects associated with exposure to multiples of PFAS (i.e., two or more co-occurring PFAS). A diverse set of resources has been developed over the past 15 + years that entails, in general, high(er)-throughput assays in cell culture (or cell-free) systems (e.g., transcriptomics; macromolecular/cellular bioactivity), *in silico* computational prediction models, alternative animal species (e.g., zebrafish), and refined short-term laboratory rodent assays and databases and platforms to collate and deliver such data to end-users. These methods, assays, and platforms are collectively referred to as NAMs. In the absence of traditional experimental animal bioassay and human epidemiological information, NAMs could potentially play a pivotal and transformational role in human health risk assessment, particularly in evaluating hazard and dose-response of PFAS that co-occur in mixtures.

Individually or in concert, NAMs such as *in vitro* cell bioactivity and *in silico* platforms (e.g., quantitative structure-activity models) might inform the identification or prediction of data that can be used in PFAS-specific hazard and dose-response assessment. For example, *in vitro* concentration-bioactivity data from resources such as ToxCast and Tox21 can be transformed into an estimated human *in vivo* exposure-response using IVIVE and rTK (Rotroff et al., 2010; Wambaugh et al., 2015; Wetmore et al., 2012, 2014). These administered human-equivalent dose datasets could potentially then be used to identify PODs (e.g., BMDs, NOAELs, LOAELs) and, with an expert-driven application of appropriate UFs, be leveraged into the derivation of corresponding noncancer toxicity values. These NAM-based toxicity values could then be converted into corresponding HBWCs and used, with exposure data, to calculate HQs for data-poor PFAS. Alternatively, particularly for RPF application, NAM-based dose-response data expressed in HEDs could be leveraged to calculate/model BMD values (e.g., $BMD_{X\text{-}AED}$) for expertly selected bioactivity (e.g., same/similar transcriptional pathway(s) and/or cellular bioactivity) to compare to that of a more data-informed member of the mixture with a similar

bioactivity profile (i.e., mixture IC). This might facilitate the derivation of RPFs for data-poor mixture components.

A critical consideration in using NAM-based hazard and concentration/dose-response data is recognizing that for some platforms or bioassays, perturbations of underlying biological pathways may not be readily identifiable as being directly related to specific apical toxic effects or even the hazard domain of interest. That is, chemical exposures may elicit a myriad of perturbations or responses at the molecular, macromolecular, or cellular level, with some alterations being critical or key to eliciting an apical toxic effect level response, whereas many other alterations may seemingly have no relationship to toxic effect(s) (e.g., general stress, housekeeping). However, the dose-response relationship associated with non-apical perturbations or effects (e.g., cell-based bioactivity) may be considered in a health effect agnostic context. Specifically, although there may not be clear qualitative linkages between non-apical biological perturbations and a specific, apical tissue- or organ-level effect, corresponding dose-response relationships for biological perturbations have been shown to provide a quantitative approximation for dose-response (e.g., POD) associated with traditional apical effects that are protective for the majority of chemicals evaluated (Paul-Friedman et al., 2020; Johnson et al., 2020; Thomas et al., 2011, 2013). The implication for use of NAM data such as *in vivo* or *in vitro* cell-based bioactivity or transcriptomics, for example, is that pathway- or cell function-based response levels (e.g., effect concentration 50 [EC_{50}], inhibitory concentration 50 [IC_{50}], or other biologically supported response levels of interest), could potentially be leveraged and applied in the mixture component approaches proposed in this chapter if the accuracy of the predicted PODs can demonstrated (e.g., HI, RPF, M-BMD).

In summary, considering the lengthy and resource-intensive processes and study protocols (e.g., OECD Test Guidelines-type studies) typically involved in generating traditional repeat-dose bioassay data for human health assessment of chemicals, incorporating NAMs could potentially serve an important role for PFAS screening and assessment, including in a mixture context. It is recognized that the practical application of NAMs in an assessment, whether for a single chemical or mixtures of chemicals, would be dependent on whether the results provide information that fits a decision context or purpose, and this may not be intuitive. It is recommended that experts in NAM data interpretation be consulted for potential integration into mixtures screening/assessment to contextualize the applicability of results appropriately and that they transparently communicate uncertainties associated with a given platform or assay output(s) in human health assessment.

5.2 Illustrative Example Application of the General HI to a Hypothetical Mixture of Five PFAS

As mentioned previously, final human health assessments with chronic oral RfDs exist for hypothetical PFAS 1–3. Based on the RfDs for PFAS 1–3 (see Table 4-3), PFAS 1 is a comprehensively studied chemical that is most potent for effect(s); PFAS 2 is also well-studied but is less potent than PFAS 1 for effect(s); and PFAS 3 has been studied and is even less potent than PFAS 1 or 2. PFAS 4 has experimental animal toxicity data available but no formal human health assessment. Finally, PFAS 5 is data-poor and was identified as having only bioactivity data available under Step 2 of the framework approach to inform hazard and dose-response (see Table 4-3). As PFAS 1–3 have existing human health assessment values, integration into the HI approach is simplified. However, for both PFAS 4 and 5, integration would necessitate *de novo*

calculation of noncancer health RfVs to develop component HQs and an overall PFAS mixture HI (Equation 5-1). For the purposes of the defined illustrative example for the hypothetical five-component PFAS mixture, this process is as follows:

5.2.1 General HI Step 1: Assemble/derive component chemical health effects-based values (e.g., Chronic oral RfDs)

PFAS 1–3: Upon review of the available information harvested in the literature search in Step 1 of the framework approach, formal human health assessments containing oral RfDs were identified (see Table 4-3). However, the critical effect on which each corresponding RfD was derived is in different effect domains: PFAS 1 critical effect = developmental effect in offspring; PFAS 2 critical effect = liver effect in adults; and PFAS 3 critical effect = thyroid hormone effect in adult females (in a repro/developmental life stage). As such, applying the general HI is optimal in this scenario and will entail using the overall RfD (or ATSDR MRL), regardless of the underlying critical health effect. If a subchronic RfD or an MRL is only available for an intermediate duration (akin to subchronic duration for EPA purposes), the user may consider the available evidence base. Additional uncertainty (e.g., subchronic-to-chronic duration) may be considered for extrapolation to a corresponding chronic duration value, unless subchronic/intermediate duration is the target.

PFAS 4: No federal, state, or other assessments with an RfV are available, but traditional hazard and dose-response (e.g., traditional experimental animal study) data were judged adequate to support derivation. Systematic review and evaluation of the animal study data led to the identification of a single best study (e.g., hypothetical 2-Gen repro/dev rat study; see Table 4-3) and multiple developmental health outcomes as candidate critical effects such as delayed growth and development at postnatal day 1 (PND 1) and decreased neonatal viability and thyroid hormone levels at PND 4. Thus, the user may choose to calculate an RfV using appropriate dose-response metrics (i.e., POD_{HED}) and the application of UFs. Appropriate characterization of hazard conclusions and qualitative and quantitative confidence and uncertainty(ies) in *de novo* derivation of RfVs for PFAS in this category is imperative. For the specific hypothetical PFAS example, the dose-response data associated with delayed growth and development in PND 1 rat offspring provided the most robust endpoint and confidence in dose-response for PFAS 4. Following BMD modeling (as per the EPA BMD guidelines [USEPA, 2012]), a lower statistical bound on a BMD (BMDL) for developmental effects of 1.06 mg/kg-day was calculated and used as the POD.

As the candidate POD for RfD derivation is identified from rats, available TK data for PFAS 4 in rats and humans should be considered for a data-informed adjustment approach for cross-species extrapolation (i.e., estimating the dosimetric adjustment factor [DAF]; Equation 5-2). In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (USEPA, 2011b), the EPA endorses a hierarchy of approaches to derive human equivalent oral exposures using data from laboratory animal species, with the preferred approach being physiologically based TK modeling. Other approaches might include using chemical-specific information without a complete physiologically based TK model. In the absence of chemical-specific models or data to inform the derivation of human equivalent oral exposures, the EPA endorses $BW^{3/4}$ as a default to extrapolate toxicologically equivalent doses of orally administered agents from laboratory animals to humans to derive an RfD under certain exposure conditions. In this illustrative hypothetical mixture example, it was determined that: (1) clearance values for

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experimental animals and humans were available and included in the dosimetric adjustment of PODs used in the derivation of noncancer human health assessment values for PFAS 1–3, and (2) kinetic data for PFAS 4 are sufficient to support a data-informed dosimetric adjustment of the rat POD. Briefly, while specific TK data needed to estimate clearance or volume of distribution in rodents or humans for PFAS 4 were not available, clearance values for humans and rats could be estimated, under the assumption that the volume of distribution in human females is equal to female adult rats (i.e., the PFAS-exposed unit leading to effects in PND 1 offspring), as follows:

Clearance = elimination rate constant (k_e) × volume of distribution (Vd)

Where k_e = (ln2 / plasma half-life) = (0.693 / plasma half-life), and Vd is assumed equivalent between female rats and humans.

Having made this assumption, the ratio of clearance values (Table 5-2) in human females to that in female rats, CL_H:CL_A, can be used to calculate the DAF, and the resulting HED can be calculated using Equation 5-2 as follows:

$$HED = POD \times \frac{CL_H}{CL_A} \quad \text{(Eqn. 5-2)}$$

Where:

POD = the rat $BMDL_{1SD}$ of 1.06 mg/kg-day

DAF = CL_H / CL_A

CL_A = 0.021 L/day-kg (female adult rat; the effect in offspring is a function of maternal intake)

CL_H = 0.000028 L/day-kg

The application of the hypothetical DAF to the rat POD results in a POD_{HED} of 0.0011 mg/kg-day. This POD_{HED} was then divided by a hypothetical composite UF of 100, resulting in an RfD for PFAS 4 = POD_{HED} / UF = 0.0011 mg/kg-day / 100 = 1 E−5 mg/kg-day.

PFAS 5: Because no final federal, state, or other RfD or MRL, or traditional hazard and dose-response data are available, NAM data streams could be surveyed and leveraged for PFAS information that might facilitate the development of a POD and, potentially, derivation of a NAM-based RfV using the application of UFs consistent with the data scenario (Judson et al., 2011; Parish et al., 2020). It is recommended that NAM data be systematically evaluated for suitability in supporting the derivation of RfVs using accepted approaches and practice. Unfortunately, no formal EPA technical guidelines currently exist to guide the approach for the use of NAM-based PODs in quantitative human health risk assessment applications. However, for the purposes of demonstrating the potential application of NAM data (e.g., *in vitro* cell bioactivity) in the hypothetical PFAS mixture evaluation, the general process within the context of this framework approach is as shown in Figure 5-1.

Table 5-2. Calculation of estimated clearance values for PFAS 4 in female rats and humans.

PFAS 4	Plasma half-life (hr)	Elimination rate constant (hr^{-1})	Volume of Distribution (L/day)*	Estimated Clearance (L/day-kg)
Female rats	33.6	0.021	1.0	0.021
Humans	24,528	0.000028	1.0	0.000028

Note:
* The value of 1.0 was used for volume of distribution (Vd) strictly for the purpose of calculating an estimated clearance value; the Vd of 1.0 is not based on empirical evidence for PFAS 4.

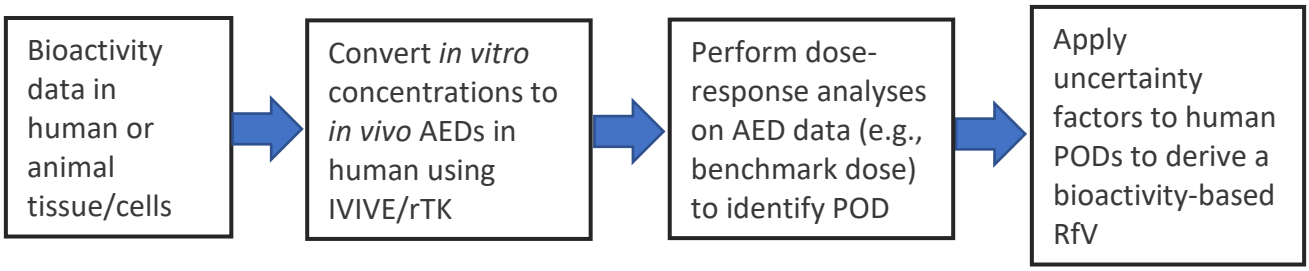


Figure 5-1. General steps to derive bioactivity-based RfV using bioactivity data in human or animal tissue/cells.

The detailed steps and mechanics of the bioactivity > IVIVE/rTK > AED process outlined in Figure 5-1 are beyond the scope of this framework document; the reader is referred instead to (Paul-Friedman et al., 2020; Wambaugh et al., 2018; Wetmore et al., 2012, 2014, 2015) for better context for the conversion of *in vitro* cell-based exposure concentrations to approximately equivalent human external exposure doses using IVIVE and rTK. In this hypothetical example, the AED identified as a NAM-based human POD for PFAS 5 is a BMD modeled off of the AED-based dose-response data for the decreased epoxide hydrolase endpoint in liver (HepaRG) cells; in practice, there are no *a priori* data-driven or default BMRs suggested for NAM data. BMR identification will be assay/platform/data-specific and should be contextualized by expert-driven analysis of the available data. As such, due to this flexibility in NAM-based data application, in the hypothetical PFAS mixture example, the $BMD_{X\text{-}AED}$ = 0.004 mg/kg-day. This human equivalent POD was then divided by a hypothetical composite UF of 100. The resulting RfD for PFAS 5 = 0.004 mg/kg-day / 100 = 4 E−5 mg/kg-day. Appropriate characterization and denoting of confidence and qualitative and quantitative uncertainty(ies) in the NAM data leveraged in POD identification and corresponding RfVs derived for PFAS in this category is imperative. Consultation with experts in the field of NAM data interpretation and risk assessment application is recommended for data-poor PFAS.

Summary of RfDs

In summary, as shown in Table 5-3, RfDs for PFAS 1–5 range from 10^{-4} to 10^{-8} mg/kg-day, with PFAS 1 being the most potent overall. Note that PFAS 4 and 5 have similar RfDs despite their different data limitations.

Table 5-3. Summary of POD_{HED}s and RfDs for hypothetical PFAS in a mixture.

	Liver POD_{HED} (mg/kg-day)	Thyroid POD_{HED} (mg/kg-day)	Develop-mental POD_{HED} (mg/kg-day)	RfD (mg/kg-day)	Basis
PFAS 1	0.044 ($BMDL_{X\text{-}HED}$)	0.24 ($BMDL_{Y\text{-}HED}$)	**0.00001 ($BMDL_{Z\text{-}HED}$)**	3 E−8	formal toxicity assessment
PFAS 2	**0.0013 ($BMDL_{X\text{-}HED}$)**	0.23 ($BMDL_{Y\text{-}HED}$)	0.0051 ($BMDL_{Z\text{-}HED}$)	1 E−5	formal toxicity assessment
PFAS 3	N/A	**0.21 ($BMDL_{Y\text{-}HED}$)**	2.1 ($BMDL_{Z\text{-}HED}$)	7 E−4	formal toxicity assessment
PFAS 4	50 ($BMDL_{X\text{-}HED}$)	N/A	**0.0011 ($BMDL_{Z\text{-}HED}$)**	1 E−5	high quality *in vivo* data
PFAS 5	**0.004 ($BMD_{X\text{-}AED}$)**[a]	N/A	N/A	4 E−5	bioactivity-based

Note:
Bold values indicate the lowest (most-sensitive) POD for the corresponding RfD derivation.
[a] Represents the NAM-based POD for *in vitro* cell bioactivity (e.g., for PFAS 5 = ↓ epoxide hydrolase activity).

5.2.2 General HI Step 2: Assemble/derive health-based media concentrations (HBWC)

Depending on the problem formulation, the user can either use the oral RfVs calculated for mixture components or leverage such values in the calculation of media-specific values, such as HBWCs for drinking water. Care should be taken to ensure that all HBWCs are applicable to the same exposure duration. In the following examples, the HBWCs are derived using chronic oral RfDs and, thus, are considered health protective values *over a lifetime* of exposure.

How to Calculate an HBWC for Drinking Water

The following equation is used to derive a noncancer HBWC. A noncancer HBWC, such as a lifetime HA or MCLG, is designed to be protective of noncancer effects over a lifetime of exposure, including for sensitive populations and life stages, and is typically based on data from chronic experimental animal toxicity and/or human epidemiological studies. The calculation of an HBWC includes an oral RfV such as an RfD (or chronic MRL or duration relevant user-provided value), body weight-based drinking water intake (DWI-BW), and a relative source contribution (RSC) factor as presented in Equation 5-3.

$$\text{Noncancer HBWC} = (\text{RfD}/(\text{DWI-BW})) * \text{RSC} \qquad \text{(Eqn. 5-3)}$$

Where:

RfD = chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime (see HI Step 1 above).

DWI-BW = the 90th percentile drinking water intake (DWI) for the selected population or life stage, adjusted for body weight (BW), in units of liters of water consumed per kilogram body weight per day (L/kg bw-day). The DWI-BW considers both direct and

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indirect consumption of drinking water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee).

RSC = relative source contribution—the percentage of the total exposure attributed to drinking water sources (USEPA, 2000a), with the remainder allocated to all other routes or sources.

When developing HBWCs, the goal is to protect all ages of the general population, including potentially sensitive populations or life stages such as children. The approach to select the DWI-BW and RSC for the HBWC includes a step to identify sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can make it difficult to identify the most sensitive population (e.g., not all windows or life stages of exposure or health outcomes may have been assessed in available studies), the critical effect and POD that form the basis for the RfD can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, infants, lactating women). In such cases, the user can select the corresponding DWI-BW for that sensitive population or life stage from the Exposure Factors Handbook (USEPA, 2019a) to derive the HBWC. In practice, when multiple populations or life stages are identified based on the critical study design and critical effect or other health effects data (from animal or human studies), the EPA selects the population or life stage with the greatest DWI-BW because it is the most health protective. This approach ensures that all populations and life stages are protected at the HBWC, and in the case of the HI approach, that each component HQ and the overall HI is protective of all populations and life stages. In the absence of information indicating a sensitive population or life stage (e.g., nondevelopmental critical effect as for PFAS 2 and 3 or NAM-based RfD as for PFAS 5), the DWI-BW corresponding to all ages of the general population may be selected (Table 5-4).

Table 5-4 shows the EPA exposure factors for DWI for some sensitive populations and life stages. Other populations or life stages may also be considered depending on the available information regarding study design and sensitivity to health effects after exposure to a contaminant.

To account for potential aggregate risk from exposures and exposure pathways other than oral ingestion of drinking water, the EPA applies an RSC when calculating HBWCs to ensure that total human exposure to a contaminant does not exceed the daily exposure associated with the RfD. When data are available for multiple sensitive populations or life stages, the most health-protective RSC is selected. The RSC represents the proportion of an individual's total exposure to a contaminant attributed to drinking water ingestion (directly or indirectly in beverages like coffee, tea, or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD is allocated to other potential exposure sources (USEPA, 2000a). The purpose of the RSC is to ensure that the level of a contaminant (e.g., HBWC), when combined with other identified potential sources of exposure for the population of concern, will not result in exposures that exceed the RfD (USEPA, 2000a).

Table 5-4. EPA exposure factors for drinking water intake.

Population or life stage	DWI-BW (L/kg bw-day)	Description of exposure metric	Source
General population	0.0338	90th percentile direct and indirect consumption of community water, consumer-only two-day average, all ages.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (USEPA, 2019a)
Infants	0.143	90th percentile direct and indirect consumption of community water, consumer-only two-day average, birth to < 1 year.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (USEPA, 2019a)
Children	0.0343	90th percentile direct and indirect consumption of community water, consumer-only two-day average, birth to < 21 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-21, NHANES 2005–2010 (USEPA, 2019a)[a]
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019a)
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019a)
Lactating women	0.0469	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[b] (USEPA, 2019a)

Notes:
CSFII = continuing survey of food intake by individuals; L/kg bw-day = liter per kilogram body weight per day.
[a] DWI-BWs are based on NHANES 2005−2010 data, also reported in the Exposure Factors Handbook. DWI-BWs for this population or life stage were calculated using the EPA's Food Commodity Intake Database, Commodity Consumption Calculator (https://fcid.foodrisk.org/percentiles).
[b] Estimates are less statistically reliable based on guidance published in NCHS (1993).

To determine the RSC, the EPA follows the Exposure Decision Tree for Defining Proposed RfD (or POD/UF) Apportionment in the EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000a). The EPA considers whether there are significant known or potential uses/sources other than drinking water, the adequacy of data and strength of evidence available for each relevant exposure medium and pathway, and whether adequate information on each source is available to quantitatively characterize the exposure profile. The RSC is developed to reflect the exposure to the general population or a sensitive population within the general population exposure.

Per the EPA's methods, in the absence of adequate data to quantitatively characterize exposure to a contaminant, the EPA typically recommends an RSC of 20%. When scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, thereby allocating the remaining 20% to other potential exposure sources (USEPA, 2000a). For the illustrative hypothetical PFAS mixture, an RSC of 0.2 (i.e., 20%) is selected as no information was identified to suggest a higher value. The calculation of HBWCs for PFAS 1–5 is presented in Table 5-5.

5.2.3 General HI Step 3: Select exposure estimates (measured water concentrations)

Select appropriate exposure estimates consistent with the problem formulation. Specifically, the user may choose to calculate or use exposure estimates for the oral route in general (i.e., total intake in mg/kg-day) or media-specific concentrations. In the hypothetical PFAS mixture example, "exposure" is represented by the drinking water monitoring data in Table 4-1.

5.2.4 General HI Step 4: Calculate PFAS mixture potency (component HQs and overall HI)

Using the median of the drinking water monitoring data (see Table 4-1) and the calculated HBWCs for PFAS 1–5 (see Table 5-5), individual component HQs are derived as shown in Table 5-6. Component HQs are expressed to two decimal places (hundredths place) and then summed across the PFAS mixture to yield the HI. The HI is rounded to one (1) significant digit.

Table 5-5. Calculation of hypothetical HBWCs for example PFAS in a mixture.

Chemical	Oral reference dose (mg/kg-day)	DWI-BW (L/kg-day)	RSC	HBWC (ng/L)
PFAS 1	3 E−8	0.0354	0.2	0.2
PFAS 2	1 E−5	0.0338	0.2	60
PFAS 3	7 E−4	0.0338	0.2	4,000
PFAS 4	1 E−5	0.0469	0.2	40
PFAS 5	4 E−5	0.0338	0.2	200

Table 5-6. Calculation of individual component HQs for the hypothetical PFAS mixture.

	Hypothetical drinking water exposure estimate (ng/L)	Hypothetical HBWC (ng/L)	Hypothetical general HQ
PFAS 1	4.8	0.2	24.00
PFAS 2	55	60	0.92
PFAS 3	172	4,000	0.04
PFAS 4	58	40	1.45
PFAS 5	120	200	0.60
Mixture general HI			**27.01 (rounded to 30)**

Note:
HQ is the DW exposure estimate / HBWC; HI is the sum of individual HQs.

5.2.5 General HI Step 5: Interpret the PFAS mixture HI

The HI (30) in the hypothetical example is significantly greater than 1, indicating potential health risks resulting from exposure to the mixture of PFAS at the measured drinking water concentrations. Further, as illustrated by the individual component HQs, PFAS 1 and 4 are risk drivers of the mixture HI with individual HQs greater than 1; PFAS 2 and 5 also appear to be contributors with HQs of 0.92 and 0.60, respectively. Assessment of PFAS 2 and 5 in isolation (individually) would indicate no/low health risk (i.e., individual HQs < 1.00), but the assessment of the binary mixture of PFAS 2 and 5 would indicate appreciable risk (HI = 1.52, rounded to 2). Conversely, with an HQ of 0.04, PFAS 3 is less influential than the other mixture components. In this hypothetical scenario, clearly PFAS 1 and 4, and potentially PFAS 2 and 5, might be prioritized for remediation activity(ies).

It should be noted that in the example PFAS mixture, the hypothetical HBWC for PFAS 1 (0.2 ng/L) is lower than its corresponding hypothetical drinking water analytical quantitation limit of 3 ng/L (see Table 4-2) by over an order of magnitude. In such cases, any detectable level (i.e., of PFAS 1) will result in an HI greater than 1 for the whole mixture.

5.3 Illustrative Example Application of the Target-Organ-Specific Hazard Index to a Hypothetical Mixture of Five PFAS

5.3.1 TOSHI Step 1: Assemble/derive component health effects endpoints (RfDs or target-organ toxicity doses)

Application of the TOSHI is essentially identical to the steps for the general HI. The critical nuance is that the use of human health/toxicity values across mixture components is effect/endpoint-specific. For some PFAS, this might be the overall RfD or MRL; for other PFAS, this may involve TTDs (i.e., an RfD for a specific health effect that may differ from the overall RfD for a given component chemical). In the TOSHI approach, there is a greater likelihood that TTDs have not been derived for effects other than the critical effect that underpins the derivation of an overall RfD for a given PFAS, although in some federal and state purviews, this practice is changing. In those instances where only an overall RfD (or ATSDR MRL) has been derived, TTDs could potentially be derived *de novo* for other health effect domains but should be accomplished with transparent characterization of qualitative and quantitative uncertainties

associated with hazard and dose-response data on a case-by-case basis. TTDs are derived identically to RfDs; however, there may be differing circumstances to consider such as type of POD (e.g., BMD vs. NOAEL or LOAEL), cross-species TK dosimetric adjustment (e.g., RfD may have been derived from a POD based on an adjustment of rat kinetics to human kinetics, whereas a TTD for the same chemical might be mouse to human resulting in a different POD_{HED}), and/or different qualitative and quantitative uncertainties. In practice, human health assessment applications, including mixtures assessment, may be more robust if TTDs are derived across all health outcome domains that are supported by evidence. For the purposes of the illustrative hypothetical PFAS mixture example, the calculation of TTDs is limited to the three selected health effect domains listed in Table 5-7. Several more TTDs could potentially be derived based on the availability of data and confidence in the evidence conclusions.

5.3.2 TOSHI Step 2: Assemble/derive health-based media concentrations (HBWC)

To calculate HBWCs for the TOSHI, the TTDs for a specific effect domain across mixture components are used in the calculation of HQs and a TOSHI. For example, a TOSHI for developmental effects ($TOSHI_{DEV}$) for the hypothetical PFAS mixture can be calculated using the developmental TTDs, appropriate DWI-BWs, and RSCs (Table 5-8). Each DWI-BW is the 90th percentile direct and indirect consumption of community water, consumer-only two-day average and was selected based on sensitive populations or life stages as identified by evaluating of each of the critical studies, including the exposure intervals. For this hypothetical example, the DWI-BW for PFAS 1, 2, and 3 is for women of childbearing age (13 to < 50 years), and the DWI-BW for PFAS 4 is for lactating women (see Table 5-4).

Table 5-7. Hypothetical TTDs for the hypothetical component PFAS; the bolded numbers represent the overall RfD for each respective PFAS.

Target Organ Toxicity Doses (mg/kg-day)					
Effect domain	PFAS 1	PFAS 2	PFAS 3	PFAS 4	PFAS 5
Liver	7 E-6	**1 E-5**	–	5 E-5	4 E-5*
Thyroid	2 E-6	4 E-4	**7 E-4**	–	–
Developmental	**3 E-8**	9 E-3	2 E-3	**1 E-5**	–

Note:
* TTD_{NAM} based on *in vitro* perturbation indicative of oxidative stress in liver cells.

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Table 5-8. Calculation of hypothetical developmental effect-specific HBWCs for hypothetical PFAS in a mixture using TTDs.

Chemical	Target-organ toxicity dose (mg/kg-day)	DWI-BW (L/kg-day)	RSC	$TOSHI_{DEV}$ HBWC (ng/L)
PFAS 1	**3 E−8**	0.0354	0.2	0.2
PFAS 2	9 E−3	0.0354	0.2	50,000
PFAS 3	2 E−3	0.0354	0.2	10,000
PFAS 4	**1 E−5**	0.0469	0.2	40
PFAS 5	--	N/A	N/A	ND

Notes:
N/A = not applicable; ND = not determined.
Bolded numbers indicate that the TTD for developmental effects is the overall RfD for that PFAS.

5.3.3 TOSHI Step 3: Select exposure estimates (measured water concentrations)

Select appropriate exposure estimates consistent with the problem formulation. Specifically, the user may choose to calculate or use exposure estimates for the oral route in general (i.e., total intake in mg/kg-day) or media-specific concentrations. In the hypothetical PFAS mixture example, "exposure" is represented by the drinking water monitoring data in Table 4-1.

5.3.4 TOSHI Step 4: Calculate PFAS mixture potency (component HQs and overall TOSHI)

Using the median of the drinking water monitoring data (see Table 4-1) and the calculated HBWCs for PFAS 1–4 derived from TTDs for the developmental effect domain (see Table 5-8), individual component HQs are derived as shown in Table 5-9.

Table 5-9. Calculation of hypothetical individual component HQs specifically for developmental effects associated with the hypothetical PFAS mixture.

Chemical	Hypothetical drinking water exposure estimate (ng/L)	Hypothetical $TOSHI_{DEV}$ HBWC (ng/L)	Hypothetical $TOSHI_{DEV}$ HQ
PFAS 1	4.8	0.2	24.00
PFAS 2	55	50,000	0.0011
PFAS 3	172	10,000	0.02
PFAS 4	58	40	1.45
PFAS 5	120	ND	ND
Mixture $TOSHI_{DEV}$			**25.47 (rounded to 30)**

Notes:
HQ is the DW exposure estimate / HBWC; HI is the sum of individual HQs; ND = not determined.
The HBWCs in this TOSHI application are derived from TTDs for the developmental effect domain.

5.3.5 TOSHI Step 5: Interpret the PFAS mixture HI

The $TOSHI_{DEV}$ of 30 in the hypothetical example indicates concern for developmental effects associated with exposure to the hypothetical PFAS mixture at the measured drinking water concentrations (see Table 4-1). While this example application shows that use of TTDs did not meaningfully diminish indication of health risk associated with the mixture (compared to a general HI approach), the individual HQs clearly demonstrate drivers (PFAS 1 and 4) and relative inerts (PFAS 2 and 3) for developmental health outcomes. The converse is possible dependent on the TTDs for different health outcomes, and differing PFAS concentrations in environmental media.

5.4 Advantages and Challenges of the General HI and TOSHI Approaches

The general HI approach provides an indication of the joint toxicity associated with co-occurrence of PFAS in environmental media, such as drinking water. One advantage of the HI formula in risk communication is that the interpretation of the results is relatively straightforward. The simplicity of the method is in taking a ratio of the exposure to hazard to indicate potential concern for a mixture of PFAS and providing an alert to specific PFAS that may be potential drivers in risk to human health (i.e., those PFAS for which the HQs have greater contribution to an HI > 1, relative to other PFAS mixture components).

Another advantage is that the "hazard" does not necessarily have to be the same for general HI (e.g., all liver or all kidney effects). Specifically, the general HI approach can be used where the individual HQ calculated for each mixture component PFAS is based on the most well-characterized, and often the most sensitive, toxic effect and corresponding noncancer RfV (e.g., oral RfD). As such, a general HI will typically represent the most health-protective indicator of mixture risk, as each component HQ is based on each mixture component's overall RfV.

Alternatively, in a TOSHI, toxicity values are aggregated by the "same" target organ endpoint/effect, and HQ (and HI) values are developed for each effect domain independently (e.g., liver-specific HI, thyroid-specific HI). Although more closely aligned to the concept of DA, the disadvantage of a TOSHI is that it can only be performed for those PFAS for which a health effect-specific RfD (e.g., TTD) is calculated. For example, for some PFAS, a given health effect might be poorly characterized or not studied at all, or, as a function of dose, may be one of the less(er) potent effects in the profile of toxicity. Another limitation is that so many PFAS lack human epidemiological or experimental animal hazard and dose-response information across a broad(er) health effect range, thus limiting the potential scope or landscape of derived TTD values. As with the general HI, a TOSHI approach might benefit from consideration of NAM data and approaches that can inform organ/tissue-specific dose-response.

The HI is an indication of appreciable risk, not an estimate of the concentration of the mixture in water that may result in adverse health outcomes after a specific period of exposure. Comparisons of HI estimates across different exposure routes/scenarios (e.g., oral versus inhalation; comparing drinking water His to soil ingestion His) can be misleading and challenging to interpret. Because the HI is based on DA, it implies that if two exposure scenarios involve the same chemicals and their HI values are the same, then with other factors being equal (e.g., exposure frequency and duration, similar health endpoints, and similar life stage), the two exposure scenarios could be judged to have the same potential for causing toxic effects. That interpretation has the strongest scientific foundation when there are only minor differences in the

component exposures (thus, same exposure route, same chemicals, and similar exposure duration for specific receptors) between the two scenarios. In addition, the magnitude of an HI, TOSHI, or an individual component HQ, should not be directly interpreted as a quantitative estimate of increased level of concern. For example, a mixture HI of 20 is not necessarily of 10-fold greater concern than a mixture HI of 2. The practical interpretation is that both mixture His would indicate an appreciable risk of health effects in exposed populations.

Another challenge of the application of the general HI and TOSHI approaches to specific media such as water is that it requires derivation of a health-based, media-specific concentration like a drinking water Health Advisory or MCLG, in addition to the underlying oral RfV (e.g., RfD, TTD). Development of these values typically requires significant expertise and resources often on a longer timeframe (i.e., years). In addition, while a formal hierarchy of preferred human health reference/toxicity values is not being proposed in this framework, there is a recognized gradation of confidence across possible PFAS values that might exist or could be derived. Specifically, it would clearly be preferable to use RfVs obtained from assessment sources that use comprehensive and transparent systematic approaches and standardized protocols. The level of confidence or certainty in such values would be greater and associated with lower levels of qualitative and quantitative uncertainty than other values.

What might be perceived as a challenge for PFAS human health assessment in general could be an opportunity to advance risk assessment science and practice. Specifically, in the case of NAMs, dose-response metrics obtained from bioactivity-based assays/platforms (or read-across) may be assigned some level of *a priori* uncertainty simply because of a lack of confidence by end-users in the interpretation and risk assessment application of such data and outputs. As mentioned previously in this framework, NAMs may represent the only opportunity to integrate a data-poor PFAS into mixtures assessment. Further, while the integration of NAMs into applications such as mixtures risk assessment was demonstrated in the hypothetical example using a POD from a specific assay type (*in vitro* cell bioactivity), available NAM data could be leveraged from a diverse assay or platform portfolio. For example, transcriptomic data from whole animals or cells *in vitro* using platforms such as BioSpyder (i.e., TempO-Seq; see https://www.biospyder.com/), microarrays, and/or RT-PCR may represent additional opportunities to integrate validated methods and data into assessment application.

A potential future improvement using NAMs such as cell bioactivity (including transcriptomics) may be the categorical integration of qualitative and quantitative information from across platforms to develop more comprehensive NAM-based hazard determinations and identification of candidate PODs (consensus lower bound BMD, cross-NAM platform mean, etc.). The end-user of this framework, in consultation with experts/practitioners in NAM development and application, would be advised to leverage NAMs when and where possible while always characterizing and transparently communicating qualitative and quantitative uncertainty(ies) along the continuum from data generation and fit-for-purpose application (Parish et al., 2020) to POD identification, RfV derivation, and subsequent HQ and HI calculations. The disadvantage of not using NAM data and approaches when applicable to a given PFAS mixture is that data-poor PFAS would not be accounted for in the HI, thus potentially underestimating mixture hazard.

In summary, in scenarios where a diverse amalgamation of different types of RfVs (i.e., deriving from different assessment sources and/or data types) are used in the calculation of HQs and His,

the respective confidence and qualitative uncertainty characterizations for each PFAS need to be transparently communicated in overall mixture hazard interpretations.

6.0 Relative Potency Factor Approach

6.1 Background on RPF Approach

RPF approaches comprise another basic dose-addition method used most commonly by the EPA in mixtures assessment. There are two key types of the RPF approach: (1) the general RPF approach that has been applied to pesticides, disinfection by-products (Simmons et al., 2004), and a few other chemical groups such as polycyclic aromatic hydrocarbons and (2) the TEF approach that was originally developed for mixtures of dioxins and DLCs. The TEF approach is considered a special case of the RPF approach wherein mixture components are known to act via an identical MOA (e.g., dioxins and DLCs and AhR activation).

For chemicals demonstrated to act via a similar MOA, or in the case of this framework, those shown to induce the same/similar health effect (see Section 3 for discussion and justification), an RPF represents the relative difference in potency between a mixture IC and other members of the mixture. The IC does not necessarily have to be the most potent member of a given mixture. Rather, an IC is typically selected because it has the highest quality and most robust toxicological database and is considered to be most representative of the type of toxicity caused by the mixture components (USEPA, 1986, 2000b). The role of the IC in the RPF approach is to serve as the point of reference for standardizing the common toxicity (i.e., scaling the potencies) of all component chemicals in the analysis. The most important consideration in selecting one mixture component over another as an IC is that *high*-quality dose-response data are available (e.g., for the common toxic effect/species/sex) for the exposure route, duration, and pathways of interest.

Further, the IC must have dose-response data for the dose range of interest; chemicals with steep slopes that cause an effect and/or induce significant toxicity at all doses tested are not ideal for IC selection. In most cases, the identification of a single best mixture component IC will be evident. However, in the event that two or more mixture components are identified as candidate ICs, the user must judge which candidate is most representative of the mixture, or subgroupings within a mixture, and has the most robust toxicity database. It should be noted that the selection of an IC can be duration-, exposure route-, and/or health outcome-specific. That is, in practical application, it is possible that different mixture components may be optimal ICs under different scenarios. For example, mixture components A and B may both be identified as candidate Ics in general; however, candidate A may be selected as the IC if it has a more robust evidence base for a specific application of interest (e.g., oral/subchronic duration). In the RPF approach, the assumption under dose additivity is that the toxicity of each mixture component chemical induces effects via a similar pathway of biological perturbation and can operationally be considered a fixed concentration or dilution of the IC (USEPA, 2000b). Mathematically, when using response-specific doses, the RPF is the ratio of the IC to that of each individual mixture component chemical (j) at a common point on the corresponding dose-response curves (e.g., human equivalent LOAELs, BMDs, or ED_X). Ideally, the dose-response functions used to calculate RPFs across mixture components would be approximately the same in exposure duration and study design (e.g., sex, species, life stage).

Further, considering the known differences in TK characteristics across PFAS (e.g., internal plasma half-life) between rodents, nonhuman primates, and humans, it is advisable to convert experimental animal dose-response data to human equivalents where possible before calculating

RPFs. Lastly, of the options for dose-response metrics to use in calculating RPFs across component PFAS, BMDs (e.g., the central tendency estimate) would be optimal. BMDs incorporate the totality of a given dose-response and facilitate the identification of a dose at a predefined BMR level (e.g., 0.5 standard deviation (SD) or 1 SD over control; 10% change in some effect/endpoint). BMD modeling would optimize the comparison of "same" as a function of dose across component PFAS for a given health effect or endpoint. It is recognized that dose-response data for chemicals are sometimes not amenable to BMD modeling. Isoeffective human equivalent LOAELs or ED_X values are suitable alternatives. No matter which dose-response metric is used, the RPF for the IC is always one. The potency ratio can be calculated for each mixture component chemical (j) as the ratio of the effect doses as shown in Equation 6-1:

$$RPF_j = \frac{EDx_{IC}}{EDx_j} \quad \text{(Eqn. 6-1)}$$

where IC refers to the index chemical.

For example, if mixture component chemical 2 is twice as potent as the IC, its LOAEL, BMD_X, or ED_X will be half as large, and the calculated RPF would be a 2. Conversely, if mixture component chemical 2 is half as potent as the IC, its LOAEL, BMD_X, or ED_X will be twice as large, and the RPF would be 0.5. In practice, the EPA determines a single RPF for the response range or dose range of interest. When data are available, RPFs can potentially be determined for more than one health effect domain and/or exposure scenario (e.g., developmental versus thyroid toxicity, shorter-term vs. chronic exposure, oral vs. inhalation exposure). As illustrated in the RPF examples in the next section, that flexibility or scenario specificity is an advantage of the general RPF approach. Once RPFs are calculated for each mixture component chemical using a common metric in Equation 6-1, ICECs are then calculated by multiplying each respective RPF_j by the corresponding component chemical's concentration (d_j), as shown in Equation 6-2:

$$ICEC_{MIX} = \sum_{j=1}^{n} d_j * RPF_j \quad \text{(Eqn. 6-2)}$$

The total mixture ICEC ($ICEC_{MIX}$) is then obtained by taking the sum of the component chemical ICECs (including that of the IC) (Equation 6-3). A numerical estimate of risk for noncancer health effects associated with exposure to the mixture of concern is then obtained by mapping the $ICEC_{MIX}$ onto the dose-response function for the IC. For example, if the IC's dose-response model is denoted f(d), then the RPF-based response to the mixture is estimated as:

$$y_{MIX} = f(ICEC_{MIX}) \quad \text{(Eqn. 6-3)}$$

where the ICEC is derived from Equation 6-3. In the context of this PFAS mixture framework, there are important modifications or adaptations of this approach to note that include: (1) use of ICECs, which are water-specific, correlates to index chemical equivalent doses (ICEDs) (USEPA, 2000b) and (2) using effect-specific HBWCs for the IC as a benchmark point to compare a mixture ICEC to rather than directly mapping the mixture ICEC onto the IC dose-response. This serves the purpose of providing the end-user a basic indication of "yes," there is potential effect-specific risk associated with the mixture (e.g., $ICEC_{MIX} \geq$ IC HBWC), or "no," there is no anticipated effect-specific risk (e.g., $ICEC_{MIX} \leq$ IC HBWC), as well as the magnitude of health effect concern and identification of potential component chemical drivers of an ICEC.

The EPA's supplementary guidelines (USEPA, 2000b) state: "The common mode-of-action assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for the application of RPFs is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. To date, the EPA has developed RPFs for only a few chemical groups, largely pesticides (organophosphorus pesticides, triazines, N-methyl carbamates, chloroacetanilides, and pyrethrins/pyrethroids), which in each case were based on MOA-level information (USEPA, 2018). However, MOA data are limited or not available for many PFAS. As such, in the interim, when using the RPF approach, it is advisable to focus the biological level of organization for component-based evaluation of potential mixtures additivity for PFAS on similarity in toxicity endpoint/effect. Further, as empirically demonstrated by Conley et al. (2022b, 2023), due to the potential variability of potency for health effects across PFAS, RPFs can vary by more than an order of magnitude. Thus, where possible, it is preferable for a given PFAS mixture to evaluate multiple common effect domains or endpoints, where and when dose-response data are available, to identify the most sensitive endpoint for evaluation of risk. Using the most sensitive endpoint(s) for the RPF analysis helps to ensure that risks are not underestimated, and providing a landscape of candidate RPFs across PFAS and health effects ensures transparent communication of mixtures risk assessment for decision-making. This approach is taken in the illustrative RPF examples below; it is consistent with previous NAS recommendations for evaluating chemicals that cause common adverse health outcomes, presumably through diverse biological pathways (NRC, 2008).

6.2 Illustrative Example Application of RPF to a Hypothetical Mixture of Five PFAS

The example application of the RPF approach incorporates hazard and dose-response information for the hypothetical five PFAS mixture presented in the HI sections above. However, in this context only dose-response data for like/similar health effect(s) are needed. Recall that PFAS 1–3 have existing hazard and dose-response data that have been formally evaluated for human health risk assessment purposes; these three PFAS also have existing HBWCs. PFAS 4 has not undergone risk assessment but has existing experimental animal assay data. Lastly, PFAS 5 is data-poor with only physicochemical, TK, and *in vitro* cell-based bioactivity data. This hypothetical example focuses on the development of RPFs for liver, thyroid, and developmental effects only (Figure 4-8), which have been reported as toxicity targets of several compounds within the broader class of PFAS (ATSDR, 2021; EFSA et al., 2020; Section 7.1 in ITRC, 2022; USEPA, 2021a, 2021b). The approach here is to use a construct that allows for a combination of PFAS with a shared, common health outcome (e.g., delayed growth and development in offspring), as opposed to a stringent requirement of the same MOA, to calculate RPFs across one or more health effect domains. Including multiple effects/domains among the constellation of PFAS effects allows for evaluation of the potential impact of differences in RPFs across PFAS in the mixture for those effects (e.g., the potency of PFAS 1 relative to PFAS 2 may be different for effects on the liver as compared to effects on the thyroid) (Mumtaz et al., 2021).

The intention is not necessarily to seek the most sensitive effects/domains; rather, it is to optimize the identification of those effects shared among the PFAS in the assessed mixture. However, for purposes of evaluating mixture risk using the RPF approach in a specific

environmental medium (e.g., drinking water), it is critical to have an IC effect-specific value or metric (e.g., an HBWC) so that the mixture ICEC can be compared to a benchmark point. For PFAS, given the limited availability of hazard effect and dose-response data, if one seeks to include several PFAS (i.e., beyond those few congeners with robust toxicity databases), the approach may be limited to a single effect domain or only those endpoints for which reasonable estimation of dose-response metrics (e.g., PODs, ED_X) for "same/similar" is possible. However, leveraging available NAM data, such as *in vitro* cell bioactivity, may provide opportunities to integrate those PFAS with poor(er) hazard and dose-response databases.

6.2.1 RPF Step 1: Assemble/Derive component health effects endpoints (select Index Chemicals, POD_{HEDs})

As PFAS 1–3 are toxicologically well-characterized and have existing HBWCs, all three are identified as candidate Ics for the mixture. PFAS 4 is also reasonably well characterized toxicologically and might be considered as a candidate IC in some RPF contexts; however, in a drinking water-specific application, another key consideration for IC selection is the existence of a quantitative benchmark such as an HBWC. This is necessary so that the ICEC for the mixture ($ICEC_{MIX}$) can be compared to the IC's corresponding HBWC to determine the potential for health risk(s). As such, PFAS 1–3 are the only candidate ICs identified for the hypothetical five-component mixture. Based on the strength of toxicological evidence (see Figure 4-8), not necessarily the quantitative potency for effect, ICs were selected as follows: Liver IC = PFAS 2; Thyroid IC = PFAS 3; and Developmental IC = PFAS 1.

The dose-response metrics for this RPF example are the same as those used above in the HI example (see Table 5-3) with the addition of a NAM-based POD for PFAS 2 (the IC for the liver effect domain). The POD_{HEDs} for three effect domains used in the calculation of the effect-specific RPFs and corresponding ICECs are presented below (Table 6-1).

Table 6-1. Summary of hypothetical POD_{HEDs} for three selected health effect domains for a mixture of five hypothetical PFAS.

	Liver POD_{HED} (mg/kg-day)	Thyroid POD_{HED} (mg/kg-day)	Developmental POD_{HED} (mg/kg-day)
PFAS 1	0.044 ($BMDL_{X\text{-}HED}$)	0.24 ($BMDL_{Y\text{-}HED}$)	**0.00001 ($BMDL_{Z\text{-}HED}$)**
PFAS 2	**0.0013 ($BMDL_{X\text{-}HED}$)**; 0.0052 ($BMD_{X\text{-}AED}$)[a]	0.23 ($BMDL_{Y\text{-}HED}$)	0.0051($BMDL_{Z\text{-}HED}$)
PFAS 3	N/A	**0.21 ($BMDL_{Y\text{-}HED}$)**	2.1 ($BMDL_{Z\text{-}HED}$)
PFAS 4	50 ($BMDL_{X\text{-}HED}$)	N/A	**0.0011 ($BMDL_{Z\text{-}HED}$)**
PFAS 5	**0.004 ($BMD_{X\text{-}AED}$)**[a]	N/A	N/A

Notes:
Bold indicates lowest POD for the corresponding RfD derivation.
[a] Represents the NAM-based POD for same *in vitro* cell bioactivity event between PFAS 2 and PFAS 5 (e.g., ↓ epoxide hydrolase activity).

6.2.2 RPF Step 2: Assemble/derive health-based media concentrations (HBWCs for the Index Chemicals)

For this illustrative RPF example, the hypothetical HBWCs are the same as those used in the General HI example (see Table 5-5). Specifically, the PFAS 1 HBWC is 0.2 ng/L (IC for developmental effects), PFAS 2 HBWC is 60 ng/L (IC for liver effects), and PFAS 3 HBWC is 4,000 ng/L (IC for thyroid effects).

6.2.3 RPF Step 3: Select exposure estimates (measured water concentrations)

Select appropriate exposure estimates consistent with the problem formulation. Specifically, the user may choose to calculate or use exposure estimates for the oral route in general (i.e., total intake in mg/kg-day) or media-specific concentrations. In the hypothetical PFAS mixture example, "exposure" is represented by the drinking water monitoring data in Table 4-1.

6.2.4 RPF Step 4: Calculate PFAS mixture potency (RPFs and ICECs for each effect domain)

Liver: Available traditional animal assay data indicate liver effects for PFAS 1, 2, and 4. PFAS 5 only has bioactivity data; however, the molecular and cellular perturbations were observed primarily in hepatocyte cell cultures (e.g., HepaRG). As such, there is increased confidence in the opportunity to integrate NAM-based information into the RPF approach specifically for the liver effect domain. Across the landscape of experimental rodent studies that inform liver toxicity for hypothetical PFAS 1, 2 and 4, several effects were noted after oral exposures such as increased absolute and relative organ weights, increased incidence of macro- and microvesicular steatosis (i.e., lipid accumulation in hepatocytes), histopathological evidence of focal hepatocellular necrosis, and increased serum ALT, AST, and ALP, indicative of hepatocyte or biliary epithelium injury, respectively. In addition, *in vitro* cell bioactivity data for PFAS 2 and 5 indicate increased pro-oxidation/oxidative stress, mitochondrial stress, and altered lipid homeostasis in the lower tested concentration range. Many of these observed cellular effects are considered Kes in signal transduction pathways leading to liver tissue alteration and injury (Figure 6-1). Of the effects observed in experimental rodents across PFAS 1, 2, and 4, histopathological evidence of a significantly *increased incidence of hepatocellular death* was common across studies. In this hypothetical example, the identified common liver effect was the effect used as the basis for deriving an oral RfD and corresponding HBWC for PFAS 2 (e.g., this liver RfD was interpreted with the highest confidence across PFAS 1, 2, and 4). As such, *increased incidence of hepatocellular death* is identified as the common effect for the liver domain for PFAS 1, 2, and 4. The liver effect-specific RPFs are calculated by dividing the selected liver effect POD_{HED} for the IC PFAS 2 by the POD_{HED} for PFAS 1 and 4 for the same effect (Table 6-2). Each RPF is multiplied by the corresponding chemical-specific measured water concentration to derive a PFAS 2 ICEC (Table 6-2). The example Mixture Total PFAS 2 $ICEC_{MIX}$ is then compared to the HBWC for PFAS 2, which is based on the effect of *increased incidence of hepatocellular death*.

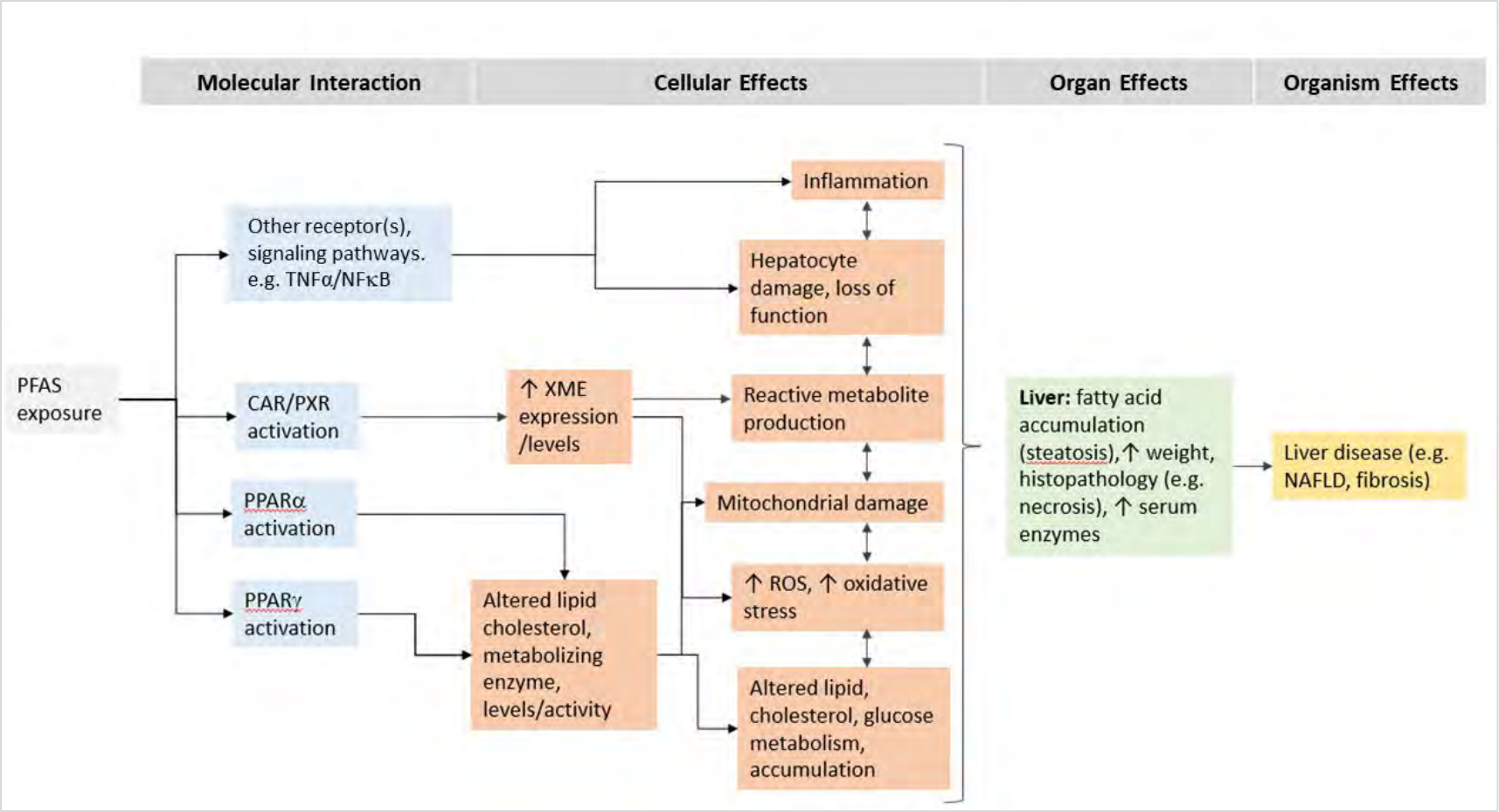


Figure 6-1. General cell signaling pathways associated with PFAS-induced liver injury. Figure sourced from Appendix A of the EPA's 2021 Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA IRIS Assessments (USEPA, 2021f).

Table 6-2. Example liver effect RPFs and ICECs for a hypothetical mixture of five PFAS.

Mixture component	Hypothetical POD_{HED} (mg/kg-day); *Increased incidence of hepatocellular death*	Hypothetical example RPF	Hypothetical exposure estimate (ng/L)	Hypothetical PFAS 2 ICEC (ng/L)
PFAS 1	0.044 ($BMDL_{X\text{-}HED}$)	0.03	4.8	0.14
PFAS 2 (IC)	0.0013 ($BMDL_{X\text{-}HED}$); 0.0052 ($BMD_{X\text{-}AED}$)[a]	1	55	55
PFAS 3	N/A	N/A	172	—
PFAS 4	50 ($BMDL_{X\text{-}HED}$)	0.00003	58	0.0017
PFAS 5	0.004 ($BMD_{X\text{-}AED}$)[a]	1.3 (RPF_{NAM})[b]	120	39[c]
Mixture total PFAS 2 ICEC (ppt)				**94**

Notes:

[a] NAM-based BMD modeled from the AED-based dose-response for the selected bioactivity event (e.g., decreased epoxide hydrolase activity, denoted as "hydrolase" in the example plots). This selected event is based on identifying the lowest (i.e., most sensitive) *common* bioactivity between the cell assay profiles for the IC (PFAS 2) and PFAS 5.

[b] RPF_{NAM} for PFAS 5 was calculated as the ratio of the $BMD_{X\text{-}AED}$ for PFAS 2 (IC) / $BMD_{X\text{-}AED}$ for PFAS 5 for the selected bioactivity event; in this example application, 0.0052 mg/kg-day / 0.004 mg/kg-day = 1.3.

[c] The ICEC for PFAS 5 was calculated by first deriving the $ICEC_{NAM}$ as follows: RPF_{NAM} × Exposure estimate for PFAS 5 = 1.3 × 120 (ng/L) = 156 ng/L; the $ICEC_{NAM}$ was then converted to an ICEC by multiplying by the ratio of the $BMDL_{X\text{-}HED}$ for PFAS 2 / $BMD_{X\text{-}AED}$ for PFAS 2 = 156 ng/L × (0.0013 / 0.0052) = 39 ng/L.

For PFAS 5, the dose-response data used in this hypothetical example RPF application, obtained from IVIVE/rTK of the *in vitro* cell bioactivity data, is identified based on the lowest bioactivity event(s)[21] common with the IC; that is, noncancer bioactivity at the lower end of the distribution for the IC is the driver for identification of "same" effect for the data-poor mixture component PFAS. In a perfect scenario, the "same" bioactivity event(s) would be shared at the level of NAM-based PODs between the IC and one or more data-poor components. However, while two or more mixture components may share a qualitatively similar profile of biological perturbations, the relative quantitative potency or dose-response at which various bioactivity events occur may be diverse. For simplification of the hypothetical PFAS mixture application, the selected bioactivity for the IC (PFAS 2) and PFAS 5 in HepaRG cells *in vitro* was identified as the same event, decreased epoxide hydrolase activity, with a $BMD_{X\text{-}AED}$ of 0.0052 mg/kg-day and $BMD_{X\text{-}AED}$ of 0.004 mg/kg-day, respectively (see Table 6-1). Importantly, epoxide hydrolases are a key component in the metabolism and detoxification of xenobiotics, particularly structures with reactive epoxide moieties; decreased hydrolase activity has been associated with increased oxidative stress, cellular/tissue inflammation, and cell death.

This NAM-based approach aims to scale the potency of the selected bioactive event for the data-poor chemical(s) to the same/similar bioactive event for the IC, where or when available data allow. The NAM-based RPF (RPF_{NAM}) is calculated by taking the ratio of the $BMD_{X\text{-}AED}$ for the

[21] The "lowest" bioactivity for noncancer application purposes should not be a potential carcinogenic event (e.g., mutagenicity or clastogenicity).

selected bioactivity event of the IC to the $BMD_{X\text{-}AED}$ for the same event associated with the data-poor mixture component chemical (see Table 6-2). The resulting RPF_{NAM} represents the relative potency between the data-poor PFAS (PFAS 5) and the IC (PFAS 2) for the selected bioactive event. This RPF_{NAM} is then multiplied by the data-poor chemical (e.g., PFAS 5) exposure metric (e.g., measured water concentration) to obtain a NAM-based ICEC ($ICEC_{NAM}$); to convert the $ICEC_{NAM}$ to a mixture ICEC that comports with the other traditional assay-based component PFAS ICECs, the $ICEC_{NAM}$ is multiplied by the ratio of $BMD_{X\text{-}HED}$ for the critical effect of the IC (in this example, the $BMD_{X\text{-}HED}$ for *increased incidence of hepatocellular death*) to the $BMD_{X\text{-}AED}$ for the bioactive event of the IC. The resulting ICEC represents the estimated contribution of PFAS 5 to the overall risk of the liver-specific effect; however, it is represented as a dose scaled for potency, relative to the IC, across different levels of biological organization (i.e., PFAS 5 *in vitro* to PFAS 2 *in vivo*). This process is illustrated in Figure 6-2.

Thyroid: Available traditional animal assay data indicate thyroid effects for PFAS 1, 2, and 3. PFAS 4 and 5 have no data available to support inclusion in the RPF analysis for this health effect domain. Applying the same approach outlined for the liver, the selected common effect was identified and hypothetically best represented the thyroid RfD for PFAS 3. Thus, the hypothetical thyroid effect-specific RPFs are calculated by dividing the selected thyroid effect POD_{HED} for the IC PFAS 3 by the POD_{HED} for PFAS 1 and 2 for the same effect (Table 6-3). Each RPF is multiplied by the corresponding chemical-specific measured water concentration to derive a PFAS 3 ICEC (Table 6-3). The example Mixture Total PFAS 3 ICEC is then compared to the HBWC for PFAS 3, which is based on the effect of decreased total serum thyroxine (TT4) and free serum thyroxine (FT4). The calculation of the thyroid-specific RPFs and corresponding ICECs are presented in Table 6-3.

Developmental: Developmental effects associated with oral exposures to PFAS 1, 2, 3, or 4 were observed in rats and mice; the studies available were predominately single-generation reproductive-developmental design however PFAS 4 also had a two-generation study in rats. PFAS 5 had no studies/data to suggest effects in the developmental domain. Based on the approaches above, the common effect was identified and hypothetically best represented by the oral RfD from and corresponding HBWC for the IC, PFAS 1. As such, *decreased body weight in offspring* was selected as the common developmental effect for the purposes of this RPF illustrative example. The developmental effect-specific RPFs are calculated by dividing the POD_{HED} for the selected effect associated with the IC PFAS 1 by the POD_{HED} for PFAS 2, 3, and 4 for the same effect (Table 6-4). Each RPF is multiplied by the corresponding chemical-specific measured water concentration to derive a PFAS 1 ICEC (Table 6-4).

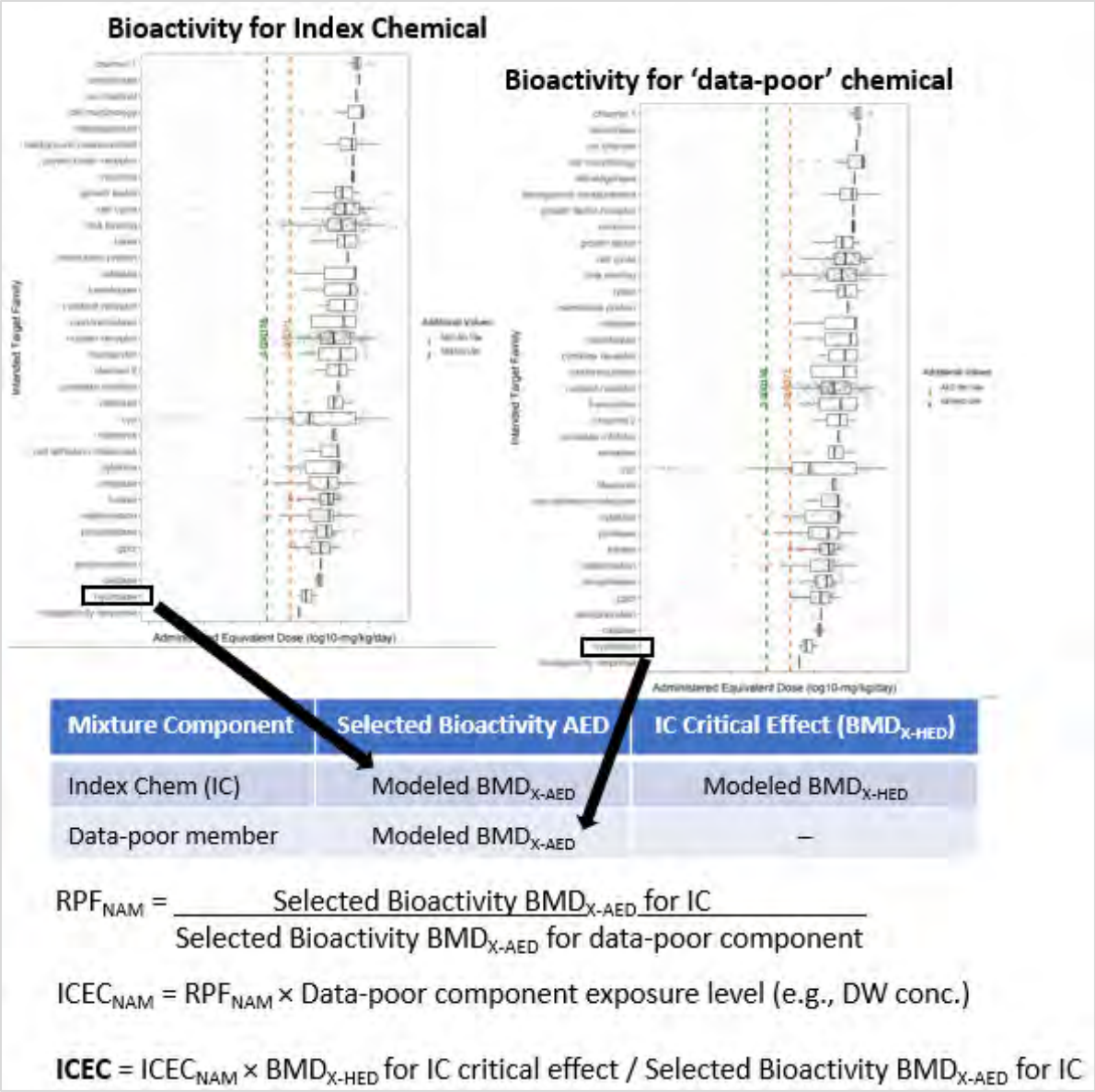


Figure 6-2. Example hypothetical process for integrating NAM-based RPFs and ICECs into mixtures assessment. Black boxes = "same" bioactive event for RPF approach.

Table 6-3. Hypothetical example thyroid effect RPFs and ICECs for a hypothetical mixture of five PFAS.

Mixture component	Hypothetical POD_{HED} (mg/kg-day); *Decreased TT4 and FT4*	Example RPF	Hypothetical exposure estimate (ng/L)	Hypothetical PFAS 3 ICEC (ng/L)
PFAS 1	0.24 ($BMDL_{Y\text{-}HED}$)	0.9	4.8	4.3
PFAS 2	0.23 ($BMDL_{Y\text{-}HED}$)	0.9	55	50
PFAS 3 (IC)	0.21 ($BMDL_{Y\text{-}HED}$)	1	172	172
PFAS 4	N/A	N/A	58	—
PFAS 5	N/A	N/A	120	—
Mixture total PFAS 3 ICEC (ppt)				**226 (230)**

Table 6-4. Hypothetical example developmental effect RPFs and ICECs for a hypothetical mixture of five PFAS.

Mixture component	Hypothetical POD_{HED} (mg/kg-day); *Decreased Body Weight in Offspring*	Hypothetical example RPF	Hypothetical exposure estimate (ng/L)	Hypothetical PFAS 1 ICEC (ng/L)
PFAS 1 (IC)	0.000010 ($BMDL_{Z\text{-}HED}$)	1	4.8	4.8
PFAS 2	0.0051 ($BMDL_{Z\text{-}HED}$)	0.002	55	0.11
PFAS 3	2.1 ($BMDL_{Z\text{-}HED}$)	5 E−6	172	0.00086
PFAS 4	0.0011 ($BMDL_{Z\text{-}HED}$)	0.009	58	0.52
PFAS 5	N/A	N/A	120	—
Mixture total PFAS 1 ICEC (ppt)				**5.4**

6.2.5 RPF Step 5: Compare PFAS mixture potency (Total $ICEC_{MIX}$) to an existing health-based value (HBWC)

In the liver-specific RPF application (see Table 6-2), the health risk(s) associated with the mixture is represented by comparing the PFAS 2 $ICEC_{MIX}$ to the IC HBWC, which is based on the specified effect for that hazard domain (e.g., for this example, increased incidence of hepatocellular death). In this hypothetical example, the PFAS 2 $ICEC_{MIX}$ of 94 ppt exceeds the PFAS 2 HBWC of 60 ppt, indicating the potential for risk of liver effects in individuals or populations exposed to a mixture of the five PFAS at the hypothetical water exposure estimates provided. Importantly, PFAS 2 and 5 appear to be drivers for the liver health risk associated with the hypothetical mixture.

In the thyroid-specific RPF application (see Table 6-3), the health risk(s) associated with the mixture is represented by comparing the mixture total PFAS 3 $ICEC_{MIX}$ to the IC HBWC, which is based on the specified effect (e.g., for this example, decreased TT4 and FT4). In this hypothetical example, the PFAS 3 $ICEC_{MIX}$ of 226 ppt is far below the PFAS 3 HBWC of 4000 ppt, indicating no apparent risk of thyroid effects in exposed individuals or populations to a mixture of the five PFAS at the hypothetical water exposure estimates provided.

In the developmental effect-specific RPF application (see Table 6-4), the health risk(s) associated with the mixture is represented by comparing the mixture total PFAS 1 $ICEC_{MIX}$ to the IC HBWC, which is based on the specified effect for hazard domain. In this hypothetical example, the PFAS 1 $ICEC_{MIX}$ of 5.4 ppt exceeds the PFAS 1 HBWC of 0.2 ppt by over an order of magnitude, indicating significant potential for health risks in developmental populations exposed to a mixture of the five PFAS at the hypothetical water exposure estimates provided.

As illustrated in the RPF examples above, PFAS can have different potencies across health effect domains. Due to the differences in TK and TD, PFAS may exhibit complex gradations of potency for different effects, which will be reflected in the corresponding RPFs. Some PFAS may be exquisitely potent for some effects and yet virtually inactive in others; however, expanding the number of PFAS and the toxicity endpoint profiles across the structural landscape will be key to illustrating such a diversity in relative potency(ies). Thus, calculating RPFs for as many endpoints/effects as possible helps to ensure that subsequent PFAS risk management strategies are health protective. In the example above, the risk would have been underestimated if the RPF analysis was limited to liver and thyroid effects: developmental effects are the risk driver in this scenario.

Further, another critical consideration illustrated in the RPF examples is the impact of component chemical concentrations. That is, in practical field application, PFAS concentrations in water, soil, or air may be drastically different depending on a number of factors (e.g., different physicochemical and environmental fate and transport properties, proximity to PFAS manufacturing or use locales, water sources [well water vs. finished drinking water], waste handling, temporal and spatial variability). In application, transparent presentation and communication of hazard and dose-response data sources, RPFs, media concentrations, ICECs, and any associated uncertainties across as many health effect domains as is practicable is ideal for RPF-based evaluation of PFAS mixtures. As mentioned previously, limitations for PFAS are the availability of human health assessment grade toxicity data, and as with many environmental mixture chemicals, dissimilarity in dose-response shapes and slopes; Section 7 offers an alternative to the RPF approach in such a scenario.

6.3 Advantages and Challenges of the Relative Potency Factor Approach

An advantage of the RPF approach is that formal toxicity or RfV derivation is unnecessary for the component chemicals. Rather, only effects/endpoints and associated dose-response metrics (e.g., NOAEL, BMD_X, ED_X) are needed to perform the exercise. While it would be ideal to conduct potency comparisons between mixture components for the same effect/endpoint using the same dose metrics from the same study design/durations, calculation of RPFs across PFAS may, in practical application, entail or necessitate the use of effect data deriving from diverse study designs and exposure durations. As such, in some cases, there may be a need to normalize or adjust available quantitative metrics such that potency comparisons are at comparable points

on a given dose-response. For example, there might be a need to selectively apply UFs in the RPF method, in particular, the LOAEL-to-NOAEL (UF_L) and/or subchronic-to-chronic duration (UF_S) factors to convert quantitative metrics to NOAELs from an estimated chronic-duration exposure. This flexibility is needed as, in some cases, effect data for mixture component PFAS may come from a variety of study designs such as reproductive/developmental in mice (e.g., GDs 1–20), less than lifetime repeat-dose (e.g., 28- or 90-days) in rats, and/or 2-year bioassays in rats. When sufficiently supported through evaluation of the available component-specific studies, such adjustments can provide the opportunity for a more 1:1 comparison of potency for a given effect (e.g., developmental body weight, increase in liver weight) among component PFAS. A critical facet of this is being transparent about POD adjustments (i.e., purpose/rationale) when applied.

RPFs were generally intended for use when mixture components are demonstrated to have similar/same MOA. This commonly presents a problem as it pertains to the practical application of RPF methodology in that a vast majority of environmental chemicals, including PFAS, have limited-to-no MOA data available. The EPA mixtures guidelines provide flexibility in using data from different levels of biological organization in dose additive approaches such as RPF. As demonstrated in this framework document, this flexibility is an advantage in that there is a greater probability of identifying effect/endpoint and associated dose-response data (e.g., effect-specific PODs) for mixture components than for MOA-type data. However, as the data for PFAS evolve, the toxicity profiles, including the number of effect types and granularity of biological perturbations (e.g., potential KE data that inform proposed MOA(s)), may eventually support MOA-based evaluations.

Another advantage is that the RPF method facilitates the calculation of an actual mixture toxicity dose or concentration estimate, as opposed to the HI, which is considered an indicator of potential health risk/toxicity. Although a given mixture ICEC is traditionally mapped to the IC's effect-specific dose-response function to arrive at a corresponding "mixture response," an advantage of the RPF approach is that the mixture ICEC may alternatively be used to inform mixture risk in the context of the relationship to a media-specific health-based value (such as an HBWC for an IC).

A clear challenge, not uniquely associated with the RPF approach, is the use of potentially disparate hazard and dose-response data (both in terms of type and confidence) across mixture components. The implicit assumption for dose-response data selection in the calculation of RPFs in this framework is that the same dose-response data that underpinned the derivation of corresponding RfVs (overall RfDs or TTDs) for use as input(s) for HQs and His would also be leveraged in RPF and/or M-BMD approaches (see Sections 6 and 7). However, although ideal, this is not an expressed requirement of the framework. The user should be afforded the flexibility to make decisions regarding suitable dose-response selection for RPF calculations on a case-by-case basis. Key to this flexibility is transparent characterization and communication of literature searching strategy and review results, hazard data selection, dose-response evaluation (e.g., BMD preferred; effect levels such as LOAELs are acceptable but ideally evaluated at isoeffective dose [ED_X], which may not be practicable), and qualitative and quantitative uncertainties or confidence in what could potentially be a diverse assembly of data/metrics to support RPF application(s).

Another challenge is that depending on data availability for the component PFAS, the effect domains used for the RPF analysis may not be the overall most sensitive out of the total constellation of common PFAS effects. In the hypothetical RPF examples shown above, the risk is indicated based on the liver and developmental RPFs but not for the thyroid effect domain. To use the RPF approach effectively, the user needs effect data for at least one common endpoint among the effects for all component PFAS in the mixture. Ideally this would include the most sensitive effect across PFAS in the mixture of interest in order to provide a conservative (health protective) risk-based scenario.

An additional potential challenge that may present an opportunity to advance the science of mixtures risk assessment is the use of NAM data. The constantly evolving information coming from alternative toxicity testing assays and platforms is important to human health assessment of environmental chemicals in general (not just for mixtures applications); however, there are inherent challenges associated with the application to hazard identification and dose-response assessment. In a PFAS mixtures assessment context, for some mixture component chemicals, NAM data (e.g., read-across or cell-based bioactivity [such as ToxCast and/or Tox21]) might be the only source(s) of evidence available to inform an RPF approach. The challenge might then be identifying and assembling "same" or "similar" effect/endpoint data compared to other PFAS in the mixture that have human epidemiological and/or experimental animal (i.e., apical (phenotypic) effect level) bioassay data. While the RPF approach affords flexibility in the selection of "effect" data, a key requirement is that the "effect" on which RPFs are based be the same. For example, one component PFAS may have histopathological evidence of multi-focal liver necrosis from *in vivo* repeat-dose rat studies, whereas another PFAS may have evidence of cytochrome c release, mitochondrial damage, and cell death in *in vitro* rat hepatocyte cell culture studies only. While in this hypothetical example NAM data clearly demonstrate hallmarks of cellular demise typically associated with necrotic (and apoptotic) cell death, pathologically consistent with cell death foci observed in whole rat liver, it may be difficult to make the case that the *in vitro*-based concentration-response data (converted to an AED) is suitable for traditional RPF calculation simply based on the interpretation of "same" effect. Further investigation is needed to evaluate the qualitative and quantitative merits of applying hazard and dose-response data from across different levels of biological organization in a component-based mixtures assessment context. This is particularly true of NAMs where the possible lumping or splitting of assay/data types to inform an integrated or more individualized interpretation of hazard and dose-response for data-poor mixture components is in its infancy; case studies using validated NAM assays and data are needed to help optimize application in mixtures risk assessment.

7.0 Mixture-BMD Approach

7.1 Background on the Mixture-BMD Approach

Given the broad range of PFAA congeners and structural diversity across the PFAS class, it is likely that for some effects used for mixture assessment, the dose-response functions (i.e., slopes) will be dissimilar across component chemicals. Using an IC in the RPF approach assumes component chemicals have similarly shaped dose-response slopes for the same health effect (and/or MOA). In addition, the HI approach requires human health assessment values, such as oral RfDs and individual HBWCs, because these metrics serve as the denominator in determining if the exposure exceeds a level estimated to be acceptable for human intake. In some cases, a PFAS mixture may contain component chemicals that do not have similarly shaped dose-response curves or have available human health assessment values (e.g., RfDs). In these cases, a third approach, called the M-BMD, can be used to estimate health risk(s) associated with mixture exposure. This approach is described in the EPA's supplementary guidelines (2000b) (Section 4.2.6) and NRC (2008) (Appendix C) and employs a DA model-based calculation of a total M-BMD that corresponds to a defined BMR (e.g., BMD_{10}) for a PFAS mixture. Similar to the RPF approach, only effects/endpoints and associated dose-response metrics (e.g., BMD_X) are needed to perform the exercise. Further, the mixture evaluation is based on a similar toxicological effect for component chemicals and the equation provided can be used to define a response-equivalent single point estimate (e.g., BMDx) or derive a full dose-response curve for the PFAS mixture of interest (i.e., by using multiple BMDx response levels for each compound in the mixture).

Because RPFs are special applications of the DA concept, such approaches can be a straightforward way of making quantitative assessments of the effects of chemicals, including PFAS. However, application of the RPF concept requires similarly shaped dose-response curves for all component chemicals for the given effect. When the response curves are dissimilar in shape, RPFs will vary with the effect levels in the mixture and thus could not be considered a "dilution" of the IC across the full dose-response range. For example, the RPF for a given PFAS may be different in the low dose range vs. the middle or high dose range, depending on slope differences with the IC. In this regard, published data (Hass et al., 2007; Howdeshell et al., 2008; Metzdorff et al., 2007; and Rider et al., 2008) reveal that chemical dose-response curves for a common effect can display very different slopes and shapes even across related structures within a given class. In contrast to the RPF approach, other DA-based equations can be used for quantitative evaluations of the effects of chemical mixtures when the slopes for a common effect differ among chemicals in the mixture.

The following discussion compares the predictions of two DA models. One assumes that the individual chemicals in the mixture have similarly shaped slopes, whereas the second DA model (described by NRC [2008]) does not require similar slopes to yield accurate predictions. As an example, these two models were recently used in a laboratory study to predict the full dose-response curve of a mixture using the ED_{50}s and slopes of each component chemical in the mixture (Gray et al., 2022). The first model, similar to the RPF method (discussed above), assumes that the chemicals in the mixture have similar dose-response slopes, indicating that the relative potencies are constant across the entire dose-response curve. Equation 7-1 is an example of such a model that uses an average slope value to calculate the joint toxicity of a mixture with

the following equation (Olmstead and LeBlanc, 2005; Rider and LeBlanc, 2005; Rider et al., 2008):

$$R = \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{D_i}{ED50_i}\right)^{\rho}}} \quad \text{(Eqn. 7-1)}$$

where R is the response to the mixture, D_i is the dose of chemical I in the mixture, $ED50_i$ is the dose of chemical I that causes a 50% response, and ρ is the average power (Hillslope) associated with the chemicals.

Because the assumption of similar slopes is not always met, DA models, like the M-BMD method described below, that do not require similarly shaped dose-response curves for the chemicals in the mixture, may provide more accurate predictions of the mixture effects. Several of these DA-based models have been previously described (for example, Altenburger et al., 2000; Kortenkamp et al., 2007; Metzdorff et al., 2007; and NRC, 2008). The M-BMD equation below (Equation 7-2) is an example of such a DA model that calculates a given effect level for the total mixture ($EDx_{mixture}$) where p_i is the proportion of chemical i in the mixture and eDx_i denotes the dose producing the given level of response for the ith chemical in the mixture:

$$eDx_{mixture} = (p_1 / eDx_1 + p_2 / eDx_2)^{-1} \quad \text{(Eqn. 7-2)}$$

When the slopes of the dose-response curves differ among chemicals in the mixture, the two DA models (i.e., Equation 7-1 and Equation 7-2) can yield different dose-response predictions (see Figure 7-1). Further, there is greater uncertainty in the accuracy of the DA mixture predictions when the assumption of similar shape among slopes is violated (NRC, 2008). The following example compares the accuracy of a DA model that assumes similar dose-response shapes (Equation 7-1) with a DA model that does not (Equation 7-2), and these model predictions are compared with a RA model. The data used in this example are from a published binary mixture study, but the chemicals are not identified (Gray et al., 2022).

In this example, two chemicals were mixed using a fixed-ratio design. The top dose of this mixture contained each chemical at a dose close to their respective ED_{50}s, but they have different dose-response shapes using nonlinear, four-parameter logistic regression (Chemical A slope = 40, Chemical B slope = 5 using linear Y axis and log10 X axis). The mixture effect described in Figure 7-1 is the percent reduction in reproductive organ weight, ranging from 0% reduction in the control (0 dose of the mixture) to complete agenesis (100% reduced).

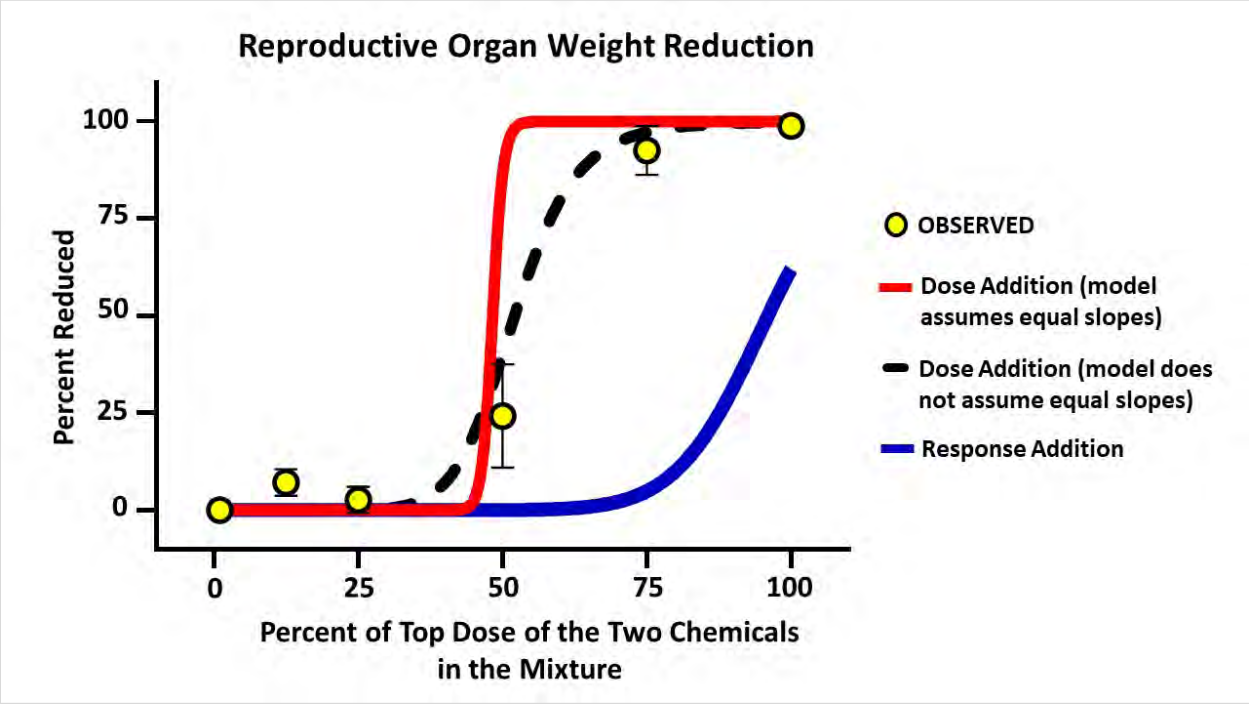


Figure 7-1. Example comparison of observed data with model predictions using two dose addition-based mixture models and an RA model for a binary mixture study (adapted from Gray et al., 2022). The two chemicals displayed individual dose-response curves with widely disparate slopes for the endpoint (reduced organ weight). The two dose addition models either assume component chemicals have similar dose-response slopes (red solid line) for the effect or have non-similarly shaped dose-response curve slopes (black dashed line). For these chemicals with disparate slopes, the dose addition model that does not assume similar slopes provided a better fit of the observed data (see Table 7-1).

The observed data were fit with the model parameters of the two DA and the RA model and Akaike Information Criteria (AIC) values were calculated to determine the best model of the observed data (Table 7-1). The lower AIC values indicate a better-fit model and, as a "rule of thumb" (Burnham and Anderson, 2004), there is little support for two of these models because the delta-AIC (the difference between the two AIC values being compared) is greater than 7.

Table 7-1. "Best model" based on AIC values.

"Best Model" based upon AIC values	
Model	**AIC**
DA - does not assume equal slopes	164.1
DA - assumes equal slopes	203.3
RA	223.4

AIC: Akaike Information Criterion

In contrast to the above example of chemicals with disparate slopes, if the dose-response curves of the component chemicals in a mixture have similar slope parameters from nonlinear regression, then there would be little or no difference between the predictions of the two DA models shown here. Hence, if sufficient dose-response information is available and the slopes are not similar, then it is preferable to model the data with the M-BMD equation (Equation 7-2) that does not assume similarly shaped curves, as stated by the NRC (2008).

Estimating PFAS mixture effects using the M-BMD method requires empirical data-driven or reasonable estimation (e.g., read-across between structures) of common endpoints for all PFAS in the mixture. Similar to the RPF approach above, ideally the dose-response functions used to calculate effect endpoints (e.g., BMD_X) across mixture components would be approximately the same in exposure duration and study design (e.g., sex, species, life stage). Further, considering the known differences in TK characteristics across PFAS (e.g., internal plasma half-life) between rodents, nonhuman primates, and humans, it is strongly recommended to convert experimental animal dose-response data to human equivalents (i.e., HEDs). Lastly, of the options for dose-response metrics to use across component PFAS, risk assessment-based PODs (e.g., $BMDL_{xHED}$) would be optimal. BMDs incorporate the totality of a given dose-response and facilitate the identification of a dose at a predefined BMR level (e.g., 0.5 SD or 1 SD over control; 10% change in the common effect/endpoint). BMD modeling would optimize the comparison of "same" as a function of dose across component PFAS for a given health effect or endpoint and identify a human health-relevant POD for M-BMD derivation. It is recognized that dose-response data for chemicals is sometimes not amenable to BMD modeling.

Importantly, the response level (i.e., BMR) for the common endpoint should be the same for all PFAS included in the calculation, for example, $BMDL_x$ for a common liver effect. In this case, the equation will produce an equivalent response metric (i.e., $BMDL_x$) for the total mixture with the given proportions of component PFAS being evaluated. In the illustrative example below, the BMDLs associated with hypothetical response levels (i.e., $BMDL_x$, $BMDL_Y$, $BMDL_Z$) estimated for each chemical in a mixture are used to determine an "M-BMD" that represents an equivalent POD for the mixture that was identified for each component PFAS. The choice of BMRs is based on expert judgment and data availability. As stated above, it is preferable to calculate the M-BMDs using HED doses rather than oral mg/kg doses administered to test animals. The equation explanation and example below will reference $BMDL_i$ as the model components using Equation 7-3 (similar to Equation 7-2), where M-BMD is the total mixture dose in mg/kg/day, a_i are the fixed proportions of the component PFAS in the mixture, and BMD_i is i^{th} chemical BMD (e.g., a BMD_X).

$$Mixture\ BMD = \left(\sum_{i=1}^{n} \frac{a_i}{BMD_i}\right)^{-1} \quad \text{(Eqn. 7-3)}$$

The equation results in a single mixture-specific M-BMD for a given BMR that could then be converted to an assessment value (e.g., oral RfD) and a corresponding HBWC for the mixture. Then, the original observed PFAS mixture concentration is compared to the estimated M-BMD-HBWC from the M-BMD equation. If the observed concentration is greater than the mixture-based HBWC, there is potential for human health risk. If the observed concentration is below the mixture-based HBWC, then the risk of health effects is not expected. Further, the calculation can be repeated at multiple BMR levels to allow for the modeling of a full mixture dose-response curve, if needed. Finally, similar to RPF, due to the potential for different effect domains to have

variable potencies across PFAS within a given mixture, the DA model should be applied across more than one effect domain for which data are available for each of the PFAS in the mixture to identify the lowest mixture-specific endpoint, which indicates the most sensitive domain for the mixture.

For consistency and comparison with the RPF illustrative example above, in the illustrative example below, the effect levels for each chemical in the hypothetical mixture of five PFAS from Tables 6-2, 6-3, and 6-4 are used to determine an "M-BMD." The previously described (Section 6.2) hypothetical response-equivalent POD values serve as the denominator values in Equation 7-3. The numerator values are the proportions of each component PFAS in the given mixture on a concentration basis. The total M-BMD is the inverse of the sum of the proportion divided by the POD for each PFAS in the mixture. The total M-BMD_X represents an equivalent BMD_X as each of the individual chemical BMDs that were used in the calculations (i.e., if the individual chemical data were $BMDL_{10}$ values, the DA calculation derives a $BMDL_{10}$ for the mixture of PFAS with those specific proportions).

The M-BMD, which is in the same units as the component chemical BMDs (e.g., human equivalent of oral dose in a rodent study such as mg/kg-day), can then be adjusted based on user-defined extrapolation factors (e.g., application of dosimetric adjustment, RSC, UFs, and life stage-specific drinking water consumption rates) to derive a unique HBWC for the total PFAS mixture (as opposed to an IC-specific HBWC as in the RPF approach). In practice, the lowest mixture-specific endpoint indicates the most sensitive effect domain for the mixture; this endpoint can then be used to derive an equivalent M-BMD-HBWC and estimation of risk. The derived M-BMD-HBWC can then be compared to the actual (measured) mixture concentration; if the actual mixture concentration exceeds the M-BMD-HBWC, there is a risk of the specific effect from exposure to that mixture at the measured concentrations.

7.2 Illustrative Example Application of the Mixture Benchmark Dose Approach to a Hypothetical Mixture of Five PFAS

7.2.1 Mixture BMD Step 1: Assemble/derive component health effects endpoints (BMDx)

Hypothetical data for the five PFAS in the illustrative example here are detailed in section 6.2 above (Note: Table 7-2 is a compilation of Hypothetical POD_{HED}s from Tables 6-2, 6-3, and 6-4 as used in the RPF example).

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Table 7-2. Summary of hypothetical POD_{HED}s for three selected health effect domains for a mixture of five hypothetical PFAS.

	Hypothetical liver POD_{HED} (mg/kg-day)	Hypothetical thyroid POD_{HED} (mg/kg-day)	Hypothetical developmental POD_{HED} (mg/kg-day)
PFAS 1	0.044 ($BMDL_{X\text{-}HED}$)	0.24 ($BMDL_{Y\text{-}HED}$)	0.00001 ($BMDL_{Z\text{-}HED}$)
PFAS 2	0.0013 ($BMDL_{X\text{-}HED}$) 0.0052 ($BMD_{X\text{-}AED}$)[a]	0.23 ($BMDL_{Y\text{-}HED}$)	0.0051 ($BMDL_{Z\text{-}HED}$)
PFAS 3	N/A	0.21 ($BMDL_{Y\text{-}HED}$)	2.1 ($BMDL_{Z\text{-}HED}$)
PFAS 4	50 ($BMDL_{X\text{-}HED}$)	N/A	0.0011 ($BMDL_{Z\text{-}HED}$)
PFAS 5	0.004 ($BMD_{X\text{-}AED}$)[a] 0.001 ($BMDL_{X\text{-}HED}$)[b]	N/A	N/A

Notes:

[a] NAM-based BMD modeled from the AED-based dose-response for the selected bioactivity event (e.g., decreased epoxide hydrolase activity, denoted as "hydrolase" in the example plots). This selected event is based on identifying the lowest (i.e., most sensitive) *common* bioactivity between the cell assay profiles for the IC (PFAS 2) and PFAS 5.

[b] Hypothetical NAM-derived Liver POD based on first calculating NAM-based relative potency for PFAS 5 = 0.0052 $BMD_{X\text{-}AED}$ / 0.004 $BMD_{X\text{-}AED}$ = 1.3 then estimating a PFAS 5 *in vivo* POD_{HED} using the POD_{HED} for PFAS 2 divided by the relative potency of PFAS 5, 0.0013 $BMDL_{X\text{-}HED}$ / 1.3 = 0.001 $BMDL_{X\text{-}HED}$.

7.2.2 Mixture BMD Step 2: Assemble/derive health-based media concentrations (HBWC)

In the case of the M-BMD approach (unlike the HI/TOSHI and RPF approaches), there is no need for pre-existing HBWC(s) because the goal of this approach is to develop a unique, mixture-specific HBWC for comparison to the Mixture Total PFAS concentration. The calculation of the M-BMD HBWC is shown in Section 7.2.4.

7.2.3 Mixture BMD Step 3: Select exposure estimates (measured water concentrations)

Select appropriate exposure estimates consistent with the problem formulation. Specifically, the user may choose to calculate or use exposure estimates for the oral route in general (i.e., total intake in mg/kg-day) or media-specific concentrations. In the hypothetical PFAS mixture example, "exposure" is represented by the drinking water monitoring data in Table 4-1.

7.2.4 Mixture BMD Step 4: Calculate PFAS mixture potency (Mixture BMD HBWC)

In this example, M-BMDs are calculated for three effect domains: Liver, Thyroid, and Developmental (Table 7-3). Application of Equation 7-3 to the example water sample in Table 4-1 is used to derive the M-BMD, as shown in Equation 7-4. This example is for the developmental domain, as it was the lowest M-BMD of the three effect domains.

(Eqn. 7-4)

$$Mixture\ BMD = \left(\sum_{i=1}^{4} \frac{a_i}{BMD_i}\right)^{-1} = \left(\frac{0.01}{0.00001} + \frac{0.13}{0.0051} + \frac{0.42}{2.1} + \frac{0.14}{0.0011} + \frac{0.29}{N/A}\right)^{-1} = 0.00087 \text{ mg/kg-day}$$

Table 7-3. M-BMD Approach: Hypothetical Water Sample and Hypothetical M-BMDs.

	Median measured water concentration (ng/L)	Mixing ratio (proportion)	Liver POD_{HED} (mg/kg/day)	Thyroid POD_{HED} (mg/kg/day)	Develop-mental POD_{HED} (mg/kg/day)
PFAS 1	4.8	0.01	0.044	0.24	0.00001
PFAS 2	55	0.13	0.0013	0.23	0.0051
PFAS 3	172	0.42	N/A	0.21	2.1
PFAS 4	58	0.14	50	N/A	0.0011
PFAS 5	120	0.29	0.0022	N/A	N/A
Mixture total	**409.8**	1.0			
M-BMD calculation			0.0025	0.38	**0.00087**[a]

Notes:
N/A = data not available.
[a] The lowest M-BMD is converted to a mixture-HBWC using Eqn. 7-3 for comparison to the measured concentration (i.e., 409.8 ng/L).

The developmental-effect produced the lowest M-BMD (i.e., 0.00087 mg/kg-day), representing the most sensitive effect domain; this value is selected for the calculation of the M-BMD HBWC. The developmental-based M-BMD is first converted to an RfD by applying UFs that are consistent with the data being used. The selection of uncertainty factors will likely be different across mixture component chemicals based on the available hazard and dose-response data. As such, there is no standard application of quantitative uncertainty for a mixture of components, although it is suggested that a user of this approach consider the composite uncertainty (UF_C) across the five areas used in EPA human health risk assessment practice: (1) Human interindividual variability (UF_H); (2) extrapolation from animal-to-human (UF_A); (3) subchronic-to-chronic duration extrapolation (UF_S); (4) LOAEL-to-NOAEL extrapolation (UF_L); and (5) database uncertainty (UF_D). In the specific context of the application of uncertainty to an M-BMD, a reasonable health-protective approach is to apply factors consistent with the data status of the most data-poor member of the mixture used in the hazard domain-specific calculation of the M-BMD selected for use in deriving the RfD. In the example illustrated in Equation 7-5, the POD from PFAS 4 is the most data-poor of the PFAS used to calculate the M-BMD for developmental effects selected for the RfD. Hypothetically, the UF_C for this PFAS 4 POD was 300. Thus:

$$\text{RfD} = \left(\frac{\text{BMD}}{\text{UF}_\text{C}}\right) = \left(\frac{0.00087\frac{\text{mg}}{\text{kg}}/\text{d}}{300}\right) = 0.000003 \text{ mg/kg-day} \qquad \text{(Eqn. 7-5)}$$

An HBWC can then be derived using Equation 7-3. In the example shown in Equation 7-6, the DWI-BW is for women of childbearing age (i.e., 90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years), and the RSC is 20% (0.2). The M-BMD HBWC is calculated as follows:

$$\text{HBWC} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC} = \left(\frac{0.000003\frac{\text{mg}}{\text{kg}}/\text{d}}{0.0354\frac{L}{kg}/d}\right) * 0.2 = 0.00002 \text{ mg/L} = 20 \text{ ng/L} \quad \text{(Eqn. 7-6)}$$

7.2.5 *Mixture BMD Step 5: Compare PFAS mixture potency (total PFAS mixture concentration) to health-based value (Mixture BMD HBWC)*

In the developmental effect-specific M-BMD application, the health risk(s) associated with the mixture is represented by comparing the mixture's total PFAS concentration (409.8 ng/L) to the M-BMD HBWC, which is based on the specified effect for hazard domain (e.g., for this example, decreased body weight in offspring). In this hypothetical example, the mixture total PFAS concentration of 409.8 ng/L exceeds the M-BMD HBWC 20 ng/L by over an order of magnitude, indicating significant potential for health risks in developmental populations exposed to a mixture of the five PFAS at the hypothetical water exposure estimates provided.

Although not shown in the above example, if the M-BMD was instead based on the liver effect domain, the M-BMD HBWC would be 48 ng/L (hypothetically assuming the same UF_C and DWI-BW were used), below the measured PFAS concentration (409.8 ng/L), indicating there is also potential for liver effects in populations exposed to the hypothetical mixture. Alternatively, if the M-BMD was based on the thyroid effect domain (again assuming the same UF_C and DWI-BW were applied to the M-BMD), the resulting M-BMD HBWC would be 7,177 ng/L, well above the measured total PFAS concentration (409.8 ng/L), indicating unlikely risk for thyroid effects among the exposed population. In practice, the composite UF_C for each health-effect domain should be estimated based on the expert judgment of the most data-poor component chemical used to derive the M-BMD. Further, depending on the specific shared health effect within a given domain, the appropriate life stage DWI-BW should be used to convert the M-BMD to an M-BMD-HBWC.

7.3 Advantages and Challenges of the Mixture BMD Approach

There are several advantages to the M-BMD approach. First, there is no *a priori* requirement for having formal human health assessment values, such as oral RfDs or chemical-specific HBWCs, for any of the individual PFAS in the mixture. The only data needs are response-equivalent effect endpoints (e.g., BMDLs) for each PFAS in the mixture for the common endpoint(s) being modeled. Another advantage is that it avoids any potential confusion that could arise from putting the mixture POD in the units of a single chemical (i.e., the IC from the RPF approach). Rather, the end result is a POD that is specific for the assortment and ratios of PFAS in the mixture being evaluated. It is important to recognize that the DA model calculation of the M-BMD is different for each PFAS mixture depending on (1) the specific PFAS in the mixture, (2) the mixing ratio, and (3) the effect or endpoint being modeled. For example, one could expect that a mixture of PFAS that has a greater concentration of a more potent compound and a lower concentration of a less potent compound would have a lower (i.e., more potent) M-BMD than a similar assortment of compounds that has a lower concentration of the more potent PFAS and a greater concentration of the less potent PFAS. It is also advantageous that the M-BMD approach does not actually require or assume that the component PFAS in a given mixture have similarly shaped dose-response curves for each effect being evaluated (reviewed in NRC [2008]). Finally, it is ideal to have well-resolved dose-response curves for each component PFAS in a mixture to estimate equivalent BMRs (e.g., BMD_x, PODx), and this is necessary if a goal is to model the entire dose-response for the mixture. However, in the absence of such data, M-BMD modeling is

also amenable to simple point estimates such as NOAELs, as long as they are toxicologically similar across component chemicals (i.e., for the same endpoint, such as increased incidence of hepatocellular death) but use of this type of point data would impede the modeling of the full mixture dose-response curve if desired.

There are also several challenges with the M-BMD approach. Similar to the RPF approach, the user needs effect data for at least one common endpoint from the constellation of PFAS effects for all components of the mixture. Ideally, this would be for one of the most sensitive—if not the most sensitive—effects across PFAS in the mixture of interest to provide a conservative (protective) risk scenario. For some mixtures that contain less well-studied PFAS there may be limited or no available dose-response data to derive component chemical BMDs to calculate the M-BMD. Another limitation is the absence of standard guidelines for selecting uncertainty factors for a mixture of components, as opposed to the procedures used to apply uncertainty factors to individual chemicals in a risk assessment. The present document provides a hypothetical example of using a composite UF for the mixture based on the composite UF of the most data-poor component in the M-BMD calculation.

A limitation that is not unique to this specific approach is that PFAS mixtures may vary over time in environmental media. As proportions of component PFAS change in the mixture, the calculations would need to be recalculated as the composition of the mixture changed from site to site or over time within the same site. However, the calculation can be readily and easily repeated for different mixing ratios and mixture concentrations once the component chemical effect endpoint values have been determined. Finally, for both the RPF and M-BMD approaches, depending on data availability for the individual compounds, the effect domains modeled may potentially not be the overall most sensitive out of the total constellation of common PFAS effects (e.g., in reality, developmental effects may be the most sensitive and would produce the lowest M-BMD, but data are only available for the component PFAS to calculate M-BMDs for liver and thyroid effects). In this example, the M-BMD HBWC is based on developmental effects because it is the most sensitive of the three assessed effect domains and, thus, is protective of the other effects (i.e., liver and thyroid). As described in the previous section, if this M-BMD analysis was instead based on the thyroid effect, the user would conclude that potential risk is unlikely.

8.0 Comparison of Component-Based Approaches

This framework document describes the conceptual bases and practical application of data-driven options for estimating the noncancer health risks associated with human exposure to mixtures of PFAS. The component-based options described are included in prior EPA mixtures guidelines (USEPA, 1986, 2000b) and/or supported by NRC (2008). Although the approaches and illustrative hypothetical examples are provided using drinking water as the exposure route, the technical basis of each approach could be readily applied or adapted to other sources of oral exposure (e.g., soil, fish/shellfish, foods). Each of the approaches included requires varying levels of data input, has relatively subtle but substantive differences in assumptions, and ultimately produces risk indications/estimations that may differ slightly based on those assumptions. Importantly, the interpretations of health risks associated with mixtures of PFAS will be highly dependent on the specific PFAS components within a given mixture and the individual concentrations or proportions of each component. Given the significant lack of toxicity data across the diverse structural landscape of compounds within the PFAS chemical class, it is likely that many users of this framework will need to incorporate information from NAMs such as *in vitro* cell bioactivity, toxicogenomic platforms, and/or structure-activity/read-across to facilitate estimation of health risk for a PFAS mixture of interest; however, *in vivo* animal or human toxicity data are strongly recommended where available.

Given the range of data-driven options presented in this framework, an important consideration is under what circumstances the different options produce similar or dissimilar indications or estimates of health risk(s). The primary basis for differing risk estimates relates to differences in data input requirements, model assumptions, and final value derivation. Both the HI and TOSHI approaches necessitate the availability (or *de novo* derivation) of health assessment values (e.g., oral RfDs) to calculate mixture component HQs. In contrast, the RPF and M-BMD approaches target dose-response data for the same/similar effect, sans derivation of health assessment values, to inform mixture risk estimates (e.g., concentrations or doses) for comparison to measured media concentrations either for an anchor/IC (such as in the RPF) or across each mixture component (such as in the M-BMD). The general HI approach allows for component PFAS in the mixture to have different health effects or endpoints as the basis for the component chemical RfVs (see Figure 4-1); thus, this approach is likely a more health-protective indicator of risk (i.e., produce a component HQ or mixture HI of > 1) since the representation of toxicity will likely be the most sensitive, compared to the RPF and M-BMD approaches where similarity in toxicity does not have to *a priori* be the most sensitive effect domain. In contrast, the TOSHI approach is more targeted and assumes the component RfVs are based on the same organ or organ system. This more narrowed focus is likely to produce a less health-protective indicator of risk than the general HI (i.e., less likely than general HI to produce HQ > 1) because the range of potential effects has been scoped to a specific target organ or organ system; for example, for some mixture components, the effect domain identified for TOSHI application may be one of the less potent across a profile of effects. This important nuance will be dependent on the availability of target organ-specific RfVs, and case-by-case interpretations of "potency" for effect will be a function of both dose-response (e.g., POD) and the uncertainty factor application. The user of the TOSHI approach would be advised to also perform the general HI for the same mixture and compare the HIs (and component HQs) across each approach. It should be noted that any component chemical HQ or mixture HI > 1 indicates potential health risks; the magnitude of HI is not an optimal comparator. On the other hand, the TOSHI and RPF approach will give essentially the

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same answer when the ratio of the POD values used to calculate the RPFs is equal to the ratio of the endpoint-specific PODs used in the derivation of RfVs used to calculate the TOSHI. The major difference between the RPF and M-BMD estimates is the RPF approach assumes similarly shaped dose-response curves, whereas the M-BMD does not. If the mixture component chemicals have similarly shaped dose-response curves for a common effect, the RPF and M-BMD calculations produce nearly identical risk estimates for the same mixture. However, if the mixture component chemicals display a range of dose-response curve shapes, then the assumptions for application of the RPF method are violated and the M-BMD approach should be used to produce a more accurate estimate of risk (NRC, 2008).

Another factor in the concordance (or not) of mixture risk estimates across the component-based approaches presented are the factors used in deriving RfVs or HBWCs from hazard data across components. The HI and TOSHI approaches are calculated based on an exposure metric divided by the component chemical RfVs or HBWCs. The input data for calculating RfV and HBWC, including the critical effect and POD (i.e., $BMDL_X$ or NOAEL or LOAEL), correction factors (i.e., UFs, DAF), and exposure route adjustments (i.e., DWI, RSC) used for each component PFAS will have impacts on the resulting risk estimates and should be carefully selected based on available data for each component PFAS. Similarly, when conducting RPF or M-BMD assessment, the PODs used across component PFAS in a mixture could potentially be derived from dissimilar response metrics (i.e., LOAEL vs. $BMDL_X$) and the resulting M-BMD-HBWC or Total ICEC comparison will be somewhat dependent on the applicability of the correction and route adjustment factors used for the M-BMD HBWC or Total ICEC derivation across all component PFAS in the mixture. For example, the correction factors and route adjustment factors may not be appropriate for all component PFAS; thus, the Total ICEC to IC RfV or HBWC comparison or M-BMD HBWC to measured concentration may be affected. Therefore, it is strongly encouraged to use comparable PODs across component PFAS where possible and select adjustment factors given careful consideration of the components for the specific PFAS mixture being evaluated. It will be key to transparently present and communicate the selection of uncertainty factors or exposure route adjustment factors and associated rationale(s), such that interpretations and conclusions of mixture risk are supportable.

A critical consideration for using the approaches in this framework document is that it may be prudent to apply each approach to the same mixture where data are available. The purpose of the comparison is not necessarily to determine which approach provides the most conservative estimate of mixture risk but rather which reflects the greatest level of confidence in the data underlying the component PFAS.

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AMIGOS BRAVOS EXHIBIT 9

United States Environmental Protection Agency

EPA-815-R-24-004

FINAL

Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS

Individual MCLGs for Three Per- and Polyfluoroalkyl Substances (PFAS):

- HFPO-DA
- PFNA
- PFHxS

Mixture MCLG for Mixtures of Four PFAS:

- HFPO-DA
- PFNA
- PFHxS
- PFBS

Maximum Contaminant Level Goals (MCLGs) for Three Individual Per- and Polyfluoroalkyl Substances (PFAS) and a Mixture of Four PFAS

Prepared by:

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

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Notices

This document has been reviewed in accordance with EPA policy and approved for publication.

This document provides a summary of information used to develop the final individual MCLGs for HFPO-DA[1], PFNA, and PFHxS and a final MCLG for mixtures of HFPO-DA, PFNA, PFHxS, and/or PFBS. Molecular formulas and Chemical Abstract Service registry numbers (CASRNs) for these four PFAS are as follows:

- HFPO-DA ($C_6F_{11}O_3^-$; CASRN 122499-17-6)
- PFNA ($C_9F_{17}CO_2^-$; CASRN 72007-68-2)
- PFHxS ($C_6F_{13}SO_3^-$; CASRN 108427-53-8)
- PFBS ($C_4F_9SO_3^-$; CASRN 45187-15-3)

These PFAS may exist in multiple forms, such as isomers or associated salts, and each form may have a separate CASRN or no CASRN at all. Additionally, these compounds have various names under different classification systems. However, at environmentally relevant pHs, these PFAS are expected to dissociate in water to their anionic (negatively charged) forms. For instance, HFPO-DA is an anionic molecule which has an ammonium salt (CASRN 62037-80-3), a conjugate acid (CASRN 13252-13-6), a potassium salt (CASRN 67118-55-2), and an acyl fluoride precursor (CASRN 2062-98-8), among other variations. At environmentally relevant pHs these all dissociate into the propanoate/anion form (CASRN 122499-17-6). Each PFAS listed has multiple variants with differing chemical connectivity, but the same molecular composition (known as isomers). Commonly, the isomeric composition of PFAS is categorized as 'linear,' consisting of an unbranched alkyl chain, or 'branched,' encompassing a potentially diverse group of molecules including at least one, but potentially more, offshoots from the linear molecule. While broadly similar, isomeric molecules may have differences in chemical properties. The final National Primary Drinking Water Regulation for PFAS covers all salts, isomers, precursors, and derivatives of the chemicals listed, including derivatives other than the anionic form which might be created or identified.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[1]The EPA notes that HFPO-DA is used in a processing aid technology developed by DuPont to make fluoropolymers without using perfluorooctanoic acid (PFOA). The chemicals associated with this process are commonly known as GenX Chemicals and the term is often used interchangeably for HFPO-DA.

Authors, Contributors, and Reviewers

Prepared by

Carlye Austin, Ph.D., DABT
Czarina Cooper, M.P.H.
Colleen Flaherty, M.S.
Brittany Jacobs, Ph.D.
Casey Lindberg, Ph.D.

Contributors

Brandi Echols, Ph.D.
Alexis Lan, M.P.H.

EPA Technical Review

Office of Chemical Safety and Pollution Prevention
Office of Children's Health Protection
Office of Land and Emergency Management
Office of Research and Development

Executive Direction

Elizabeth Behl
Eric Burneson, P.E.

Formatting by

Tetra Tech, Inc
ICF

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Abbreviations and Acronyms

AFFF	aqueous film-forming foam
AMAP	Arctic Monitoring and Assessment Programme
AOP	adverse outcome pathway
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	bioaccumulation factor
BDL	below the detection limit
BMD	benchmark dose
CASRN	Chemical Abstract Service registry number
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CTEPP	Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants
DA	dose addition
DF	detection frequency
DWI-BW	body weight-adjusted drinking water intake
DWTP	drinking water treatment plant
DWR	durable water repellent
E	duration-relevant exposure
EEE	electrical and electronic equipment
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
FAO	Food and Agriculture Organization Area
FCM	food contact materials
FDA	U.S. Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GCA	groundwater contamination area
GenX chemicals	hexafluoropropylene oxide dimer acid (HFPO-DA) and HFPO-DA ammonium salt
GD	gestational day
g/L	grams per liter
HA	health advisory
HBWC	health-based water concentration
HED	human equivalent dose
HDPE	high-density polyethylene
HFPO	hexafluoropropylene oxide
HI	hazard index
HQ	hazard quotient
IA	integrated addition
IRIS	Integrated Risk Information System
K+PFBS	potassium perfluorobutane sulfonate
L/kg/day	liters per kilogram body weight per day
LAS	land application site
LB	lower bound
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantitation
MAMA	Methods Advancement for Milk Analysis
MCLG	Maximum Contaminant Level Goal
MDL	method detection limit

MF	modifying factor
mg/kg/day	milligrams per kilogram body weight per day
mg/L	milligrams per liter
MOA	mode of action
MRL	minimal risk level
mRNA	messenger ribonucleic acid
MSW	municipal solid waste
MQL	method quantification limit
NAS	National Academy of Sciences
NCDHHS	North Carolina Department of Health and Human Services
NCDEQ	North Carolina Department of Environmental Quality
ng/g dw	naanograms per gram dry weight
ng/kg	nanograms per kilogram
ng/L	nanograms per liter
NHANES	National Health and Nutrition Examination Survey
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no-observed-adverse-effect level
NPDWR	National Primary Drinking Water Regulation
NRSA	National Rivers and Streams Assessment
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
ORD	Office of Research and Development
PECO	Population, Exposure, Comparator, and Outcome
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutanesulfonic acid
PFC	perfluorochemicals
PFCA	perfluoroalkyl carboxylic acids
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
pg/g	picograms per gram
pg/L	picograms per liter
pg/m^3	picograms per cubic meter
PHG	provisional health goal
PND	postnatal day
POD	point of departure
POP	persistent organic pollutants
PPARα	peroxisome proliferator-activated receptor alpha
ppt	parts per trillion
PSA	prostate-specific antigen
PWS	public water system
RA	response addition
RfD	reference dose
RfV	reference value
RPF	relative potency factor
RSC	relative source contribution
SAB	Science Advisory Board
SDWA	Safe Drinking Water Act

SLEA	Screening Level Exposure Assessment
SPM	suspended particulate matter
TOFMS	time-of-flight mass spectrometry
TOSHI	target-organ-specific hazard indices
TRI	Toxics Release Inventory
TSCATS	Toxic Substances Control Act Test Submissions
TTD	target organ toxicity dose
UB	upper bound
UCMR	Unregulated Contaminant Monitoring Rule
UCMR 3	third Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
UF_H	human interindividual variability uncertainty factor
UF_S	extrapolation from subchronic-to-chronic exposure duration uncertainty factor
µg/kg	micrograms per kilogram
µg/L	micrograms per liter
$\mu g/m^2$	micrograms per square meter
WOS	Web of Science
WWTP	wastewater treatment plant

1 Introduction and Background

1.1 Purpose

Section 1412(a)(3) of the Safe Drinking Water Act (SDWA) requires the Administrator of the U.S. Environmental Protection Agency (EPA) to propose a Maximum Contaminant Level Goal (MCLG) simultaneously with the National Primary Drinking Water Regulation (NPDWR). The MCLG is set, as defined in Section 1412(b)(4)(A), at "the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety." The MCLG incorporates a margin of safety to reflect scientific uncertainty and, in some cases, the particular susceptibility of some groups (e.g., children) within the general population. Consistent with SDWA 1412(b)(3)(C)(i)(V), in developing the MCLG, the EPA considers "the effects of the contaminant on the general population and on groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other subpopulations that are identified as likely to be at greater risk of adverse health effects due to exposure to contaminants in drinking water than the general population." Other factors considered in determining MCLGs include health effects data for drinking water contaminants and potential sources of exposure other than drinking water. MCLGs are not regulatory levels and are not enforceable.

The purpose of this document is to provide a summary of the health effects and exposure information and analyses and to describe the derivation of the EPA's final MCLGs for the following per- and polyfluoroalkyl substances (PFAS), for which the EPA is finalizing a NPDWR: hexafluoropropylene oxide dimer acid (HFPO-DA) (also known as GenX chemicals)[2], perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), and perfluorobutane sulfonic acid (PFBS).[3] The EPA is finalizing individual MCLGs for HFPO-DA, PFNA, and PFHxS. The EPA is also finalizing a PFAS mixture MCLG for mixtures of two or more of four PFAS—HFPO-DA, PFNA, PFHxS, and PFBS—that accounts for dose-additive health effects when these PFAS co-occur in drinking water. The PFAS mixture MCLG is based on a hazard index (HI) approach, a commonly used component-based mixtures risk assessment method (see Section 1.4 and USEPA, 2024a). This document summarizes key elements (e.g., reference doses (RfDs)) from recently published, peer-reviewed, publicly available human health toxicity assessments for HFPO-DA (USEPA, 2021c), PFBS (USEPA, 2021d), PFNA (ATSDR, 2021), and PFHxS (ATSDR, 2021) that the EPA used to develop MCLGs for HFPO-DA, PFNA, and PFHxS and an MCLG for mixtures of two or more of these PFAS plus PFBS. The MCLG represents the level below which adverse health effects over a lifetime of exposure are not expected to occur, including for sensitive populations and life stages, and with an adequate margin of safety. This document is not intended to be an exhaustive description of all health effects or modeled endpoints (i.e., human health toxicity assessment) nor is it a drinking water health advisory (HA).

[2]The EPA notes that HFPO-DA is used in a processing aid technology developed by DuPont to make fluoropolymers without using PFOA. The chemicals associated with this process are commonly known as GenX chemicals and the term is often used interchangeably for HFPO-DA along with its ammonium salt.

[3]Note: The EPA is also finalizing individual MCLGs for two other PFAS: perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) (see USEPA, 2024c)).

1.2 Occurrence and Co-Occurrence of PFAS in Drinking Water

Improved analytical monitoring and detection methods have enabled detection of the occurrence and co-occurrence of multiple PFAS in drinking water, ambient surface waters, aquatic organisms, and other environmental media (see Appendices A through D and USEPA, 2024d). The two PFAS perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have historically been target analytes, and the focus of many environmental monitoring studies. More recent monitoring studies, however, have focused on additional PFAS via advanced analytical instruments/methods and nontargeted analysis (De Silva et al., 2021; McCord et al., 2020; McCord and Strynar, 2019).

The EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect occurrence data nationwide for contaminants that are suspected to be present in drinking water. Between 2013 and 2015, the EPA's third UCMR (UCMR 3) required all large public water systems (PWSs) (each serving more than 10,000 people) and a statistically selected, nationally representative sample of 800 small PWSs (each serving 10,000 people or fewer) to monitor for 30 unregulated contaminants in drinking water, including PFNA, PFHxS, and PFBS. In addition to the UCMR 3 data collection, many states have undertaken more recent efforts to monitor for PFAS in both source and finished drinking water using newer analytical methods and reflecting lower reporting limits than those in UCMR 3. These results and other peer-reviewed studies show continued PFAS occurrence and co-occurrence in multiple geographic locations (USEPA, 2024b; Cadwallader et al., 2022). These data also show certain PFAS (including PFNA, PFHxS, and PFBS) measured at lower concentrations and significantly greater frequencies than were measured under UCMR 3. Additionally, these state monitoring data include results for HFPO-DA (which was not included in the suite of PFAS analyzed in UCMR 3) and demonstrate HFPO-DA occurrence (and co-occurrence with other PFAS) in drinking water. From 2023–2025, monitoring data for 29 PFAS including HFPO-DA, PFBS, PFNA, and PFHxS are being collected under UCMR 5. These drinking water occurrence and co-occurrence data for HFPO-DA, PFNA, PFHxS, PFBS, as well as additional PFAS are detailed in the EPA's *PFAS Occurrence and Contaminant Background Support Document for the Final PFAS NPDWR* (USEPA, 2024b).

1.3 Dose Additivity for PFAS Mixtures

1.3.1 Overview of Scientific Support

Dose additivity means that when two or more chemicals (in this case, PFHxS, PFNA, HFPO-DA, and/or PFBS) exist in one mixture, the risk of adverse health effects following exposure to the mixture is equal to the sum of the individual doses or concentrations scaled for potency (USEPA, 2000a). Studies with PFAS and other classes of chemicals support the health-protective conclusion that toxicologically similar chemicals (i.e., those that elicit similar observed adverse effects following individual exposure, even if at different exposure levels) should be assumed to act in a dose-additive manner when present in a mixture unless data demonstrate otherwise. Experimental data demonstrate that PFAS elicit similar adverse health effects on several of the same biological systems and functions including thyroid hormone signaling, lipid synthesis and metabolism, development, and immune and liver function. Thus, exposure to these PFAS, at doses that individually would not likely result in adverse health effects, when combined in a mixture may pose health risks.

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Numerous published studies across multiple chemical classes, biological effects, and study designs support a dose-additive mixture assessment approach for PFAS because they demonstrate that experimentally observed responses to exposure to PFAS mixtures and other chemical mixtures are consistent with modeled predictions of dose additivity (see the EPA's *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances* (PFAS) (hereafter "PFAS Mixtures Framework;" (USEPA, 2024a)). Since the EPA's draft PFAS Mixtures Framework underwent SAB review in 2021−2022, new studies from the EPA and others have provided robust evidence of combined toxicity of PFAS in mixtures, corroborating and confirming earlier findings (USEPA, 2024a; e.g., Conley et al., 2023; Conley et al., 2022). Additionally, the National Academies of Sciences, Engineering, and Medicine (NASEM, 2022) recently recommended that clinicians apply an additive approach for evaluating patient levels of PFAS currently measured in the National Health and Nutrition Examination Survey (NHANES) in order to protect human health from additive effects from PFAS co-exposure.

Data from *in vivo* studies that rigorously tested accuracy of Dose Additivity (DA), Integrated Addition (IA), and Response Additivity (RA) model predictions of mixtures with components that disrupted the same pathways (i.e., were toxicologically similar) demonstrated that DA models provided predictions that were better than or equal to IA and RA predictions of the observed mixture effects (Section 3.2 in USEPA, 2024a). In some circumstances the different additivity models provide highly similar predictions of mixture effects and thus are essentially equally effective. In situations where the models provide very different predictions, experimental data have demonstrated that DA-based models consistently provide more accurate predictions of observed mixture effects than RA or IA. This strongly supports the use of dose additivity as the default method for estimating mixture effects of compounds that are toxicologically similar. The National Academy of Sciences (NAS) conclusions on phthalates (and related chemicals) (NRC, 2008) and systematic reviews of the published literature (USEPA, 2024a; Martin et al., 2021; Boobis et al., 2011) support dose additivity as the default model for estimating mixture effects in some circumstances, even when the mixtures included chemicals with diverse MOAs (but the same target organs/effects). Systematic reviews of mixture studies with chemical classes other than PFAS also indicate that departures from dose additivity are uncommon and rarely exceed minor deviations (~2-fold) from predictions based on dose additivity (Martin et al., 2021; Boobis et al., 2011). Boobis et al. (2011) examined literature from 1990 to 2008 that discussed synergy in mammalian test systems, with an emphasis on "low dose" studies. They found that of the 11 available studies with synergy data that reported the magnitude of the difference between the dose-additive estimates of toxicity and observed toxicity, six studies reported magnitudes of synergy that were generally small, and the authors concluded that deviations from dose additivity at low doses were not common. Additionally, Martin et al. (2021) reviewed more than 1,200 mixture studies and concluded that there was little evidence for synergy (greater than additive effects) or antagonism (less than additive effects) among chemicals in mixtures, and that dose additivity should be considered as the default model. This supports the health-protective conclusion that a mixture of PFHxS, PFNA, HFPO-DA, and/or PFBS should be assumed to act in a dose-additive manner unless data demonstrate otherwise.

Although some available *in vitro* studies do not provide conclusive evidence of dose additivity for PFAS mixtures, their results also do not justify drawing a conclusion other than dose additivity. For example, a study on PFAS cytotoxicity in a human liver cell line (Ojo et al., 2020)

reported synergistic effects of mixtures of perfluoroalkyl acids (PFAAs; a type of PFAS) compared to a dose addition model, but also reported evidence of antagonistic effects. Other *in vitro* studies that have assessed PFAS mixture-based effects do not report these results; that is, they do not offer strong evidence for synergistic or antagonistic effects, particularly at environmentally relevant concentrations. For example, Wolf et al. (2014) evaluated *in vitro* PPARα activation and reported that effects seen following exposure to combinations of different PFAS were consistent with dose additivity in the lower tested concentration ranges. Wolf et al. (2014) also reported slightly greater than additive effects at higher test concentrations (approximately 500 parts per billion to over 800 parts per million); however, in environmental media such as drinking water, PFAS are not likely to occur at these higher concentrations (e.g., see USEPA, 2024b). Carr et al. (2013) reported slightly less than additive effects for *in vitro* PPARα activation of binary mixtures of PFAAs including PFOA, PFNA, PFOS, and PFHxS. Addicks et al. (2023) evaluated mRNA transcription in primary human liver spheroids exposed to seven different PFAS mixtures and found that all tested mixtures produced effects that were consistent with effects predicted using dose addition. To summarize, the available *in vitro* data do not support a conclusion other than dose additivity for PFAS mixtures.

Available *in vivo* data on this subject similarly support dose additivity. Two studies with PFAS mixtures in zebrafish reported no indications of synergy (Menger et al., 2020; Ding et al., 2013). Additionally, recent EPA Office of Research and Development (ORD) studies provide robust evidence that PFAS behave in a dose-additive manner (Gray et al., 2024; Conley et al., 2023; Conley et al., 2022). For example, results of a developmental toxicity study of exposure to PFOA and PFOS mixtures in rats showed that the observed results for almost all tested endpoints were consistent with dose additivity (Conley et al., 2022). Likewise, a rat developmental study of a PFAS mixture of PFOS, HFPO-DA, and Nafion byproduct 2 (an emerging polyfluoroethersulfonic acid compound recently detected in human serum (Kotlarz et al., 2020)) found that multiple tested endpoints in both parental females and offspring conformed to dose additivity and no endpoints demonstrated synergy (Conley et al., 2023).

Additionally, as described in the final PFAS Mixtures Framework (USEPA, 2024a), over the past two decades, many *in vivo* experimental animal studies have been published in which toxicity of chemical mixtures has been systematically evaluated (e.g., Conley et al., 2023; Conley et al., 2022; Martin et al., 2021; Hass et al., 2017; Howdeshell et al., 2015; Moser et al., 2012; Rider et al., 2010; Kortenkamp and Haas, 2009; Rider et al., 2009; Rider et al., 2008; Crofton et al., 2005; Moser et al., 2005; Walker et al., 2005; Gennings et al., 2004; Altenburger et al., 2000). These studies span different chemical classes, proposed MOAs, and health outcomes, but they generally show that chemicals in mixtures typically act dose additively. Even when mixture components with different MOAs/adverse outcome pathways (AOPs) are combined, they induce toxic effects consistent with dose additivity (Rider et al., 2009). This concept was further articulated in the National Research Council's 2008 report *Phthalates and cumulative risk assessment: The tasks ahead* (NRC, 2008), wherein that expert panel provided significant evidence that mixture components that elicit similar adverse health effects individually will demonstrate dose additivity when combined in a mixture, regardless of similarity in MOA.

This evidence base supports the longstanding recommendation in EPA chemical mixtures guidance for dose additivity as a default approach for evaluation of mixture toxicity (USEPA,

2000a, 1986). This position is further supported and articulated in the newly published EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023b).

1.3.1.1 Science Advisory Board Support

The EPA's conclusions regarding dose additivity of PFAS were supported by the SAB during its 2021–2022 review of the EPA's draft *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances*. The EPA directly asked the SAB for feedback on PFAS dose additivity as part of its review of technical materials supporting development of the PFAS MCLG and NPDWR. Specifically, the EPA asked the SAB to, "[p]lease comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity" (USEPA SAB, 2022). The SAB strongly supported the scientific soundness of this approach when evaluating PFAS and concurred that it was a health protective conclusion. For example, the SAB said:

> "The SAB supports dose additivity based on a common outcome, instead of a common mode of action as a health protective default assumption and does not propose another default approach." (USEPA SAB, 2022)
>
> "…The information included in the draft framework supports the conclusion that toxicological interactions of chemical mixtures are frequently additive or close to additive. It also supports the conclusion that dose additivity is a public health protective assumption that typically does not underestimate the toxicity of a mixture..." (USEPA SAB, 2022)
>
> "The SAB Panel agrees with use of the default assumption of dose additivity when evaluating PFAS mixtures that have similar effects and concludes that this assumption is health protective." (USEPA SAB, 2022)
>
> "…dose additivity can provide an estimate of composite effects." (USEPA SAB, 2022)

While the SAB also noted that there remain some questions about PFAS interaction in mixtures (USEPA SAB, 2022), the available data justify an approach that accounts for PFAS dose additivity. As described above, studies that have assessed PFAS mixture-based effects do not provide support for a conclusion other than dose additivity (i.e., they do not offer strong evidence for synergistic/antagonistic effects) (USEPA, 2024a).

1.3.2 Toxicological Similarity of PFHxS, PFNA, HFPO-DA, and PFBS

1.3.2.1 Background on Concept of "Toxicological Similarity"

This concept and application of dose additivity for "toxicologically similar components" in mixtures assessment is consistent with EPA mixtures guidance (USEPA, 2000a, 1986) and the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023b). Specifically, the EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 2000a) notes that although the shared MOA metric for application of dose addition is optimal, MOA data are not always available and that toxicological similarity in the context of mixtures risk assessment can be based on adverse

effects observed at the organ or system level (USEPA, 2000a). This concept is further described in the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023b): "The primary criterion for choosing between dose addition and response addition methods is toxicological similarity among the chemicals in the mixture [(USEPA, 2000a)]. "Toxicological similarity" is used here as an overarching concept with a wide range of specificity across levels of biological organization, allowing similarity judgments to be tailored to both the specific goals of the mixture risk assessment and the availability of hazard and dose-response information across components." Unless there are available data that suggest deviation(s) from dose additivity, mixture chemicals that are "toxicologically similar" (e.g., same/similar effect or profile of effect[s], regardless of differences in potencies) prototypically behave dose additively. This concept is depicted in Figure 1-1 below, which shows that dose additivity is the logical default approach for "toxicologically similar" components and that component-based mixture assessment approaches including hazard index (HI), relative potency factor (RPF), and mixture-benchmark dose (Mixture-BMD) are options for mixture assessment in such cases (see Section 1.5).

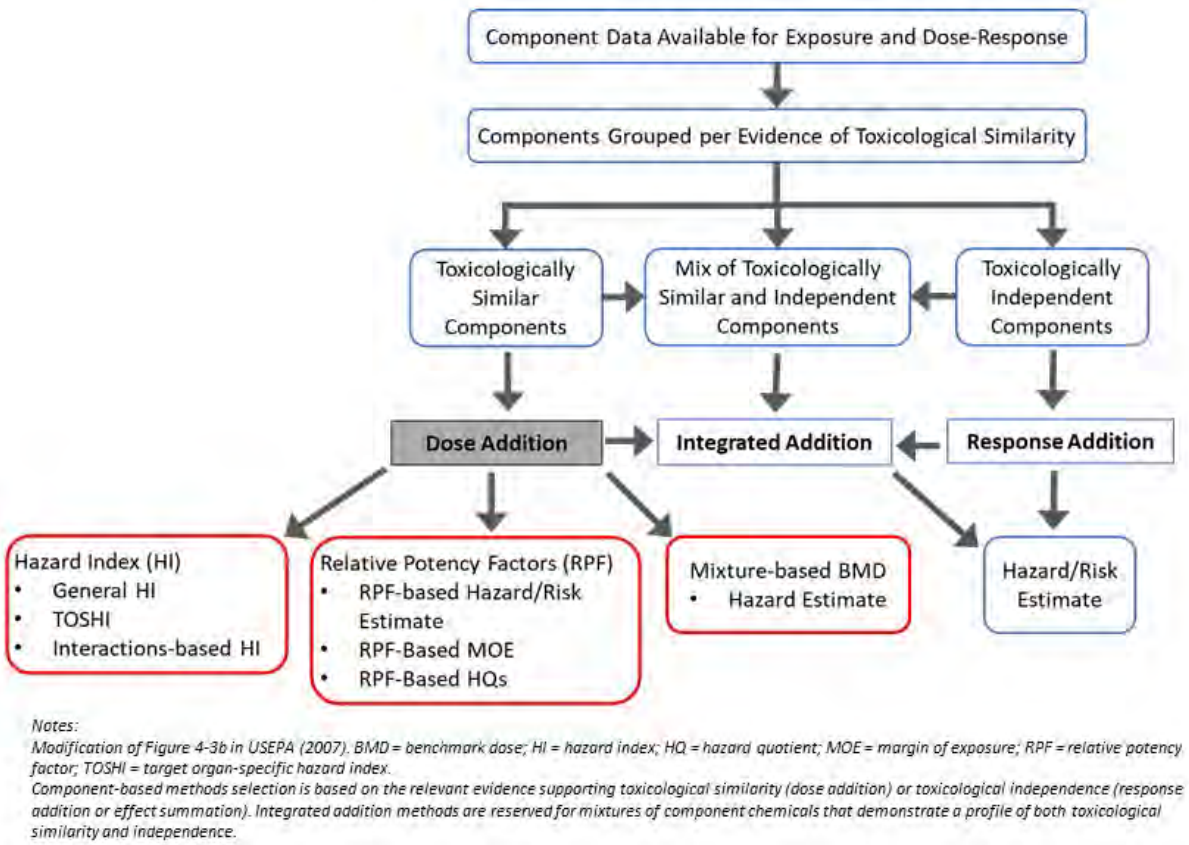


Figure 1-1. Flow chart for evaluating chemical mixtures using component-based additive methods. (Reproduction of Figure 2-1 from USEPA, 2024a).

1.3.2.2 Overview of Scientific Support

The EPA's approach is to evaluate risks from exposure to mixtures of PFAS based on similar adverse health effects (but with differing potencies for effect(s)) of the individual PFAS mixture components, rather than similar MOA. MOA describes key changes in cellular or molecular

events that may cause functional or structural changes that lead to adverse health effects and can be a useful metric by which risk can be assessed. It is considered a key determinant of chemical toxicity, and chemicals can often be classified by their type of toxicity pathway(s) or MOA(s). PFAS are an emerging chemical class, and MOA data are limited or entirely lacking for many PFAS. Although similarities among some PFAS have been shown at the level of molecular and cellular perturbations, no conserved MOAs have been identified across PFAS for noncancer health effects assessed thus far. Therefore, the EPA's approach for assessing risks of PFAS mixtures is based on the conclusion that PFAS that are "toxicologically similar"—that is, elicit the same or similar adverse health effects (but with differing potencies for effect(s))—will produce dose-additive effects from co-exposures (see USEPA, 2024a).

Available epidemiological and animal toxicological data demonstrate that exposure to each of these four PFAS (PFHxS, PFNA, HFPO-DA, and PFBS) is associated with many of the same or similar adverse health endpoints and outcomes, and thus they are "toxicologically similar" (see Table 1-1, Table 1-2, and Table 1-3 below). Further, these four PFAS are well-studied PFAS for which the EPA or ATSDR has developed human health assessments and toxicity reference values (i.e., reference doses (RfDs), minimal risk levels (MRLs)). Available animal toxicological and/or epidemiological studies demonstrate that PFHxS, PFNA, HFPO-DA, and PFBS are documented to affect at least five (5) of the same major health outcomes: lipids, developmental, immune, endocrine, and hematologic (Table 1-1). Similarly, according to the 2023 Interagency PFAS Report to Congress (US OSTP, 2023), available animal toxicological data show that PFHxS, PFNA, HFPO-DA, and PFBS significantly affect at least eight (8) of the same major health effect domains: body weight, respiratory, hepatic, renal, endocrine, immunological, reproductive, and developmental (Table 1-2). Furthermore, numerous *in vivo* and *in vitro* studies demonstrate that these four PFAS share many common health effects across diverse health outcome categories (e.g., developmental, immunological, and endocrine), and that they induce some of the same effects at the molecular level along biological pathways. Table 1-3 below shows specific endpoints shared across these four PFAS, including toxicologically relevant molecular perturbations (*in vitro*), and health effects (*in vivo*) from oral repeated-dose studies in rats and/or mice (note that this table is a summary of select studies for illustrative purposes and should not be construed to represent a systematic review or MOA analysis).

Table 1-1. Affected health outcomes in animal toxicological and/or epidemiological studies for the four PFAS included in the HI MCLG (adapted from Table 6-7 in USEPA, 2024d).

Health Outcome	HFPO-DA	PFNA	PFHxS	PFBS
Lipids	X	X	X	X
Developmental	X	X	X	X
Hepatic	X	X	X	-
Immune	X	X	X	X
Endocrine	X	X	X	X
Renal	X	-	-	X
Hematologic	X	X	X	X

Notes: (X) Health outcome examined, evidence of association; (-) health outcome examined, no evidence of association.

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Table 1-2. Affected health endpoints in animal toxicity studies for the four PFAS included in the HI MCLG (adapted from Table 4 in US OSTP, 2023).

Health Endpoint	HFPO-DA	PFNA	PFHxS	PFBS
Body weight	X	X	X	X
Respiratory	X	X	X	X
Cardiovascular			X	X
Gastrointestinal		X	X	X
Hematological	X		X	X
Musculoskeletal			X	X
Hepatic	X	X	X	X
Renal	X	X	X	X
Dermal	X			
Ocular	X			X
Endocrine	X	X	X	X
Immunological	X	X	X	X
Neurological	X		X	X
Reproductive	X	X	X	X
Developmental	X	X	X	X
Other noncancer	X	X		

Notes: (X) Health outcome examined, evidence of association.

Table 1-3. Specific Endpoints Affected by One or More of the Four PFAS Included in the HI MCLG.

Endpoint	HFPO-DA	PFNA	PFHxS	PFBS
Molecular/Cellular Perturbations				
PPAR alpha binding/activation	Evans et al. (2022); Nielsen et al. (2021)	Evans et al. (2022); Nielsen et al. (2021); Rosenmai et al. (2018); Wolf et al. (2012)	Evans et al. (2022); Nielsen et al. (2021); Rosenmai et al. (2018); Wolf et al. (2012)	Evans et al. (2022); Rosenmai et al. (2018); Wolf et al. (2012)
PPAR gamma binding/activation	Evans et al. (2022); Houck et al. (2021)	Evans et al. (2022); Houck et al. (2021)	Evans et al. (2022); Houck et al. (2021)	Evans et al. (2022)
Liver gene induction (PPAR signaling pathway)	Conley et al. (2019); Blake et al. (2022)	NTP (2019b); Rosen et al. (2017); Rosen et al. (2013)	NTP (2019c); Rosen et al. (2017); Rosen et al. (2013); Chang et al. (2018)	NTP (2019c); Rosen et al. (2013)
Liver gene induction (CAR signaling pathway)	-	NTP (2019b)	NTP (2019c)	NTP (2019c)

Endpoint	HFPO-DA	PFNA	PFHxS	PFBS
Serum bile salts/acids (increased)	DuPont (2010c)	NTP (2019b)	-	NTP (2019c)
Serum globulin (reduced)	DuPont (2009); DuPont (2008b); DuPont (2008a)	NTP (2019b)	NTP (2019c)	NTP (2019c)
Serum albumin:globulin (increased)	DuPont (2009); DuPont (2008b); DuPont (2008a)	NTP (2019b)	NTP (2019c); Butenhoff et al. (2009)	NTP (2019c)
Health Effects				
Serum lipids (reduced cholesterol and/or triglycerides)	DuPont (2008b); DuPont (2009); DuPont (2008a)	NTP (2019b)	NTP (2019c); Chang et al. (2018); Butenhoff et al. (2009)	NTP (2019c)
Serum liver enzymes (increased ALT, AST, and/or ALKP)	DuPont (2008b); DuPont (2010c)	NTP (2019b)	-	NTP (2019c)
Serum thyroid hormones (reduced T4, T3)	Conley et al. (2019)	NTP (2019b)	NTP (2019c); Gilbert et al. (2021)	NTP (2019c)
Liver weight (increased)	DuPont (2008b); Blake et al. (2020); Conley et al. (2021); Conley et al. (2019); DuPont (2009); Rushing et al. (2017); DuPont (2008a)	NTP (2019b); Das et al. (2015)	NTP (2019c); Chang et al. (2018)	NTP (2019c); Lieder et al. (2009b)
Liver histopathology (nonneoplastic effects)	DuPont (2008b); DuPont (2010c); DuPont	NTP (2019b)	Chang et al. (2018); NTP (2019c)	NTP (2019c)

Endpoint	HFPO-DA	PFNA	PFHxS	PFBS
	(2008a); NTP (2019a)			
Thymus weight (reduced)	DuPont (2009)	NTP (2019b)	-	NTP (2019c)
Spleen weight (reduced)	-	NTP (2019b)	-	NTP (2019c); Lieder et al. (2009a)
Kidney weight (increased)	DuPont (2009); DuPont (2008b)	NTP (2019b)	NTP (2019c)	NTP (2019c)
Reduced fetal/pup bodyweight	Conley et al. (2021); DuPont (2010a); DuPont (2010b)	Das et al. (2015)	-	Feng et al. (2017)
Reduced fetal/pup survival	Conley et al. (2021)	Das et al. (2015)	Chang et al. (2018)	-
Reduced adult bodyweight	DuPont (2013)	NTP (2019b)	-	NTP (2019c); Lieder et al. (2009a)
Overt toxicity (lethality)	DuPont (2009)	NTP (2019b)	-	NTP (2019c)

Note: (-) indicates no statistically significant effect reported by study authors of cited studies at dose levels and dose interval used and/or effect not measured in cited studies.

1.3.2.3 Science Advisory Board Support

The SAB strongly supported the EPA's decision to focus on similarity of adverse health effects rather than similarity of MOA to assess risks of exposure to PFAS mixtures during its 2021–2022 review of the EPA's draft PFAS Mixtures Framework. Specifically, the EPA asked the SAB, "If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s)" (USEPA SAB, 2022). The SAB strongly supported the EPA's approach of using a similar toxicity endpoint/health effect instead of a common MOA as a default approach for evaluating mixtures of PFAS using dose additivity and did not recommend an alternative methodology. The SAB panel stated that:

> "The Panel agreed with use of a similar toxicity endpoint/health effect instead of a common MOA as a default approach for evaluating mixtures of PFAS. This approach makes sense because multiple physiological systems and multiple MOAs can contribute

> to a common health outcome. Human function is based on an integrated system of systems and not on single molecular changes as the sole drivers of any health outcome. The Panel concluded that rather than the common MOA, as presented in the EPA draft mixtures document, common physiological outcomes should be the defining position" (USEPA SAB, 2022).
>
> "Furthermore, many PFAS, including the four used in the examples in the draft EPA mixtures document and others, elicit effects on multiple biological pathways that have common adverse outcomes in several biological systems (e.g., hepatic, thyroid, lipid synthesis and metabolism, developmental and immune toxicities)" (USEPA SAB, 2022).

1.3.3 Summary

The available scientific evidence supports the conclusion that PFAS that elicit similar adverse health effects following individual exposure (even if with differing potencies for effect(s)) should be assumed to act in a dose-additive manner when in a mixture unless data demonstrate otherwise. This means that individual PFAS, each at doses that are not anticipated to result in adverse health effects, when combined in a mixture may result in adverse health effects. (For a more complete discussion of the evidence supporting dose additivity as the default approach for assessing mixtures of PFAS, see the final PFAS Mixtures Framework (USEPA, 2024a)). The EPA's conclusions regarding PFAS dose additivity were supported by the SAB during its review of the EPA's draft PFAS Mixtures Framework (USEPA SAB, 2022) and are consistent with longstanding agency chemical mixtures guidance (USEPA, 2000a, 1986) and a recent EPA white paper (USEPA, 2023b). The SAB also strongly supported the EPA's default assumption of dose additivity in the absence of other information and the EPA's approach of using similar toxicity endpoints/health effects instead of a common MOA for evaluating mixtures of PFAS (USEPA SAB, 2022). This approach of basing the concept of toxicological similarity on same/similar adverse effects in the absence of adequate MOA information is also consistent with the EPA's chemical mixtures guidance (USEPA, 2000a, 1986) and the EPA Risk Assessment Forum's *Advances in Dose Addition for Chemical Mixtures: A White Paper* (USEPA, 2023b).

1.4 General Hazard Index (HI) Approach for PFAS Mixtures

1.4.1 Background/Overview

The EPA's final determination that mixtures of the four PFAS "may have an adverse effect on the health of persons" is based on the health-protective conclusion that chemicals that are toxicologically similar (i.e., have similar observed adverse health effects, regardless of potency differences) following individual exposure should be assumed to act in a dose-additive manner when in a mixture unless data demonstrate otherwise (see Section 1.3 and USEPA, 2024a). This means that where drinking water contains any combination of two or more of the four PFAS that are the subject of the NPDWR—PFHxS, PFNA, HFPO-DA, and PFBS—the hazard associated with each PFAS in the mixture must be added together to determine whether the mixture exceeds a level of public health concern.

The SDWA requires the agency to establish a health-based MCLG set at "a level at which no known or anticipated adverse effects on the health of persons occur and which allows for an adequate margin of safety." The MCLG "incorporates a margin of safety to reflect scientific

uncertainty and, in some cases, the particular susceptibility of some groups (e.g., children) within the general population" (see S. Rep. No. 169, 104th Cong., 1st Sess. (1995) at 3). In the context of this NPDWR, the general HI is the approach used to determine if a mixture of two or more of four PFAS in drinking water—PFHxS, PFNA, HFPO-DA, and PFBS—exceeds the level of health concern with a margin of safety. A general HI equal to 1 is the MCLG for any mixture of these four PFAS.

Based on the scientific record, each of these four PFAS has a health-based water concentration (HBWC), which is set at the level below which adverse effects are not anticipated to occur and allows for an adequate margin of safety (see Section 2). The general HI approach accounts for the measured drinking water concentration of each of the four PFAS in the mixture, and the toxicity (represented by the HBWC) of each of the four PFAS. The general HI is derived by first calculating the ratio of the measured concentration of each of the four PFAS to its toxicity (the HBWC) to yield a "hazard quotient" (HQ) for each of the four PFAS. HQs are then added together to account for the dose-additive health concerns that these PFAS present. Adding the four HQs together yields the general HI. If the general HI exceeds 1, then the hazard from the combined amounts of the four PFAS present together in drinking water exceeds a level of public health concern.

The EPA has determined that in the context of SDWA, the general HI is an appropriate methodology for determining the level at and below which there are no known or anticipated adverse human health effects with an adequate margin of safety with respect to certain PFAS mixtures in drinking water. The general HI approach is the most practical approach for establishing an MCLG for PFAS mixtures that meets the statutory requirements outlined in Section 1412(b)(1)(A) of SDWA. As noted above, the general HI assesses the exposure level of each component PFAS relative to its HBWC, which is based on the most sensitive known adverse health effect (based on the weight of evidence) and considers sensitive population(s) and life stage(s) as well as potential exposure sources beyond drinking water. The general HI also accounts for dose-additive health concerns by summing the hazard contributions from each mixture component. In this way, the general HI approach ensures that mixtures of two or more of these four PFAS are not exceeding the level below which there are no known or anticipated adverse health effects and allows for an adequate margin of safety.

1.4.2 Consideration of Mixtures Assessment Approaches and Selection of General HI Approach

In selecting an approach to develop the MCLG for mixtures of two or more of four PFAS—PFHxS, PFNA, HFPO-DA, and PFBS—the EPA followed its *Guidelines for the Health Risk Assessment of Chemical Mixtures* (USEPA, 1986), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA, 2000a), and *Risk Assessment Guidance for Superfund* (e.g., USEPA, 1991b). As described below, the EPA first considered whether data were available for the whole mixture or a "sufficiently similar" mixture, per agency guidance (USEPA, 2000a, 1986), and then considered several mixture component-based assessment methods (USEPA, 2024a), ultimately selecting the general HI approach for PFAS mixture MCLG derivation.

The EPA's guidance documents (USEPA, 2000a, 1991b, 1986) propose a hierarchy of mixtures assessment approaches, with the preferred approach being evaluation of health risk using hazard and dose-response data for a specific whole mixture of concern, or alternatively, a "sufficiently similar" mixture. Whole-mixture data are rare; there are often many chemical combinations and proportions in the environment (e.g., parent chemicals, metabolites, and/or abiotic degradants), introducing a level of complexity that complicates evaluation and characterization. The exponential diversity of PFAS co-occurring in different combinations and proportions makes whole-mixture evaluations complex and unfeasible. Due to differing fate and transport properties, biotic (metabolism) and abiotic (degradation) processes, pH, ultraviolet radiation, media temperature, and so on, chemicals commonly co-occur in the environment in an array of parent species, metabolites, and/or degradants, making characterization and evaluation of any given mixture complicated. In controlled experimental study designs, whole mixtures can be assembled with defined component membership and proportions, but the relevance of toxicity associated with exposure to a defined mixture in a laboratory setting may not be translatable to environmental mixtures of different component combinations and proportions across time and space in environmental media. The complexities associated with the diversity of PFAS co-occurring in different component proportions (see USEPA, 2024b) make evaluating each unique whole mixture of PFAS intractable. This is why component-based mixture assessment approaches are considered particularly useful and appropriate for addressing human exposure(s) to mixtures of PFAS (see Sections 5–7 in USEPA, 2024a). For a more detailed discussion on whole-mixture and component-based approaches for PFAS risk assessment, please see the final *PFAS Mixtures Framework* (USEPA, 2024a).

The EPA considered several component-based assessment approaches to develop an MCLG for mixtures of PFHxS, PFNA, HFPO-DA, and/or PFBS under an assumption of dose additivity, including the general HI, the target organ-specific HI (TOSHI), the Relative Potency Factor (RPF) approach, and the mixture-benchmark dose (mixture-BMD) approach (USEPA, 2024a). As part of the technical support materials for the PFAS NPDWR, the EPA's draft PFAS Mixtures Framework (USEPA, 2021f) was submitted to the SAB for expert review. The SAB supported the EPA's proposed component-based approaches under the assumption of dose additivity in the absence of information to support a conclusion other than dose additivity (see Section 1.3). Following the SAB review, the EPA addressed the SAB's recommendations. Then, the EPA solicited public comment on the draft PFAS Mixtures Framework as part of the proposed NPDWR (88 FR 18638; USEPA (2023c)). The EPA evaluated potential component-based mixture assessment options, and ultimately proposed using the general HI approach as the most appropriate option based on available data and consistent with the statutory definition of MCLG.

The EPA first considered a "whole mixture" approach. Although use of data from whole mixtures or "sufficiently similar mixtures" is ideal in a theoretical sense, it is not practical, possible, or necessary for evaluating mixtures of PFAS in drinking water. Instead, the EPA is using the general HI approach, a longstanding component-based mixtures assessment approach which was endorsed by the SAB in the context of assessing risk associated with exposure to PFAS mixtures in drinking water (USEPA SAB, 2022), as discussed below. The goal of this component-based mixtures assessment approach is to approximate what the whole-mixture toxicity would be if the whole mixture could be tested and relies on toxicity information for each individual component in a mixture (USEPA, 2000a). A whole-mixture approach for regulating

mixtures of these four PFAS in drinking water is not possible because it would entail developing a single toxicity reference value (e.g., RfD) for one specific mixture of PFHxS, PFNA, HFPO-DA, and PFBS with defined proportions of each PFAS. Toxicity studies are typically conducted with only one test substance to isolate that particular substance's effects on test organisms, and whole-mixture data are exceedingly rare. There are no known whole-mixture studies for PFHxS, PFNA, HFPO-DA, and PFBS, and even if they were available, a toxicity reference value derived from such a study (i.e., a single RfD for a specific mixture of these four PFAS) would only be directly applicable to that specific mixture. Thus, a more flexible approach is necessary—one that considers the potential for the four PFAS to co-occur in different combinations and at different concentrations across time and space. The general HI approach affords this flexibility; the general HI indicates risk from exposure to a mixture and is useful to ensure a health-protective MCLG for PFAS mixtures that can be spatially and/or temporally variable. Given the variability of PFAS occurrence in drinking water across the nation (USEPA, 2024b), the general HI allows the EPA to regulate mixtures of these PFAS in drinking water by taking into account site-specific data at each PWS. HQs for the four different PFAS are expected to differ depending on the actual measured concentrations of each of the four PFAS at each PWS. The general HI approach thus allows for flexibility beyond a one-size-fits-all approach and is tailored to address risk at each PWS. Furthermore, the EPA's application of the general HI approach accounts for the dose additivity that was the basis for the EPA's final determination to regulate mixtures of two or more of these PFAS.

The EPA considered the two main types of HI approaches: 1) the general HI, which allows for component chemicals in the mixture to have different health effects or endpoints as the basis for their toxicity reference values (e.g., RfDs, minimal risk levels), and 2) the TOSHI, which relies on toxicity reference values based on the same specific target organ or system effects (e.g., effects on the liver or thyroid; effects on developmental or reproductive systems) (USEPA, 2000a). The general HI approach uses the most health-protective RfD (or minimal risk level) available for each mixture component, irrespective of whether the RfDs for all mixture components are based on effects in the same target organs or systems. These "overall" RfDs (as they are sometimes called) are protective of all other adverse health effects because they are based on the most sensitive known endpoints as supported by the weight of the evidence. As a result, this approach is protective of all types of toxicity/adverse effects, and thus ensures that the MCLG is the level at and below which there are no known or anticipated adverse human health effects with an adequate margin of safety with respect to certain PFAS mixtures in drinking water.

The TOSHI produces a less health protective indicator of risk than the general HI because the basis for the mixture component toxicity reference values has been limited to a specific target organ or system effect, which may occur at higher exposure levels than other effects (i.e., be a less sensitive endpoint). In other words, a TOSHI may not be health protective compared to the general HI if available data for a mixture component show effects in other organs at lower exposure levels compared to the critical effect observed in the target organ used for the TOSHI. Additionally, since a TOSHI relies on toxicity reference values aggregated for the same specific target organ or system endpoint/effect, an absence or lack of data on the specific target organ or system endpoint/effect for a mixture component may result in that component not being adequately accounted for in this approach (thus, underestimating health risk of the mixture). A TOSHI can only be derived for those PFAS for which the same target organ or system

endpoint/effect-specific RfDs have been calculated. For example, a TOSHI based on changes in thyroid effects illustrates why the target organ-specific approach underestimates risk in the context of these four PFAS in drinking water. To develop a thyroid effects-based TOSHI for mixtures of these four PFAS, only those PFAS with chronic toxicity reference values based on thyroid effects—PFHxS (MRL) and PFBS (RfD)—would be included in the TOSHI calculation; HFPO-DA and PFNA have chronic toxicity reference values based on other effects (i.e., liver and developmental effects, respectively) and thus would not be included in a thyroid effects-based TOSHI. Although thyroid effects are not the basis for the RfDs for HFPO-DA and PFNA, studies have shown that these two PFAS significantly affect the thyroid; for example, both have been shown to significantly affect serum thyroid hormone levels (reduced T4, T3) (Conley et al., 2019; NTP, 2019b). According to the Interagency Report to Congress on PFAS, "Multiple studies on diverse species (developing rodents and fish) suggest that some PFAS (e.g., PFOS, PFOA, ***PFNA, GenX chemicals*****,** PFHxS, PFDA, PFBA, PFBS, PFHxA) interfere with thyroid hormone signaling pathways and thyroid homeostasis through various mechanisms, including regulation of hepatic glucuronidation enzymes and deiodinases in the thyroid gland" (emphasis added, US OSTP, 2023). Therefore, a thyroid-specific HI that excluded HFPO-DA and PFNA would underestimate the dose additivity concerns for thyroid effects from the total mixture.

Many PFAS have data gaps in epidemiological or animal toxicological dose-response information for multiple types of health effects, thus limiting derivation of target organ-specific toxicity reference values; target organ-specific toxicity reference values for the same target for all four PFAS are not currently available for PFHxS, PFNA, HFPO-DA, and PFBS. The EPA's guidance recognizes the potential for organ- or system-specific data gaps and supports use of overall RfDs in a general HI approach, stating, "The target organ toxicity dose (TTD) is not a commonly evaluated measure and currently there is no official EPA activity deriving these values, as there is for the RfD and RfC" … "Because of their much wider availability than TTDs, standardized development process including peer review, and official stature, the RfD and RfC are recommended for use in the default procedure for the HI" (USEPA, 2000a). Even if target organ-specific toxicity reference values (TTDs) were available for PFHxS, PFNA, HFPO-DA, and PFBS, the general HI approach would still be more appropriate for this specific application because it is protective of all adverse health effects rather than just those associated with a specific organ or system, consistent with the statutory definition of MCLG.

Although these four PFAS elicit many of the same adverse health effects, the most sensitive known endpoint for each of the four PFAS is different, and thus the toxicity reference values used to calculate the HBWCs in the general HI approach are different. Epidemiological and/or experimental animal studies have demonstrated that exposure to PFHxS, PFNA, HFPO-DA, and PFBS individually is associated with many of the same observed adverse health effects (e.g., effects on lipids, as well as developmental, immune, endocrine, and hematologic endpoints; see Section 1.3), but with differing potencies for effect(s). In other words, two or more PFAS may elicit the same adverse effects, but at different exposure levels; for example, liver effects are associated with all four PFAS (PFHxS, PFNA, HFPO-DA, and PFBS) but HFPO-DA is the only one of the four for which liver effects represent the most sensitive known endpoint and serve as the basis for its toxicity reference value (i.e., RfD). The fact that the toxicity reference values (i.e., RfDs or MRLs) for the four PFAS are based on different health endpoints does not mean that the four PFAS are not toxicologically similar; rather, it means that based on the available data, the most sensitive endpoint currently known is different for each of these PFAS. The

general HI approach uses the most health-protective toxicity reference value available for each of the four PFAS to derive HBWCs, irrespective of whether they are based on effects in the same target organs or systems. Since each RfD (or MRL) is based on the most sensitive known endpoint based on the weight of evidence (i.e., toxicity reference value selection is not limited to a specific organ or system), this approach is protective of all other adverse health effects. This approach of allowing for component chemicals in the mixture to have different health effects or endpoints as the basis for their toxicity reference values is consistent with EPA guidance (see examples in USEPA, 2000a; USEPA, 1991b) and was supported by SAB (see Section 1.4.2.1).

The general HI is a well-established methodology that has been used for several decades in at least one other regulatory context to account for dose additivity in mixtures assessments. The EPA routinely uses the HI approach to consider the risks from multiple contaminants of concern in the Remedial Investigations and Feasibility Studies for cleanup sites on the Superfund National Priorities List under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Noncarcinogenic effects are summed to provide an HI that is compared to an acceptable index, generally 1. This approach assumes dose additivity in the absence of information on a specific mixture. These assessments of hazards from multiple chemical exposures are important factors to help inform the selection of remedies that are ultimately captured in the Superfund Records of Decision.

1.4.2.1 Science Advisory Board Support

The EPA directly asked the SAB about the utility and scientific defensibility of the general HI approach (in addition to other methods, including TOSHI) during the SAB's 2021–2022 review of the EPA's draft *Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS*. Specifically, the EPA asked the SAB to "Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative;" and "Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment" (USEPA SAB, 2022). In its report (USEPA SAB, 2022), the SAB stated its support for the general HI approach:

> "The HI methodology is a reasonable approach for estimating the potential aggregate health hazards associated with the occurrence of chemical mixtures in environmental media. The HI is an approach based on dose additivity (DA) that has been validated and used by the EPA…. This approach is mathematically straightforward and may readily identify mixtures of potential toxicological concern, as well as identify chemicals that drive the toxicity within a given mixture." (USEPA SAB, 2022).
>
> "In general, the screening level Hazard Index (HI) approach, in which Reference Values (RfVs) for the mixture components are used regardless of the effect on which the RfVs are based, is appropriate for initial screening of whether exposure to a mixture of PFAS poses a potential risk that should be further evaluated. Toxicological studies to inform human health risk assessment are lacking for most members of the large class of PFAS, and mixtures of PFAS that commonly occur in environmental media, overall. For these reasons, the HI methodology is a reasonable approach for estimating the potential aggregate health hazards associated with the occurrence of chemical mixtures in environmental media. The HI is an approach based on dose additivity (DA) that has been

> validated and used by the EPA. The HI does not provide quantitative risk estimates (i.e., probabilities) for mixtures, nor does it provide an estimate of the magnitude of a specific toxicity. This approach is mathematically straightforward and may readily identify mixtures of potential toxicological concern, as well as identify chemicals that drive the toxicity within a given mixture." (USEPA SAB, 2022).

The SAB recognized the need for regulatory agencies to make decisions in the face of uncertainty to reduce exposures to PFAS. The SAB stated,

> "Given the agency's desire to support fit-for-purpose approaches, not every PFAS mixture scenario will be one that warrants a tiered or hierarchical approach. In some instances, an HI or target-organ-specific hazard indices (TOSHI) might provide enough information for decision-making about PFAS (or other chemicals) contamination in drinking water (or other media). Tiered approaches that require increasingly complex information before reaching a final decision point can be extremely challenging for data-poor chemicals such as PFAS. Data gaps identified in a such tiered methodologies could result in a bottleneck through which these chemicals may never emerge…" (USEPA SAB, 2022).

1.5 Establishment of Individual MCLGs for PFHxS, HFPO-DA, PFNA, and/or PFBS

The EPA has determined that sufficient information is available to satisfy the statutory requirements for individual regulation of PFHxS, HFPO-DA, and PFNA (in addition to PFOA and PFOS). To support this determination, the EPA carefully examined the health effects information from available peer-reviewed final human health assessments as well as published studies, reviewed PFAS drinking water occurrence data collected as part of the UCMR 3 and state-led monitoring efforts, and considered public comments received. The EPA finds that oral exposure to PFHxS, HFPO-DA, or PFNA individually may lead to adverse health effects in humans; that each of these three PFAS have a substantial likelihood of occurring in finished drinking water with a frequency and at levels of public health concern; and that, in the sole judgment of the Administrator, regulation of PFHxS, HFPO-DA, and PFNA individually presents a meaningful opportunity for health risk reductions for persons served by PWSs.

The agency is deferring the final individual regulatory determination for PFBS to further consider whether occurrence information supports a finding that there is substantial likelihood that PFBS will individually occur in PWSs and at a level of public health concern. Therefore, no individual MCLG for PFBS is being established at this time. However, when evaluating PFBS in mixture combinations with PFHxS, PFNA, and/or HFPO-DA, the EPA has determined that based on the best available information, PFBS does meet all three statutory criteria for regulation when a part of these mixtures, including that it is anticipated to have dose-additive adverse health effects; there is a substantial likelihood of its co-occurrence in combinations with PFHxS, PFNA, and/or HFPO-DA with a frequency and at levels of public health concern; and that there is a meaningful opportunity for health risk reduction by regulating mixture combinations of these four PFAS (USEPA, 2023c). Therefore, although the agency is deferring the individual final regulatory determination for PFBS, PFBS is included in the final determination to regulate mixture combinations containing two or more of PFHxS, PFNA, HFPO-DA, and PFBS. The

establishment of individual MCLGs for PFHxS, PFNA, and HFPO-DA as well as an HI MCLG for mixtures of two or more of PFHxS, PFNA, HFPO-DA, and PFBS addresses potential health risks related to individual PFAS exposure as well as dose additive adverse health effects from exposure to mixtures of two or more of these four PFAS.

1.6 Overview of Individual MCLG and Mixture Hazard Index (HI) MCLG Approaches

To establish an MCLG for an individual contaminant, the EPA assesses the peer-reviewed science examining cancer and noncancer health effects associated with oral exposure to the contaminant. For contaminants determined to be known or likely human carcinogens (USEPA, 2005) with a linear carcinogenic MOA (i.e., where there is a proportional relationship between dose and carcinogenicity at low concentrations) or for which there is insufficient information to determine that a carcinogen has a threshold dose below which no carcinogenic effects have been observed, the EPA has a longstanding practice of establishing the MCLG at zero (see USEPA (1998); USEPA (2000c); USEPA (2001); see S. Rep. No. 169, 104th Cong., 1st Sess. (1995) at 3). For contaminants determined to be known or likely human carcinogens but with a nonlinear carcinogenic MOA,[4] contaminants that are designated as having suggestive evidence of carcinogenic potential in humans (USEPA, 2005), and noncarcinogenic contaminants, the EPA typically establishes the MCLG based on a noncancer toxicity reference value (RfV) that represents the best available science (e.g., EPA RfD or ATSDR MRL).

An MCLG that is based on noncancer effects is designed to be protective of noncancer effects over a lifetime of exposure with an adequate margin of safety, including for sensitive populations and life stages, consistent with SDWA 1412(b)(3)(C)(i)(V) and 1412(b)(4)(A). The inputs for a noncancer MCLG include an oral noncancer toxicity RfV (e.g., RfD or MRL), body weight-adjusted drinking water intake (DWI-BW), and a relative source contribution (RSC), as presented in Equation 1:

$$\boldsymbol{MCLG} = \left(\frac{Oral\ RfV}{DWI\text{-}BW}\right) * RSC \qquad \text{(Eqn. 1)}$$

Where:

RfV = Chronic toxicity reference value (EPA RfD or ATSDR MRL). An RfD is an estimate of a daily exposure to the human population (including sensitive populations) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 2002). An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure (ATSDR, 2021).

DWI-BW = Body weight-adjusted drinking water intake – an exposure factor for the 90th percentile body weight-adjusted drinking water intake for the identified population or life stage, in units of liters of water consumed per kilogram body weight per day

[4] A carcinogen with a nonlinear MOA is a chemical agent for which the associated cancer response does not increase in direct proportion to the exposure level and for which there is scientific evidence demonstrating a threshold level of exposure below which there is no appreciable cancer risk (USEPA, 2005).

(L/kg/day). The DWI-BW considers both direct and indirect consumption of drinking water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee). Chapter 3 of the EPA's *Exposure Factors Handbook* (USEPA, 2019b) provides the most up-to-date DWI-BWs for various populations or life stages within the U.S. general population based on publicly available, peer-reviewed data such as from the National Health and Nutrition Examination Survey (NHANES).

RSC = Relative source contribution – the percentage of the total exposure attributed to drinking water sources (USEPA, 2000b), with the remainder of the exposure allocated to all other routes or sources. The purpose of the RSC is to ensure that the level of a contaminant allowed by one criterion (e.g., MCLG), when combined with other identified sources of exposure common to the population and contaminant of concern, will not result in exposures that exceed the RfD. The RSC is derived by applying the Exposure Decision Tree approach (USEPA, 2000b).

The EPA's approach to DWI-BW selection includes a step to identify the sensitive population(s) or life stage(s) (i.e., those that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant, including the adverse health effects observed in the toxicity study on which the RfD/minimal risk level was based (known as the critical effect within the critical or principal study). Although data gaps can complicate identification of the most sensitive population (e.g., not all windows or life stages of exposure and/or health outcomes may have been assessed in available studies), the critical effect and point of departure (POD) that form the basis for the RfD (or minimal risk level) can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure window, may identify a sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In such cases, the EPA can select the corresponding DWI-BW for that sensitive population or life stage from the Exposure Factors Handbook (USEPA, 2019b). DWI-BWs in the Exposure Factors Handbook are based on information from publicly available, peer-reviewed studies, and were updated in 2019. In the absence of information indicating a sensitive population or life stage, the DWI-BW corresponding to the general population may be selected. Following this approach, the EPA selected appropriate DWI-BWs for each of the four PFAS included in the HI MCLG (see Section 2). The EPA did consider infants as a sensitive life stage for all four PFAS; however, the agency did not select the infant DWI-BW because the exposure intervals of the critical studies supporting the chronic toxicity reference values did not correspond to infants. Instead, the exposure intervals were relevant to other sensitive target populations (i.e., lactating women or women of childbearing age) or the general population.

The EPA applies an RSC to account for potential aggregate risk from exposure routes and exposure pathways other than oral ingestion of drinking water to ensure that an individual's total exposure to a contaminant does not exceed the daily exposure associated with toxicity (i.e., threshold level or RfD). Application of the RSC in this context is consistent with EPA methods (USEPA, 2000b) and long-standing EPA practice for establishing drinking water MCLGs and NPDWRs (e.g., see USEPA, 2010, 2004; USEPA, 1989). The RSC represents the proportion of an individual's total exposure to a contaminant that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee, tea, or soup, as well as from dietary items prepared with drinking water) relative to other exposure pathways. The remainder of the exposure equal to the RfD (or MRL) is allocated to other potential exposure sources (USEPA,

2000b). The purpose of the RSC is to ensure that the level of a contaminant (e.g., MCLG) in drinking water, when combined with other identified potential sources of exposure for the population of concern, will not result in total exposures that exceed the RfD (or MRL) (USEPA, 2000b). This ensures that the MCLG under SDWA meets the statutory requirement that it is a level of a contaminant in drinking water at or below which no known or anticipated adverse effects on human health occur and allowing an adequate margin of safety.

To determine the RSCs for the four PFAS, the agency assessed the available scientific literature on potential sources of human exposure other than drinking water (see Section 2). The EPA conducted literature searches and reviews for each of the four PFAS to identify potential sources of exposure and physicochemical properties that may influence occurrence in environmental media (see Appendices A–D). Considering this exposure information, the EPA followed its longstanding, peer-reviewed Exposure Decision Tree Approach in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000b) to determine the RSC for each PFAS. The EPA carefully evaluated studies that included information on potential exposure to these four PFAS (PFHxS, PFNA, HFPO-DA, and PFBS) via sources other than drinking water, such as food, soil, sediment, and air. For each of the four PFAS, the findings indicated that there are significant known or potential uses/sources of exposure beyond drinking water ingestion (e.g., food, indoor dust) (USEPA, 2000b), but that data are insufficient to allow for quantitative characterization of the different exposure sources (Box 8A in USEPA, 2000b). The EPA's Exposure Decision Tree approach states that when there are insufficient environmental and/or exposure data to permit quantitative derivation of the RSC, the recommended RSC for the general population is 20 percent (Box 8B in USEPA, 2000b). This means that 20 percent of the exposure equal to the RfD is allocated to drinking water, and the remaining 80 percent is attributed to all other potential exposure sources.

In the general HI approach, a HQ is calculated as the ratio of human exposure (E) to a health-based RfV for each mixture component chemical (i) (USEPA, 1986). The HI involves the use of RfVs for each PFAS mixture component (in this case, PFHxS, HFPO-DA, PFNA, and/or PFBS) which are based on the most sensitive known health outcomes and which are expected to be protective of all other adverse health effects observed after exposure to the individual PFAS. This approach, which protects against all adverse effects and not just a single adverse outcome/effect, is a conservative risk indicator and appropriate for MCLG derivation. The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Equation 2). For example, if E is the oral intake rate (milligrams per kilogram per day (mg/kg/day)), then the RfV could be the RfD or MRL, which have the same units. Alternatively, the exposure metric can be a media-specific metric such as a measured water concentration (e.g., nanograms per liter or ng/L) and the RfV can be an HBWC (e.g., ng/L). The component chemical HQs are then summed across the mixture to yield the HI (Equation 2). A mixture HI exceeding 1 indicates potential risk for a given environmental medium or site. The HI provides an indication of: (1) concern for the overall mixture and (2) potential driver PFAS (i.e., those PFAS mixture components with high(er) HQs). For a detailed discussion of PFAS dose additivity and the HI approach, see the PFAS Mixtures Framework (USEPA, 2024a).

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{E_i}{RfV_i} \qquad \text{(Eqn. 2)}$$

Where:

HI = Hazard index

HQ_i = Hazard quotient for chemical i

E_i = Exposure, i.e., dose (mg/kg/day) or occurrence concentration, such as in drinking water (in milligrams per liter or mg/L), for chemical i

RfV_i = Reference value (e.g., oral RfD or MRL) (mg/kg/day), or corresponding HBWC; e.g., an MCLG for chemical i (in mg/L)

The HBWCs/MCLGs are based on the best available science and data collected by accepted methods (see Section III in the USEPA, 2023c). Specifically, peer-reviewed, publicly available toxicity assessments are available for HFPO-DA (USEPA, 2021c), PFBS (USEPA, 2021d), PFNA (ATSDR, 2021), and PFHxS (ATSDR, 2021) that provide the oral toxicity values (i.e., RfD or MRL) used to calculate the HBWCs; the EPA selected the corresponding DWI-BW for the relevant sensitive population or life stage from the Exposure Factors Handbook (USEPA, 2019b) based on the best available, peer-reviewed science from publicly available, peer-reviewed studies taking into account the relevant sensitive population(s) or life stage(s); and the RSCs are based on the best available, peer-reviewed science or best available methods taking into account the relevant sensitive population(s) or life stage(s) (USEPA, 2000b).

2 Calculating the Health-Based Water Concentrations for HFPO-DA, PFBS, PFNA, and PFHxS for the HI MCLG

2.1 HFPO-DA

HFPO-DA is a shorter-chain PFAS that was intended to be a replacement for the longer-chained PFOA. In water, HFPO-DA and its various salts dissociate to form the HFPO-DA anion (HFPO−) as a common analyte.

The HBWC for HFPO-DA that the agency is using in the HI MCLG was derived in part from information from the agency's 2021 human health toxicity assessment of HFPO-DA (specifically the chronic RfD of 3E-06 mg/kg/day) (USEPA, 2021c). Summaries of key information from the HFPO-DA toxicity assessment of (i.e., information about the RfD), as well as information about the DWI-BW and RSC that were used to derive the HBWC for HFPO-DA, are presented in the following sections. Based on this information, an HBWC of 10 ng/L for HFPO-DA is used in the HI MCLG for mixtures of two or more of HFPO-DA, PFBS, PFNA, and PFHxS (see Section 3.2).

2.1.1 Toxicity

The HBWC for HFPO-DA is derived from a chronic RfD that is based on liver effects (specifically, a constellation of liver lesions including cytoplasmic alteration, single-cell and focal necrosis, and apoptosis) observed in parental female mice following oral exposure to HFPO-DA from pre-mating through lactation (53–64 days) (USEPA, 2021c).

As described in the EPA's human health toxicity assessment of HFPO-DA, oral toxicity studies in rodents exposed to HFPO-DA report a range of adverse effects. Repeated-dose oral exposure of rats and mice resulted in liver toxicity (e.g., increased relative liver weight, hepatocellular hypertrophy, apoptosis, and single-cell/focal necrosis), kidney toxicity (e.g., increased relative kidney weight), immune system effects (e.g., antibody suppression), hematological effects (e.g., decreased red blood cell count, hemoglobin, and hematocrit), reproductive/developmental effects (e.g., increased number of early deliveries, placental lesions, changes in maternal gestational weight gain, and delays in genital development in offspring), and cancer (e.g., liver and pancreatic tumors) (USEPA, 2021c).

The most sensitive noncancer effects observed among the available data were the adverse effects on liver, which were observed in both male and female mice and rats across a range of exposure durations and dose levels, including the lowest tested dose levels and shortest exposure durations (USEPA, 2021c). Noncancer liver effects formed the basis for the chronic RfD of 3E-06 mg/kg/day, which the EPA used to derive the HBWC for HFPO-DA. As described in the HFPO-DA toxicity assessment, to develop the chronic RfD for HFPO-DA, the EPA derived a human equivalent dose (HED) of 0.01 mg/kg/day from a no-observed-adverse-effect level (NOAEL) of 0.1 mg/kg/day for liver effects observed in the identified critical study (an oral

reproductive/developmental toxicity study in mice (DuPont, 2010b)). The EPA then applied a composite uncertainty factor (UF) of 3,000 (i.e., 10× for intraspecies variability (UF_H), 3× for interspecies differences (UF_A), 10× for extrapolation from a subchronic-to-chronic dosing duration (UF_S), and 10× for database deficiencies (UF_D)) to yield the chronic RfD (USEPA, 2021c).

The EPA determined that there is suggestive evidence of carcinogenic potential following oral exposure to HFPO-DA in humans, but the available data are insufficient to derive a cancer risk concentration in water for HFPO-DA (USEPA, 2021c).

2.1.2 Exposure Factor

To select an appropriate DWI-BW for use in derivation of the HBWC for HFPO-DA, the EPA considered the HFPO-DA exposure interval used in the oral reproductive/developmental toxicity study in mice that was the basis for chronic RfD derivation (the critical study). In this study, parental female mice were dosed from pre-mating through lactation, corresponding to three potentially sensitive human adult life stages that may represent critical windows of exposure for HFPO-DA: women of childbearing age (13 to < 50 years), pregnant women, and lactating women (Table 3-63 in USEPA, 2019b). Of these three, the DWI-BW for lactating women (0.0469 L/kg/day) is the highest (see Table 2-1), and therefore anticipated to be protective of the other two sensitive life stages. Therefore, the EPA used the DWI-BW for lactating women to calculate the HBWC for HFPO-DA in the HI MCLG.

Table 2-1. EPA Exposure Factors for Drinking Water Intake for Different Candidate Sensitive Populations or Life Stages, Based on the Critical Effect and Study for HFPO-DA.

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)
Lactating women	**0.0469**	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[a] (USEPA, 2019b)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day; DWI-BW = body weight-adjusted drinking water intake; NHANES = National Health and Nutrition Examination Survey. The DWI-BW used to calculate the HFPO-DA HBWC is in bold.

[a] Estimates are less statistically reliable based on guidance published in the Joint Policy on Variance Estimation and Statistical Reporting Standards on NHANES III and CSFII Reports: HNIS/NCHS Analytical Working Group Recommendations (NCHS, 1993).

2.1.3 Relative Source Contribution

The EPA conducted literature searches and reviews for HFPO-DA to identify potential sources of exposure and physicochemical properties that may influence occurrence in environmental media. Based on the physical properties, detected levels, and limited available exposure information for HFPO-DA, multiple non-drinking water sources (e.g., foods, indoor dust, air, soil, and sediment) are potential exposure sources (see Appendix A). Following the EPA's Exposure Decision Tree approach (USEPA, 2000b), potential sources other than drinking water ingestion were identified, but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources. Thus, the EPA used an RSC of 0.20 for HFPO-DA. This means that 20% of the RfD is attributed to drinking water, and the remaining 80% is attributed to all other potential exposure sources. As explained above (Section 1.6), applying the RSC ensures that an individual's total exposure to HFPO-DA from all sources does not exceed the daily exposure associated with the RfD, consistent with agency methodology (USEPA, 2000b) and longstanding practice for establishing drinking water MCLGs and NPDWRs.

2.1.4 Derivation of HFPO-DA HBWC

The HBWC for HFPO-DA is calculated as follows and summarized in Table 2-2:

$$\textbf{HFPO–DA HBWC} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC}$$

$$= \left(\frac{0.000003\ \frac{\text{mg}}{\text{kg/day}}}{0.0469\ \frac{\text{L}}{\text{kg/day}}}\right) * 0.2$$

$$= 0.00001\ \frac{\text{mg}}{\text{L}}$$

$$= 0.01\ \frac{\mu\text{g}}{\text{L}}$$

$$= 10\ \frac{\text{ng}}{\text{L}}\ \text{or parts per trillion (ppt)}$$

Table 2-2. HFPO-DA HBWC – Input Parameters and Value

Parameter	Value	Units	Source
Chronic oral RfD	3E-06	mg/kg/day	Final RfD based on liver effects (constellation of liver lesions as defined by the NTP Pathology Working Group) in parental female mice exposed to HFPO-DA by gavage from pre-mating through lactation (53–64 days) (USEPA, 2021c; DuPont, 2010b).
DWI-BW	0.0469	L/kg/day	90th percentile 2-day average, consumer-only estimate of combined direct and indirect community water ingestion for lactating women (age 13 to <50 years) based on 2005–2010 NHANES data (USEPA, 2019b).
RSC	0.2	N/A	Based on a review of the available scientific literature on HFPO-DA, potential exposure routes and sources exist but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources (see Appendix A).
HFPO-DA HBWC = 0.00001 mg/L or 10 ppt			

Notes: RfD = reference dose; DWI-BW = body weight-adjusted drinking water intake; HBWC = health-based water concentration; HFPO-DA = hexafluoropropylene oxide dimer acid; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; NTP = National Toxicology Program; RSC = relative source contribution.

2.2 PFBS

PFBS and its potassium salt (K^+PFBS) are shorter-chain PFAS that were developed as "safer" replacements for the longer-chained PFOS (USEPA, 2021d). In water, K^+PFBS dissociates to the deprotonated anionic form of PFBS (PFBS-) and the K^+ cation at environmental pH levels (pH 4–9). These three PFBS chemical forms are referred to collectively as PFBS.

The HBWC that the agency is using for the HI MCLG was derived from information in the agency's 2021 human health toxicity assessment for PFBS, specifically the chronic RfD of 3E-04 mg/kg/day based on thyroid effects observed in newborn mice born to mothers that had been orally exposed to PFBS throughout gestation (USEPA, 2021d). Summaries of key information from the PFBS toxicity assessment (i.e., information about the RfD), as well as information about the DWI-BW and RSC that were used to derive the HBWC for PFBS, are presented in the following sections. Based on this information, an HBWC of 2,000 ng/L for PFBS is used in the HI MCLG for mixtures of two or more of HFPO-DA, PFBS, PFNA, and PFHxS (see Section 3.2).

2.2.1 Toxicity

The HBWC for PFBS was derived using a chronic oral RfD based on thyroid effects seen in an oral toxicity study in mice (USEPA, 2021d).

The EPA's human health toxicity assessment for PFBS (USEPA, 2021d) considered all publicly available human, animal, and mechanistic studies of PFBS exposure and effects. The assessment

identified associations between PFBS exposure and thyroid, developmental, and kidney effects based on studies in animals. The limited evidence for thyroid or kidney effects in human studies was equivocal, and no studies evaluating developmental effects of PFBS in humans were available. Human and animal studies evaluated other health effects following PFBS exposure including effects on the reproductive system, liver, and lipid and lipoprotein homeostasis, but the evidence did not support clear associations between exposure and effect (USEPA, 2021d).

The most sensitive noncancer effect observed was an adverse effect on the thyroid (i.e., decreased serum total thyroxine) seen in newborn mice (postnatal day (PND) 1) born to mothers that had been orally exposed to K^+PFBS throughout gestation (USEPA, 2021d; Feng et al., 2017). This critical effect was the basis for the chronic RfD of 3E-04 mg/kg/day which the EPA used to derive the HBWC for PFBS (USEPA, 2021d). As described in the PFBS toxicity assessment, to develop the chronic RfD for PFBS,[5] the EPA derived an HED of 0.095 mg/kg/day from BMD modeling of the critical effect in mice. The EPA then applied a composite UF of 300 (i.e., 10× for UF_H, 3× for UF_A, and 10× for UF_D) to yield the chronic RfD (USEPA, 2021d). The EPA did not apply an additional UF to adjust for subchronic-to-chronic duration (i.e., UF_S) because the critical effects were observed during a developmental life stage[6] (USEPA, 2002).

There were no human or animal studies identified that evaluated the potential carcinogenicity of PFBS (USEPA, 2021d).

2.2.2 Exposure Factor

To select an appropriate DWI-BW for use in deriving the HBWC, the EPA considered the PFBS exposure interval used in the developmental toxicity study in mice that was the basis for chronic RfD derivation. In this study, pregnant mice were exposed throughout gestation, which is relevant to two human adult life stages: women of childbearing age (13 to < 50 years) who may be or become pregnant, and pregnant women and their developing embryo or fetus (Table 3-63 in USEPA, 2019b). Of these two, the EPA selected the DWI-BW for women of childbearing age (0.0354 L/kg/day) to derive the HBWC for PFBS because it is higher and therefore more health protective (see Table 2-3).

[5] Data for K^+PFBS were used to derive the chronic RfD for the free acid (PFBS), resulting in the same value (3E-04 mg/kg/day), after adjusting for differences in molecular weight between K+ PFBS (338.19) and PFBS (300.10) (USEPA, 2021d).

[6] As stated in USEPA (2002), "…This is because it is assumed that most endpoints of developmental toxicity can be caused by a single exposure. If, however, developmental effects are more sensitive than those seen after longer-term exposures, then even the chronic RfD/RfC should be based on such effects to reduce the risk of potential greater sensitivity in children. Because the standard studies currently conducted for developmental toxicity involve repeated exposures, data are not often available on which endpoints may be induced by acute, subacute, subchronic, or chronic dosing regimens and, therefore, on which should be used in setting various duration reference values."

Table 2-3. EPA Exposure Factors for Drinking Water Intake for Different Candidate Sensitive Populations or Life Stages, Based on the Critical Effect and Study for PFBS.

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Women of childbearing age	**0.0354**	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day; DWI-BW = body weight-adjusted drinking water intake; NHANES = National Health and Nutrition Examination Survey. The DWI-BW used to calculate the PFBS HBWC is in bold.

2.2.3 Relative Source Contribution

The EPA conducted literature searches and reviews for PFBS to identify potential sources of exposure and physicochemical properties that may influence occurrence in environmental media. Based on the physical properties, detected levels, and available exposure information for PFBS, multiple non-drinking water sources (seafood including fish and shellfish, other foods, indoor air, and some consumer products) are potentially significant exposure sources (see Appendix B). Following the EPA's Exposure Decision Tree approach (USEPA, 2000b), potential sources other than drinking water ingestion were identified, but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources. Thus, the EPA used an RSC of 0.20 for PFBS. This means that 20% of the RfD is attributed to drinking water, and the remaining 80% is attributed to all other potential exposure sources. As explained above (Section 1.6), applying the RSC ensures that an individual's total exposure to PFBS from all sources does not exceed the daily exposure associated with the RfD, consistent with agency methodology (USEPA, 2000b) and longstanding practice for establishing drinking water MCLGs and NPDWRs.

2.2.4 Derivation of PFBS HBWC

The HBWC for PFBS is calculated as follows and summarized in Table 2-4:

$$\textbf{PFBS HBWC} = \left(\frac{\text{RfD}}{\text{DWI-BW}}\right) * \text{RSC}$$

$$= \left(\frac{0.0003 \ \frac{\text{mg}}{\text{kg/day}}}{0.0354 \frac{\text{L}}{\text{kg/day}}}\right) * 0.2$$

$$= 0.0017 \frac{\text{mg}}{\text{L}} \left(\text{rounded to } 0.002 \frac{\text{mg}}{\text{L}}\right)$$

$$= 2 \frac{\mu\text{g}}{\text{L}}$$

$$= 2{,}000 \frac{\text{ng}}{\text{L}} \text{ or ppt}$$

Table 2-4. PFBS HBWC – Input Parameters and Value

Parameter	Value	Units	Source
Chronic RfD	3E-04	mg/kg/day	Final RfD based on critical effect of decreased serum total thyroxine in newborn mice after gestational exposure (USEPA, 2021d; Feng et al., 2017).
DWI-BW	0.0354	L/kg/day	90th percentile 2-day average, consumer-only estimate of combined direct and indirect community water ingestion for women of childbearing age (13 to <50 years) based on 2005−2010 NHANES data (USEPA, 2019b).
RSC	0.2	N/A	Based on a review of the available scientific literature on PFBS, potential exposure routes and sources exist but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources (see Appendix B).
PFBS HBWC = 0.002 mg/L or 2,000 ppt			

Note: RfD = reference dose; DWI-BW = body weight-adjusted drinking water intake; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; HBWC = health-based water concentration; PFBS = perfluorobutanesulfonic acid; RSC = relative source contribution.

2.3 PFNA

PFNA has been used as a processing aid in the production of fluoropolymers, primarily polyvinylidene fluoride, which is a plastic designed to be temperature resistant and chemically nonreactive (USEPA, 2020b; NJSWQI, 2017; Prevedouros et al., 2006). PFNA has been used since the 1950's in a wide variety of industrial and consumer products. It has also been used in aqueous film-forming foam (AFFF) for fire suppression (USEPA, 2020b; Laitinen et al., 2014).

ATSDR has published a toxicological profile for a group of PFAS including PFNA and has developed an intermediate-duration oral MRL for PFNA (ATSDR, 2021). The EPA's derived HBWC for PFNA (described below) is based on the ATSDR MRL (ATSDR, 2021), a DWI-BW (selected by the EPA) that corresponds to this MRL, and an RSC determined by the EPA. There is no published EPA human health toxicity assessment for PFNA at the time of this writing; however, the EPA's Integrated Risk Information System (IRIS) program is developing a human health toxicity assessment for PFNA, which is expected to be finalized in 2024 (USEPA, 2023a, 2021e). The EPA's IRIS assessment will use systematic review methods to evaluate the

epidemiological and toxicological literature for PFNA, including consideration of relevant mechanistic evidence (USEPA, 2021e).

2.3.1 Toxicity

The HBWC for PFNA is based on an ATSDR intermediate-duration oral MRL that was based on developmental effects seen in mice after oral PFNA exposure (ATSDR, 2021).

Studies of oral PFNA exposure in rodents have reported adverse effects on the liver, development, and reproductive and immune systems (ATSDR, 2021). The most sensitive noncancer effects and basis for the ATSDR intermediate-duration oral MRL were decreased body weight gain and impaired development (i.e., delayed eye opening, preputial separation, and vaginal opening) in mice born to mothers that were treated with PFNA from gestational days (GDs) 1–17 (with presumed continued indirect exposure of offspring via lactation), and monitoring until PND 287 (ATSDR, 2021). The study reporting these effects (Das et al., 2015) was selected by ATSDR as the principal study for MRL derivation. To derive the MRL, an HED of 0.001 mg/kg/day was calculated from the NOAEL of 1 mg/kg/day identified in the study. ATSDR applied a total UF of 30 (i.e., 10× for UF_H and 3× for UF_A) and a modifying factor (MF) of 10× for database deficiencies to account for the small number/limited scope of studies examining PFNA toxicity following intermediate-duration exposure. The resulting intermediate-duration oral MRL was 3E-06 mg/kg/day (ATSDR, 2021). The EPA did not apply an additional UF_S to calculate the HBWC because the critical effects were observed during a developmental life stage[6] (USEPA, 2002). Toxicological assessments from other sources (e.g., states) report toxicity reference values based on animal studies for PFNA that are in the same range, providing additional support (USEPA, 2021e).

The carcinogenic potential of PFNA has been examined in three epidemiological studies. No consistent associations between serum PFNA levels and breast cancer or prostate cancer were found (ATSDR, 2021). The EPA has not yet completed a final evaluation and classification of the carcinogenicity of PFNA.

2.3.2 Exposure Factor

Based on the life stages of exposure in the principal study from which the intermediate-duration MRL was derived (i.e., directly to maternal animals during gestation, and indirectly to offspring during gestation and lactation), the EPA identified three potentially sensitive life stages that may represent critical windows of exposure for PFNA: women of childbearing age (13 to <50 years), pregnant women, and lactating women (Table 3-63 in USEPA, 2019b). The DWI-BW for lactating women (0.0469 L/kg/day; 90th percentile direct and indirect consumption of community water, consumer-only 2-day average) was selected to calculate the HBWC for PFNA because it is the highest of the three DWI-BWs and is anticipated to be protective of the other two sensitive life stages (see Table 2-5).

Table 2-5. EPA Exposure Factors for Drinking Water Intake for Different Candidate Sensitive Populations and Life Stages, Based on the Critical Effect and Study for PFNA.

Population	DWI-BW (L/kg bw-day)	Description of Exposure Metric	Source
Women of childbearing age	0.0354	90th percentile direct and indirect consumption of community water, consumer-only two-day average, 13 to < 50 years.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)
Pregnant women	0.0333	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010 (USEPA, 2019b)
Lactating women	**0.0469**	90th percentile direct and indirect consumption of community water, consumer-only two-day average.	2019 Exposure Factors Handbook Chapter 3, Table 3-63, NHANES 2005–2010[a] (USEPA, 2019b)

Notes: L/kg bw-day = liters of water consumed per kilogram body weight per day; DWI-BW = body weight-adjusted drinking water intake; NHANES = National Health and Nutrition Examination Survey. The DWI-BW used to calculate the PFNA HBWC is in bold.

2.3.3 Relative Source Contribution

The EPA conducted literature searches and reviews for PFNA to identify potential sources of exposure and physicochemical properties that may influence occurrence in environmental media. Based on the physical properties, detected levels, and available exposure information for PFNA, multiple non-drinking water sources (fish and shellfish, non-fish food, some consumer products, indoor dust, and air) are potentially significant exposure sources (see Appendix C). Following the Exposure Decision Tree approach (USEPA, 2000b), potential sources other than drinking water ingestion were identified but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources. Thus, the EPA used an RSC of 0.20 for PFNA. This means that 20% of the RfV is attributed to drinking water, and the remaining 80% is attributed to all other potential exposure sources. As explained above (Section 1.6), applying the RSC ensures that an individual's total exposure to PFNA from all sources does not exceed the daily exposure associated with the RfV, consistent with agency methodology (USEPA, 2000b) and longstanding practice for establishing drinking water MCLGs and NPDWRs.

2.3.4 Derivation of PFNA HBWC

The HBWC for PFNA is calculated as follows and summarized in Table 2-6:

$$\textbf{PFNA HBWC} = \left(\frac{\text{RfV}}{\text{DWI-BW}}\right) * \text{RSC}$$

$$= \left(\frac{0.000003 \ \frac{\text{mg}}{\text{kg/day}}}{0.0469 \frac{\text{L}}{\text{kg/day}}}\right) * 0.2$$

$$= 0.000014 \ \frac{\text{mg}}{\text{L}} \left(\text{rounded to } 0.00001 \ \frac{\text{mg}}{\text{L}}\right)$$

$$= 0.01 \ \frac{\mu\text{g}}{\text{L}}$$

$$= 10 \ \frac{\text{ng}}{\text{L}} \text{ or ppt}$$

Table 2-6. PFNA HBWC – Input Parameters and Value

Parameter	Value	Units	Source
RfV	3E-06[a]	mg/kg/day	Based on decreased body weight gain and delayed eye opening, preputial separation, and vaginal opening in mouse offspring after gestational and presumed lactational exposure (ATSDR, 2021; Das et al., 2015).
DWI-BW	0.0469	L/kg/day	90th percentile 2-day average, consumer-only estimate of combined direct and indirect community water ingestion for lactating women (13 to <50 years) based on 2005−2010 NHANES data (USEPA, 2019b).
RSC	0.2	N/A	Based on a review of the current scientific literature on PFNA, potential exposure routes and sources exist but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources (see Appendix C).
PFNA HBWC = 0.00001 mg/L or 10 ppt			

Notes: RfV = chronic toxicity reference value; DWI-BW = body weight-adjusted drinking water intake; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; PFNA = perfluorononanoic acid; RSC = relative source contribution.

[a] Note that ATSDR MRLs and EPA RfDs are not identical (e.g., intermediate-duration MRL vs. chronic RfD; the EPA and ATSDR may apply different uncertainty/modifying factors) and are developed for different purposes. In this case, the EPA did not apply an additional UF_S to calculate the HBWC for PFNA because the critical effect is identified in a developmental population (USEPA, 2002).

2.4 PFHxS

PFHxS has been used in laboratory applications and as a raw material or a precursor for the manufacture of PFAS/perfluoroalkyl sulfonate-based products, though production of PFHxS in

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the United States was phased out by its major manufacturer in 2002 (Sigma-Aldrich, 2014 as cited in NCBI, 2022; Backe et al., 2013; Buck et al., 2011; OECD, 2011). PFHxS has also been used in firefighting foam and carpet treatment solutions, and it has been used as a stain and water repellant (Garcia and Harbison, 2015 as cited in NCBI, 2022).

ATSDR has published a toxicological profile for a group of PFAS including PFHxS and has calculated an intermediate-duration oral MRL for PFHxS (ATSDR, 2021). The EPA's derived HBWC for PFHxS (described below) is based on the ATSDR MRL (ATSDR, 2021), a DWI-BW (selected by the EPA) that corresponds to the MRL, and an RSC determined by the EPA. There is no published EPA human health toxicity assessment for PFHxS at the time of this writing; however, the EPA's IRIS program is developing a human health toxicity assessment for PFHxS, which is expected to be finalized in 2024 (USEPA, 2023a, 2021e). The EPA's IRIS assessment will use systematic review methods to evaluate the epidemiological and toxicological literature for PFHxS, including consideration of relevant mechanistic evidence (USEPA, 2021e).

2.4.1 Toxicity

The HBWC for PFHxS is derived using an ATSDR intermediate-duration oral MRL based on thyroid effects seen in male rats after oral PFHxS exposure (ATSDR, 2021).

Toxicity studies of oral PFHxS exposure to animals have reported health effects on the liver, thyroid, and development (ATSDR, 2021). The most sensitive noncancer effect observed was thyroid follicular epithelial hypertrophy/hyperplasia in parental male rats that had been exposed for 42–44 days, identified in the principal developmental toxicity study selected by ATSDR (NOAEL of 1 mg/kg/day for this effect) (ATSDR, 2021; Butenhoff et al., 2009). This critical effect was the basis for the ATSDR intermediate-duration oral MRL which the EPA used to derive the HBWC for PFHxS. An HED of 0.0047 mg/kg/day was calculated from the NOAEL of 1 mg/kg/day identified in the principal study. ATSDR applied a total UF of 30 (i.e., 10× for UF_H and 3× for UF_A) and an MF of 10× for database deficiencies to yield an intermediate-duration oral MRL of 2E-05 mg/kg/day (ATSDR, 2021). To calculate the HBWC, the EPA applied an additional UF_S of 10, per agency guidelines (USEPA, 2002), because the effect is not in a developmental population (i.e., thyroid follicular epithelial hypertrophy/hyperplasia in parental male rats). The resulting adjusted chronic reference value is 2E-06 mg/kg/day. Toxicological assessments from other sources (e.g., states) report toxicity reference values based on animal studies for PFHxS that are in the same range, providing additional support (USEPA, 2021e).

The carcinogenic potential of PFHxS has been examined in four epidemiological studies (ATSDR, 2021). Bonefeld-Jørgensen et al. (2014) reported a significant negative correlation between serum PFHxS levels (mean concentration 1.2 ng/mL) and breast cancer risk among Danish women. However, a study in Greenland found a significant, positive association between high serum levels of PFHxS and breast cancer risk (Wielsøe et al., 2017). The median serum PFHxS concentration among cases in that study was 2.52 ng/mL and serum levels ranged from 0.19 ng/mL to 23.40 ng/mL (Wielsøe et al., 2017). Hardell et al. (2014) found a statistically significant interaction between above-median PFHxS concentrations and increased risk for prostate cancer among men with genetics as a risk factor (first-degree relative). Prostate-specific antigen (PSA) levels were not associated with serum PFHxS levels (mean concentration 3.38 ng/mL) in men 20–49 or 50–69 years of age (Ducatman et al., 2015). The EPA has not yet completed a final evaluation and classification of the carcinogenicity of PFHxS.

2.4.2 Exposure Factor

No sensitive population or life stage was identified for DWI-BW selection for PFHxS because the critical effect on which the ATSDR MRL was based (thyroid alterations) was observed in adult male rats. Since this exposure life stage does not correspond to a sensitive population or life stage, a DWI-BW for adults within the general population (0.034 L/kg/day; 90th percentile direct and indirect consumption of community water, consumer-only 2-day average, adults 21 years and older) was selected for HBWC derivation (USEPA, 2019b).

2.4.3 Relative Source Contribution

The EPA conducted literature searches and reviews for PFHxS to identify potential sources of exposure and physicochemical properties that may influence occurrence in environmental media. Based on the physical properties, detected levels, and available exposure information for PFHxS, multiple non-drinking water sources (fish and shellfish, non-fish food, some consumer products, indoor dust, and soil) are potentially significant exposure sources (see Appendix D). Following the Exposure Decision Tree approach (USEPA, 2000b), potential sources other than drinking water ingestion were identified but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources. Thus, the EPA used an RSC of 0.20 for PFHxS. This means that 20% of the RfV is attributed to drinking water, and the remaining 80% is attributed to all other potential exposure sources. As explained above (Section 1.6), applying the RSC ensures that an individual's total exposure to PFHxS from all sources does not exceed the daily exposure associated with the RfV, consistent with agency methodology (USEPA, 2000b) and longstanding practice for establishing drinking water MCLGs and NPDWRs.

2.4.4 Derivation of PFHxS HBWC

The HBWC for PFHxS is calculated as follows and summarized in Table 2-7:

$$\textbf{PFHxS HBWC} = \left(\frac{\text{RfV}}{\text{DWI-BW}}\right) * \text{RSC}$$

$$= \left(\frac{0.000002\ \frac{\text{mg}}{\text{kg/day}}}{0.034\ \frac{\text{L}}{\text{kg/day}}}\right) * 0.2$$

$$= 0.000012\ \frac{\text{mg}}{\text{L}}\ \left(\text{rounded to } 0.00001\ \frac{\text{mg}}{\text{L}}\right)$$

$$= 0.01\ \frac{\mu\text{g}}{\text{L}}$$

$$= 10\ \frac{\text{ng}}{\text{L}}\ \text{or ppt}$$

Table 2-7. PFHxS HBWC – Input Parameters and Value

Parameter	Value	Units	Source
RfV	2E-06[a]	mg/kg/day	Based on thyroid follicular epithelial hypertrophy/hyperplasia in parental male rats (exposed 42–44 days) (ATSDR, 2021; Butenhoff et al., 2009).
DWI-BW	0.034	L/kg/day	90th percentile 2-day average, consumer-only estimate of combined direct and indirect community water ingestion for adults 21 years and older based on 2005−2010 NHANES data (USEPA, 2019b).
RSC	0.2	N/A	Based on a review of the current scientific literature on PFHxS, potential exposure routes and sources exist but the available information is limited and does not allow for the quantitative characterization of the relative levels of exposure among these different sources (see Appendix D).
PFHxS HBWC = 0.00001 mg/L or 10 ppt			

Notes: RfV = chronic toxicity reference value; DWI-BW = body weight-adjusted drinking water intake; N/A = not applicable; NHANES = National Health and Nutrition Examination Survey; RSC = relative source contribution; PFHxS = perfluorohexanesulfonic acid.

[a] Note that ATSDR MRLs and EPA RfDs are not identical (e.g., intermediate-duration MRL vs. chronic RfD; the EPA and ATSDR may apply different uncertainty/modifying factors) and are developed for different purposes. The EPA applied an additional UF of 10 to the ATSDR MRL for PFHxS to account for subchronic-to-chronic duration (i.e., UF_S) yielding a chronic reference value of 2E-06 mg/kg/day, which was used to calculate the HBWC for PFHxS (USEPA, 2002).

3 Derivation of MCLGs

3.1 Individual MCLGs for HFPO-DA, PFNA, and PFHxS

The EPA is setting the individual MCLGs for HFPO-DA, PFHxS, and PFNA at 10 ng/L based on their respective RfVs, DWI-BWs, and RSCs (see Section 2, specifically Sections 2.1, 2.3, and 2.4). The MCLG for each of these PFAS is set, as defined in Section 1412(b)(4)(A), at "the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety." Each of these MCLGs (shown in Table 3-1 below) is set at the same level as the HBWCs derived in Section 2.

Table 3-1. Individual MCLGs

PFAS	MCLG (ng/L or ppt)
HFPO-DA	10
PFHxS	10
PFNA	10

Notes: HFPO-DA = hexafluoropropylene oxide dimer acid; MCLG = maximum contaminant level goal; PFAS = per- and polyfluoroalkyl substances; PFHxS = perfluorohexanesulfonic acid; PFNA = perfluorononanoic acid; ng/L = nanograms per liter; ppt = parts per trillion.

3.2 PFAS Mixtures Hazard Index MCLG

In consideration of the known toxic effects, potential dose additivity, and occurrence and likely co-occurrence of these PFAS in drinking water, the EPA is finalizing an HI of 1 (unitless) as the MCLG for any mixture of two or more of PFHxS, PFNA, HFPO-DA, and PFBS. As described in Section 1.6, a mixture HI can be calculated when HBWCs for a set of PFAS are available or can be calculated. HQs are calculated for each of the mixture components by dividing the measured component PFAS concentration in water (e.g., expressed as ng/L) by the relevant HBWC (e.g., expressed as ng/L), as shown in the equation below. Component HQs are summed across the PFAS mixture to yield the HI MCLG. A PFAS mixture HI MCLG greater than 1 (i.e., rounded to one significant digit) indicates an exceedance of the health-protective level and indicates potential human health risk for noncancer effects from oral exposure to the PFAS mixture in water. For more details, please see USEPA (2024a). The final PFAS mixture HI MCLG for mixtures of two or more of HFPO-DA, PFBS, PFNA, and PFHxS is calculated as follows:

$$HI\ MCLG = \left(\frac{[HFPO\text{-}DA_{ng/L}]}{[HFPO\text{-}DA_{HBWC}]}\right) + \left(\frac{[PFBS_{ng/L}]}{[PFBS_{HBWC}]}\right) + \left(\frac{[PFNA_{ng/L}]}{[PFNA_{HBWC}]}\right) + \left(\frac{[PFHxS_{ng/L}]}{[PFHxS_{HBWC}]}\right) = 1$$

$$HI\ MCLG = \left(\frac{[HFPO\text{-}DA_{ng/L}]}{[10\ ng/L]}\right) + \left(\frac{[PFBS_{ng/L}]}{[2000\ ng/L]}\right) + \left(\frac{[PFNA_{ng/L}]}{[10\ ng/L]}\right) + \left(\frac{[PFHxS_{ng/L}]}{[10\ ng/L]}\right) = 1$$

Where:

$[PFAS_{ng/L}]$ = the measured component PFAS concentration in water (in ng/L) and

$[PFAS_{HBWC}]$ = the HBWC of a component PFAS.

In summary, although current weight of evidence suggests that PFAS vary in their precise structure and function, exposure to different PFAS can result in the same or similar adverse health effects; as a result, PFAS co-exposures are likely to result in dose-additive effects and therefore the conclusion of dose additivity is appropriate (see Section 1.3 and also USEPA, 2024a). While individual PFAS can pose a potential risk to human health if the exposure level exceeds the chemical-specific toxicity reference value (RfD or MRL) (i.e., individual PFAS HQ >1.00), mixtures of PFAS at lower individual PFAS concentrations can result in dose-additive adverse health effects. For example, if the individual HQs for PFHxS, HFPO-DA, PFNA, and PFBS were each 0.90, that would indicate that the measured concentration of each individual PFAS in drinking water is below the level of appreciable risk for each individual PFAS (recall that an RfV, such as an oral RfD, represents an estimate at which no appreciable risk of deleterious effects exists). However, the overall HI for that mixture would be 4 (i.e., 3.6, sum of four HQs of 0.90, rounded to 4), indicating risk. Thus, setting an MCLG based on the concentration of an individual PFAS without considering the potential dose-additive effects from other PFAS in a mixture would not provide a sufficiently protective MCLG with an adequate margin of safety. To account for dose-additive noncancer effects associated with co-occurring PFAS to protect against health impacts from multi-chemical exposures to mixtures of two or more of PFHxS, HFPO-DA, PFNA, and PFBS, the agency is finalizing use of the HI approach for the MCLG for mixtures of two or more of these four PFAS. Consistent with the statutory requirement under 1412(b)(4)(A) of SDWA, establishing the MCLG for mixtures of two or more of PFHxS, HFPO-DA, PFNA, and PFBS at an HI equal to 1 ensures that the MCLG is set at a level at which there are no known or anticipated adverse effects on the health of persons and which ensures an adequate margin of safety.

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Appendix A. HFPO-DA: Summary of Occurrence in Water and Detailed Relative Source Contribution

A.1. Occurrence in Water

HFPO-DA can enter the aquatic environment through industrial discharges, runoff into surface water, and leaching into groundwater from soil and landfills (USEPA, 2021c). HFPO-DA is water-soluble, with solubilities of greater than 751 grams per liter (g/L) and greater than 739 g/L for HFPO-DA and its ammonium salt, respectively, at 20°C (USEPA, 2021c). Volatilization from water surfaces is expected to be an important fate process for HFPO-DA and its ammonium salt (USEPA, 2021c).

A.1.1. Ground Water

Pétré et al. (2021) quantified the mass transfer of PFAS, including HFPO-DA, from contaminated groundwater to five tributaries of the Cape Fear River. All sampling sites were located within 5 km of a manufacturing plant known known to be a major source of PFAS contamination. HFPO-DA and another fluoroether (perfluoro-2-[perfluoromethoxy] propanoic acid) together accounted for 61% of the total quantified PFAS. The study authors calculated that approximately 32 kg/year of PFAS is discharged from contaminated groundwater to the five tributaries. These data indicate that the discharge of contaminated groundwater has led to long-term contamination from HFPO-DA in surface water and could lead to subsequent impacts on downstream drinking water (Pétré et al., 2021).

A.1.2. Surface Water

Chemours has reported that HFPO-DA has been discharged into the Cape Fear River for several decades as a byproduct of other manufacturing processes (NCDEQ, 2017). An exposure assessment of the Chemours Fayetteville Works Facility located in Bladen County, North Carolina evaluated HFPO-DA in surface water data collected between July 2017 and October 2019 at four locations in the Cape Fear River (one upstream, one facility-adjacent location, and two downstream), one pond located in the facility site, and one offsite pond (Geosyntec, 2019). HFPO-DA was detected in surface water at the six sampled locations where mean (range) concentrations were 5 ng/L (n=1), 23.37 ng/L (2.1-160 ng/L; n=26), 133.6 ng/L (8.6-580 ng/L; n= 17), 16.38 ng/L (2.14-76 ng/L; n=79), 800 ng/L (730-940 ng/L; n=4), and 303.3 ng/L (290-310 ng/L; n=3), respectively (Geosyntec, 2019). Additionally, several other studies evaluated the occurrence of HFPO-DA in surface waters in North America (see Table A-1). As noted in the EPA's human health toxicity assessment for HFPO-DA (USEPA, 2021c), HFPO-DA was first detected in North Carolina's Cape Fear River and its tributaries in the summer of 2012 (Pritchett et al., 2019; Strynar et al., 2015). Since that finding, U.S. studies of surface waters, some of which are source waters for PWSs, have reported results of sampling efforts from contaminated areas near the Cape Fear River (McCord et al., 2018; Sun et al., 2016) and in Ohio and West Virginia (Galloway et al., 2020).

In studies of the Cape Fear River basin by McCord et al. (2018) and Sun et al. (2016), surface water concentrations of HFPO-DA ranged from below the North Carolina Department of Health and Human Services (NCDHHS) provisional health goal (PHG) of 140 ng/L to a maximum level of 4,560 ng/L. Sun et al. (2016) analyzed surface water from two sites upstream of a drinking water treatment plant (DWTP) and one site downstream. They reported a median HFPO-DA concentration of 304 ng/L with a maximum of 4,560 ng/L in the source water of the plant. HFPO-DA levels did not exceed the quantitation limit (10 ng/L) at the two upstream locations. In source water samples collected from the Cape Fear River near a DWTP downstream of a fluorochemical manufacturer, McCord et al. (2018) reported initial HFPO-DA concentrations of approximately 700 ng/L. After the manufacturer diverted waste stream emissions from one of its manufacturing lines, the measured concentrations decreased to levels below the NCDHHS PHG (140 ng/L).

In Ohio and West Virginia, Galloway et al. (2020) sampled rivers and streams located upstream, downstream, and downwind to the north and northeast of the Chemours Washington Works facility outside Parkersburg, West Virginia. The downwind sampling was intended to explore potential airborne deposition. Some of the downstream sampling sites were in the vicinity of landfills. Reported levels of HFPO-DA in these waters ranged from non-detectable levels to a maximum of 227 ng/L. The highest HFPO-DA concentrations were measured downwind of the facility (i.e., to the northeast). The study observed an exponentially declining trend of HFPO-DA concentrations in surface water with distance from the facility in this direction and attributed its occurrence in surface water to air dispersion of emissions from the facility. The most distant site where HFPO-DA was detected was 24 km north of the facility.

In one study of sites located in highly industrialized commercial waterways (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources), Pan et al. (2018) detected HFPO-DA in 100% of samples from sites in the Delaware River (n=12), reporting median and maximum concentrations of 2.02 ng/L and 8.75 ng/L, respectively, in surface waters.

Globally, HFPO-DA occurrence has been reported in surface waters from Germany (Pan et al., 2018; Heydebreck et al., 2015), China (Li et al., 2020a; Pan et al., 2018; Song et al., 2018; Pan et al., 2017; Heydebreck et al., 2015), the Netherlands (Pan et al., 2018; Gebbink et al., 2017; Heydebreck et al., 2015), the United Kingdom (Pan et al., 2018), South Korea (Pan et al., 2018), and Sweden (Pan et al., 2018). HFPO-DA was also detected with a mean concentration of 30 picograms per liter (pg/L; 0.030 ng/L) in Artic seawater samples, suggesting long-range transport (Joerss et al., 2020).

In one study of surface water collected from industrialized areas in Europe (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources), Pan et al. (2018) reported HFPO-DA detections in 100% of samples from the Thames River in the United Kingdom (n=6 sites), the Rhine River in Germany and the Netherlands (n=20 sites), and the Malaren Lake in Sweden (n=10 sites). Across these three river systems, median HFPO-DA concentrations ranged from 0.90 to 1.38 ng/L and the highest concentration detected was 2.68 ng/L. In another study, Heydebreck et al. (2015) detected HFPO-DA at 17% of sampling locations on the industrialized non-estuarine reaches of the Rhine River, with a maximum concentration of 86.08 ng/L; however, HFPO-DA was not detected at locations on the Elbe River.

Gebbink et al. (2017) evaluated surface water samples upstream and downstream of a fluorochemical production plant in the Netherlands and reported only one of three samples upstream of the plant with detectable HFPO-DA concentrations (22 ng/L; method quantification limit [MQL] = 0.2 ng/L). Downstream of the fluorochemical plant, HFPO-DA was detected in 100% of samples, with a mean concentration of 178 ng/L and a range of 1.7 to 812 ng/L. Vughs et al. (2019) analyzed surface water from 11 water suppliers in the Netherlands and Belgium, some of which were in the vicinity of a fluoropolymer manufacturing plant. The authors reported HFPO-DA detections in 77% of surface water samples (n=13) with a mean concentration of 2.2 ng/L and a maximum of 10.2 ng/L; however, only three samples in the study had HFPO-DA concentrations exceeding 1 ng/L.

Of the five studies conducted in China, one study evaluated surface water samples from an industrialized region (authors did not indicate whether sampling sites were in the vicinity of known PFAS point sources) (Pan et al., 2018), one study evaluated surface water river and reservoir samples in an industrialized river basin with potential PFAS point sources (Li et al., 2020a), and three studies examined samples from sites along the Xiaoqing river at locations upstream, downstream, or in the vicinity of known PFAS sources (Song et al., 2018; Pan et al., 2017; Heydebreck et al., 2015). HFPO-DA was detected in freshwater systems sampled in all five studies, though HFPO-DA concentrations appeared to be positively correlated with proximity to known PFAS point sources. Song et al. (2018), Pan et al. (2017), and Heydebreck et al. (2015) sampled sites in the Xiaoqing River system, including one of its tributaries, nearby a known fluoropolymer production facility. These three studies reported maximum HFPO-DA concentrations of 9,350, 2,060, and 3,060 ng/L, respectively. HFPO-DA concentrations in samples collected upstream of the facility did not exceed 3.64 ng/L. Other Chinese freshwater systems evaluated in the other two studies (Li et al., 2020a; Pan et al., 2018) generally reported maximum concentrations like those from the upstream Xiaoqing River system sites (≤ 10.3 ng/L), except for one site in Tai Lake which was reported to have a maximum HFPO-DA concentration of 143 ng/L. Similarly, in a study that sampled an industrialized river in South Korea (authors did not report whether sampling sites were in the vicinity of known PFAS point sources), HFPO-DA was found in 100% of samples and the maximum concentration found was 2.49 ng/L (Pan et al., 2018).

Table A-1. Compilation of Studies Describing HFPO-DA Occurrence in Surface Water

Study	Location	Site Details	Results
North America			
Sun et al. (2016)	United States (North Carolina, Cape Fear River Basin)	Source waters of three community drinking water treatment plants, two upstream and one downstream of a PFAS manufacturing plant (LOQ = 10 ng/L)	Community A (upstream): DF 0% Community B (upstream): DF NR, median (range) = ND (ND-10 ng/L) Community C (downstream): DF NR, mean = 631 ng/L, median (range) = 304 (55–4,560) ng/L
McCord et al. (2018)	United States (North Carolina, Cape Fear River Basin)	Source water of a drinking water treatment plant near the industrial waste outfall of a fluorochemical manufacturer, before and after the manufacturer diverted a waste stream (exact values NR, estimated values from Figure 3)	Before waste diversion (estimated): DF NR, measured concentration = ~ >700 ng/L After waste diversion (estimated): DR NR, measured concentration = < 140 ng/L
Galloway et al. (2020)	United States (Ohio and West Virginia, Ohio River Basin)	Rivers and tributaries located upstream, downstream, and downwind of a fluoropolymer production facility; some sample locations potentially impacted by local landfills	DF = 21/24 unique sites with detections > LOQ, median[a] (range) = 46.7 (ND–227) ng/L
Europe			
Gebbink et al. (2017)	The Netherlands	Upstream and downstream of the Dordrecht fluorochemical production plant; two control sites	Control sites: DF 0% Upstream of plant (n=3): DF[a] 33%, point = 22 ng/L Downstream of plant (n=13): DF 100%, mean[a] (range) = 178 (1.7–812) ng/L (MQL = 0.2)

Study	Location	Site Details	Results
Vughs et al. (2019)	The Netherlands and Belgium	Thirteen surface water samples collected from eleven water suppliers, some near a fluoropolymer manufacturing plant. The study did not map the distribution of reported concentrations by geographic location or with respect to distance from the fluoropolymer manufacturing plant.	DF 77%, mean (range) = 2.2 (ND–10.2) ng/L (LOQ = 0.2 ng/L)
Asia			
Pan et al. (2017)	China (Xiaoqing River and tributary)	Upstream and downstream of a fluoropolymer production plant in an industrialized region	Upstream of plant in the Xiaoqing River (n=6): DF[a] 100%, median[a] (range) = 2.10 (1.61–3.64) ng/L Tributary directly receiving plant effluent (n=4): DF[a] 100%, median[a] (range) = 1,855 (2.34-2,060) ng/L Downstream of plant in the Xiaoqing River receiving tributary waters (n=8): DF[a] 100%, median[a] (range) = 311 (118–960) ng/L
Song et al. (2018)	China (Xiaoqing River)	Near the Dongyue group industrial park, including a fluoropolymer production plant	DF NR, mean, median (range) = 519, 36.7 (<LOQ–9,350) ng/L (n=25 sites; LOQ=0.24 ng/L)
Li et al. (2020)	China (Hai River Basin)	40 surface water samples from 8 rivers and 3 reservoirs – many of the rivers flowed through industrialized areas, some with potential PFAS point sources	DF[b] 80%, mean (range) = 0.316 (<MDL–2.6) ng/L (MDL = 0.0132 ng/L)
Multiple Continents			

Study	Location	Site Details	Results
Heydebreck et al. (2015)	Germany (Elbe and Rhine Rivers), the Netherlands (Rhine-Meuse delta)	All sampling locations in industrialized areas	Rhine River (n=23): DF[a] 17%, range = ND–86.08 ng/L Elbe River (n=22): DF 0%
	China (Xiaoqing River)	Some sampling locations were downstream of PFAS point sources	Xiaoqing River (n=20): DF[a] 65%, range = ND–3,060 ng/L
Pan et al. (2018)	United States (Delaware River)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Delaware River (n=12): DF 100%, mean, median (range) = 3.32, 2.02 (0.78–8.75) ng/L
	United Kingdom (Thames River), Germany and the Netherlands (Rhine River), Sweden (Malaren Lake)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Thames River (n=6): DF 100%, mean, median (range) = 1.12, 1.10 (0.70–1.58) ng/L Rhine River (n=20): DF 100%, mean, median (range) = 0.99, 0.90 (0.59–1.98) ng/L Malaren Lake (n=10): DF 100%, mean, median (range) = 1.47, 1.38 (0.88–2.68) ng/L

Study	Location	Site Details	Results
	South Korea (Han River), China (Liao, Huai, Yellow, Yangtze, and Pearl Rivers; Chao and Tai Lakes)	Sampling sites along industrialized river systems that were not proximate to known point sources of PFAS from fluorochemical facilities	Han River (n=6): DF 100%, mean, median (range) = 1.38, 1.16 (0.78–2.49) ng/L Liao River (n=6): DF 100%, mean, median (range) = 1.44, 0.88 (0.62–4.51) ng/L Huai River (n=9): DF 100%, mean, median (range) = 1.66, 1.40 (0.83–3.62) ng/L Yellow River (n=15): DF 67%, mean, median (range) = 1.01, 1.30 (< LOQ–1.74) ng/L Yangtze River (n=35): DF 94%, mean, median (range) = 0.73, 0.67 (< LOQ–1.54) ng/L Pearl River (n=13): DF 100%, mean, median (range) = 1.51, 0.70 (0.21–10.3) ng/L Chao Lake (n=13): DF 100%, mean, median (range) = 1.92, 1.81 (0.93–3.32) ng/L Tai Lake (n=15): DF 100%, mean, median (range) = 14.0, 0.77 (0.38–143.7) ng/L (LOQ = 0.05 ng/L; MDL = 0.38 ng/L)
	All locations	Sampling sites were not proximate to known point sources of any fluorochemical facilities	All locations (n=160): DF 96%, mean, median (range) = 2.55, 0.95 (0.18–144) ng/L (LOQ = 0.05 ng/L; MDL = 0.38 ng/L)

Notes:
DF = detection frequency; LOQ = limit of quantification; ND = not detected.; ng/L = nanograms per liter; NR = not reported; MQL = method quantification limit; MDL = method detection limit.
[a] The DF, median, and/or mean was not reported in the study and was calculated in this synthesis. Mean values were only calculated if DF = 100%.

[b] The DF in Li et al. (2020a) was reported as 82.5% in the main article. The DF of 80% shown in this table is based on the supporting information data, which show only 32/40 samples with data > MDL.
[c] The Xiaoqing River results reported in Heydebreck et al. (2015) included samples from Laizhou Bay. The EPA considered freshwater samples only.

A.2. RSC for HFPO-DA, Literature Search and Screening Methodology

The EPA applies an RSC to the RfD when calculating an MCLG based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (USEPA, 2000b). The EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. The EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000b). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (USEPA, 2000b). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (USEPA, 2000b). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively-derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (USEPA, 2000b). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources

of exposure, changes to exposure characteristics over time, and data inadequacies (USEPA, 2000b).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of 20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (USEPA, 2000b). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

A.2.1. Literature Search and Screening

In support of the EPA's human health toxicity assessment for HFPO-DA (USEPA, 2021c), literature searches were conducted of four databases (PubMed, Toxline, Web of Science (WOS), and Toxic Substances Control Act Test Submissions (TSCATS)) to identify publicly available literature using CASRN, synonyms, and additional relevant search strings (see USEPA (2021c) for details). Due to the limited search results, additional databases were searched for information on physicochemical properties, health effects, toxicokinetics, and mechanism of action. The initial date-unlimited database searches were conducted in July 2017 and January/February 2018, with updates completed in February 2019, October 2019, and March 2020. In addition, information on toxicokinetics; acute, short-term, subchronic, and chronic toxicity; developmental and reproductive toxicity; neurotoxicity; immunotoxicity; genotoxicity; and cancer in animals was submitted with premanufacture notices to the EPA by DuPont/Chemours, the manufacturer of HFPO-DA, as required under the Toxic Substances Control Act pursuant to a consent order (USEPA, 2009b) or reporting requirements (15 U.S.C. § 2607.8(e)). The results of the literature searches of publicly available sources and submitted studies from DuPont/Chemours are available through the EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

The HFPO-DA literature search results and all studies submitted by DuPont/Chemours were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and non-human (i.e., those not identified as being in humans) studies. Studies identified as human studies were further categorized into seven major PFAS pathway categories (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) as well as an additional category for Human Exposure Measures. Non-human studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill.

Application of the SWIFT-Review tags identified 52 studies for title and abstract screening. An additional three references were identified through gray literature sources that were included to supplement the search results. Title and abstract screening to determine relevancy followed the populations, exposures, comparators, and outcomes (PECO) criteria in Table A-2:

Table A-2. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults (including women of childbearing age) and/or children in the general populations from any country
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater, human blood/serum/urine, indoor air, landfill, sediment, soil, surface water (freshwater), wastewater/biosolids/sludge
Comparator	Not applicable
Outcome	Measured concentrations of HFPO-DA (or measured emissions from food packaging and consumer products only)

The title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree if a study should be included or excluded, a third reviewer was consulted to make a final decision. The title and abstract screening resulted in 24 studies tagged as relevant (i.e., data on occurrence of HFPO-DA in one of the media of interest were presented in the study) that were further screened with full-text review using the same inclusion criteria. Of these 24 studies, 4 contain only human biomonitoring data and are not discussed further here. Based on full-text review, 15 studies were identified as relevant and are summarized below. At the full-text review stage, two additional studies were identified as only containing biomonitoring data.

A.2.2. Additional Screening

To supplement the primary literature database, the EPA also searched the following publicly available gray literature sources in February 2022 for information related to relative exposure of HFPO-DA for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- USEPA (2021c) *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"*;
- ATSDR's *Toxicological Profiles*;
- Centers for Disease Control and Prevention's (CDC's) national reports on human exposures to environmental chemicals;
- EPA's CompTox Chemicals Dashboard;
- EPA's fish tissue studies;
- EPA's Toxics Release Inventory;
- Relevant documents submitted under the Toxic Substances Control Act and relevant reports from the EPA's Office of Chemical Safety and Pollution Prevention;

- U.S. Food and Drug Administration's (FDA's) *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada;
- National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science data collections;
- National Science Foundation direct and indirect food and/or certified drinking water additives;
- PubChem compound summaries;
- Relevant sources identified in the relative source contribution discussions (Section 5) of the EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA)/Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water*; and
- Additional sources, as needed.

The EPA has included available information from these gray literature sources for HFPO-DA relevant to its uses, chemical and physical properties, and for occurrence in ambient or indoor air, foods (including fish and shellfish), soil, dust, and consumer products. The EPA has also included available information specific to HFPO-DA below on any regulations that may restrict HFPO-DA levels in media (e.g., water quality standards, air quality standards, food tolerance levels).

The EPA incorporated six references (Li et al., 2022; Burkhard, 2021; Feng et al., 2021; Straková et al., 2021; Semerád et al., 2020; Geosyntec, 2019) that were not identified in the EPA's RSC literature search strategy; these references were provided by Chemours as part of their outreach to the EPA on uses and sources for HFPO-DA in April 2022 and/or through the public comment period for the proposed PFAS NPDWR in 2023.

A.3. Summary of Potential Exposure Sources of HFPO-DA Other than Water

A.3.1. Dietary Sources

HFPO-DA was included in a suite of individual PFAS selected as part of PFAS-targeted reexaminations of samples collected for the U.S. Food and Drug Administration's (FDA's) Total Diet Study (US FDA, 2022b, 2022a, 2021b, 2021a, 2020b, 2020a); however, it was not detected in any of the food samples tested. It should be noted that FDA indicated that the sample sizes were limited and that the results should not be used to draw definitive conclusions about PFAS levels or presence in the general food supply (US FDA, 2022c). HFPO-DA was not detected in cow milk samples collected from a farm with groundwater known to be contaminated with PFAS; however, it was detected in produce (collard greens, cabbage) collected from an area near a PFAS production plant in FDA studies of the potential exposure to the U.S. population to PFAS (US FDA, 2021c, 2018). HFPO-DA was detected at low levels in 14% of vegetable garden crops (endive, beets, celery, lettuce, and tomatoes) grown near a PFAS manufacturing facility in the Netherlands (NCDEQ, 2018c; Mengelers et al., 2018). An exposure assessment of the Chemours Fayetteville Works Facility located in Bladen County, North Carolina evaluated HFPO-DA in fish fillet tissue samples collected between July and September 2019 at five locations within the Cape Fear River (Geosyntec, 2019). HFPO-DA was detected in three

samples of largemouth bass from the Bladen Bluffs location which is approximately eight miles downstream of the Fayetteville Works Facility at concentrations of 68,000 ng/kg, 54,000 ng/kg, and 24,000 ng/kg (Geosyntec, 2019). HFPO-DA was not detected in fish (largemouth bass, catfish, bluegill, or redbreast sunfish) collected from the other sites located upstream, adjacent to the site, 4 miles downstream, or in an on-site pond.

Feng et al. (2021) measured HFPO-DA in food samples collected from up to ten home gardens or farms in villages within 15 km of a large fluoropolymer facility located on the Dongzhulong River in Shandong Province, China. The authors detected HFPO-DA in wheat (mean concentration: 5.53 nanograms per gram dry weight [ng/g dw]; range: 2.27–9.19 ng/g dw; detection frequency [DF] 100%), maize (mean concentration: 1.17 ng/g dw; range: not detected (ND)–1.94 ng/g dw; DF 80%), and vegetable samples (mean concentration: 20.1 ng/g dw; range: ND–67.2 ng/g dw; DF 82%). In fish collected at two sites along the Dongzhulong River, HFPO-DA was detected at concentrations of 43.9 and 3.23 ng/g dw at sites approximately 3 km and 15 km downstream of the fluoropolymer facility, respectively. HFPO-DA was not found in eggs (home-produced and store-bought), store-bought meat or seafood, or milk from domestic goats (Feng et al., 2021). Except for the fish sampled at two sites, the study did not report HFPO-DA concentrations in food according to sampling location or proximity to the fluoropolymer facility.

HFPO-DA was not a target chemical in the EPA's National Lake Fish Tissue Study or the EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study, nor in the EPA's 2008–2009 or 2013–2014 National Rivers and Streams Assessment studies (USEPA, 2021e, 2020a; Stahl et al., 2014; USEPA, 2009a). HFPO-DA was detected in a redear sunfish fillet composite sample collected from a privately-owned lake near a PFAS manufacturing facility in North Carolina at a concentration of 270 nanograms per kilogram (ng/kg) (wet weight tissue) (USEPA, 2021c; NCDEQ, 2018c). HFPO-DA was not included in the NOAA's National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). Burkhard (2021) identified a single bioaccumulation factor (BAF) in muscle tissue/fillet reported in the literature of 4.07 L/kg wet weight (reported as a logBAF of 0.61 L/kg).

A.3.2. Food Contact Materials

In an analysis performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology in Prague, Czech Republic, HFPO-DA was not detected in 42 samples of disposable food packaging and tableware purchased from six different European countries between May and December 2020 (LOQ = 1.7 mg/kg) (Straková et al., 2021).

A.3.3. Consumer Products

Although no specific studies on the occurrence of HFPO-DA in consumer products were identified, DuPont began transitioning to GenX processing aid technology in 2009 to work toward eliminating long-chain PFAS as part of the company's commitment under the 2010/2015 PFOA Stewardship Program (USEPA, 2021c). It is unknown if HFPO-DA in consumer products have increased as a result of this transition.

A.3.4. Indoor Dust

Feng et al. (2021) detected HFPO-DA in indoor dust samples taken from homes from 10 villages within 15 km of a large fluoropolymer facility in Shandong Province, China, at concentrations

ranging from ND to 841 ng/g (mean concentration 159 ng/g; DF 72%). Contaminated dust was found in homes as far as 15 km from the fluoropolymer facility and HFPO-DA concentrations were highest in homes nearest to the facility. Although only one study on the occurrence of HFPO-DA in indoor dust was identified, other PFAS have been detected in indoor dust and on window films (ATSDR, 2021).

A.3.5. Air

PFAS have been released to air from wastewater treatment plants, waste incinerators, and landfills (USEPA, 2016a). HFPO-DA could be transported in the vapor phase or with particulates (USEPA, 2021c). When released to air or volatilized from water, HFPO-DA is stable and short- and long-range transport has occurred (D'Ambro et al., 2021; Galloway et al., 2020). Galloway et al. (2020) analyzed HFPO-DA concentrations in soil samples downwind of and surface water samples upstream of the Chemours Washington Works facility outside of Parkersburg, West Virginia, and results suggest atmospheric transport of HFPO-DA emissions. Additionally, a study that modeled the atmospheric transport of a PFAS mixture containing HFPO-DA from a fluoropolymer manufacturing facility in North Carolina (D'Ambro et al., 2021) predicted that only 2.5% of total HFPO-DA (consisting of HFPO-DA and HFPO-DA fluoride) would be deposited within 150 km of the facility (USEPA, 2021c).

HFPO-DA is persistent in air (half-lives longer than 6 months), and not readily broken down by biodegradation, direct photolysis, or hydrolysis (USEPA, 2021c). In the vapor phase, HFPO-DA is expected to undergo hydroxyl radical-catalyzed indirect photolysis slowly, with a predicted average hydroxylation rate of 8.50 x 10-13 cubic centimeters (cm^3)/molecule - second (USEPA, 2022f, 2022e, 2021c). Based on a measured vapor pressure of 2.7 mm Hg at 20°C for HFPO-DA, volatilization is expected to be an important fate process for this chemical (USEPA, 2021c). EPA's Toxics Release Inventory reported release data for HFPO-DA in 2020 (USEPA, 2022c). HFPO-DA is not listed as a hazardous air pollutant (USEPA, 2022d).

HFPO-DA has been identified in air emissions. North Carolina Department of Environmental Quality (NCDEQ) estimates for the Chemours Fayetteville Works plant, located in the North Carolina Cape Fear watershed, indicate that annual emissions of HFPO-DA could have exceeded 2,700 pounds per year during the reporting period (2017–2018) (NCDEQ, 2018a). Rainwater samples collected within a seven-mile radius of this facility were reported to have detectable levels of HFPO-DA (NCDEQ, 2018b), with the highest concentration of 810 ng/L found in a rainwater sample collected five miles from the facility. The three samples collected seven miles from the plant had HFPO-DA concentrations ranging from 45.3 to 60.3 ng/L (NCDEQ, 2018b).

A.3.6. Soil

When HFPO-DA is deposited on or applied to soil, it is expected to run off into surface waters or rapidly leach to groundwater (USEPA, 2021c). PFAS can also be taken up from contaminated soil by plants (ATSDR, 2021). No specific studies on the occurrence of HFPO-DA in biosolids were identified. An exposure assessment of the Chemours Fayetteville Works Facility located in Bladen County, North Carolina evaluated HFPO-DA in soil samples collected between July and September 2019 at twelve offsite locations (Geosyntec, 2019). HFPO-DA was detected in two of four surface soil samples (0 to 0.5 ft depth) collected within a 2.5 km radius of the facility at concentrations of 2,600 ng/kg and 360 ng/kg. HFPO-DA was also detected in two of four

subsurface soil samples (4 to 4.5 ft depth) collected within a 2.5 km radius of the facility at concentrations of 430 ng/kg and 590 ng/kg, and in one of four subsurface samples (excluding duplicates) collected within a 5 km radius to the facility at a concentration of 400 ng/kg (Geosyntec, 2019). HFPO-DA was not detected in surface or subsurface soil samples collected in a 10 km radius to the facility.

Two peer-reviewed studies reported HFPO-DA concentrations in soil. In the United States, Galloway et al. (2020) analyzed 13 soil samples for HFPO-DA at locations in Ohio and West Virginia that were upstream and downwind of the Chemours Washington Works facility in order to evaluate HFPO-DA contamination due to atmospheric deposition. HFPO-DA was detected in 5 out of 13 samples, with a maximum concentration of 8.14 ng/g dw. In China, Li et al. (2020b) collected and analyzed residential soil samples throughout the country from 31 provincial-level administrative regions (consisting of 26 provinces, 4 municipalities, and 1 special administrative region). HFPO-DA was detected in 40.5% of soil samples at concentrations up to 967 picograms per gram (pg/g) dw and a mean level of 19.1 pg/g dw. PFOA was detected in these soils more frequently (96.6%) and at higher mean levels (354 pg/g dw), leading the authors to conclude that HFPO-DA consumption was still limited at the national scale of China, despite its use as a PFOA replacement.

One study measured concentrations of HFPO-DA in and/or on grass and leaves collected from sites various distances from a fluoropolymer manufacturing plant in the Netherlands (Brandsma et al., 2019). HFPO-DA concentrations ranged from 86 ng/g in leaves from a site closest to the plant to ND furthest from the plant. A similar pattern was observed for grass samples, except the maximum HFPO-DA concentration was lower (27 ng/g). The study authors note that it hadn't rained for five days prior to sample collection.

Semerád et al. (2020) investigated occurrence of HFPO-DA in sewage sludge from 43 facilities in the Czech Republic. HFPO-DA was detected in 7 of 43 samples at concentrations ranging from 0.3 to 1.2 ng/g dw. The authors raised concerns about the agricultural use of sludge containing PFAS for growing crops.

A.3.7. Sediment

HFPO-DA is expected to remain in water and exhibit low partitioning to sediment (USEPA, 2021c). One study evaluated the occurrence of HFPO-DA in sediments from the North and Baltic Seas in Europe, and reported that HFPO-DA was not detected in any of the 24 sediment samples taken in the North and Baltic Seas in the vicinity of Germany (Joerss et al., 2019). An additional four studies analyzed sediments in China (Li et al., 2022; Li et al., 2020b; Wang et al., 2019a; Song et al., 2018). Of the four studies, Wang et al. (2019a) analyzed sediment from the South China Sea coastal region in the area of the highly industrialized Pearl River Delta and reported that HFPO-DA was below the LOQ in all 53 samples. Li et al. (2020) analyzed 20 sediment samples from eight rivers and three reservoirs in the Hai River Basin in the vicinity of several industrialized areas. HFPO-DA was reportedly detected at minimal levels, but the authors did not report actual concentrations. Song et al. (2018) analyzed concentrations of HFPO-DA in 24 sediment samples from the Xiaoqing River in the vicinity of a fluoropolymer production facility. The study reported a maximum HFPO-DA concentration in sediment of 22.3 ng/g dw, with median and mean levels below the LOQ. Li et al. (2022) also analyzed sediment samples

from five sites of the Xiaoqing River estuary and reported a mean HFPO-DA concentration of 0.23 ng/g dw.

A.4. Recommended RSC

The EPA followed the Exposure Decision Tree approach to determine the RSC for HFPO-DA (USEPA, 2000b). The EPA first identified three potential populations of concern (Box 1): pregnant women, lactating women, and women of childbearing age (see Section 2.1.2). However, limited information was available regarding specific exposure of these populations to HFPO-DA in different environmental media. The EPA considered exposures in the general U.S. population as likely being applicable to these two populations. Second, the EPA identified several relevant HFPO-DA exposures and pathways (Box 2), including dietary consumption, incidental oral consumption via dust and soil or dermal exposure via soil and dust, and inhalation exposure via ambient air. Several of these may be potentially significant exposure sources. Third, the EPA determined that there was inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, a study from China indicates that exposure via dust may be a significant pathway for HFPO-DA. At the time of the literature search, the EPA was unable to identify studies assessing HFPO-DA concentrations in dust samples from the U.S. and therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, based on the studies summarized in the sections above, there are significant known or potential uses/sources of HFPO-DA other than drinking water (Box 6), though there is not information available on each source to make a characterization of exposure (Box 8A). For example, the EPA identified physico-chemical properties of HFPO-DA indicating that volatilization may be an important fate process for this chemical. There is evidence in the literature of atmospheric transport of HFPO-DA and occurrence in rainwater. However, monitoring data describing the occurrence of HFPO-DA in ambient air is limited to a single report from an area located nearby a known point source of this chemical. The levels of HFPO-DA in ambient air at this source may not be representative of the U.S. as a whole. Therefore, it is not possible to determine whether ambient air can be considered a major or minor contributor to total HFPO-DA exposure in the U.S. general population. Similarly, it is not possible to determine whether the other potentially significant exposure sources such as vegetables and soil should be considered major or minor contributors to total HFPO-DA exposure. Given these considerations, following recommendations of the Exposure Decision Tree (USEPA, 2000b), the EPA recommends an RSC of 20% (0.20) for HFPO-DA.

Appendix B. PFBS: Summary of Occurrence in Water and Detailed Relative Source Contribution

B.1. Occurrence in Water

PFBS can enter the aquatic environment through releases from manufacturing sites, industrial uses, fire/crash training areas, and wastewater treatment facilities, as well as from the use of contaminated biosolids (USEPA, 2021d; ATSDR, 2021). PFBS has a high solubility in water (52.6 g/L at 22.5–24 °C for the potassium salt) and high mobility in the environment (log Koc 1.2 to 2.7) (ECHA, 2019). A literature review of physicochemical properties and environmental monitoring data for PFBS by the Norwegian Environment Agency determined that volatilization from water is negligible, but that the presence of PFBS in ambient air can result from direct emissions or transport of droplets in contaminated water (Arp and Slinde, 2018). PFBS has been found in rain as well as in snow/ice in the Arctic and Antarctic (Arp and Slinde, 2018).

The EPA collected information about PFBS occurrence in water (described below). To better understand PFBS sources and occurrence patterns in water, this section includes studies conducted within and outside the United States. Overall, studies that analyzed water from sites receiving inputs from or in proximity to known sources of PFAS (as reported by study authors) did not provide a consistent pattern of detection; increased PFBS detection frequencies (DFs) or concentrations were not only observed in studies of sites with known sources of PFAS contamination from point sources. Specifically, DFs of 0% were reported at some sites with known, suspected, or historic PFAS contamination, and DFs of 100% were reported at some sites with no known point sources of PFAS contamination. However, the maximum reported PFBS concentrations in groundwater and surface water were measured at sites with known PFAS contamination from AFFF usage (Anderson et al., 2016).

B.1.1. Ground Water

Several studies evaluated the occurrence of PFBS in raw groundwater in the United States or Europe (see Table B-1). Most of the available studies sampled from groundwaters known or suspected to be contaminated with PFAS through various sources, as reported by the study authors. Importantly, some of these groundwaters are known to be used as input sources for PWSs.

The EPA identified four U.S. studies assessing PFBS concentrations in groundwater at sites known to be contaminated with PFAS from the use of AFFF (Steele et al., 2018; Eberle et al., 2017; Anderson et al., 2016; Moody et al., 2003). Of the three studies that reported PFBS detections, two reported DFs of 78.26% and 100% (Eberle et al., 2017; Anderson et al., 2016); the third study did not report a PFBS DF across sample sites but indicated a range of PFBS concentrations (ND–48 ng/L) (Steele et al., 2018). The fourth study, which analyzed groundwater from the decommissioned Wurtsmith Air Force Base, did not detect PFBS at any of the ten sites sampled, though other PFAS were detected (Moody et al., 2003). However, a case study published by the Association of State and Territorial Solid Waste Management Officials reported quantifiable levels of PFBS in four of seven samples tested from the Wurtsmith Air

Force Base; one site sampled directly below the fire training area was reported to have a PFBS concentration of 4,100 ng/L (ASTSWMO, 2015).

Additionally, PFBS has been detected at concentrations ranging from 0.00211 ng/L to 0.0261 ng/L in groundwater wells (100% well DF) at a site near the 3M Cottage Grove perfluorochemical manufacturing facility in Minnesota (ATSDR, 2021; 3M, 2007). Lee et al. (2015) evaluated urban shallow groundwater contaminated by wastewater effluent discharge and reported a DF of 20% (1 of 5 shallow sites) and a maximum PFBS level of 36.3 ng/L. In contrast, Procopio et al. (2017) collected groundwater from 17 sampling sites (53 total across all water types sampled), some of which were located downstream of an industrial facility that used materials containing PFOA. PFBS was not detected in groundwater collected from any of the sampling locations. Post et al. (2013) assessed raw water from PWS intakes in New Jersey; these intake locations were selected to represent New Jersey geographically and they were not necessarily associated with any known PFAS release. PFBS was detected pre-treatment in 1 of 18 systems at a concentration of 6 ng/L (minimum reporting level = 5 ng/L). Lindstrom et al. (2011) analyzed water from 13 wells intended for uses other than drinking water (e.g., livestock, watering gardens) in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer. PFBS was detected in three of the wells (mean concentration 10.3 ng/L; range: ND–76.6 ng/L).

Of the 10 identified studies conducted in Europe, seven studies evaluated groundwater samples from sites with known or suspected PFAS releases associated with AFFF use, fluorochemical manufacturing, or other potential emission sources including landfill/waste disposal sites, skiing areas, or areas of unspecific industries that use PFAS in manufacturing (e.g., metal plating) (Dauchy et al., 2019; Høisæter et al., 2019; Gobelius et al., 2018; Dauchy et al., 2017; Gyllenhammar et al., 2015; Wagner et al., 2013; Dauchy et al., 2012). All of these studies reported PFBS detections in at least one sample or site, though only two studies (both conducted in the vicinity of areas with known AFFF usage) reported PFBS concentrations ≥ 100 ng/L (Dauchy et al., 2019; Gyllenhammar et al., 2015). The remaining three studies of the 10 identified did not provide information on whether there were potential sources of PFAS at the sampling locations or were designed to be regionally, nationally, or internationally representative (Barreca et al., 2020; Boiteux et al., 2012; Loos et al., 2010). At these sites, PFBS was detected infrequently (DFs 4 to 18%) with a maximum concentration of 25 ng/L across the three studies.

Table B-1. Compilation of Studies Describing PFBS Occurrence in Groundwater

Study	Location	Site Details	Results
North America			
Lee et al. (2015)	United States (California)	Samples from 5 urban shallow groundwater wells with wastewater contamination	DF[a] 20%, range = ND–36.3 ng/L

Study	Location	Site Details	Results
Appleman et al. (2014)	United States (New Jersey)	Samples from 5 New Jersey groundwater source waters for PWSs impacted by upstream wastewater effluent discharge	DF[a] 100%, mean[a] (range) = 2.4 (0.43–3.7) ng/L
Post et al. (2013)	United States (New Jersey)	Raw water from 18 public drinking water system groundwater intakes	DF 6%, range = ND–6 ng/L
Steele et al. (2018)	United States (Alaska)	Military base contaminated with PFAS from AFFF use (4 wells sampled once per month for 8 months)	DF[a] NR, range = ND–48 ng/L
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, VA)	Former fire training site, site characterization and pretreatment groundwater samples	Site characterization: DF 100%, mean[a] (range) = 3,700 (1,100–13,000) ng/L (10 wells) Pretreatment: DF 100%, mean[a] (range) = 3,400 (1,200–5,000) ng/L (5 wells, 2 laboratory samples/well)
Anderson et al. (2016)	United States (national)	Ten active U.S. Air Force installations with historic AFFF release	DF 78.26%, median of detects (range) = 200 (ND–110,000) ng/L
Moody et al. (2003)	United States (Oscoda, MI)	Groundwater plume at former Wurtsmith Air Force Base; firefighting training area active from 1952 to 1993	DF 0%
Procopio et al. (2017)	United States (New Jersey)	Samples collected from temporary wells in a small area of an industrial/business park located within the Metedeconk River Watershed	DF 0%

Study	Location	Site Details	Results
Lindstrom et al. (2011)	United States (Alabama)	Samples from 13 wells used for purposes aside from drinking water (e.g., livestock, watering gardens, washing), located in areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 23%, mean (range) = 10.3 (ND–76.6) ng/L
Europe			
Barreca et al. (2020)	Italy (Lombardia region)	Groundwater sampling stations representative of region	DF 18%[a], concentrations NR
Boiteux et al. (2012)	France (national)	Raw water from 2 sampling campaigns of DWTPs, some sites possibly affected by industrial or commercial releases	DF 4%, range = ND–9 ng/L
Loos et al. (2010)	23 European countries	Monitoring stations were not necessarily representative of surrounding area or contaminated	DF 15.2%, range = ND–25 ng/L
Gobelius et al. (2018)	Sweden (national)	Sampling locations selected based on potential vicinity of PFAS hot spots and importance as a drinking water source area	DF 26%[a] (triplicate samples removed), range = ND–22 ng/L
Dauchy et al. (2012)	France (unspecified)	Raw water from 2 DWTPs supplied by alluvial wells; DWTPs located 15 km downstream of fluorochemical manufacturing facility	DF[a] 40%, range = ND–4 ng/L

Study	Location	Site Details	Results
Høisæter et al. (2019)	Norway (unspecified)	Samples from 19 sampling campaigns of 5 pumping wells placed to intercept a groundwater contamination plume originating from a firefighting training facility that ceased usage of PFAS- and fluorotelomer-based AFFF 15 years prior	Detections reported but DF and concentrations not provided
Dauchy et al. (2019)	France (unspecified)	Samples collected over 2 campaigns from 6 areas (13 monitoring wells) of a firefighter training site	DF[a] 77%, range = ND–750 ng/L
Dauchy et al. (2017)	France (unspecified)	Samples collected near 3 sites (A, C, D) impacted by the use of AFFF. Site A results describe 1 sampling location with 2 sampling events. Site C results describe a single sampling location and event. Site D results describe 5 sampling locations, each with a single sampling event	Site A: DF[a] 100% mean[a] = 8 ng/L Site C: point = 6 ng/L Site D: DF[a] 20%, range = ND–59 ng/L

Study	Location	Site Details	Results
Gyllenhammar et al. (2015)	Sweden (Uppsala)	Samples from local aquifers extracted by 21 production wells, 6 observation wells or 1 private well located in the vicinity of a potential AFFF point source (military airport). Results for all well sites were not provided.	Site 1 (production well): DF 0% (n = NR) Site 3 (observation wells): DF 100%, median = 100 ng/L (n = 3) Site 5 (observation well): DF 0% (n = NR) Site 6 (production well): DF 0% (n = NR) Site 7 (observation well): DF 100%, median = 35 ng/L (n = 3) Site 8 (production well): DF[a] 91%, median = 13 ng/L (n = 103) Site 10 (production well): DF[a] 2%, median = ND (n = 50)
Wagner et al. (2013)	Germany (unspecified)	Samples (n = 3) taken downstream from a site contaminated by AFFF from firefighting activities	DF[a] 100%, concentrations NR

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; DWTP = drinking water treatment plant; km = kilometer; ND = not detected; ng/L = nanogram per liter; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; NR = not reported; WWTP = wastewater treatment plant.
[a] The DF and/or mean was calculated using point data. Means were calculated only when DF = 100%.

B.1.2. Surface Water

Studies evaluating the occurrence of PFBS in surface water in North America or Europe are summarized in Table B-2. Broadly, studies either targeted surface waters used as drinking water sources, surface waters known to be contaminated with PFAS (as reported by the study authors), or surface waters over a relatively large geographic area (i.e., statewide) with some or no known point sources of PFAS.

Zhang et al. (2016) identified major sources of surface water PFAS contamination by collecting samples from 37 rivers and estuaries in the northeastern United States (metropolitan New York area and Rhode Island). PFBS was detected at 82% of sites and the range of PFBS concentrations was ND to 6.2 ng/L. Appleman et al. (2014) collected samples of surface water that were impacted by wastewater effluent discharge in several states. PFBS was detected in 64% of samples from 11 sites with a range of PFBS concentrations from ND to 47 ng/L. Several other studies from North America (four from the United States and two from Canada) evaluated

surface waters from sites for which authors did not indicate whether sites were associated with any specific, known PFAS releases (Pan et al., 2018; Yeung et al., 2017; Subedi et al., 2015; Veillette et al., 2012; Nakayama et al., 2010). Nakayama et al. (2010) also collected samples across several states, but no specific source of PFAS was identified. The DF in the Nakayama et al. (2010) study was 43% with median and maximum PFBS levels of 0.71 and 84.1 ng/L, respectively. Pan et al. (2018) sampled surface water sites in the Delaware River and reported a 100% DF, though PFBS levels were relatively low (0.52 to 4.20 ng/L); Yeung et al. (2017) reported results for a creek (PFBS concentration of 0.02 ng/L) and a river (no PFBS detected) in Canada. Veillette et al. (2012) analyzed surface water from an Arctic lake and detected PFBS at concentrations ranging from 0.011 to 0.024 ng/L. Subedi et al. (2015) evaluated lake water potentially impacted by septic effluent from adjacent residential properties, and detected PFBS in only one sample at a concentration of 0.26 ng/L.

Additional available studies assessed surface water samples at U.S. sites contaminated with PFAS from nearby PFAS manufacturing facilities (ATSDR, 2021; Galloway et al., 2020; Newsted et al., 2017; Newton et al., 2017) or facilities that manufacture products containing PFAS (Procopio et al., 2017; Zhang et al., 2016; Lasier et al., 2011). A few of these studies identified potential point sources of PFAS contamination, including industrial facilities (e.g., textile mills, metal plating/coating facilities), airports, landfills, and wastewater treatment plants (WWTPs) (Galloway et al., 2020; Zhang et al., 2016). Among these sites, DFs (0 to 100%) and PFBS levels (ND to 336 ng/L) varied. In general, DFs that ranged from 0 to 3% were associated with samples collected upstream of PFAS point sources, and higher DFs (up to 100%) and PFBS concentrations were associated with samples collected downstream of point sources. An additional study (Lindstrom et al., 2011) sampled pond and stream surface water in areas impacted by up to 12 years of field applications of biosolids contaminated by a fluoropolymer manufacturer, and the maximum and mean PFBS concentrations were 208 and 26.3 ng/L, respectively.

Another group of studies from the United States evaluated sites known to be contaminated from military installations with known or presumed AFFF use (Anderson et al., 2016; Post et al., 2013; Nakayama et al., 2007). The highest PFBS levels reported among these available studies were from Anderson et al. (2016) who performed a national study of 40 AFFF-impacted sites across 10 military installations and reported a maximum PFBS concentration of 317,000 ng/L. Lescord et al. (2015) examined PFAS levels in Meretta Lake, a Canadian lake contaminated with runoff from an airport and military base, which are likely sources of PFAS from AFFF use. The authors reported a 70-fold higher mean PFBS concentration for the contaminated lake versus a control lake. In addition to AFFF, Nakayama et al. (2007) identified industrial sources, including metal-plating facilities and textile and paper production, as contributing to the total PFAS contamination in North Carolina's Cape Fear River Basin. Nakayama et al. (2007) reported a PFBS DF of 17% and PFBS concentrations ranging from ND to 9.41 ng/L at these sites.

The EPA identified additional studies evaluating surface water samples from sites in Europe with known or suspected PFAS releases associated with AFFF use (Mussabek et al., 2019; Gobelius et al., 2018; Dauchy et al., 2017) or fluorochemical manufacturing (Bach et al., 2017; Boiteux et al., 2017; Gebbink et al., 2017; Valsecchi et al., 2015). PFBS levels were comparable at the AFFF-impacted sites (< 300 ng/L overall). Of the four study sites potentially contaminated based on proximity to fluorochemical manufacturing sites, two (from studies conducted in France) did not have PFBS detections (Bach et al., 2017; Boiteux et al., 2017). PFBS levels were low at most

sampling locations of the remaining two studies (up to approximately 30 ng/L) except for the site in River Brenta in Italy (maximum PFBS concentration of 1,666 ng/L) which is also impacted by nearby textile and tannery manufacturers (Valsecchi et al., 2015).

Eight studies in Europe evaluated areas close to urban areas, commercial activities, or industrial activities (e.g., textile manufacturing) (Lorenzo et al., 2015; Zhao et al., 2015; Boiteux et al., 2012; Eschauzier et al., 2012; Rostkowski et al., 2009) and/or wastewater effluent discharges (Wilkinson et al., 2017; Lorenzo et al., 2015; Labadie and Chevreuil, 2011; Möller et al., 2010). Among these sites, DFs varied (0 to 100%) and PFBS levels were < 250 ng/L overall.

Ten studies conducted in Europe evaluated sites with no known fluorochemical point source of contamination (Barreca et al., 2020; Pan et al., 2018; Loos et al., 2017; Shafique et al., 2017; Munoz et al., 2016; Eriksson et al., 2013; Wagner et al., 2013; Ahrens et al., 2009b; Ahrens et al., 2009a; Ericson et al., 2008b). Pan et al. (2018) analyzed surface water from sites in the United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). None of the sites sampled were proximate to known sources of PFAS, but PFBS was detected in all three water bodies. Concentrations of PFBS ranged from 0.46 to 146 ng/L; the highest level (146 ng/L) was detected in the Rhine River and was more than 20 times greater than any maximum level found in the other water bodies. In the remaining nine studies, reported PFBS levels ranged from ND to 26 ng/L, except for one study in Italy that reported a PFBS DF of 39% and levels in the µg/L range at three out of 52 locations within the same river basin: Legnano (16,000 ng/L), Rho (15,000 ng/L), and Pero (3,400 ng/L) (Barreca et al., 2020).

Table B-2. Compilation of Studies Describing PFBS Occurrence in Surface Water

Study	Location	Site Details	PFBS Results
North America			
Yeung et al. (2017)	Canada (Ontario; Mimico Creek, Rouge River)	Two water samples at each of the sites	Mimico Creek: point = 0.020 ng/L Rouge River: DF 0%
Subedi et al. (2015)	United States (New York; Skaneateles Lake)	Lake water along the shoreline of residences that use an enhanced treatment unit for onsite wastewater treatment	DF[a] 4% (n=28); single detection value = 0.26 ng/L
Appleman et al. (2014)	United States (Wisconsin, Oklahoma, Alaska, California, Alabama, Colorado, Ohio, Nevada, Minnesota, New Jersey)	Raw surface waters from 11 sites, some impacted by upstream wastewater effluent discharge	DF[a] 64% (n=25); range = ND–47 ng/L (MRL = 0.3)
Veillette et al. (2012)	Canada (Ellesmere Island, Nunavut)	A lake near the northwest coast with no known sources of PFAS	DF[a] 100%, mean (range) = 0.016 (0.011–0.024) ng/L

Study	Location	Site Details	PFBS Results
Nakayama et al. (2010)	United States (Illinois, Iowa, Minnesota, Missouri, Wisconsin; Upper Mississippi River Basin and Missouri River Basin)	88 sampling sites from tributaries and streams	DF 43%, median (range) = 0.71 (ND–84.1) ng/L
Galloway et al. (2020)	United States (Ohio and West Virginia; Ohio River Basin)	Rivers and tributaries 58 km upstream to 130 km downwind of a fluoropolymer production facility, some sample locations potentially impacted by local landfills	DF NR, range[a] = ND–28.0 ng/L
Newsted et al. (2017)	United States (Minnesota; Upper Mississippi River Pool 2)	Upstream and downstream of 3M Cottage Grove facility outfall, which is a source of PFAS	Upstream: DF[a] 3%, point = 4.2 ng/L Downstream: DF[a] 67%, range = ND–336.0 ng/L
Procopio et al. (2017)	United States (New Jersey; Metedeconk River Watershed)	Downstream of suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs	DF[a] 5%, range = ND–100 ng/L
Newton et al. (2017)	United States (Decatur, Alabama; Tennessee River)	6 sites upstream and 3 sites downstream of fluorochemical manufacturing facilities	Upstream: DF 0% Downstream: DF[a] 100%, mean[a] (range) = 69 (10–160) ng/L
Zhang et al. (2016)	United States (Rhode Island, New York Metropolitan Region)	Rivers and creeks, some sampling locations downstream from industrial activities, airport, textile mills, and WWTP. PFAS are used for water resistant coating in textiles.	DF[a] 85%, range = ND–6.181 ng/L

Study	Location	Site Details	PFBS Results
Lescord et al. (2015)	Canada (Resolute Bay, Nunavut)	One lake (Meretta) contaminated with runoff from an airport, which is a known source of PFAS; one control lake (9 Mile)	Meretta: DF NR, mean = 4.9 ng/L 9 Mile: DF NR, mean = 0.07 ng/L
Lasier et al. (2011)	United States (Georgia; Coosa River watershed)	Upstream (sites 1 and 2) and downstream (sites 3–8) of a land-application site where effluents from carpet manufacturers (suspected of producing wastewaters containing perfluorinated chemicals) are processed at a WWTP and the treated WWTP effluent is sprayed onto the site. Site 4 was downstream of a manufacturing facility for latex and polyurethane backing material.	Upstream Sites 1 and 2: DF 0% Downstream Site 3: DF NR, mean = 205 ng/L Site 4: DF NR, mean = 260 ng/L Site 5: DF NR, mean = 125 ng/L Site 6: DF NR, mean = 134 ng/L Site 7: DF NR, mean = 122 ng/L Site 8: DF NR, mean = 105 ng/L
Anderson et al. (2016)	United States (national)	Ten U.S. Air Force installations with historic AFFF release	DF 80.00%, median (range) = 106 (ND–317,000) ng/L
Post et al. (2013)	United States (New Jersey)	6 rivers and 6 reservoirs from public drinking water system intakes, some sites may include nearby small industrial park and civil-military airport	DF 17%, range = ND–6 ng/L
Nakayama et al. (2007)	United States (North Carolina; Cape Fear River Basin)	80 sampling sites in river basin; some sites near industrial areas and Fort Bragg and Pope Air Force Base with suspected use of AFFF at the Air Force Base	DF 62%, mean (range) = 2.58 (ND–9.41) ng/L

Study	Location	Site Details	PFBS Results
Lindstrom et al. (2011)	United States (Alabama)	32 surface water samples (ponds and streams) from areas with historical land application of fluorochemical industry-impacted biosolids	DF[a] 63%, range = ND–208 ng/L
Bradley et al. (2020)	United States (Lake Michigan)	Untreated Lake Michigan water from treatment plant intake (4 sites)	DF 29%, range = ND–0.5 ng/L
Europe			
Barreca et al. (2020)	Italy (Lombardia Region)	Rivers and streams with no known fluorochemical sources	DF[a] 39%, range = ND–16,000 ng/L
Loos et al. (2017)	Austria, Bulgaria, Croatia, Moldova, Romania, Serbia, Slovakia (Danube River and tributaries)	Some sampling locations downstream of major cities	DF 94%, mean (range) = 1.6 (ND–3.7) ng/L
Wilkinson et al. (2017)	England (Greater London and southern England; Hogsmill River, Chertsey Bourne River, Blackwater River)	50 m upstream and 250 m and 1,000 m downstream from WWTP effluent outfalls	Upstream: DF NR, mean = 20.4 ng/L Downstream 250 m: DF NR, mean = 40.3 ng/L Downstream 1,000 m: DF NR, mean = 41.1 ng/L
Shafique et al. (2017)	Germany (Leipzig, Pleiße-Elster River, Saale River, and Elbe River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Pleiße-Elster: DF NR, mean = 1.2 ng/L Saale: DF NR, mean = 7.5 ng/L Elbe: DF NR, mean = 4.3 ng/L
Munoz et al. (2016)	France (Seine River)	Two sites downstream of Greater Paris and one site unaffected by the Greater Paris region	DF 70%, range = ND–3.1 ng/L

Study	Location	Site Details	PFBS Results
Lorenzo et al. (2015)	Spain (Guadalquivir River Basin, Ebro River Basin)	Guadalquivir sampling locations included downstream of WWTPs, near industrial areas, near a military camp, or through major cities; Ebro sampling locations included nearby ski resorts and downstream of WWTP and industrial areas	Guadalquivir: DF 8%, mean (range) = 10.1 (ND–228.3) ng/L Ebro: DF 0%
Zhao et al. (2015)	Germany (Elbe River and lower Weser River)	Some sampling sites near Hamburg city and industrial plants	Elbe: DF 100%, mean (range) = 7.4 (0.24–238) ng/L Weser: DF 100%, mean (range) = 1.41 (0.75–1.85) ng/L
Eriksson et al. (2013)	Denmark (Faroe Islands)	Lakes Leitisvatn, Havnardal, Kornvatn, and Á Mýranar with no known point sources of any fluorochemical facilities	Leitisvatn: DF 0% Havnardal Lake: DF 0% Kornvatn Lake: DF 0% Á Mýranar: DF 0%
Wagner et al. (2013)	Germany (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean[b] (range[b]) = 18 (9–26) ng/L
Boiteux et al. (2012)	France (national)	Rivers; some locations may have upstream industrial sources	DF 1%, range = ND–5 ng/L
Eschauzier et al. (2012)	The Netherlands (Amsterdam; Lek Canal, tributary of Rhine River)	Downstream of an industrial point source in the German part of the Lower Rhine	DF[a] 100%, mean (range) = 35 (31–42) ng/L
Labadie and Chevreuil (2011)	France (Paris; River Seine)	Urban stretch of the River Seine during a flood cycle, sampling location under the influence of two urban WWTPs and two major combined sewer overflow outfalls	DF 100%, mean (range) = 1.3 (0.6–2.6) ng/L

Study	Location	Site Details	PFBS Results
Möller et al. (2010)	Germany (Rhine River watershed)	Upstream and downstream of Leverkusen, where effluent of a WWTP treating industrial wastewater was discharged; other major rivers and tributaries	Rhine upstream Leverkusen: DF 100%, mean (range) = 3.19 (0.59–6.58) ng/L Rhine downstream Leverkusen: DF 100%, mean (range) = 45.4 (15.0–118) ng/L River Ruhr: DF 100%, mean (range) = 7.08 (2.87–11.4) ng/L River Moehne: point = 31.1 ng/L Other tributaries: DF 100%, mean (range) = 2.84 (0.22–6.82) ng/L
Ahrens et al. (2009b)	Germany (Elbe River)	Sampling sites in Hamburg city (sites 16–18) and from Laurenburg to Hamburg (sites 19–24)	Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.6 (1.1–2.5) ng/L Laurenburg to Hamburg: Dissolved: DF[a] 100%, mean (range) = 1.1 (0.53–1.5) ng/L
Ahrens et al. (2009a)	Germany (Elbe River)	Sampling locations 53 to 122 km (sites 1 to 9)[c] upstream of estuary mouth of Elbe River	DF NR; range of mean (for different locations) = 1.8–3.4 ng/L
Rostkowski et al. (2009)	Poland (national)	Rivers, lakes, and streams in northern and southern Poland, some southern locations near chemical industrial activities	North: DF[a] 60%, range = ND–10 ng/L South: DF[a] 73%, range = ND–16.0 ng/L
Ericson et al. (2008b)	Spain (Tarragona Province; Ebro River, Francolí River, Cortiella River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	Ebro site 1: DF 0% Ebro site 2: DF 0% Francolí: DF 0% Cortiella: DF 0%
Bach et al. (2017)	France (southern)	Upstream and downstream from discharge point that receives wastewater from an industrial site with two fluoropolymer manufacturing facilities	Upstream: DF 0% Downstream: DF 0%

Study	Location	Site Details	PFBS Results
Boiteux et al. (2017)	France (northern)	River samples from upstream and downstream of an industrial WWTP that processes raw sewage from fluorochemical manufacturing facility	Upstream: DF 0% Downstream: DF 0%
Gebbink et al. (2017)	The Netherlands (Dordrecht)	Upstream and downstream of Dordrecht fluorochemical production plant; two control sites	Control sites: DF[a] 100%, mean[a] (range) = 17 (12–22) ng/L Upstream: DF[a] 100%, mean[a] (range) = 19.7 (18–21) ng/L Downstream: DF[a] 100%, mean[a] (range) = 21 (16–27) ng/L
Valsecchi et al. (2015)	Italy (Po River Basin, Brenta River Basin, Adige River Basin, Tevere River Basin, and Arno River Basin)	Two river basins (Po and Brenta) which receive discharges from two chemical plants that produce fluorinated polymers and intermediates; three river basins (Adige, Tevere, Arno) with no known point sources of any fluorochemical facilities	Po: DF[a] 56%, range = ND–30.4 ng/L Brenta: DF[a] 100%, mean[a] (range) = 707 (23.1–1,666) ng/L Adige: DF[a] 20%, range = ND–4.3 ng/L Tevere: DF 0% Arno: DF[a] 58%, range = ND–31.4 ng/L
Mussabek et al. (2019)	Sweden (Luleå)	Samples from lake and pond near a firefighting training facility at the Norrbotten Air Force Wing known to use PFAS-containing AFFF	Lake: DF NR, mean = 200 ng/L Pond: DF NR, mean = 150 ng/L
Gobelius et al. (2018)	Sweden (national)	Sampling locations selected based on potential vicinity of PFAS hot spots and importance as a drinking water source area, some sites include firefighting training sites at airfields and military areas	DF[a] 29%, range = ND–299 ng/L

Study	Location	Site Details	PFBS Results
Dauchy et al. (2017)	France (unspecified)	Samples collected near 3 sites (B, C, D) impacted by the use of firefighting foams	Site B: DF 0% Site C: DF 0% Site D: DF[a] 30%, range = ND–138 ng/L
Multiple Continents			
Pan et al. (2018)	United States (Delaware River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 2.19 (0.52–4.20) ng/L
	United Kingdom (Thames River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 5.06 (3.26–6.75) ng/L
	Germany and the Netherlands (Rhine River)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 21.9 (0.46–146) ng/L
	Sweden (Mälaren Lake)	Sampling sites were not proximate to known point sources of any fluorochemical facilities	DF[a] 100%, mean (range) = 1.43 (0.75–1.92) ng/L

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; km = kilometer; m = meter; ND = not detected; ng/L = nanogram per liter; NR = not reported; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; WWTP = wastewater treatment plant; µg/L = microgram per liter.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] For Wagner et al. (2013), PFBS concentrations were calculated using the fluorine concentrations reported in Table 4 from the study.

[c] Freshwater locations determined as sites with conductivity < 1.5 milliSiemens/cm.

B.2. RSC for PFBS, Literature Search and Screening Methodology

The EPA applies an RSC to the RfD when calculating an MCLG based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (USEPA, 2000b). The EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared

with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. The EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000b). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (USEPA, 2000b). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (USEPA, 2000b). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively-derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (USEPA, 2000b). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies (USEPA, 2000b).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of 20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (USEPA, 2000b). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

B.2.1. Literature Search and Screening

In 2020, the EPA conducted a literature search to evaluate evidence for pathways of human exposure to eight PFAS (PFOA, PFOS, PFBA, PFBS, PFDA, perfluorohexanoic acid (PFHxA), PFHxS, and PFNA) (Holder et al., 2023). This search was not date limited and spanned the

information collected across the Web of Science (WOS), PubMed, and ToxNet/ToxLine (now ProQuest) databases. The results of the PFBS literature search of publicly available sources are available through the EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2610.

The 654 literature search results for PFBS were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and nonhuman (i.e., those not identified as human) studies. Studies identified as human studies were further categorized into seven major PFAS pathway categories (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) as well as an additional category for Human Exposure Measures. Nonhuman studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill. Only studies published between 2003 and 2020 were considered. Application of the SWIFT-Review tags identified 343 peer-reviewed papers matching these criteria for PFBS.

The 343 articles were screened to identify studies reporting measured occurrence of PFBS in human matrices and media commonly related to human exposure (human blood/serum/urine, drinking water, food, food contact materials, consumer products, indoor dust, indoor and ambient air, and soil). For this synthesis, additional screening was conducted to identify studies relevant to surface water (freshwater only) and groundwater using a keyword[7] search for water terms.

Following the Populations, Exposures, Comparators, and Outcomes (PECO) criteria outlined in Table B-3, the title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree whether a study should be included or excluded, a third reviewer was consulted to make a final decision. The title and abstract screening resulted in 191 unique studies being tagged as relevant (i.e., having data on occurrence of PFBS in exposure media of interest) that were further screened with full-text review using the same inclusion criteria. After additional review of the evidence collected by Holder et al. (2023), 87 studies originally identified for other PFAS also contained information relevant to PFBS. Based on full-text review, 147 studies were identified as having relevant, extractable data for PFBS from the United States, Canada, or Europe for environmental media, not including studies with only human biomonitoring data. Of these 147 studies, 130 were identified from Holder et al. (2023), where primary data were extracted into a comprehensive evidence database. Parameters of interest included sampling dates and locations, numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics. Seventeen of the 147 studies were identified in this synthesis as containing primary data on only surface water and/or groundwater.

The evidence database of Holder et al. (2023) additionally identified 18 studies for which the main article was not available for review. As part of this synthesis, 17 of the 18 studies could be retrieved. An additional three peer-reviewed references were identified through gray literature sources that were included to supplement the search results. The combined 20 studies underwent

[7] Keyword list: water, aquifer, direct water, freshwater, fresh water, groundwater, ground water, indirect water, lake, meltwater, melt water, natural water, overland flow, recreation water, recreational water, river, riverine water, riverwater, river water, springwater, spring water, stream, surface water, total water, water supply

full-text screening using the inclusion criteria in Table B-3. Based on full-text review, four studies were identified as relevant.

Table B-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults and/or children in the general population and populations in the vicinity of PFAS point sources from the United States, Canada, or Europe
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater[a], human blood/serum/urine, indoor air, landfill, sediment, soil, surface water[a] (freshwater), wastewater/biosolids/sludge
Comparator	Not applicable
Outcome	Measured concentrations of PFBS (or measured emissions from food packaging and consumer products only)

Notes: PFBS = perfluorobutane sulfonic acid
[a] Surface water and groundwater were not included as relevant media in Holder et al. (2023). Studies were re-screened for these two media in this synthesis.

Using the screening results from the evidence database and this synthesis, a total of 151 peer-reviewed studies were identified as relevant.

B.2.2. Additional Screening

The EPA also searched the following publicly available gray literature sources for information related to relative exposure of PFBS for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- USEPA (2021d). *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)*;
- ATSDR's *Toxicological Profiles;*
- CDC's national reports on human exposures to environmental chemicals;
- The EPA's CompTox Chemicals Dashboard;
- The EPA's fish tissue studies;
- The EPA's Toxics Release Inventory;
- Relevant documents submitted under the Toxic Substances Control Act and relevant reports from the EPA's Office of Chemical Safety and Pollution Prevention;
- U.S. Food and Drug Administration's (FDA's) *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada;
- National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science data collections;
- National Science Foundation direct and indirect food and/or certified drinking water additives;
- *Throwaway Packaging, Forever Chemicals: European wide survey of PFAS in disposable food packaging and tableware* (Straková et al., 2021);

- PubChem compound summaries;
- Relevant sources identified in the relative source contribution discussions (Section 5) of the EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA)/Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water*; and
- Additional sources, as needed.

The EPA has included available information from these gray literature sources for PFBS relevant to its uses, chemical and physical properties, and for occurrence in ambient or indoor air, foods (including fish and shellfish), soil, dust, and consumer products. The EPA has also included available information specific to PFBS below on any regulations that may restrict PFBS levels in media (e.g., water quality standards, air quality standards, food tolerance levels).

B.3. Summary of Potential Exposure Sources of PFBS Other than Water

B.3.1. Food

PFBS was included in a suite of individual PFAS selected as part of PFAS-targeted reexaminations of samples collected for the U.S. Food and Drug Administration's (FDA's) Total Diet Study (US FDA, 2022b, 2022a, 2021b, 2021a, 2020b, 2020a); however, it was not detected in any of the food samples tested. It should be noted that FDA indicated that the sample sizes were limited and that the results should not be used to draw definitive conclusions about PFAS levels or presence in the general food supply (US FDA, 2022c). PFBS was detected in cow milk samples collected from a farm with groundwater known to be contaminated with PFAS, as well as in produce (collard greens) collected from an area near a PFAS production plant, in FDA studies of the potential exposure of the U.S. population to PFAS (US FDA, 2021c, 2018). Maximum residue levels for PFBS were not found in the Global MRL Database (Bryant Christie Inc, 2022).

In addition to efforts by FDA, peer-reviewed studies conducted in North America, Europe, and across multiple continents analyzed PFBS in food items obtained from home, recreational, or commercial sources (see Table B-4). Food types evaluated include fruits and vegetables, grains, meat, seafood, dairy, and fats/other (e.g., eggs, spices, and oils), with seafood showing the highest levels of PFBS detected. PFBS was not detected in any of the eight studies that analyzed human milk for PFAS (not shown in Table B-4)—one in the United States (von Ehrenstein et al., 2009) and seven in Europe (Abdallah et al., 2020; Nyberg et al., 2018; Cariou et al., 2015; Lankova et al., 2013; Beser et al., 2011; Kärrman et al., 2010; Kärrman et al., 2007).

Of the studies conducted in North America, four U.S. studies (Scher et al., 2018; Byrne et al., 2017; Blaine et al., 2014; Schecter et al., 2010) found PFBS in at least one food item. Locations and food sources varied in these studies. In Schecter et al. (2010), PFBS was detected in cod samples but not in any of the other foods collected from Texas grocery stores. Scher et al. (2018) detected PFBS in plant parts (leaf and stem samples) analyzed from garden produce collected at homes in Minnesota within a GCA impacted by a former 3M PFAS production facility (PFBS concentrations ranged from ND to 0.065 nanograms per gram [ng/g]). The authors suggested that

the PFBS detections in plant parts were likely associated with PFAS present in irrigation water that had accumulated in produce. Blaine et al. (2014) found PFBS in radish, celery, tomato, and peas that were grown in soil amended with industrially impacted biosolids. They also found PFBS in these crops grown in soil that had received municipal biosolid applications over 20 years. In unamended control soil samples, PFBS was only detected in radish root with an average value of 22.36 ng/g (Blaine et al., 2014). In a similar study conducted by Blaine et al. (2013), PFBS was found in lettuce, tomato, and corn grown in industrially impacted biosolids-amended soils in greenhouses. Young et al. (2012) analyzed 61 raw and retail milk samples from 17 states for PFAS, but PFBS was not detected.

Based on the available data collected to date, seafood (including fish and shellfish) has been found to contain the highest concentrations of PFBS out of all food types examined. Burkhard (2021) identified 16 studies reporting BAFs for PFBS and calculated a median (standard deviation) bioaccumulation factor (BAF) in muscle tissue/fillet of 22.39 ± 6.92 L/kg wet weight (reported as a logBAF of 1.35 ± 0.84 L/kg). Several large-scale sampling efforts have been conducted by the EPA and other agencies to determine PFAS levels in fish. In the EPA's 2013–2014 National Rivers and Streams Assessment (NRSA), PFBS was detected at concentrations between the quantitation limit (1 ng/g) and the method detection limit (0.1 ng/g) at 0.571 ng/g in a largemouth bass fish fillet sample collected from Big Black River, Mississippi; 0.475 ng/g in a smallmouth bass fillet composite collected from Connecticut River, New Hampshire; and 0.148 ng/g in a walleye fillet composite collected from Chenango River, New York (USEPA, 2020a). Notably, PFBS was not detected in any fish species sampled in the 2008–2009 NRSA (Stahl et al., 2014). PFBS was also detected at a concentration of 0.36 ng/g in a smallmouth bass fillet composite collected from Lake Erie, New York in the EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study (USEPA, 2021g). PFBS has been detected in Irish pompano, silver porgy, grey snapper, and eastern oyster from the St. Lucie Estuary in the National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). PFBS was not a target chemical in the EPA's National Lake Fish Tissue Study (USEPA, 2009a).

Several peer-reviewed publications that examined PFBS concentrations in fish and shellfish are also available. As mentioned previously, Schecter et al. (2010) detected PFBS in cod samples. Mean PFBS levels in cod from this study (0.12 ng/g wet weight [ww]) were much lower than maximum levels detected in Alaska blackfish obtained from the Suqi River, Alaska in remote locations upstream and downstream of a former (unnamed) defense site (59.2 ng/g ww) (Byrne et al., 2017). In this study, blackfish were considered sentinel species but are not among the traditional fish consumed in the area. The authors noted that the presence of PFAS in fish from remote sites is suggestive of atmospheric deposition. In two additional studies from North America, PFBS was not detected in samples of farmed and wild-caught seafood (Chiesa et al., 2019; Young et al., 2013).

The European Food Safety Authority (EFSA) reported the presence of PFBS in various food and drink items, including fruits, vegetables, cheese, and bottled water (EFSA, 2012). For average adult consumers, the estimated exposure ranges for PFBS were 0.03−1.89 nanograms per kilogram body weight per day (ng/kg bw-day) (minimum) to 0.10−3.72 ng/kg bw-day (maximum) (EFSA, 2012). Of the studies conducted in Europe, 12 found PFBS in at least one food type (Table B-4). Eight of the 12 studies included food samples obtained solely from

markets (Scordo et al., 2020; Sznajder-Katarzyńska et al., 2019; Surma et al., 2017; D'Hollander et al., 2015; Pérez et al., 2014; Eschauzier et al., 2013; Hlouskova et al., 2013; Domingo et al., 2012). Across studies, PFBS detections were found in seafood; other animal products such as meat, dairy, and eggs; fruits and vegetables; tap water-based beverages such as coffee; sweets; and spices.

Papadopoulou et al. (2017) analyzed duplicate diet samples with PFBS detected in only one solid food sample (ND−0.001 ng/g; DF 2%; food category unspecified). Eriksson et al. (2013) evaluated foods that were farmed or freshly caught in the Faroe Islands, and only detected PFBS in cow milk (0.019 ng/g ww) and packaged dairy milk (0.017 ng/g ww) samples among the products analyzed. In eight of the European studies where PFBS was not detected, foods were primarily obtained from commercial sources, but wild-caught seafood was also included.

Two of the 12 European studies examined both market-bought and fresh-caught fish, and PFBS was detected in seafood from both sources (Vassiliadou et al., 2015; Yamada et al., 2014). Yamada et al. (2014) found higher PFBS in fresh-caught river fish samples (0.16 ng/g ww maximum) versus fresh or frozen market samples (0.03 ng/g ww maximum) in France. Vassiliadou et al. (2015) detected PFBS in raw shrimp (from Greek markets) but did not detect PFBS in either fried shrimp, raw hake (from Greek fishing sites), or fried hake.

In summary, in Europe and North America, PFBS has been detected in multiple food types, including fruits, vegetables, meats, seafoods, and other fats. Several large-scale fish tissue sampling efforts conducted by the EPA and others indicate that fish consumption may be an important PFBS exposure source. Future large-scale sampling efforts by FDA and others may help to similarly elucidate PFBS concentrations in other food types. Although several U.S. studies have evaluated PFBS in meats, fats/oils, fruits, vegetables, and other non-seafood food types, many of these sampling efforts were localized to specific cities or markets and/or used relatively small sample sizes. Broader-scale sampling efforts will be helpful in determining the general levels of PFBS contamination in these food types, as well as the impact of known PFAS contamination sources on PFBS concentrations in foods.

Table B-4. Compilation of Studies Describing PFBS Occurrence in Food

Study	Location and Source	Food Types	Results
North America			
Schecter et al. (2010)	United States (Texas) Grocery stores	Dairy, fruits and vegetables, grain, meat, seafood, fats/other	**Cod: DF NR, mean = 0.12 ng/g ww** ND in salmon, canned sardines, canned tuna, fresh catfish fillet, frozen fish sticks, tilapia, cheeses (American, mozzarella, Colby, cheddar, Swiss, provolone, and Monterey jack), butter, cream cheese, frozen yogurt, ice cream, whole milk, whole milk yogurt, potatoes, apples, cereals, bacon, canned chili, ham, hamburger, roast beef, sausages, sliced chicken breast, sliced turkey, canola oil, margarine, olive oil, peanut butter, eggs
Byrne et al. (2017)	United States (Alaska) Upstream/downstream of former defense site (Suqi River)	Seafood	**Blackfish: DF 48%, range = ND–59.2 ng/g ww** Highest concentration was upstream
Scher et al. (2018)	United States (Minnesota) Home gardens Near former 3M PFAS production facility, homes within and outside a GCA	Fruits and vegetables	Within GCA: **Leaf: DF 6%, max = 0.061 ng/g** **Stem: DF 4%, max = 0.065 ng/g** ND in floret, fruit, root, seed Outside GCA: ND
Blaine et al. (2014)	United States (Midwestern) Greenhouse study, unamended controls	Fruits and vegetables	**Radish root: DF NR, mean = 22.36 ng/g** ND in celery shoot, pea fruit
Blaine et al. (2013)	United States (Midwestern) Greenhouse and field studies, unamended controls	Fruits and vegetables, grain	ND in corn, lettuce, tomato in unamended soil.
Young et al. (2013)	United States (Maryland, Mississippi, Tennessee, Florida, New York, Texas, Washington, D.C.) Retail markets	Seafood	ND in crab, shrimp, striped bass, farm raised catfish, farm raised salmon
Young et al. (2012)	United States (17 states) Retail markets	Dairy	ND in retail cow's milk

Study	Location and Source	Food Types	Results
Europe			
Domingo et al. (2012)	Spain (Catalonia) Local markets, small stores, supermarkets, big grocery stores	12 food categories	**Vegetables: DF NR, mean = 0.013 ng/g fw** **Fish and seafood: DF NR, mean = 0.054 ng/g fw** ND in meat and meat products, tubers, fruits, eggs, milk, dairy products, cereals, pulses, industrial bakery, oils
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Various supermarkets and retail stores	8 food categories	Categories included cereals, pulses and starchy roots, tree-nuts, oil crops and vegetable oils, vegetables and fruits, meat and meat products, milk, animal fats, dairy products, and eggs, fish and seafood, and others such as candies or coffee **Spain: DF 3.2%, range = ND–13 ng/g (primarily fish, oils)** **Serbia: DF 5.2%, range = ND–0.460 ng/g (primarily meat and meat products, cereals)**
D'Hollander et al. (2015)	Belgium, Czech Republic, Italy, Norway PERFOOD study; items from 3 national retail stores of different brands and countries of origin	Fruit, cereals, sweets, salt	**Sweets: DF[a] 25%, range = ND–0.0016 ng/g** **Fruit: DF[a] 19%, range = ND–0.067 ng/g** ND in cereals, salt
Hlouskova et al. (2013)	Belgium, Czech Republic, Italy, Norway Several national supermarkets	Pooled milk/dairy products, meat, fish, hen eggs	**DF 5%, mean (range) = 0.00975 (0.006–0.012) ng/g**
Eriksson et al. (2013)	Denmark Farm, dairy farm, fish from Faroe Shelf area	Dairy, fruits and vegetables, seafood	**Milk:** **Farmer (Havnardal): point = 0.019 ng/g ww** **Diary (Faroe Island): point = 0.017 ng/g ww;** ND or NQ in 4 samples ND in yogurt, creme fraiche, potatoes, farmed salmon, wild-caught cod, wild-caught saithe

Study	Location and Source	Food Types	Results
Sznajder-Katarzyńska et al. (2019)	Poland Markets	Dairy	**All dairy: sum PFBS = 0.04 ng/g** **Butter: range = 0.01–0.02 ng/g** ND in camembert-type cheese, cottage cheese, milk, natural yogurt, sour cream, kefir (bonny clabber)
Yamada et al. (2014)	France Freshwater fish from 6 major French rivers; fresh and frozen fish from markets	Seafood	**Freshwater fish: DF NR, range = 0.06–0.16 ng/g ww** **Fresh or frozen fish: DF NR, range = 0.02–0.03 ng/g ww**
Vassiliadou et al. (2015)	Greece Local fish markets, mariculture farm, fishing sites	Seafood	**Hake: raw mean = 0.45 ng/g ww, fried mean = 0.83 ng/g ww** **Shrimp: raw mean = 1.37 ng/g ww** ND in raw, fried, and grilled anchovy, bogue, picarel, sand smelt, sardine, squid, striped mullet, raw and fried mussel, fried shrimp, and grilled hake
Eschauzier et al. (2013)	The Netherlands (Amsterdam) Cafés, universities, supermarkets	Fats/other	**Brewed coffee (manual): mean (range) = 1.6 (1.3–2.0) ng/L** **Brewed coffee (machine): mean (range) = 2.9 (ND–9.8) ng/L** **Cola: mean (range) = 7.9 (ND–12) ng/L**
Surma et al. (2017)	Spain, Slovakia Source NR	Fats/other	**Spices: ND–1.01 ng/g** Spain: Detected in anise, star anise, fennel, coriander, cinnamon, peppermint, parsley, thyme, laurel, cumin, and oregano ND in white pepper, cardamon, clove, nutmeg, allspice, vanilla, ginger, garlic, black paper, and hot pepper (mild and hot) Slovakia: ND in anise, star anise, white pepper, fennel, cardamom, clove, coriander, nutmeg, allspice, cinnamon, vanilla, and ginger

Study	Location and Source	Food Types	Results
Papadopoulou et al. (2017)	Norway A-TEAM project: food and drinks collected by participants as duplicate diet samples	Solid foods (11 food categories), liquid foods (5 drinks)	**Solid foods (unspecific food category): DF 2%, range = ND–0.001 ng/g** ND in liquid foods (coffee, tea and cocoa, milk, water, alcoholic beverages and soft drinks)
Scordo et al. (2020)	Italy Supermarkets	Fruits	**Olives: DF[a] 100%, mean[a] (range) = 0.294 (0.185–0.403) ng/g dw** ND in strawberries
Ericson et al. (2008a)	Spain Local markets, large supermarkets, grocery stores	18 food categories	ND in all categories: veal, pork, chicken, lamb, white fish, seafood, tinned fish, blue fish, whole milk, semi-skimmed milk, dairy products, vegetables, pulses, cereals, fruits, oil, margarine, and eggs
Noorlander et al. (2011)	The Netherlands Several Dutch retail store chains with nationwide coverage	15 food categories	ND in all categories: flour, fatty fish, lean fish, pork, eggs, crustaceans, bakery products, vegetables/fruit, cheese, beef, chicken/poultry, butter, milk, vegetable oil, and industrial oil
Jogsten et al. (2009)	Spain (Catalonia) Local markets, large supermarkets, grocery stores	Fruits and vegetables, meat, seafood, fats/other	ND in lettuce, raw, cooked, and fried meat (veal, pork, and chicken), fried chicken nuggets, black pudding, lamb liver, pate of pork liver, foie gras of duck, “Frankfurt” sausages, home-made marinated salmon, and common salt
Sznajder-Katarzyńska et al. (2018)	Poland Markets	Fruits and vegetables	ND in apples, bananas, cherries, lemons, oranges, strawberries, beetroots, carrots, tomatoes, potatoes, and white cabbage
Falandysz et al. (2006)	Poland Gulf of Gdañsk, Baltic Sea south coast	Meat, seafood	ND in eider duck, cod
Barbosa et al. (2018)	Belgium, France, the Netherlands, Portugal Various markets	Seafood	ND in raw and steamed fish (*P. platessa, M. australis, M. capenis, K. pelamis*, and *M. edulis*)

Study	Location and Source	Food Types	Results
Hölzer et al. (2011)	Germany Fish from Lake Möhne and river Möhne, contaminated with PFCs from use of polluted soil conditioner on agricultural lands; retail trade, wholesale trade, supermarkets, and producers	Seafood	Lake Möhne /River Möhne: ND in cisco, eel, perch, pike, and roach Trade/markets: ND in eel, pike/perch, and trout
Jörundsdóttir et al. (2014)	Iceland Collected during biannual scientific surveys, commercially produced	Seafood	ND in anglerfish, Atlantic cod, blue whiting, lemon sole, ling, lumpfish, plaice, and pollock
Rivière et al. (2019)	France Based on results of national consumption survey	Seafood, fats/other	ND in infant food, vegetables, non-alcoholic beverages, dairy-based desserts, milk, mixed dishes, fish, ultra-fresh dairy products, meat, poultry and game
Lankova et al. (2013)	Czech Republic Retail market	Fats/other	ND in infant formula
Zafeiraki et al. (2016a)	Greece, the Netherlands Home and commercially produced	Fats/other	ND in chicken eggs
Gebbink et al. (2015)	Sweden Major grocery chain stores, market basket samples	12 food categories	ND in all categories: dairy products, meat products, fats, pastries, fish products, egg, cereal products, vegetables, fruit, potatoes, sugar and sweets, soft drinks
Herzke et al. (2013)	Belgium, Czech Republic, Italy, Norway PERFOOD study: items from 3 national retail stores of different brands per location	Vegetables	ND for all vegetables
Zafeiraki et al. (2016b)	The Netherlands Local markets and slaughterhouses	Meat	ND for horse, sheep, cow, pig, and chicken liver

Study	Location and Source	Food Types	Results
Multiple Continents			
Chiesa et al. (2019)	United States (Pacific Ocean) Wholesale fish market	Seafood	ND in wild-caught salmon
	Canada Wholesale fish market	Seafood	ND in wild-caught salmon
	Norway Wholesale fish market	Seafood	ND in farm salmon
	Scotland Wholesale fish market	Seafood	ND in wild-caught and farm salmon

Notes: DF = detection frequency; dw = dry weight; fw = fresh weight; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; ng/L = nanogram per liter; NR = not reported; PFAS = per- and polyfluoroalkyl substances; NQ = not quantified; µg/L = microgram per liter; ww = wet weight.
Bold indicates detected levels of PFBS in food.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

B.3.2. Food Contact Materials

PFBS is not authorized for use in food packaging in the United States; however, PFBS has been detected in food packaging materials in the few available studies that investigate this potential route of exposure (USEPA, 2021d; ATSDR, 2021). In one report from the United States, PFBS was detected in fast-food packaging (7/20 samples) although the concentrations detected were not reported (Schaider et al., 2017). Additionally, in an analysis performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology in Prague, Czech Republic, PFBS was not detected in 42 samples of disposable food packaging and tableware purchased from six different European countries between May and December 2020 (LOQ = 1.7 mg/kg) (Straková et al., 2021).

The EPA identified five peer-reviewed studies in Europe (conducted in Poland, Norway, Greece, Czech Republic, and Germany) analyzed the occurrence of PFBS in food packaging or food contact materials (FCMs), such as baking papers and fast-food boxes and wrappers. Surma et al. (2015) measured levels of 10 perfluorinated compounds in three different brands of common FCMs commercially available in Poland, including wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3). PFBS was detected in one brand of baking paper at 0.02 picograms per square centimeter (pg/cm^2), but PFBS was not detected at or below the LOQ in all other FCMs. Vestergren et al. (2015) analyzed paper plates (n = 2), paper cups (n = 1), baking covers (n = 1), and baking molds (n = 1) purchased from retail stores in Tromsø and Trondheim, Norway. PFBS was detected in one paper plate at 6.9 pg/cm^2.

The remaining three studies did not detect PFBS in FCMs. Zafeiraki et al. (2014) analyzed FCMs made of paper, paperboard, or aluminum foil collected from a Greek market. PFBS was

not detected in any of the samples of beverage cups (n = 8), ice cream cups (n = 1), fast-food paper boxes (n = 8), fast-food wrappers (n = 6), paper materials for baking (n = 2), microwave bags (n = 3), and aluminum foil bags/wrappers (n = 14). The study concluded that the use of perfluorinated compound alternatives such as fluorophosphates and fluorinated polyethers in the local manufacturing process potentially explains the low levels of other PFAS (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluoroheptanoic acid [PFHpA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and perfluorododecanoic acid [PFDoDA]) detected in the sampled FCMs. Vavrouš et al. (2016) analyzed 15 samples of paper FCMs acquired from a market in the Czech Republic. FCMs included paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220°C (n = 3), and paper filters for coffee preparation (n = 3). PFBS was not detected in any samples. Kotthoff et al. (2015) analyzed 82 samples for perfluoroalkane sulfonate (PFSA) and perfluoroalkyl carboxylic acid (PFCA) compounds in 10 consumer products including individual paper-based FCMs (n = 33) from local retailers in Germany in 2010. PFBS was not detected in paper-based FCMs.

Overall, the single available studies conducted in the United States indicate that PFBS may be present in food packaging materials; however, further research is needed to understand which packaging materials generally contain PFBS at the highest concentrations and with the greatest frequency. There are also uncertainties related to data gaps on topics that may influence whether food packaging is a significant PFBS exposure source in humans, including differences in transfer efficiency from different packaging types directly to humans or indirectly through foodstuffs.

B.3.3. Consumer Products

Several studies examined a range of consumer products and found multiple PFAS, including PFBS, at various levels (van der Veen et al., 2020; Zheng et al., 2020; Schultes et al., 2018; Bečanová et al., 2016; Favreau et al., 2016; Gremmel et al., 2016; Kotthoff et al., 2015; Vestergren et al., 2015; Liu et al., 2014). Two of the studies collected consumer products in the United States, five purchased consumer products in Europe, and two studies did not report the purchase location(s) of the consumer products that were tested.

Zheng et al. (2020) determined the occurrence of ionic and neutral PFAS in items collected from childcare environments in the United States. Nap mats (n = 26; 20 polyurethane foam, 6 vinyl cover samples) were collected from seven Seattle childcare centers. PFBS was detected in 5% of nap mat samples at a maximum concentration of 0.04 ng/g. Liu et al. (2014) analyzed the occurrence of PFAS in commonly used consumer products (carpet, commercial carpet-care liquids, household carpet/fabric-care liquids, treated apparel, treated home textiles, treated non-woven medical garments, floor waxes, membranes for apparel, and thread-sealant tapes) purchased from retail outlets in the United States. PFBS was detected in 100% of commercial carpet/fabric-care liquids samples (n = 2) at concentrations of 45.8 and 89.6 ng/g, in 75% of household carpet/fabric-care liquids and foams samples (n = 4) at concentrations up to 911 ng/g, in one treated apparel samples (n = 2) at a concentration of 2 ng/g, in the single treated floor wax and stone/wood sealant sample (143 ng/g, n = 2), and in the single apparel membrane sample

(30.7 ng/g, n = 2). PFBS was not detected in treated home textile and upholstery (n = 2) or thread-sealant tapes and pastes (n = 2).

van der Veen et al. (2020) examined the effects of weathering on PFAS content in durable water-repellent clothing collected from six suppliers in Sweden (1 pair of outdoor trousers, 7 jackets, 4 fabrics for outdoor clothes, 1 pair of outdoor overalls). Two pieces of each of the 13 fabrics were cut. One piece of each fabric was exposed to elevated ultraviolet radiation, humidity, and temperature in an aging device for 300 hours (assumed lifespan of outdoor clothing); the other was not aged. Both pieces of each fabric were analyzed for ionic PFAS (including PFBS) and volatile PFAS. In general, aging of outdoor clothing resulted in increased perfluoroalkylated acid (PFAA) levels of 5-fold or more. For 8 of 13 fabrics, PFBS was not detected before or after aging. For three fabrics, PFBS was detected before and after aging, increasing approximately 3- to 14-fold in the aged fabric (i.e., from 43 to 140 micrograms per square meter [µg/m^2], 45 to 350 µg/m^2, and 9.6 to 130 µg/m^2 respectively for the 3 fabrics). For the remaining two fabrics, PFBS was not detected prior to aging but was detected afterward at concentrations of 0.57 and 1.7 µg/m^2, respectively. The authors noted that possible explanations for this could be weathering of precursor compounds (e.g., fluorotelomer alcohols) to PFAAs such as PFBS or increased extractability due to weathering.

Kotthoff et al. (2015) analyzed 82 samples for PFSA and PFCA compounds in outdoor textiles (n = 3), gloves (n = 3), carpets (n = 6), cleaning agents (n = 6), impregnating sprays (n = 3), leather (n = 13), wood glue (n = 1), ski wax (n = 13), and awning cloth (n = 1). Individual samples were bought from local retailers or collected by coworkers of the involved institutes or local clubs in Germany. The age of the samples ranged from a few years to decades. PFBS was detected in outdoor textiles (level not provided), carpet samples (up to 26.8 µg/m^2), ski wax samples (up to 3.1 micrograms per kilogram [µg/kg]), leather samples (up to 120 µg/kg), and gloves (up to 2 µg/kg). Favreau et al. (2016) analyzed the occurrence of 41 PFAS in a wide variety of liquid products (n = 132 consumer products, 194 total products), including impregnating agents, lubricants, cleansers, polishes, AFFFs, and other industrial products purchased from stores and supermarkets in Switzerland. PFBS was not detected in impregnation products (n = 60), cleansers (n = 24), or polishes (n = 18). PFBS was detected in 13% of a miscellaneous category of products (n = 23) that included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances, with mean and maximum concentrations of 998 and 2,992 parts per million (ppm), respectively (median = ND).

The remaining two European studies from Norway (Vestergren et al., 2015) and Sweden (Schultes et al., 2018) did not detect PFBS in the consumer products analyzed. Vestergren et al. (2015) analyzed furniture textile, carpet, and clothing samples (n = 40) purchased from retail stores in Tromsø and Trondheim, Norway, while Schultes et al. (2018) determined levels of 39 PFAS in 31 cosmetic products collected in Sweden. Both studies found measurable concentrations of at least one PFAS; however, PFBS was not detected in any of the samples.

Of the two studies for which purchase location(s) were not specified, Gremmel et al. (2016) determined levels of 23 PFAS in 16 new outdoor jackets since it has been shown that outdoor jackets emit PFAS to the air as well as into water during washing. The jackets were selected based on factors such as fabric and origin of production (primarily Asia, with some origins not specified). PFBS (concentration of 0.51 µg/m^2) was only detected in one large hardshell jacket made of 100% polyester that was polyurethane-coated and finished with Teflon® (production

origin unknown). Bečanová et al. (2016) analyzed 126 samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment (EEE), and plastics); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, facade materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) for 15 target PFAS, including PFBS. The condition (new versus used) and production year of the samples varied; the production year ranged from 1981 to 2010. The origin(s) of production were not specified. PFBS was detected in 31/55, 9/54, 7/10, and 6/7 household equipment, building materials, car interior, and WEEE samples, respectively. The highest level was 11.4 µg/kg found in a used 1999 screen associated with WEEE.

In summary, in the few studies available from North America and Europe, PFBS was detected in a wide range of consumer products including clothing, household textiles and products, children's products, and commercial/industrial products. However, there is some uncertainty in these results as the number and types of products tested in each study were often limited in terms of sample size. While there is evidence indicating PFBS exposure may occur through the use of or contact with consumer products, more research is needed to understand the DF and concentrations of PFBS that occur in specific products, as well as how the concentrations of PFBS change in these products with age or weathering.

B.3.4. Indoor Dust

Dust ingestion may be an important exposure source of PFAS including PFBS (ATSDR, 2021), though it should be noted that dust exposure may also occur via inhalation and dermal routes. The EPA identified several studies conducted in the United States, Canada, various countries in Europe, and across multiple continents that analyzed PFBS in dust of indoor environments (primarily in homes, but also schools, childcare facilities, offices, and vehicles; see Table B-5). Most of the studies sampled dust from areas not associated with any known PFAS activity or release. PFBS concentrations in dust measured in these studies ranged from ND to 170 ng/g with three exceptions: two studies (Kato et al., 2009; Strynar and Lindstrom, 2008) reported maximum PFBS concentrations > 1,000 ng/g in dust from homes and daycare centers, and a third study (Huber et al., 2011) reported a PFBS concentration of 1,089 ng/g in dust from a storage room that had been used to store "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years."

Of the two available studies that measured PFBS in dust from vehicles, one (in the United States) detected no PFBS (Fraser et al., 2013) and the other (in Ireland) reported a DF of 75% and PFBS concentrations ranging from ND to 170 ng/g (Harrad et al., 2019).

One U.S. study, Scher et al. (2019) evaluated indoor dust from 19 homes in Minnesota within a GCA impacted by the former 3M PFAS production facility. House dust samples were collected from both interior living rooms and entryways to the yard. The DFs for PFBS were 16% and 11% for living rooms and entryways, respectively, and a maximum PFBS concentration of 58 ng/g was reported for both locations.

Haug et al. (2011) indicated that house dust concentrations are likely influenced by a number of factors related to the building (e.g., size, age, floor space, flooring type, ventilation); the residents or occupants (e.g., number of people, housekeeping practices, consumer habits such as

buying new or used products); and the presence and use of certain products (e.g., carpeting, carpet or furniture stain-protective coatings, waterproofing sprays, cleaning agents, kitchen utensils, clothing, shoes, cosmetics, insecticides, electronic devices). In addition, the extent and use of the products affects the distribution patterns of PFAS in dust of these buildings.

At this time, there is uncertainty regarding the extent of human exposure to PFBS through indoor dust compared with other exposure pathways.

Table B-5. Compilation of Studies Describing PFBS Occurrence in Indoor Dust

Study	Location	Site Details	Results
North America			
Zheng et al. (2020)	United States (Seattle, Washington and West Lafayette, Indiana)	Childcare facilities (20 samples from 7 facilities in Seattle and 1 in West Lafayette)	DF 90%, mean (range) = 0.34 (ND–0.86) ng/g
Byrne et al. (2017)	United States (St. Lawrence Island, Alaska)	Homes (49)	DF 16%, median = ND; 95th percentile = 1.76 ng/g
Fraser et al. (2013)	United States (Boston, Massachusetts)	Homes (30); offices (31); vehicles (13)	Homes: DF 3% (single detection), range = ND–4.98 ng/g Offices: DF 10%, range = ND–12.0 ng/g Vehicles: DF 0%
Knobeloch et al. (2012)	United States (Great Lakes Basin, Wisconsin)	Homes (39)	DF 59%, median (range) = 1.8 (ND–31) ng/g
Strynar and Lindstrom (2008)	United States (Cities in North Carolina and Ohio)	Homes (102) and daycare centers (10); samples had been collected in 2000–2001 during EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study	DF 33%, mean (range) = 41.7 (ND–1,150) ng/g

Study	Location	Site Details	Results
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility; 19 homes within the GCA	Entryway: DF 11%, median (range) = ND (ND–58 ng/g) Living room: DF 16%, median (range) = ND (ND–58 ng/g)
Kubwabo et al. (2005)	Canada (Ottawa)	Homes (67)	DF 0%
Europe			
de la Torre et al. (2019)	Spain (unspecified), Belgium (unspecified), Italy (unspecified)	Homes (65)	Spain: DF 52%, median (range) = 0.70 (ND–12.0) ng/g Belgium: DF 27%, median (range) = 0.40 (ND–56.7) ng/g Italy: DF 18%, median (range) = 0.40 (ND–11.6) ng/g
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick counties)	Homes (32); offices (33); cars (31); classrooms (32)	Homes: DF 81%, mean (range) = 17 (ND–110) ng/g Offices: DF 88%, mean (range) = 19 (ND–98) ng/g Cars: DF 75%, mean (range) = 12 (ND–170) ng/g Classrooms: DF 97%, mean (range) = 17 (ND–49) ng/g
Giovanoulis et al. (2019)	Sweden (Stockholm)	Preschools (20)	DF 0%
Winkens et al. (2018)	Finland (Kuopio)	Homes (63 children's bedrooms)	DF 12.7%, median (range) = ND (ND–13.5) ng/g
Padilla-Sánchez and Haug (2016)	Norway (Oslo)	Homes (7)	DF 14% (single detection), range = ND–3 ng/g
Jogsten et al. (2012)	Spain (Catalonia)	Homes (10)	DF 60%, range = ND–6.5 ng/g
Haug et al. (2011)	Norway (Oslo)	Homes (41)	DF 22%, mean (range) = 1.3 (0.17–9.8) ng/g

Study	Location	Site Details	Results
Huber et al. (2011)	Norway (Tromsø)	Homes (7; carpet, bedroom, sofa); one office; one storage room that had been used for storage of "highly contaminated PFC [polyfluorinated compounds] samples and technical mixtures for several years"	All homes: DF NR, median = 1.1 ng/g Living room: DF[a] 57%, range = ND–10.6 ng/g Carpet, bedroom, sofa: DF 0% Office: point = 3.8 ng/g Storage room: point = 1,089 ng/g
D'Hollander et al. (2010)	Belgium (Flanders)	Homes (45); offices (10)	Homes: DF 47%, median = 0 ng/g dw Offices: DF NR, median = 0.2 ng/g dw
Multiple Continents			
Kato et al. (2009)	United States (Atlanta, Georgia), Germany (unspecified), United Kingdom (unspecified), Australia (unspecified)	Homes (39)	DF 92.3%, median (range) = 359 (ND–7,718) ng/g
Karásková et al. (2016)	United States (unspecified)	Homes (14)	DF 60%, mean (range) = 1.4 (ND–2.6) ng/g
	Canada (unspecified)	Homes (15)	DF 55%, mean (range) = 1.6 (ND–5.8) ng/g
	Czech Republic (unspecified)	Homes (12)	DF 37.5%, mean (range) = 3.6 (ND–14.4) ng/g

Notes: DF = detection frequency; GCA = groundwater contamination area; ND = not detected; ng/g = nanogram per gram; NR = not reported; dw = dry weight

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

B.3.5. Air

PFAS have been released to air from WWTPs, waste incinerators, and landfills (USEPA, 2016a). ATSDR (2021) noted that PFAS have been detected in particulates and in the vapor phase in air and can be transported long distances via the atmosphere; they have been detected at low concentrations in areas as remote as the Arctic and ocean waters. However, EPA's Toxic Release Inventory did not report release data for PFBS in 2020 (USEPA, 2022c). In addition, PFBS is not listed as a hazardous air pollutant (USEPA, 2022d).

B.3.5.1. Indoor Air

No studies from the U.S. reporting levels of PFBS in indoor air were identified from the peerr-reviewed or gray literature. However, the EPA identified studies from Europe that are summarized below. These three studies were conducted in Norway (Barber et al., 2007), Spain (Jogsten et al., 2012), and Ireland (Harrad et al., 2019).

In Norway, neutral and ionic PFAS were analyzed in four indoor air samples collected from homes in Tromsø (Barber et al., 2007). PFBS levels were below the limit of quantitation. The authors noted that measurable amounts of other ionic PFAS were found in indoor air samples, but levels were not significantly elevated above levels in outdoor air. In Spain, Jogsten et al. (2012) collected indoor air samples (n = 10) from selected homes in Catalonia and evaluated levels of 27 perfluorinated chemicals (PFCs). PFBS was not detected.

In Ireland, Harrad et al. (2019) measured eight target PFAS in air from cars (n = 31), home living rooms (n = 34), offices (n = 34), and school classrooms (n = 28). PFBS was detected in all four indoor microenvironments, at DFs of 53%, 90%, 41%, and 54% in samples from homes, cars, offices, and classrooms, respectively. The mean (maximum) concentrations were 22 (270) picograms per cubic meter (pg/m^3) in homes, 54 (264) pg/m^3 in cars, 37 (313) pg/m^3 in offices, and 36 (202) pg/m^3 in classrooms.

There is some evidence from European studies indicating PFBS exposure via indoor air. However, further research is needed to understand the DF and concentrations of PFBS that occur in indoor environments in the United States.

B.3.5.2. Ambient Air

Similar to studies on indoor PFBS air concentrations, no studies from the U.S. reporting levels of PFBS in ambient air were identified from the peer-reviewed or gray literature. Four studies conducted across Europe (Harrad et al., 2020; Jogsten et al., 2012; Beser et al., 2011; Barber et al., 2007) and one study conducted in Canada (Ahrens et al., 2011) analyzed ambient air samples for PFBS. Two of the studies (Harrad et al., 2020; Barber et al., 2007) found detectable levels of PFBS in outdoor air. Barber et al. (2007) collected air samples from four field sites in Europe (one semirural site [Hazelrigg] and one urban site [Manchester] in the United Kingdom, one rural site from Ireland, and one rural site from Norway) for analysis of neutral and ionic PFAS. The study authors did not indicate whether any of the sites had a history of local PFAS-related activities (e.g., AFFF usage, PFAS manufacturing or use). PFBS was detected in the particle phase of outdoor air samples during one of the two sampling events in Manchester at 2.2 pg/m^3 and one of the two sampling events in Hazelrigg at 2.6 pg/m^3. PFBS was not detected above the method quantification limit at the Ireland and Norway sites. Harrad et al. (2020) measured PFBS

in air near 10 Irish municipal solid waste landfills located in non-industrial areas. Air samples were collected upwind and downwind of each landfill. PFBS was detected in more than 20% of the samples, with mean concentrations (ranges) at downwind and upwind locations of 0.50 (< 0.15–1.4) pg/m^3 and 0.34 (< 0.15–1.2) pg/m^3, respectively. Beser et al. (2011) and Jogsten et al. (2012) did not detect PFBS in ambient air samples in Spain. Beser et al. (2011) analyzed fine airborne particulate matter ($PM_{2.5}$) in air samples collected from five stations located in Alicante province, Spain (3 residential, 1 rural, 1 industrial) to determine levels of 12 ionic PFAS. PFBS was below the method quantification limit at all five locations. Jogsten et al. (2012) did not detect PFBS in ambient air samples collected outside homes in Catalonia, Spain.

In the one study identified from North America, Ahrens et al. (2011) determined levels of PFAS in air around a WWTP and two landfill sites in Canada. PFBS was not detected in any sample above the method detection limit.

PFBS has been detected in Artic air in one study, with a DF of 66% and mean concentration of 0.1 pg/m^3 (Arp and Slinde, 2018; Wong et al., 2018).

As with exposure to PFBS via indoor air, there is some evidence from European studies indicating PFBS is present in some ambient air samples. Further research is needed to understand the DF and concentrations of PFBS that occur in ambient environments in the United States.

B.3.6. Soil

PFBS can be released into soil from manufacturing facilities, industrial uses, fire/crash training sites, and biosolids containing PFBS (USEPA, 2021d; ATSDR, 2021). The EPA identified 16 studies that evaluated the occurrence of PFBS and other PFAS in soil, conducted in the United States, Canada, or Europe (see Table B-6). Two U.S. studies and two Canadian studies (Cabrerizo et al., 2018; Venkatesan and Halden, 2014; Blaine et al., 2013; Dreyer et al., 2012) were conducted in areas not reported to be associated with any known PFAS release or were experimental studies conducted at research facilities. At these sites, PFBS levels were low (≤ 0.10 ng/g) or below detection limits in non-amended or control soils. Two U.S. studies by Scher et al. (2019; 2018) evaluated soils at homes in Minnesota within and outside of a GCA impacted by a former 3M PFAS production facility; for sites within the GCA, one of the studies reported a DF of 10% and a 90th percentile PFBS concentration of 0.02 ng/g, and the other reported a DF of 9% and a maximum PFBS concentration of 0.017 ng/g. For sites outside of the GCA, the DF was 17% and the maximum PFBS concentration was 0.031 ng/g. Three U.S. studies and one Canadian study analyzed soils potentially impacted by AFFF used to fight fires—one at U.S. Air Force installations with historic AFFF use (Anderson et al., 2016), two at former fire training sites (Nickerson et al., 2020; Eberle et al., 2017), and another at the site of a train derailment and fire in Canada (Mejia-Avendaño et al., 2017). In these four studies, DFs ranged from 35 to 100%. PFBS concentrations in the study of the U.S. Air Force installations ranged from ND–79 ng/g, and PFBS concentrations ranged from ND–58.44 ng/g at one fire training site (Nickerson et al., 2020). The study of the other fire training site measured PFBS pre-treatment (0.61–0.6.4 ng/g) and post-treatment (0.07–0.83 ng/g) (Eberle et al., 2017). The DFs and range of PFBS concentrations measured in soils at the site of the train derailment were 75% DF and ND–3.15 ng/g, respectively, for the AFFF run-off area (measured in 2013, the year of accident) and 36% DF and ND–1.25 ng/g, respectively, at the burn site and adjacent area (measured in 2015) (Mejia-Avendaño et al., 2017).

Of the six European studies, one study (Harrad et al., 2020) analyzed soil samples collected upwind and downwind of 10 municipal solid waste landfills in Ireland and found PFBS levels to be higher in soils from downwind locations. Based on the overall study findings, however, the authors concluded there was no discernible impact of the landfills on concentrations of PFAS in soil surrounding these facilities. Grønnestad et al. (2019) investigated soils from a skiing area in Norway to elucidate exposure routes of PFAS into the environment from ski products, such as ski waxes. The authors found no significant difference in mean total PFAS in soil samples from the Granåsen skiing area and the Jonsvatnet reference area but noted that the skiing area samples were dominated by long-chain PFAS (C8–C14; ≥ 70%) and the reference area samples were dominated by short-chain PFAS (> 60%), which included PFBS. A study in Belgium (Groffen et al., 2019) evaluated soils collected at a 3M fluorochemical plant in Antwerp and at four sites located at increasing distances from the plant. PFBS levels were elevated at the plant site and decreased with increasing distance from the plant. The other three studies analyzed soil samples from areas near firefighting training sites in Norway and France, and reported PFBS concentrations varying from ND to 101 ng/g dry weight (Dauchy et al., 2019; Skaar et al., 2019; Hale et al., 2017).

A U.S. study of biosolid samples from 94 WWTPs across 32 states and the District of Columbia detected PFBS in 60% of samples at a mean concentration (range) of 3.4 (2.5–4.8) ng/g (Venkatesan and Halden, 2013). As mentioned, PFBS has been detected in drinking water wells, food types, and plant samples from soils or fields that have received biosolids applications that were industrially impacted (Blaine et al., 2014; Blaine et al., 2013; Lindstrom et al., 2011).

In summary, results of some available studies suggest that proximity to a PFAS production facility or a site with historical AFFF use or firefighting is correlated with increased PFBS soil concentrations compared to soil from sites not known to be impacted by PFAS. However, few available studies examined PFBS concentrations in soils not known to have nearby sources of PFBS. Additional research is needed that quantifies ambient levels of PFBS in soils in the United States.

Table B-6. Compilation of Studies Describing PFBS Occurrence in Soil

Study	Location	Site Details	Results
North America			
Venkatesan and Halden (2014)	United States (Baltimore, Maryland)	Control (nonamended) soil from Beltsville Agricultural Research Center	DF 0%
Blaine et al. (2013)	United States (Midwestern)	Urban and rural full-scale field study control (nonamended) soil	Urban control: DF NR, mean = 0.10 ng/g Rural control: DF NR, mean = ND
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within a GCA	DF 10%, median (p90) = ND (0.02) ng/g

Study	Location	Site Details	Results
Scher et al. (2018)	United States (Twin Cities metropolitan region, Minnesota)	Near former 3M PFAS production facility, homes within and outside a GCA	Within GCA: DF 9%, median (range) = ND (ND–0.17 ng/g) Outside GCA: DF 17%, median (range) = ND (ND–0.031 ng/g)
Anderson et al. (2016)	United States (unspecified)	Ten U.S. Air Force installations with historic AFFF release, surface and subsurface soils	Surface soil: DF 35%, median (range) = 0.775 (ND–52.0) ng/g Subsurface soil: DF 35%, median (range) = 1.30 (ND–79.0) ng/g
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Firefighting training site, pre- and posttreatment	Pretreatment: DF 60%, range = 0.61–6.4 ng/g Posttreatment: DF 100%, range = 0.07–0.83 ng/g
Nickerson et al. (2020)	United States (unspecified)	Two AFFF-impacted soil cores from former fire-training areas	Core E: DF[a] 91%, range = ND–27.37 ng/g dw Core F: DF 100%, range = 0.13–58.44 ng/g dw
Cabrerizo et al. (2018)	Canada (Melville and Cornwallis Islands)	Catchment areas of lakes	DF 100%, mean[a] (range) = 0.0024 (0.0004–0.0083) ng/g dw
Dreyer et al. (2012)	Canada (Ottawa, Ontario)	Mer Bleue Bog Peat samples (core samples)	Detected once at 0.071 ng/g in 1973 sample and not considered for further evaluation
Mejia-Avendaño et al. (2017)	Canada (Lac-Mégantic, Quebec)	Site of 2013 Lac-Mégantic train accident (oil and AFFF runoff area [sampled 2013], burn site and adjacent area [sampled 2015])	Background: DF NR, mean = 0.035 ng/g dw 2013: DF 75%, mean range = ND–3.15 ng/g dw 2015: DF 36%, mean range = ND–1.25 ng/g dw
Europe			
Harrad et al. (2020)	Ireland (multiple cities)	10 landfills, samples collected upwind and downwind	Downwind: DF NR, mean (range) = 0.0059 (ND–0.044) ng/g dw Upwind: DF NR, mean (range) = 0.0011 (ND–0.0029) ng/g dw
Grønnestad et al. (2019)	Norway (Granåsen, Jonsvatnet)	Granåsen (skiing area); Jonsvatnet (reference site)	Skiing area: DF 0%[b] Reference area: DF 70%, mean (range) = 0.0093 (ND–0.0385 ng/g dw)

Study	Location	Site Details	Results
Groffen et al. (2019)	Belgium (Antwerp)	3M perfluorochemical plant and 4 sites with increasing distance from plant	Plant: DF 92%, mean (range) = 7.84 (ND–33) ng/g dw Vlietbos (1 km from plant): DF 90%, mean (range) = 2.79 (ND–7.04) ng/g dw 2.3 km, 3 km, 11 km from plant: DF 0%
Dauchy et al. (2019)	France (unspecified)	Firefighting training site, samples collected in 6 areas collected up to 15-m depth; in areas 2 and 6, foams used more intensely and/or before concrete slab was built	Areas 1, 3, 4, and 5 combined: DF[a] 0–10%, range = ND–7 ng/g dw, across all depths Area 2: DF[a] 35%, range = ND–82 ng/g dw, across all depths Area 6: DF[a] 55%, range = ND–101 ng/g dw, across all depths
Skaar et al. (2019)	Norway (Ny-Ålesund)	Research facility near firefighting training site	Background: DF 0% Contaminated: DF 100%, mean[a] (range) = 4.9 (2.64–7.13) ng/g dw
Hale et al. (2017)	Norway (Gardermoen)	Firefighting training site	DF 0%

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; dw = dry weight; GCA = groundwater contamination area; km = kilometer; ND = not detected; ng/g = nanogram per gram; NR = not reported; PFAS = per- and polyfluoroalkyl substances; p90 = 90th percentile

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

[b] Grønnestad et al. (2019) reported a DF = 10% but a range, mean, and standard deviation of < LOQ.

B.4. Recommended RSC

The EPA followed the Exposure Decision Tree approach to determine the RSC for PFBS (USEPA, 2000b). The EPA first identified two potential populations of concern (Box 1): pregnant women and their developing fetuses and women of childbearing age (see Section 2.2.2). However, limited information was available regarding specific exposure of these populations to PFBS in different environmental media. The EPA considered exposures in the general U.S. population as likely being applicable to these two populations. Second, the EPA identified several relevant PFBS exposures and pathways (Box 2), including dietary consumption, incidental oral consumption via dust, consumer products, and soil or dermal exposure via soil, consumer products, and dust, and inhalation exposure via indoor or ambient air. Several of these may be potentially significant exposure sources. Third, the EPA determined that there was inadequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, studies from Canada and Europe indicate that indoor and ambient air may be a significant source of exposure to PFBS. At the time of the literature search, the EPA was unable to identify studies assessing PFBS concentrations in

indoor or ambient air samples from the U.S. and therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, based on the studies summarized in the sections above, there are significant known or potential uses/sources of PFBS other than drinking water (Box 6), though there is not information available on each source to make a characterization of exposure (Box 8A). For example, there are several studies from the U.S. indicating that PFBS may occur in dust sampled from various microenvironments (e.g., homes, offices, daycare centers, vehicles). However, the majority of studies sampled in only one location and few studies examined dust samples outside of the home (e.g., one study from the U.S. assessed PFBS occurrence in dust sampled from vehicles). Additionally, though several studies from around the U.S. measured PFBS concentrations in dust from houses, the detection frequencies in these studies varied widely (from 3% to 59%) and may be a result of uncertainties including home characteristics, behaviors of the residents, and the presence or absence of PFBS-containing materials or products (Haug et al., 2011). Therefore, it is not possible to determine whether dust can be considered a major or minor contributor to total PFBS exposure. Similarly, it is not possible to determine whether the other potentially significant exposure sources such as seafood and consumer products should be considered major or minor contributors to total PFBS exposure. Given these considerations, following recommendations of the Exposure Decision Tree (USEPA, 2000b), the EPA recommends an RSC of 20% (0.20) for PFBS.

Appendix C. PFNA: Summary of Occurrence in Water and Detailed Relative Source Contribution

C.1. Occurrence in Water

The use and production of PFNA could result in its release to the aquatic environment through various waste streams (NCBI, 2022b). PFNA has an estimated water solubility of 62.5 µg/L (6.25×10^{-2} mg/L) at 25°C and when released to surface water, it is expected to adsorb to suspended solids and sediment (NCBI, 2022b). Volatilization from water surfaces is not expected to be an important fate process for PFNA (NCBI, 2022b).

C.1.1. Groundwater

Several peer-reviewed studies were identified that examined PFNA occurrence in groundwater sources from the U.S. In three studies, sampling locations included sites known or suspected to contain PFAS but not related to AFFF use (Procopio et al., 2017; Post et al., 2013; Lindstrom et al., 2011). Procopio et al. (2017) evaluated groundwater samples from a small area of an industrial/business park located within the South Branch Metedeconk River watershed. Two sampling events were conducted as part of a source trackdown study to identify potential sources of PFAS contamination after elevated PFOA levels were discovered at a raw surface water intake of the Brick Township Municipal Utilities Authority. Samples were collected following the installation of 16 temporary monitoring wells by the New Jersey Geological and Water Survey or a contract driller during August 2013 and June and July 2014. PFNA was detected in 32% of samples (n = 19), with concentrations ranging from < 5 to 63 ng/L; a mean concentration was not reported. The maximum PFNA level found (63 ng/L) occurred at a well located in the middle of the industrial/business park where the highest PFAAs were detected in the study. Based on the results of all PFAAs analyzed, the authors concluded a strong likelihood of a groundwater plume of PFAS contamination resulting from the suspected illicit discharge of liquid waste to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs. Post et al. (2013) evaluated raw groundwater samples from public drinking water system intakes in two sampling campaigns. Between August 2009 and February 2010, groundwater samples from 18 drinking water systems were obtained from 1 confined well (sunk into an aquifer located between two impermeable strata) and 17 unconfined wells in the upper unconfined aquifer that were chosen to represent New Jersey geographically. The sampled locations included one site with a nearby industrial facility that previously used large quantities of PFNA. PFNA was found in 5 of 18 samples with concentrations ranging from not detected (ND) to 96 ng/L. The maximum PFNA concentration was at the site with the nearby industrial facility. Sampling was also conducted in 2010–2013 from unconfined wells of two additional public drinking water systems with groundwater known to be contaminated by PFOA. Four wells were sampled at the first system and one well at the second system. PFNA was detected in all five wells. Sample detection frequencies were not reported but concentrations ranged from ND to 16 ng/L across the four wells in the first system and from 24 to 72 ng/L in the second system. Lindstrom et al. (2011) analyzed well water samples from 13 wells used for livestock, watering gardens, and washing in Decatur, Alabama. The samples were collected in

February 2009 from farms that had applied PFC-contaminated biosolids to local agricultural fields as a soil amendment for at least 12 years. PFNA was below the LOQ (10 ng/L) in all 13 wells.

Three studies from the U.S. evaluated groundwater potentially impacted by wastewater (Boone et al., 2019; Appleman et al., 2014; Quiñones and Snyder, 2009). In the first study, Boone et al. (2019), evaluated 17 PFAS in source and treated waters collected in 2010–2012. Of the three groundwater sources evaluated, PFNA was detected in two out of three samples at levels of 1.25 and 0.156 ng/L. In Appleman et al. (2014), authors assessed source water from five utilities in New Jersey from November 2011 to September 2012, the majority of which were selected because they were either known from previous monitoring or expected to contain detectable PFAS because they were impacted by upstream wastewater effluent discharge. PFNA was detected in six of seven groundwater samples. The study did not report an average value for PFNA, but concentrations ranged from below the method RL (0.5 ng/L) to 47 ng/L. In the third study, Quiñones and Snyder (2009) examined levels of eight PFAS at two sites in Nevada that were highly impacted from treated wastewater. Mean PFNA levels at the two sites were 6.9 and 5.7 ng/L at sites 1 (n = 7) and 2 (n = 8), respectively.

The remaining three U.S. studies identified addressed sites of current and/or historic use of AFFFs (Steele et al., 2018; Eberle et al., 2017; Anderson et al., 2016). Anderson et al. (2016) assessed 40 sites across 10 active Air Force installations throughout the continental United States and Alaska between March and September 2014. Installations were included if there was known historic AFFF release in the period 1970–1990. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. The selected sites were not related to former fire training areas and were characterized according to volume of AFFF release—low (n = 24), medium (n = 100), and high (n = 25). Across all sites, the PFNA detection frequency was 46.38% and the median concentration at sites with detectable levels was 105 ng/L. PFNA was detected only at low- and medium-volume release sites with detection frequencies of 37.5% and 40.6%, respectively, and mean concentrations of 300 and 900 ng/L, respectively. Authors noted that given PFNA is not present in 3M AFFF formulations, there may be some degree of telomer-based AFFF contamination. Steele et al. (2018) investigated a contaminated military base in Alaska and former Pease Air Force Base (the latter being historical, secondary data). Authors reported the primary source of contamination for the Alaska military base to be from prior legacy AFFF use and wells were selected for sampling based on historical data that indicated PFOS and PFOA contamination. Well samples at the Alaska base were collected monthly from July 2016 to March 2017 to determine if monthly variations in PFAS concentrations existed. For four wells, PFNA was detected one to three times during the monthly sampling at concentrations ranging from 0.91 to 6.6 ng/L. PFNA was not detected in any of the eight monthly timepoints in two other wells. A seventh well was only sampled in July 2016 and reported a PFNA concentration of 1.3 ng/L. The authors found that PFAS concentrations did not vary significantly on the scale of weeks or months. Eberle et al. (2017) collected groundwater samples first in April and December 2012 as part of a screening/site characterization analysis. Additional samples were collected in 2013–2014 before and after a pilot scale field test at a former fire training site at Joint Base Langley-Eustis, Virginia. Monthly fire training activities were conducted at the site from 1968 to 1980 and irregular fire training activities continued until 1990. Of the data reported, samples collected for site characterization showed PFNA was detected in all wells (seven deep, three shallow)

sampled. PFNA was also detected in all pre-treatment samples (n = 5), ranging from approximately 100 to 1,500 ng/L.

The EPA also identified studies from Canada and Europe reporting the occurrence of PFNA in groundwater, which are briefly summarized below. Detailed results from each study are presented in Table C-1. PFNA was detected in groundwater samples collected in 2010 from the Highland Creek watershed in Canada at concentrations ranging from 0.071 ng/L to 0.54 ng/L (Meyer et al., 2011, as cited in NCBI, 2022). In this study, the authors reported that none of the sampling sites receive water that is impacted by known PFAS point sources (Meyer et al., 2011). In Europe, PFNA investigations in groundwater were conducted in France (Dauchy et al., 2019; Bach et al., 2017; Boiteux et al., 2017; Dauchy et al., 2017; Gellrich et al., 2013; Boiteux et al., 2012; Dauchy et al., 2012), Germany (Gellrich et al., 2013), Ireland (Harrad et al., 2020), Italy (Barreca et al., 2020; Ciofi et al., 2018; Gellrich et al., 2013), Malta (Sammut et al., 2019), Norway (Høisæter et al., 2019), Spain (Jurado-Sánchez et al., 2013; Llorca et al., 2012) and Sweden (Gobelius et al., 2018; Gyllenhammar et al., 2015). Loos et al. (2010) also collected groundwater from 164 groundwater monitoring stations of participating European Union laboratories, within 23 countries, in the fall of 2008. They detected PFNA in 15.2% of these collected samples with concentrations up to 10 ng/L. Gellrich et al. (2013) also collected samples in multiple countries along the Rhine River, collecting both river filtrate and combined groundwater and percolated water from the Rhine riverbed in Germany, France, and Italy. In this campaign, they did not detect PFNA from the Rhine River or riverbed groundwater.

In the studies conducted in countries along the Mediterranean Sea, there was little-to-no detected PFNA in groundwater sources. In Spain, no PFNA was detected in well water samples collected by Jurado-Sánchez et al. (2013) in the southeast of the country nor by Llorca et al. (2012) in Barcelona. In the Lombardia region of Italy, Barreca et al. (2020) detected PFNA in 3% of 130 collected groundwater samples across 57 sampling stations in 2018. Ciofi et al. (2018) collected groundwater samples at 12 locations across Tuscany, including Siena, Florence, and Prato. One grab sample was collected at each of these 12 locations, detecting PFNA at each with concentrations between <0.26–4.8 ng/L (Ciofi et al., 2018). Sammut et al. (2019) collected groundwater from ten boreholes across the island country in 2015-2016, which were sites used by the Malta Water Services Corporation for both water extraction and quality analysis sampling. Across these ten boreholes, they detected PFNA in one borehole at a concentration of 0.90 ng/L (Sammut et al., 2019).

The EPA identified a number of studies reporting PFNA measurements within France (Dauchy et al., 2019; Bach et al., 2017; Boiteux et al., 2017; Dauchy et al., 2017; Gellrich et al., 2013; Boiteux et al., 2012; Dauchy et al., 2012). Boiteux et al. (2012) analyzed raw water from drinking water treatment plants distributed across 100 French departments, representing approximately 20% of the national water supply flow in 2009 and 2010. In their first sampling campaign in 2009, they detected PFNA in 6% of collected samples, with a maximum concentration of 14 ng/L. In their second sampling campaign in 2010, they did not detect PFNA at a limit of detection of 1.3 ng/L (Boiteux et al., 2012). In 2013, Bach et al. (2017) and Boiteux et al. (2017) evaluated the PFNA concentration in alluvial wells that are influent groundwater to drinking water treatment plants in southern and northern France, respectively. Both Bach et al. (2017) and Boiteux et al. (2017) sampled groundwater downstream from industrial sites which produce fluoropolymers and fluorotelomer-based products. In southern France, Bach et al. (2017) detected FPNA in 86–100% of collected samples, with concentrations from <4 pg/m^3 (the

limit of quantification) to 37 pg/m^3. Alternatively, in northern France, Boiteux et al. (2017) did not detect PFNA in any of the sampled alluvial wells.

A number of studies focused specifically on investigating sites where PFAS-containing material had been heavily used. In Norway, Høisæter et al. (2019) analyzed groundwater at a firefighting training site that extensively used PFAS-containing materials until their ban in 2011. Five monitoring wells were sampled in 2016, totaling 19 sampling campaigns, which detected a mean concentration of approximately 950 ng/L in the monitoring wells at the site. Dauchy et al. (2019) investigated a similar firefighting training facility with heavy PFAS use in France in 2015–2016 but detected no groundwater contamination. Neighboring Norway, in Sweden, Gyllenhammar et al. (2015) sampled monitoring, private, and production wells for four drinking water treatment plants downstream of a military airport with firefighting training activities. Unlike the findings of Høisæter et al. (2019) in Norway, they found no detectable PFNA across the wells they sampled in 2012–2014 (Gyllenhammar et al., 2015). Gobelius et al. (2018) also sampled at "PFAS hot spots" (e.g., firefighter training sites, sewage treatment plants, landfills) across Sweden, detecting PFNA in 27% of samples with concentrations between <0.08–66 ng/L. In Ireland, Harrad et al. (2020) collected groundwater samples from boreholes downgradient from ten municipal solid waste landfills across the country, which accepted municipal waste, non-hazardous industrial waste, construction and demolition waste, and biomedical waste. Across these sites, they detected PFNA in 10% of groundwater samples, with concentrations ranging from <0.1–0.22 ng/L.

Dauchy et al. (2012) sampled raw water from monitoring groundwater wells at a fluoropolymer manufacturing plant and at two drinking water treatment plants downstream of the manufacturing plant and many other domestic and industrial activities. At the fluoropolymer manufacturing plant, three of the four sampled wells reported detectable levels of PFNA from 21–724 ng/L. Downstream, at the drinking water treatment plants, five of five sampled monitoring wells contained detectable PFNA ranging from 13–35 ng/L (Dauchy et al., 2012). Dauchy et al. (2017) also conducted sampling campaigns in late 2014 through early 2015, investigating a previously operated oil refinery, a military airport, and a training center for firefighters. These sites were selected due to their heavy use of fluorosurfactant-based foams, and samples were collected from groundwater monitoring wells. PFNA was detected in the oil storage depot, between 11–12 ng/L in October 2014 and March 2015. During this period, no PFNA was detected in the military airport or firefighter training center groundwater (limit of quantification=4 ng/L) (Dauchy et al., 2017).

Table C-1. Summary of Studies Reporting the Occurrence of PFNA in Groundwater

Study	Location	Site Details	Results
United States			
Procopio et al. (2017)	United States (New Jersey)	Groundwater from an industrial/business park located within the South Branch Metedeconk River watershed, where there was suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs. Samples were collected following the installation of 16 temporary monitoring wells by the NJ Geological and Water Survey or a contract driller during August 2013 (sampling event #7) and June–July 2014 (sampling event #8). Samples were taken from the upper 1.5 m (5 ft) of the water table from each well, except for one "profile well" in which samples were collected at three different depths (3.7–4.6, 6.7–7.6, and 10.7–11.6 m below grade; 12–15, 22–25, and 35–38 ft below grade, respectively).	n = 19, DF[a] 32%, range = <5–63 ng/L (minimum reporting level = 5 ng/L)
Post et al. (2013)	United States (New Jersey)	Raw water collected from public drinking water system intakes in two sampling campaigns. In the first sampling campaign, samples from 18 drinking water systems were collected between August 2009 and February 2010 from 1 confined well (sunk into an aquifer located between two impermeable strata) and 17 unconfined wells in the upper unconfined aquifer; sites were chosen to represent NJ geographically and included 1 site with a nearby industrial facility that previously used large quantities of PFNA (site 5). In the second sampling campaign, samples from two drinking water systems (PWS-A and PWS-B) were collected in 2010–2013 from five unconfined wells. Groundwater at these two systems were known to be contaminated by PFOA.	1st sampling campaign: n = 18, DF 28%, range = ND–96 ng/L 2nd sampling campaign: PWS-A, WF1A: n = 5, DF NR, range = ND–6 ng/L PWS-A, WF1B: n = 4, DF NR, range = ND–12 ng/L PWS-A, WF2A: n = 9, DF NR, range = ND–16 ng/L PWS-A, WF2B: n = 9, DF NR, range = ND–7 ng/L PWS-B: n = 8, DF NR, range = 24–72 ng/L (minimum reporting level = 5 ng/L)
Lindstrom et al. (2011)	United States (Decatur, Alabama)	Thirteen samples collected in February 2009 from 13 wells located on farms with historical land application of PFC-contaminated biosolids to local agricultural fields between 1995 and 2008. Biosolids obtained from local municipal WWTP where sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers,	n = 13, DF (frequency of quantification) 0% (LOQ = 10 ng/L)

Study	Location	Site Details	Results
		fluorocarbon fibers, polymers, polymer films, and resins.	
Boone et al. (2019)	United States (unspecified)	Three groundwater sites used as source waters for three DWTPs, collected in 2010−2012; some locations with known or suspected sources of wastewater in the source water, but study did not differentiate which locations had known or suspected sources.	n = 3, DF[a] 67%, range = ND–1.25 ng/L (LCMRL = 0.094 ng/L)
Appleman et al. (2014)	United States (New Jersey)	Groundwater source water for five DWTPs, sampled November 2011 to September 2012. Majority of the utilities were selected because they were either known from previous monitoring or expected based on their source waters to contain detectable PFAS (i.e., impacted by upstream wastewater effluent discharge). Two sites were sampled twice and three sites were sampled only once.	n = 7, DF[a] 86%, range = <Method RL–47 ng/L (method reporting limit = 0.5 ng/L)
Quiñones and Snyder (2009)	United States (Nevada)	Samples collected in 2008 from two groundwater sites in Las Vegas Wash, Nevada that were highly impacted from treated wastewater.	Site 1: n = 7, DF NR, mean (maximum) = 6.9 (8.8) ng/L Site 2: n = 8, DF NR, mean (maximum) = 5.7 (8.9) ng/L (method reporting limit = 1.0 ng/L)
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between 1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were not specifically controlled for in the site selection process; active remedies had not been applied at any of the sites selected. Approximately ten samples were collected between March and September 2014 at each site; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases. Groundwater	Overall: n = 149, DF 46.38%, median (maximum) = 105 (3,000) ng/L *Breakdown by site group:* Emergency Response (low-volume release): n = 24, DF 37.5%, mean (range) = 300 (57–450) ng/L Hangars and Buildings (medium-volume release): n = 100, DF 40.6%, mean (range) = 900 (22–10,000) ng/L Testing and Maintenance (high-volume release): n = 25, DF 0% (median reporting limit = 18 ng/L) *Minimum of detected values reported *Median calculated using quantified detections

Study	Location	Site Details	Results
		samples were collected from existing monitoring wells and temporary monitoring wells installed with direct push technology.	*Non-detects were substituted with ½ the reporting limit
Steele et al. (2018)	United States (Alaska)	Monthly samples collected from a military installation during July 2016–March 2017; six wells from around the installation were sampled each month, along with a seventh well that was only sampled in July 2016. PFAS contamination predominately from prior legacy AFFF use. Wells selected based on historical sample data indicating PFAS contamination.	Data for July, August, September, October, November, December, January, and February, respectively: Well A: 1, ND, ND, ND, ND, ND, ND, ND ng/L Well B: 1.2, ND, ND, ND, ND, ND, ND, ND ng/L Well D: 1.3 ng/L (no values provided for other months) Well E: ND, ND, ND, ND, ND, ND, ND, ND ng/L Well F: ND, ND, ND, ND, ND, ND, ND, ND ng/L DK: 4.8, ND, ND, ND, 6.4, 6.6, ND, ND ng/L FG: 0.91, 0.91, ND, ND, ND, ND, ND, ND ng/L (method detection limit not reported)
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Pilot testing area in former fire training area (Training Site 15) at Joint Base Langley-Eustis where monthly fire training activities were conducted from 1968 to 1980 in a zigzag pattern burn pit. Facility was abandoned in 1980 but irregular fire training activities using an above-ground germed burn pit continued until 1990. Groundwater samples collected for screening/site characterization (April and December 2012), and for pre- (April 2013) and post- (October 2013 and February 2014) in situ chemical oxidation treatment using a peroxone activated persulfate (OxyZone) technology. Treatment was conducted in Test Cell 1 over 113 days (April through August 2013). Pre-treatment samples were collected from 14 wells screened in the deep zone, and 3 wells screened in the shallow zone. Post-treatment samples were collected from the same wells as the pre-treatment samples with an additional three wells (two shallow, one deep) sampled. Wells EC-1, EC-2, EC-3, EC-4, I-	Screening/site characterization: EC-1 (deep, sentry): 100 ng/L EC-2 (deep, sentry): 600 ng/L I-1 (deep): 700 ng/L I-2 (deep, sentry): 400 ng/L I-4 (deep): 900 ng/L I-5 (shallow): 100 ng/L I-6 (shallow): 200 ng/L MW-2904 (deep): 100 ng/L U-16D (deep): 1,700 ng/L U-16S (shallow): 200 ng/L Pre-treatment (values reported for two different laboratories): EC-2: 900; 500 ng/L EC-3: 1,500; 700 ng/L I-1: 200; 300 ng/L I-2: 200; 100 ng/L I-4: 1,700; 800 ng/L

Study	Location	Site Details	Results
		2, and I-3 were sentry wells to monitor the possible migration of oxidants and contaminants outside Test Cell 1. No PFNA data reported for post-treatment samples.	(LOQ not reported)
Europe			
Bach et al. (2017)	France (southern)	Samples were collected from alluvial wells that provide source water for two DWTPs. The two DWTPs are located on both sides of a river, ~15 km downstream from an industrial site where two facilities produce fluoropolymers; the industrial site discharges its effluents at three points along a river. The alluvial wells are located along the river, with wells for the first DWTP (DWTP A) located on the left shore and alluvial wells for the second DWTP (DWTP B) located on the right shore, on an island formed by a backwater. Sample collection occurred in April, July, October, and December 2013.	Alluvial wells for DWTP A: April 2013: n = 7, DF[a] 86%, range = <4–25 ng/L July 2013: n = 7, DF[a] 86%, range = <4–25 ng/L October 2013: n = 7, DF[a] 86%, range = <4–37 ng/L December 2013: n = 7, DF[a] 86%, range = <4–30 ng/L Alluvial wells for DWTP B: April 2013: n = 8, DF[a] 100%, mean[a] (range) = 8.13 (4–17) ng/L July 2013: n = 8, DF[a] 88%, range = <4–15 ng/L October 2013: n = 7, DF[a] 100%, mean[a] (range) = 9.43 (5–15) ng/L December 2013: n = 8, DF[a] 100%, mean[a] (range) = 7.38 (4–10) ng/L (LOQ = 4 ng/L) *DF represents frequency of quantification
Boiteux et al. (2017)	France (northern)	Samples were collected in four sampling campaigns (May, July, October, and December 2013) from alluvial wells that provide source water for two DWTPs. The two DWTPs (A and B) are located downstream of an industrial WWTP that processes raw sewage from a facility that manufactures fluorotelomer-based products and side-chain-fluorinated polymers used in firefighting foams and stain repellents. DWTP A is located 15 km downstream from the WWTP and is supplied by five alluvial wells. DWTP B is located 20 km downstream of the WWTP and is supplied by four alluvial wells.	DWTP A: May 2013: n = 5, DF 0% July 2013: n = 5, DF 0% October 2013: n = 5, DF 0% December 2013: n = 5, DF 0% DWTP B: May 2013: n = 4, DF 0% July 2013: n = 4, DF 0% October 2013: n = 4, DF 0% December 2013: n = 4, DF 0% (LOQ = 4 ng/L) *DF represents frequency of quantification
Dauchy et al. (2012)	France (unspecified)	Raw water sampled in June 2010 from four monitoring wells at a fluoropolymer manufacturing	Fluoropolymer manufacturing plant:

Study	Location	Site Details	Results
		plant (P13, P14, P15, P01). Groundwater flowed from well P14 to P01 and well P15 is nearest to the polyvinylidene fluoride production area. Raw water resources also collected from two DWTPs (five sampling sites – DWA-1, DWA-2, DWA-3, DWA-4, DWB-1); the first DWTP (DWA) is supplied by four alluvial wells, and the second DWTP (DWB) is supplied by one alluvial well. The two DWTPs are located on both sides of a river, 15 km downstream of fluorochemical manufacturing facility. The river receives wastewater from many domestic and industrial activities.	P13: not quantifiable due to dilution or matrix effects P14: n = NR, DF NR, 21 ng/L P15: n = NR, DF NR, 342 ng/L P01: n = NR, DF NR, 724 ng/L DWA: DWA-1: n = NR, DF NR, 13 ng/L DWA-2: n = NR, DF NR, 35 ng/L DWA-3: n = NR, DF NR, 30 ng/L DWA-4: n = NR, DF NR, 33 ng/L DWB: DWB-1: n = NR, DF NR, 21 ng/L (LOQ = 4 ng/L) *Study did not indicate whether concentrations reported were point values or means
Harrad et al. (2020)	Ireland (multiple cities)	Groundwater samples collected between November 2018 and January 2019 from ten municipal solid waste landfills at two sampling points down-gradient from the main body of each landfill. Each sampling point consisted of a borehole leading down to water reservoirs at a minimum depth of 5 m below ground level. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction and demolition, and biomedical waste.	n = 10, DF[a] 10%, range = <0.1–0.22 ng/L (LOD = <0.1 ng/L) *Non-detects replaced by ½ LOD
Gobelius et al. (2018)	Sweden (national)	Sampling conducted between May and August 2015, with the majority in July and a few samples in September and November 2015. Samples were collected in 21 regional counties by the County Administration Boards. Sampling locations selected based on potential vicinity of PFAS hot spots (i.e., fire training sites, unspecific industry, sewage treatment plant effluent, landfill/waste disposal, skiing, and urban areas) and/or importance as a drinking water source. Sample numbers varied for each county and sampling sites were spread unevenly across Sweden.	n = 161, DF[a] 27%, range = <0.08–66 ng/L (method detection limit = 0.084 ng/L)

Study	Location	Site Details	Results
Boiteux et al. (2012)	France (national)	Raw water from DWTPs distributed across 100 French departments to represent ~20% of the national water supply flow; samples collected during two sampling campaigns in July–September 2009 (first campaign) and June 2010 (second campaign – focused on sites from first sampling campaign that had PFC levels >LOQ). Some sites possibly affected by industrial or commercial releases.	Overall: n = 196, DF (frequency of quantification) 3%, maximum = 14 ng/L *1st Sampling Campaign* n = 163, DF 6%, mean, median (maximum) = <1, <1 (14) ng/L *2nd Sampling Campaign* n = 33; results not reported (LOD = 1.3 ng/L, LOQ = 4 ng/L)
Loos et al. (2010)	23 European countries	Groundwater collected from 164 groundwater monitoring stations of participating European Union Member State laboratories during an 8-week window in Fall 2008. There were no strict selection criteria for the sampling sites such as “representative” or “contaminated”. Most monitoring stations were “official” monitoring stations also used for drinking water abstraction.	n = 164, DF 15.2%, mean, median (maximum) = 0, 0 (10) ng/L (LOD = 0.4 ng/L)
Dauchy et al. (2019)	France (unspecified)	Samples collected in two sampling campaigns in and around site where fluorosurfactant-based foams have been used extensively. From 1969 to 1984, the site was an oil refinery, with the exact location of the firefighting training area, frequency of training sessions, and history of firefighting training activities unknown. From 1987 to date, it has been a large training area for firefighters. First sampling campaign collected 13 samples from 9 monitoring wells and 4 springs in June 2015. Second sampling campaign collected from four monitoring wells in October 2016. Monitoring wells MW-1 to MW-5 were located upgradient from the firefighter training site around a landfill site. Monitoring well MW-11 and springs SW-A, SW-B, and SW-D located downgradient from the landfill or firefighter training site but not in the direction of groundwater flow. Monitoring wells MW-6 to MW-13 and spring SW-C were located downgradient from the firefighter training site in the direction of groundwater flow.	Upgradient: Monitoring wells: n = 5, DF 0% Downgradient but not in the direction of groundwater flow: Monitoring wells: n = 1, DF 0% Spring water: n = 3, DF 0% Downgradient in the direction of groundwater flow: Monitoring wells: n = 7, DF 0% Spring water: n = 1, DF 0% (LOQ = 4 ng/L)
Høisæter et al. (2019)	Norway (unspecified)	Firefighting training site with an airport that extensively used AFFF containing PFOS since the early 1990s until 2001 when it was replaced by fluorotelomer containing AFFF. All PFAS containing	n = 19, DF NR, mean* = 950 ng/L (LOD/LOQ not reported) *Mean estimated from Figure 4b in the paper

Study	Location	Site Details	Results
		firefighting foams was banned at the airport in 2011. Groundwater samples collected in 2016 at five pumping wells installed down gradient of the site to intercept and pump and treat the plume spreading from the firefighting training site. A total of 19 sampling campaigns were performed.	
Dauchy et al. (2017)	France (unspecified)	Samples collected in the vicinity of three sites (A, C, D) where fluorosurfactant-based foams are or were being heavily used. Site A is an oil storage depot located in a river port. In June 1987, a large explosion occurred in the depot and the fire was extinguished by applying a large amount of fluorosurfactant-based foams. Two groundwater samples were collected in October 2014 and March 2015 from a monitoring well located in the center of the depot. The water table lies 2.5 – 3.5 m below the ground. Site C is a military airport, with the exact location of the training area, frequency of the training sessions, and history of the firefighting training activities unknown. The well supplying the DWTP was sampled in March 2015. Site D is a training center for firefighters. From 1969 to 1984, the site was an oil refinery. Starting in 1987, the site became a training area for firefighters, with exercises carried out directly on the soil. From the 1990s, some exercise areas were covered with concrete. In November 2014, groundwater samples were collected from five springs.	Site A: October 2014: n = 1, point = 11 ng/L March 2015: n = 1, point = 12 ng/L Site C: n = 1, DF 0% Site D: n = 5, DF[a] 0% (LOQ = 4 ng/L)
Gyllenhammar et al. (2015)	Sweden (Uppsala)	Three observation well sites (Tuna backar: n = 3 wells; Svartbäcken: n = 1 well, Librobäck: n = 2 wells;) were sampled from September 2012 to January 2013. Four DWTP production well sites (Storvad: n = 9 wells; Galgbacken: n = 1 well; Stadsträdgården and Kronåsen: n = 6 wells; Sunnersta: n = 5 wells) were sampled from July 2012 to February 2014. One private well (Klastorp) was sampled in September 2012.	Observation wells: Tuna backar: n = 3, DF 0% Librobäck: n = 4, DF 0% Svartbäcken: n = 3, DF 0% Production wells: Storvad: n = 12, DF 0% Galgbacken: n = 7, DF 0% Stadsträdgården and Kronåsen: n = 103, DF 0% Sunnersta: n = 50, DF 0% Private well: Klastorp: n = 1, DF 0%

Study	Location	Site Details	Results
		All wells located downstream of a military airport with firefighting training activities up to the year 2003. It is not known when the usage of AFFF started.	(method detection limit = 10 ng/L)
Barreca et al. (2020)	Italy (Lombardia region)	Fifty-seven groundwater sampling stations throughout the region. Samples collected in 2018.	n = 130, DF[a] 3%, range NR (LOQ = 1 ng/L)
Sammut et al. (2019)	Malta	Groundwater collected from ten boreholes at different areas on the island during November and December 2015 and January 2016. Collection sites were the most commonly used extraction sites by the Malta Water Services Corporation for water extraction as well as for sampling for water quality analysis.	n = 10, DF 10%, range = ND–0.90 ng/L (LOD = 0.02 ng/L; LOQ = 0.04 ng/L)
Ciofi et al. (2018)	Italy (Tuscany)	Groundwater samples were collected at 12 locations. Sampling year was not reported. Each sample was collected from the phreatic layer with a mean depth between 10–75 m: GW-1: Siena, 10 m GW-2, GW-3, GW-4: Florence, 15 m GW-5: Prato, 75 m GW-6: Prato, 71 m GW-7: Prato, 70 m GW-8: Prato, 61 m GW-9: Florence, 17 m GW-10, GW-11, GW-12: Florence, 10 m	GW-1: n = 1, point = <0.28 ng/L GW-2: n = 1, point = <0.28 ng/L GW-3: n = 1, point = <0.28 ng/L GW-4: n = 1, point = <0.29 ng/L GW-5: n = 1, point = <0.33 ng/L GW-6: n = 1, point = <0.46 ng/L GW-7: n = 1, point = 3.2 ng/L GW-8: n = 1, point = 1.3 ng/L GW-9: n = 1, point = 4.8 ng/L GW-10: n = 1, point = <0.27 ng/L GW-11: n = 1, point = <0.26 ng/L GW-12: n = 1, point = <0.27 ng/L (MDL = 0.26–0.46 ng/L) *method detection limit varied by sample and was provided for 9 of 12 samples
Gellrich et al. (2013)	Germany (Hesse); France; Italy	Untreated water samples for preparation of mineral water included seven from Hesse, three from France, and four from Italy. The supplying waterworks obtain their untreated water either from Rhine river filtrate, a mixture of ground water and percolation water from the Rhine riverbed, drawn from wells 30–50 m deep or from wells in their closer vicinity. Sampling year not reported.	n = 14, DF (frequency of quantification) 0% (LOQ = 1 ng/L)

Study	Location	Site Details	Results
Jurado-Sánchez et al. (2013)	Spain (southeast)	Well water samples were collected and analyzed using a newly developed analytical method. Authors did not report sampling details or sampling year.	n = NR, DF 0% (LOD = 0.1 ng/L)
Llorca et al. (2012)	Spain (Barcelona)	Well water samples from two different sites were collected from the North of Barcelona metropolitan area in 2011.	n = 2, DF 0% (method LOD = 1.9; method LOQ = 6.3 ng/L)

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; DWTP = drinking water treatment plant; ft = feet; m = meter; ND = not detected; ng/L = nanogram per liter; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; NR = not reported; LOD = limit of detection; LOQ = limit of quantification; LCMRL = lowest concentration minimum reporting level; WWTP = wastewater treatment plant.

[a] The DF and/or mean was calculated using point data. Means were calculated only when DF = 100%.

C.1.2. Surface Water

The EPA identified many studies that reported on the occurrence of PFNA in surface water in both the U.S. and internationally. Overall, most U.S. studies reported PFNA detected in at least one surface water sample site in each study. Concentrations of PFNA in 12 remote and urban Minnesota surface water samples, including samples collected from Lake Michigan, ranged from <0.3 ng/L to 3.1 ng/L (<0.0003 µg/L to 0.0031 µg/L) (Simcik and Dorweiler, 2005, as cited in ATSDR, 2021). PFNA was detected in 38% of eight surface water samples from U.S. streams in the Great Lakes basin collected during 1994 to 2000 at concentrations between 0.03 ng/L and 0.4 ng/L (0.00003 µg/L and 0.0004 µg/L) (Klecka et al., 2010, as cited in NCBI, 2022). PFNA was detected in six locations in the Delaware River at concentrations ranging from 1.65 ng/L to 976 ng/L (0.00165 µg/L to 0.976 µg/L) in 2007 to 2009 (DRBC, 2013, as cited in ATSDR, 2021).

Three studies investigated surface water upstream and downstream of fluoropolymer facilities, with some sites also downstream of other potential PFAS sources (e.g., landfills, WWTPs) (Galloway et al., 2020; Newsted et al., 2017; Newton et al., 2017). Galloway et al. (2020) assessed several rivers and tributaries along the Ohio River in three sampling trips in 2016. The sampling sites ranged from upstream, downstream, and north/northeast of a fluoropolymer facility and known PFAS containing landfills. In June 2016, samples were collected on a 188 km stretch of the Ohio River, from 130 km downstream to 58 km upstream of the facility, and tributaries that pass near known PFAS-containing landfills. In July 2016, samples were collected from lakes, rivers, and creeks to the north and northeast of the facility as far as 16 km downwind. The December 2016 trip expanded the collection radius to more than 48 km downwind to the north and northeast of the facility. PFNA was detected in 92% of samples (n = 26) in June 2016, however all detects were below the LOQ (10 ng/L). From the second sampling trip, PFNA was not detected in any sample in July 2016 (n = 25). Finally, in December 2016, PFNA was detected at levels above the LOQ in one sample at 24.2 ng/L, detected but below the LOQ in 31 samples, and not detected in 8 samples. In Newsted et al. (2017), surface water samples were collected in August 2011 from a 3-mile section of the Upper Mississippi River: ten sampling reaches (three samples each) in an area between Ford Dam (between Minneapolis and St. Paul) and Hastings Dam (near Hastings) and which had been subject to 10–15 years of actions to reduce PFAS contamination from 3M Cottage Grove plant and other commercial/industrial entities. PFNA was detected in one sample from reach 10, immediately downstream the 3M Cottage Grove facility outfall, at a concentration of 2.0 ng/L. PFNA in all other samples was below the LOQ (2.0 ng/L). Newton et al. (2017) investigated surface water upstream and downstream of facilities that manufactured or used fluorinated materials along the Tennessee River near Decatur, Alabama. Six sampling sites were located upstream of the manufacturing facilities and three sites were downstream. Among the upstream sites, three were also upstream of a WWTP. All samples were collected in October 2015. PFNA was below the LOQ (10 ng/L) in all nine samples from the nine different sampling sites.

In four U.S. studies, sampling locations included surface waters potentially impacted by current and/or historic use of AFFFs (Anderson et al., 2016; Post et al., 2013; Nakayama et al., 2010; Nakayama et al., 2007). Anderson et al. (2016) assessed 40 sites across 10 active Air Force installations throughout the continental United States and Alaska between March and September 2014. Installations were included if there was known historic AFFF release in the period 1970–

1990. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. The selected sites were not related to former fire training areas and were characterized according to volume of AFFF release—low (n = 2), medium (n = 32), and high (n = 2). PFNA was detected only at medium-volume release sites (11.4% detection frequency; mean concentration of 15,400 ng/L). Across all 36 sites, the detection frequency was 36.00% with a median concentration of detects of 96 ng/L. Authors noted that given PFNA is not present in 3M AFFF formulations, there may be some degree of telomer-based AFFF contamination. Post et al. (2013) evaluated raw surface water samples from 12 public drinking water system intakes collected between August 2009 and February 2010. Six rivers and six reservoirs, including two reservoirs in Atlantic County near a civil-military airport with possible AFFF use, were selected to represent New Jersey geographically. PFNA was below the minimum RL (5 ng/L) in all six river samples. In reservoir samples, PFNA was detected in 67% of samples (n = 6) at a maximum level of 19 ng/L. At the two reservoir sites near the civil-military airport, PFNA was below the minimum RL (5 ng/L) at one, and the other found PFNA at 5 ng/L. Two studies from Nakayama et al. (2010; 2007) assessed surface water samples from the Upper Mississippi River, Missouri River, and Cape Fear River Basins. In Nakayama et al. (2010), a large-scale evaluation of the Upper Mississippi River Basin and portion of the Missouri River Basin was conducted to provide preliminary PFC data given the importance of the two basins in supplying drinking water. Between the two basins, 173 samples were collected across 88 sampling sites in March–August 2008 by several different agencies—Minnesota Pollution Agency, Wisconsin Department of Natural Resources, Illinois Environmental Protection Agency, and the EPA Region 7 Water Quality Monitoring Team. Overall, the detection frequency of PFNA was 87% with a median concentration of 0.71 ng/L. Authors reported higher PFC concentrations adjacent to chemical manufacturers, downstream of WWTPs receiving waste from those types of manufacturers, and near an airport with historic use of firefighting foams. In Nakayama et al. (2007), one hundred surface water samples were taken from 80 sites selected to reflect water quality throughout the basin. PFNA was detected in 89.9% of samples with mean and median concentrations of 33.6 and 5.70 ng/L, respectively. The highest concentrations were found in the middle reaches of the Cape Fear River and its two major tributaries. The authors noted possible sources of PFCs to the basin included firefighting foam from nearby air force bases and commercial/industrial facilities.

Three studies conducted in the U.S. examined surface water near or downstream of land application sites where PFC-contaminated WWTP effluent or biosolids were applied (Lasier et al., 2011; Lindstrom et al., 2011; Konwick et al., 2008). In Lindstrom et al. (2011), authors analyzed surface water samples from ponds and streams in Decatur, Alabama. The samples were collected in February 2009 from farms that had applied PFC-contaminated biosolids to local agricultural fields as a soil amendment for at least 12 years. The biosolids were obtained from a local municipal WWTP where authors noted that sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers, fluorocarbon fibers, polymers, polymer films, and resins, although specific sources could not be characterized. PFNA was detected in 28% of samples (n = 32), with levels ranging from below the LOQ (10 ng/L) to 286 ng/L. The remaining two studies (Lasier et al., 2011; Konwick et al., 2008) evaluated surface water upstream and downstream of a land application site (LAS) in Georgia, where treated WWTP effluent was sprayed. The WWTP processed effluents from multiple carpet manufacturers who were reported to use significant quantities of PFCs. Lasier et al. (2011) sampled along the Conasauga, Oostanaula, and Coosa Rivers during summer 2008; samples

included two sites upstream (sites 1 and 2) and six sites downstream (sites 3–8) of the LAS. Additionally, site 2 was downstream of a local airport, and site 4 was downstream of a manufacturing facility of latex and polyurethane back material—inputs for the carpet manufacturers. PFNA was below the MDL (0.005 ng/g) at both of the sites upstream. Mean concentrations for sites 3–8 were 35, 44, 17, 21, 20, and 21 ng/L, respectively. Authors reported highest concentrations downstream of the land application and backing-material sites and then decreased concentrations increasingly downstream as a result of dilution. Konwick et al. (2008) sampled from the Conasauga River near Dalton, Georgia in March 2006 at one location upstream, two downstream, and one at the LAS. Sampling was also conducted in January 2005 at one freshwater location in the Altamaha River, a river remote from the carpet industry, and from four different streams and ponds located in Dalton, Georgia. PFNA was detected at all sites. Mean PFNA concentrations for the four sites along the Conasauga River were 32.8, 201.6, 369, and 284 ng/L for the upstream, LAS site, and two downstream locations, respectively (n = 5 at each site), with a pattern of increasing concentration with distance downstream of the LAS before a decrease in concentration at the final site. Authors suggested sorption to sediments, particularly organic carbon, as a possible reason for the decrease in PFNA concentration at the final site. At the single freshwater site in the Altamaha River, PFNA was detected in one of three samples at a concentration of 0.6 ng/L. The range of PFNA concentrations in ponds and streams near Dalton were: 11.1–12.2, 40.6–41.0, 4.8–6.3, and 2.1–2.5 ng/L for sites 1, 2, 3, and 4, respectively (n = 2 at each site).

Three studies evaluated surface water potentially impacted by wastewater (Boone et al., 2019; Subedi et al., 2015; Appleman et al., 2014). Boone et al. (2019) evaluated 17 PFAS in source and treated waters collected in 2010–2012. Authors attempted to select locations with known or suspected sources of wastewater in the source water, but ultimately the site selection was dependent upon the willingness of DWTPs to participate. The study did not differentiate which locations had known or suspected sources. Of the 22 surface water sources evaluated (16 river and 6 lake/reservoir), PFNA was detected in all samples (n = 22), with a mean concentration of 2.93 ng/L. Subedi et al. (2015) collected 28 lake water samples from 3 sampling events in August–September 2012 and four sampling events in May–September 2013 from Skaneateles Lake. Sites were selected to be along the shoreline of homes that use an enhanced treatment unit for onsite wastewater treatment. Wastewater effluents were identified as a source of contamination to the lake. PFNA was detected in 57% of samples with mean and median concentrations of 0.36 and 0.26 ng/L, respectively. Appleman et al. (2014) assessed source water from 11 utilities in Alaska, Alabama, Colorado, Nevada, New Jersey, Ohio, Oklahoma, and Wisconsin from August 2011 to May 2012, the majority of which were selected because they were either known from previous monitoring or expected to contain detectable PFAS because they were impacted by upstream wastewater effluent discharge. Authors evaluated the utilities and their effectiveness for removing PFAS. The study did not report an average concentration for PFNA, but PFNA was detected in 14 of 25 samples (from 7 of 11 utilities) with a maximum concentration of 5.7 ng/L.

In two studies, surface water samples were collected from locations with potential sources of PFAS that were not related to AFFF use (Procopio et al., 2017; Zhang et al., 2016). Procopio et al. (2017) evaluated samples collected between September 2011 and July 2014 from the Metedeconk River. Eight sampling events were conducted as part of a source trackdown study to identify potential sources of PFAS contamination after elevated PFOA levels were discovered at

a raw surface water intake of the Brick Township Municipal Utilities Authority. In all 56 samples, PFNA was below the minimum laboratory RL (5 ng/L). Zhang et al. (2016) conducted analyses to determine major sources of surface water PFAS contamination. Freshwater sample collection sites included 22 sites in the state of Rhode Island (sampled June 2014) and 6 sites in the New York Metropolitan Area (sampled October 2014). Surface water sites were creeks and rivers in urban and rural locations. PFNA was detected at all sites with a mean of 1.914 ng/L (n = 28). Authors identified potential PFAS sources at these sites to be metal coating plating; paint, coating, adhesive manufacturing; paper manufacturing; petroleum coal products manufacturing; printing activity; printing ink manufacturing; semiconductor manufacturing; sewage treatment; textile mills; waste management including landfills, and airports.

Of the remaining four U.S.-based studies (Boone et al., 2014; Quiñones and Snyder, 2009)|(Pan et al., 2018; Kim and Kannan, 2007), Boone et al. (2014) analyzed PFCs in water samples collected from the surface of the Mississippi River at a low flow level (2.95 ft) in September 2010 and a high flow level (8.32 ft) in June 2009. PFNA levels were 1.800 and 3.30 ng/L at low and high flow levels, respectively. In Quiñones and Snyder (2009), surface water samples from the Boulder Basin, Hoover Dam, and the lower Colorado River were collected in 2008. Mean PFNA levels at all sites were below the method RL (1.0 ng/L). Kim and Kannan (2007) sampled two urban lakes in Albany, New York during five sampling trips from February–November 2006. The lakes, Washington Park Lake and Rensselaer Lake, are located in downtown Albany and receive surface runoff from nearby roadways and residential areas during stormwater runoff. PFNA was detected in Washington Park Lake (n = 6) at mean and median concentrations of 1.99 and 2.14 ng/L, respectively, and in Rensselaer Lake (n = 5) at mean and median concentrations of 1.35 and 1.45 ng/L, respectively. Overall, PFNA was detected in 81.8% of the 11 total samples. Finally, in a multicontinental study, Pan et al. (2018) assessed surface water samples from several countries including the United States (Delaware River), United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). Twelve samples were collected in September–December 2016 along the Delaware River that spanned seven cities—Trenton, Bristol, Philadelphia, Chester, Delaware, Smyrna, and Frederica. Authors noted that all sampling sites were along the main stream of the rivers and not proximate to known point sources of any fluorochemical facilities. PFNA was detected in all samples from the Delaware River with a mean concentration of 2.51 ng/L and were similar to levels found in the Thames River.

The EPA also identified studies from Canada and Europe reporting the occurrence of PFNA in surface water, which are briefly summarized below. Detailed results from each study are presented in Table C-2. Most Canadian and European studies reported PFNA detected in at least one surface water sample site in each study. Concentrations of PFNA in creek and river samples measured throughout Canada ranged from <125 pg/L to 3,000 pg/L (D'Eon J et al., 2009 as cited in NCBI, 2022). Also, PFNA concentrations ranged from 0.80 ng/L to 2.4 ng/L in surface water samples collected from Highland Creek watershed, Canada in 2010 (Meyer et al., 2011 as cited in NCBI, 2022). Concentrations of PFNA in lake water samples collected from four lakes on Cornwallis Island, Canada from 2003 to 2005 ranged from not detected to 6.1 ng/L (Stock et al., 2007 as cited in NCBI, 2022).

Several studies in Europe sampled surface water from sites in proximity to fluoropolymer facilities or in locations with current or past AFFF usage. Four studies investigated surface water

in proximity to fluoropolymer facilities. One study in France (Boiteux et al., 2017) reported no detections, but the other three, from France, the Netherlands, and Italy, respectively reported at least one site or sampling event with detections from 0.49 ng/L to 2,637 ng/L (Bach et al., 2017; Gebbink et al., 2017; Valsecchi et al., 2015). In the study by Bach et al. (2017), the maximum level detected of 2,637 ng/L was from a sampling area in a river downstream from two facilities that produce fluoropolymers. Four additional studies included sampling locations potentially impacted by current or past AFFF usage (Mussabek et al., 2019; Skaar et al., 2019; Gobelius et al., 2018; Dauchy et al., 2017). The reported PFNA detection frequencies ranged from 0% to 89% with varying maximum levels from 1.81 ng/L to 26 ng/L.

Many European and Canadian studies sampled sites where there was no known PFAS manufacturing or use of PFAS-heavy materials (e.g., AFFF). In some cases, PFNA was not detected or concentrations were not reported (Barreca et al., 2020; Yeung et al., 2017; Lescord et al., 2015; Jurado-Sánchez et al., 2013; Villaverde-de-Sáa et al., 2012; Möller et al., 2010) , and in other cases there was 100% (or near 100%) detection frequency of PFNA (Zhao et al., 2015; Eschauzier et al., 2012; Kovarova et al., 2012; Labadie and Chevreuil, 2011; Ahrens et al., 2009a). Maximum PFNA concentrations reported among all PFNA detections in these studies ranged from low (e.g., 0.18 ng/L in Zhao et al. (2015)) to very high (e.g., 8,100 ng/L in Kovarova et al. (2012)). Several studies with high maximum detections of PFNA used sampling locations near or potentially near wastewater treatment plants or other industrial activity (Wilkinson et al., 2017; Lorenzo et al., 2015; Boiteux et al., 2012; Llorca et al., 2012). The remaining studies did not report details on the area surrounding sampling locations and how nearby activities may have impacted the results (Ciofi et al., 2018; Munoz et al., 2018; Loos et al., 2017; Shafique et al., 2017; Eriksson et al., 2013; Veillette et al., 2012; Ahrens et al., 2009b; Rostkowski et al., 2009; Ericson et al., 2008b); the detected concentrations in these studies ranged from 0.057 ng/L to 9.89 ng/L.

Table C-2. Summary of Studies Reporting the Occurrence of PFNA in Surface Water

Study	Location	Site Details	Results
United States			
Galloway et al. (2020)	United States (Ohio; West Virginia)	Rivers and tributaries near a fluoropolymer facility sampled throughout three trips on June, July, and December 2016. In June 2016, samples were collected on a 188 km stretch of the Ohio River, from 130 km downstream to 58 km upstream of the facility, and tributaries that pass near known PFAS-containing landfills. In July 2016, samples were collected from lakes, rivers, and creeks to the north and northeast of the facility as far as 16 km downwind. The December 2016 trip expanded the collection radius to more than 48 km downwind to the north and northeast of the facility.	June 2016: n = 26; DF[a] 92%* *PFNA was detected but below the LOQ in 24 samples, and ND in 2 samples July 2016: n = 25; DF[a] 0% December 2016: n = 40; DF[a] 80%*, range = <LOQ–24.2 ng/L *PFNA was detected above the LOQ in 1 sample, detected but below the LOQ in 31 samples, and ND in 8 samples (LOQ = 10 ng/L)
Newsted et al. (2017)	United States (Minnesota)	Ten sampling reaches (three samples each) spanned 3 miles of the Mississippi River within Pool 2, an area between Ford Dam (between Minneapolis and St. Paul) and Hastings Dam (near Hastings) and subject to ongoing PFAS reduction efforts by the Minnesota Pollution Control Agency for 10–15 years. Surface water samples were collected in August 2011.	Upstream of 3M Cottage Grove facility: n = 27, DF 0% Downstream of 3M Cottage Grove facility: DF[a] 33%, range = <LOQ–2.0 ng/L (LOQ = 2.0 ng/L)
Newton et al. (2017)	United States (Decatur, Alabama)	Samples collected in October 2015 from nine sites along the Tennessee River. Three sites were downstream of facilities that manufacture or use fluorinated materials and three sites were upstream. Among the upstream sites, three were also upstream of a WWTP.	Upstream: n = 6, DF 0% Downstream: n = 3, DF 0% (LOQ = 10 ng/L)
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between 1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were	Overall: n = 36, DF 36.00%, median (maximum) = 96 (10,000) ng/L *Breakdown by site:* Emergency Response (low-volume release): n = 2, DF 0% Hangars and Buildings (medium-volume release): n = 32, DF 11.4%, mean (range) = 15,400 (480–59,000) ng/L Testing and Maintenance (high-volume release):

Study	Location	Site Details	Results
		not specifically controlled for in the site selection process; active remedies had not been applied at any of the sites selected. Approximately ten samples were collected between March and September 2014 at each site; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases. Surface water sample locations included engineered storm water channels, engineered AFFF ponds, and natural streams.	n = 2, DF 0% (median reporting limit = 17 ng/L) *Median calculated using quantified detections *Non-detects were substituted with ½ the reporting limit
Post et al. (2013)	United States (New Jersey)	Raw water collected from 12 public drinking water system intakes between August 2009 and February 2010 from 6 rivers and 6 reservoirs. Sites were chosen to represent NJ geographically and included two reservoir sites near a civil-military airport with possible AFFF use.	Overall: n = 12, DF 33%, range = ND–19 ng/L Rivers: n = 6, DF 0% Reservoirs: n = 6, DF 67%, range = <5–19 ng/L (minimum reporting limit = 5 ng/L)
Nakayama et al. (2010)	United States (Illinois; Iowa; Minnesota; Missouri; Wisconsin)	Eighty-eight sampling sites collected between March and August 2008 from tributaries and streams in the Upper Mississippi River Basin and a portion of the Missouri River Basin. Samples were collected by the Minnesota Pollution Agency, Wisconsin Department of Natural Resources, Illinois Environmental Protection Agency, and U.S. EPA Region 7 Water Quality Monitoring Team. Each agency selected sampling sites with the intention of providing preliminary PFC data to the individual regions. Sampling sites included locations adjacent to chemical manufacturers, downstream of WWTPs receiving waste from those types of manufacturers, and near an airport with historic use of firefighting foams.	n = 173, DF 87%, median (range) = 0.71 (ND–72.9) ng/L (LOD = 0.02 ng/L) *ND data points were substituted with LOD/sqrt(2) = 0.014 ng/L
Nakayama et al. (2007)	United States (North Carolina)	Eighty sampling sites in river basin during spring 2006. The sites were selected to reflect water quality throughout the basin. Possible sources of PFCs include use of firefighting foam from Fort Bragg and Pope Air Force Base, metal-plating facilities, textile, and paper production, and other industries.	n = 100, DF 89.9%, mean, GM, median (range) = 33.6, 9.73, 5.70 (<LOQ–194) ng/L (LOQ = 1 ng/L, LOD = 0.05 ng/L) *Values below the LOQ were excluded from the calculation of the mean and GM
Lindstrom et al. (2011)	United States (Decatur, Alabama)	Samples collected in February 2009 from ponds and streams located on farms with historical land application of PFC-contaminated biosolids to local	n = 32, DF (frequency of quantification)[b] 28%, range = <LOQ–286 ng/L (LOQ = 10 ng/L)

Study	Location	Site Details	Results
		agricultural fields between 1995 and 2008. Biosolids obtained from local municipal WWTP where sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers, fluorocarbon fibers, polymers, polymer films, and resins.	
Lasier et al. (2011)	United States (Georgia)	Upstream (sites 1 and 2) and downstream (sites 3–8) of a land application site were sampled along the Conasauga, Oostanaula, and Coosa Rivers during summer 2008, where effluents from carpet manufacturers (suspected of producing wastewaters containing perfluorinated chemicals) are processed at a WWTP and the treated WWTP effluent is sprayed onto the site. Additionally, site 2 was downstream of a local airport and site 4 was downstream of a manufacturing facility for latex and polyurethane backing material.	Upstream: Sites 1 and 2: DF 0% Downstream: Site 3: DF NR, mean = 35 ng/L Site 4: DF NR, mean = 44 ng/L Site 5: DF NR, mean = 17 ng/L Site 6: DF NR, mean = 21 ng/L Site 7: DF NR, mean = 20 ng/L Site 8: DF NR, mean = 21 ng/L (MDL = 0.005 ng/g, LOQ = 0.010 ng/g) *Half of the MDL was used when measured concentrations were below the MDL *Concentrations measured in triplicate samples
Konwick et al. (2008)	United States (Georgia)	Samples collected in March 2006 from the Conasauga River near Dalton, a major carpet manufacturing city, where there is high use of PFAAs in the carpet industry. Four sites on the Conasauga River were sampled: one location upstream, two downstream, and one at the site of a land application system where treated wastewater (approximately 87% industrial source) is sprayed. One freshwater site on the Altamaha River, a reference site away from Dalton, was sampled in January 2005. Four ponds and streams in Dalton were also sampled in January 2005.	Conasauga River: Site 1 (upstream): n = 5, DF[a] 100%, mean (range) = 32.8 (12.3–75.4) mg/L Site 2 (at site): n = 5, DF[a] 100%, mean (range) = 201.6 (136–248) mg/L Site 3 (downstream): n = 5, DF[a] 100%, mean (range) = 369 (280–456) mg/L Site 4 (downstream): n = 5, DF[a] 100%, mean (range) = 284 (190–366) mg/L Altamaha River: Site 1 (freshwater): n = 3, DF[a] 33%, range = <0.6–0.6 ng/L Dalton streams/ponds: Site 1: n = 2, DF[a] 100%, range = 11.1–12.2 ng/L Site 2: n = 2, DF[a] 100%, range = 40.6–41.0 ng/L Site 3: n = 2, DF[a] 100%, range = 4.8–6.3 ng/L

Study	Location	Site Details	Results
			Site 4: n = 2, DF[a] 100%, range = 2.1–2.5 ng/L (LOD = 0.6 ng/L)
Boone et al. (2019)	United States (unspecified)	Twenty-two surface waters (16 rivers and 6 lakes/reservoirs) used as source waters for 22 DWTPs, collected in 2010−2012; some locations with known or suspected sources of wastewater in the source water, but study did not differentiate which locations had known or suspected sources.	n = 22, DF[a] 100%, mean[a] (range) = 2.93 (0.117–41.4) ng/L (LCMRL = 0.094 ng/L)
Subedi et al. (2015)	United States (New York)	Lake water along the shoreline of residences that use an enhanced treatment unit for onsite wastewater treatment; samples were collected ~40 ft from the lakeshore about 2 ft below surface. Sampling occurred August–September 2012 (three sampling events) and May–September 2013 (four sampling events). Wastewater effluents identified as source of contamination.	n = 28, DF[a] 57%, mean, median (range) = 0.36, 0.26 (ND–1.21) ng/L (LOQ = 0.2 ng/L) *Data points <LOQ were substituted with ½ LOQ and NDs were substituted with zero
Appleman et al. (2014)	United States (Wisconsin; Oklahoma; Alaska; Alabama; Colorado; Ohio; Nevada; New Jersey)	Surface water source water for 11 DWTPs, sampled August 2011 to May 2012; majority of the utilities selected because they were either known from previous monitoring or expected based on their source waters to contain detectable PFAS (i.e., impacted by upstream wastewater effluent discharge). Each site was sampled between one and four times.	n = 25, DF[a] 56%, range = <0.5–5.7 ng/L (Method reporting limit = 0.5 ng/L)
Procopio et al. (2017)	United States (New Jersey)	Surface water from the Metedeconk River, where there was suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs. Majority of samples collected from a 4.0 km (2.5 mile) reach of the South Branch Metedeconk River, although samples from the North Branch were also collected in sampling event 1. Samples were collected in eight sampling events in September and December 2011; February, July, September, and December 2012; June and August 2013; and June and July 2014.	n = 56, DF 0% (Minimum reporting limit = 5 ng/L)
Zhang et al. (2016)	United States (Rhode Island; New York)	River and creek samples from 22 sites in Rhode Island collected in June 2014 and from six sites in the NY Metropolitan Area collected in October 2014. Both urban and rural locations were sampled.	Overall: n = 28, DF[a] 100%, mean[a] (range) = 1.914 (0.104–13.986) ng/L

Study	Location	Site Details	Results
			Rhode Island: n = 22, DF[a] 100%, mean[a] (range) = 1.868 (0.104–13.986) ng/L Urban: n = 10, DF[a] 100%, mean[a] (range) = 2.576 (0.308–13.986) ng/L Rural: n = 12, DF[a] 100%, mean[a] (range) = 1.278 (0.104–7.235) ng/L New York Metropolitan Area (all urban sites): n = 6, DF[a] 100%, mean[a] (range) = 2.084 (0.151–6.658) ng/L (LOD = 0.04 ng/L)
Boone et al. (2014)	United States (New Orleans, Louisiana)	Surface samples from the Mississippi River collected in June 2009 when the river was at a high flow level (8.32 ft) and in September 2010 when the river was at a low flow level (2.95 ft).	Low flow (2.95 ft): mean based on a primary and duplicate sample = 1.800 ng/L High flow (8.32 ft): mean based on four replicates = 3.30 ng/L (DL = 0.047 ng/L, LCMRL = 0.110 ng/L)
Quiñones and Snyder (2009)	United States (Arizona; Nevada)	Samples collected in 2008 from three sites in Boulder Basin, one site in Hoover Dam, and two sites from the lower Colorado River. PFC occurrence had not been previously determined or reported for these sites.	n = 40, DF NR (Method RL = 1.0 ng/L) *Mean values at all sites were <Method RL; Figure 3 in the paper shows the maximum value at one lower Colorado River site is >Method reporting limit
Kim and Kannan (2007)	United States (Albany, New York)	Samples collected from two urban lakes—Washington Park and Rensselaer Lake—during five sampling trips from February–November 2006. Both lakes are located in downtown Albany and receive surface runoff from nearby roadways and residential areas during stormwater runoff.	Total: n = 11, DF 81.8%, mean, median (range) = 1.70, 1.63 (ND–3.51) ng/L Washington Park Lake: n = 6, DF NR, mean, median (range) = 1.99, 2.14 (<LOQ–3.51) ng/L Rensselaer Lake: n = 5, DF NR, mean, median (range) = 1.35, 1.45 (ND–2.73) ng/L (LOQ = 0.25 ng/L) *Non-detects were set to zero; values below the LOQ were set to ½ LOQ
Canada			
Lescord et al. (2015)	Canada (Cornwallis Island, Nunavut)	Six lakes (Meretta, Resolute, Char, Small, North, and 9 Mile) located on Cornwallis Island and near the Inuit community of Resolute Bay were sampled weekly in July to August in 2010 and biweekly in July to August 2011. Two lakes (Meretta and Resolute) are	Meretta: n = 5, DF >0% Resolute: n = 5, DF >0% *Concentrations and summary statistics were not reported in tables or text; Figure 2 shows non-zero concentrations of PFNA in Meretta

Study	Location	Site Details	Results
		approximately 0.5 km downstream from a local airport, where wastewater from the airport and military base was discharged with little treatment into the Meretta catchment from 1949 to 1998.	and Resolute. Concentrations in Char, Small, North, and 9 Mile lakes are unclear (method detection limit = 0.0018 ng/L)
Yeung et al. (2017)	Canada (Ontario)	River water samples were collected from Mimico Creek and Rouge River in November 2014 and analyzed using two methods. Reference method used ultra performance liquid chromatograph and a newly developed method used ultra performance convergence chromatograph for separation.	Results presented as reference method, new method: Mimico: n = 1, point = <2, <5 ng/L Rouge: n = 1, point = <2, <5 ng/L (MLOQ for reference method not reported; MLOQ for new method reported in Table S3 as 200 ng/L but based on Table S7, this should likely be 2 ng/L) *Results in Table S7 are presented in ng/L but based on a comparison to Figure 4, Table S7 should be in ng/mL
Veillette et al. (2012)	Canada (Ellesmere Island, Nunavut)	Lake catchment area located on the northwest coast of the island. Surface water was collected from the center of the lake, the littoral zone (30 m from the delta), the delta, and lake inflow and outflow in July 2007, May 2008, and August 2008. Samples were collected at depths of 2 m (underneath the ice cover), 10 m (the bottom of the mixed layer), and 32 m (in the monimolimnion).	n = 11, DF[a] 100%, mean (range) = 0.118 (0.057–0.192) ng/L (method detection limit = 0.009 ng/L)
Europe			
Bach et al. (2017)	France (southern)	Grab water samples were collected from six locations along the shore of a river in April, July, October, and December 2013. The river selected for the study receives effluent at three points along the river from an industrial site where two facilities produce fluoropolymers. The first facility has been active since the 1960s, with production including PTFE synthesis from the beginning of the 1960s to 1985 with PFOA as a processing aid; more recently, PVDF has been synthesized since the early 1970s with fluorotelomer sulfonic acid (6:2 FTSA) or PFNA as a processing aid. The second facility, established in 2002, produced fluoropolymers with PFOA as a processing aid until 2008 when it was replaced with PFHxA. Samples were collected starting ~1.3 km upstream from the industrial site and covered ~15 km of the river.	Upstream: Sampling point #1: n = 1, point = <4 ng/L for April, July, October, and December 2013 Downstream: Sampling point #2: n = 1, point = 209, 2,637, 15, and 12 ng/L for April, July, October, and December 2013 Sampling point #3: n = 1, point = <4, <4, <4, and <4 ng/L for April, July, October, and December 2013 Sampling point #4: n = 1, point = 9, 42, <4, and <4 ng/L for April, July, October, and December 2013

Study	Location	Site Details	Results
		Samples (point #1 to #6) were collected from upstream to downstream.	Sampling point #5: n = 1, point = <4, <4, <4, and <4 ng/L for April, July, October, and December 2013 Sampling point #6: n = 1, point = <4, 87, <4, and <4 ng/L for April, July, October, and December 2013 (LOQ = 4 ng/L)
Boiteux et al. (2017)	France (northern)	Grab water samples were collected from seven locations along a river in May, July, October, and December 2013. The river selected for the study receives wastewater from an industrial WWTP that treats raw sewage coming from a facility that manufactures fluorotelomer-based products and side-chain-fluorinated polymers used in firefighting foams and stain repellents. Samples were collected starting ~1.2 km upstream of the WWTP discharge and encompassed ~65 km of the river. Samples were collected from upstream to downstream.	Upstream: Sampling point #1: n = 1, DF 0% for May, July, October, and December 2013 Downstream: Sampling points #3, 4, 5, 7, 9, 11: n = 1, DF 0% for May, July, October, and December 2013 (LOQ = 4 ng/L) *DF represents frequency of quantification
Gebbink et al. (2017)	The Netherlands (Dordecht)	River water samples collected in October 2016 at sites downstream (R1−R13) and upstream (R14−R16) of the Dordrecht fluorochemical production plant. Samples (R17−R18) were also collected from different waterbodies at control sites.	Control sites: n = 2, DF[a] (frequency of quantification) 100%, mean[a] (range) = 0.9 (0.8–1.0) ng/L Upstream: n = 3, DF[a] (frequency of quantification) 100%, mean[a] (range) = 0.75 (0.54–0.92) ng/L Downstream: n = 13, DF[a] (frequency of quantification) 100%, mean[a] (range) = 0.67 (0.49–1.0) ng/L (minimum quantification level = 0.03 ng/L)
Valsecchi et al. (2015)	Italy (River Basins Po, Brenta, Adige, Tevere, and Arno)	Five river basins were sampled between 2008 and 2013. Two river basins (Po and Brenta) receive discharges from two chemical plants that produce fluorinated polymers and intermediates; two river basins (Tevere and Adige) are not impacted by relevant industrial activities; and one river basin (Arno) has textile and tannery districts located along parts of the river. In total, 20 rivers were sampled at their basin closure stations. Rivers Arno, Tevere, and Po were also sampled along the course of the river.	Po: n = 105, DF[a] 64%, range = <LOD–70.3 ng/L Brenta: n = 5, DF[a] 40%, range = <LOD–1.4 ng/L Adige: n = 5, DF 0% Tevere: n = 7, DF 0% Arno: n = 19, DF[a] 95%, range = <LOD–30.1 ng/L (LOD = 0.5 ng/L)

Study	Location	Site Details	Results
Skaar et al. (2019)	Norway (Ny-Ålesund; Lake Linnévatnet area)	Freshwater samples were collected from Ny-Ålesund (research facility) in June 2016 and from the Lake Linnévatnet area (background site) in March 2014 and from April to June 2015. Surface water in Ny-Ålesund was contaminated by a local firefighting training site. Lake Linnévatnet receives water from meltwater of the adjacent glaciers and has few potential pollution sources.	Ny-Ålesund: Background: n = 7, DF 0% Contaminated: n = 3, DF[b] 67%, range = <0.02–1.81 ng/L Lake Linnévatnet: Background: n = 20, DF[b] 65%, range = <0.03–0.16 ng/L (LOD = 0.021 ng/L; LOQ = 0.085 ng/L)
Mussabek et al. (2019)	Sweden (Luleå)	Samples from a man-made lake and pond approximately 500 m southwest from a firefighting training facility at the Norrbotten Air Force Wing collected in October 2015. The training facility has been active since 1941 and has used PFAS-containing AFFFs in the last decades. The lake and pond lie above a groundwater reservoir with high permeable soil and were selected because they are isolated water bodies receiving PFAS contamination and can potentially impact groundwater.	Lake: n = 2, DF NR, mean = <0.5 ng/L Pond: n = 2, DF NR, mean = <0.5 ng/L (LOD = 0.5 ng/L)
Gobelius et al. (2018)	Sweden (national)	Sampling conducted between May and August 2015, with the majority in July and a few samples in September and November 2015. Samples were collected in 21 regional counties by the County Administration Boards. Sampling locations selected based on potential vicinity of PFAS hot spots (i.e., fire training sites, unspecific industry, sewage treatment plant effluent, landfill/waste disposal, skiing, and urban areas) and/or importance as a drinking water source. Sample numbers varied for each county and sampling sites were spread unevenly across Sweden. Surface water samples collected approximately 10 cm below the water surface.	n = 281, DF[a] 89%, range = <0.08–26 ng/L (MDL = 0.084 ng/L) *Two types of water (i.e., surface water and recipient water [surface water]) included
Dauchy et al. (2017)	France (unspecified)	Samples collected in the vicinity of three sites (B, C, D) where fluorosurfactant-based foams are or were being heavily used. Site B is an international civilian airport built in 1974. The exact location of the training area, frequency of training sessions, and history of firefighting training activities are unknown. In November 2014, surface water samples were collected in the only river running alongside the airport.	Site B: n = 5, DF 0% Site C: n = 9, DF 0% Site D: n = 2, DF 0% (LOQ = 4 ng/L)

Study	Location	Site Details	Results
		Downstream from the airport, this river joins two other rivers, which were also sampled. Site C is a military airport, with the exact location of the training area, frequency of the training sessions, and history of the firefighting training activities unknown. In April 2014, surface water samples were collected in several rivers surrounding the military base. Site D is a training center for firefighters. From 1969 to 1984, the site was an oil refinery. Starting in 1987, the site became a training area for firefighters, with exercises carried out directly on the soil. From the 1990s, some exercise areas were covered with concrete. In November 2014, two surface water samples were collected from the river receiving effluent from a WWTP at the site, one upstream and one downstream of the discharge pipe.	
Ciofi et al. (2018)	Italy (Tuscany)	Surface water samples were collected at 13 locations. Sampling year was not reported. SW-1: Arno river before the "Canale Maestro della Chiana" (Arezzo), receiving agricultural runoff and untreated urban wastewater SW-2: Arno river after the "Canale Maestro della Chiana" (Arezzo) SW-3: Arno river before entering in Florence SW-4: Arno river after the discharge of the Florence WWTP SW-5: Arno river after the confluence of the Bisenzio river SW-6: Arno river after the city of Empoli (Florence) SW-7: Arno river after receiving the WWTP effluent from the leather industrial district of Santa Croce (Pisa) SW-8: Arno river in the proximity of the mouth (Pisa) SW-9: Bisenzio river before the confluence with Arno river (Florence) SW-10: Serchio river in the proximity of the mouth (Lucca) SW-11: East area of the coastal lake "Massaciuccoli" (Lucca)	SW-1: n = 1, point = <0.23 ng/L SW-2: n = 1, point = <0.21 ng/L SW-3: n = 1, point = <0.25 ng/L SW-4: n = 1, point = <0.21 ng/L SW-5: n = 1, point = <0.22 ng/L SW-6: n = 1, point = <0.22 ng/L SW-7: n = 1, point = <0.20 ng/L SW-8: n = 1, point = <0.25 ng/L SW-9: n = 1, point = 2.7 ng/L SW-10: n = 1, point = <0.23 ng/L SW-11: n = 1, point = <0.19 ng/L SW-12: n = 1, point = <0.70 ng/L SW-13: n = 1, point = <0.27 ng/L (MDL = 0.19–0.27 ng/L; MQL = 0.70 ng/L) *MDL/MQL varied by sample. MDL provided for 11 of 13 samples; minimum quantitation level provided for 1 of 13 samples

Study	Location	Site Details	Results
		SW-12: West area of the coastal lake "Massaciuccoli" (Lucca) SW-13: Central area of the artificial lake "Bilancino" (Florence)	
Munoz et al. (2018)	France (Marnay-sur-Sein; Bougival; Triel-sur-Seine)	Surface water along the Seine River was collected during four sampling campaigns between September 2011 and December 2012, each conducted in a different season. For each campaign, two to four samples were collected over a one-month period. Three sampling sites were investigated: Marnay-sur-Sein, located 200 km upstream from Paris, was selected as a reference site, non-affected by the Greater Paris region; Bougival, situated 40 km downstream from Paris, was chosen to investigate the impact of Greater Paris on PFAS levels; Triel-sur-Seine, another 40 km further downstream, was selected to assess the global influence of the Paris urban area, including other inputs such as WWTPs.	n = 36, DF 97%, range = ND–2.0 ng/L (LOD = 0.02–0.3 ng/L for all PFAS) *PFNA concentrations were not reported in text or table by site but relative abundance by site is available in Figure 1
Wilkinson et al. (2017)	England (Greater London and southern England)	Three rivers selected due to their accessibility, receiving only STW effluent outfalls (i.e., no confluence with another major river in the study area) and accessibility of river headwater sampling for the Hogsmill and Blackwater Rivers. Each river sampled received inputs from at least one STW. Three STWs discharge into the Blackwater River, one STW discharges into the Hogsmill River, and one STW discharges to Chertsey Bourne River. Headwaters were evaluated for the Hogswill and Blackwater Rivers. Water samples were collected 50 m upstream and 250 m and 1,000 m downstream from STW effluent outfalls. In total, samples were collected on 3–4 separate occasions from 23 sites. Sampling dates were not reported.	Headwaters: n = 6, DF NR, mean = 2.75 ng/L Upstream: n = 19, DF NR, mean = 16.7 ng/L Downstream 250 m: n = 19, DF NR, mean = 32.5 ng/L Downstream 1,000 m: n = 19, DF NR, mean = 23.9 ng/L (LOD = 0.23 ng/L; LOQ = 0.75 ng/L)
Lorenzo et al. (2015)	Spain (Guadalquivir River Basin; Ebro River Basin)	Surface water was collected from the Guadalquivir River and its main tributaries and from the Ebro River and its main tributaries in October 2010. Guadalquivir sampling locations included downstream of WWTPs, near industrial areas, near a military camp, or through major cities; Ebro sampling locations included nearby ski resorts and downstream of WWTP and industrial areas.	Guadalquivir: n = 24, DF 8%, mean (range) = 5.1 (6.8–116.1) ng/L Ebro: n = 24, DF 8%, mean (range) = 0.5 (4.8–7.9) ng/L *Minimum reported is the lowest amount quantified

Study	Location	Site Details	Results
			*Mean was calculated with not detected concentrations as zeros (MQL = 0.4 ng/L)
Zhao et al. (2015)	Germany (Elbe River)	Four sampling campaigns conducted in February, April, August, and October 2011 to represent the four seasons. Freshwater samples (sites E619 to E689 with salinity <1 PSU) were collected at nine locations in the river Elbe. Some sampling sites were near Hamburg city and experienced occasional discharge of wastewater from industrial plants,	February: n = 8, DF[a] 100%, mean[a] (range) = 0.23 (0.16–0.43) ng/L April: n = 9, DF[a] 100%, mean[a] (range) = 0.18 (0.16–0.23) ng/L August: n = 9, DF[a] 100%, mean[a] (range) = 0.24 (0.19–0.36) ng/L October: n = 8, DF[a] 100%, mean[a] (range) = 0.15 (0.12–0.18) ng/L (MDL = 0.03 ng/L)
Boiteux et al. (2012)	France (national)	Raw water from rivers used as source water for DWTPs. Sites distributed across 100 French department to represent ~20% of the national water supply flow; samples collected during two sampling campaigns in July–September 2009 (first campaign) and June 2010 (second campaign – focused on sites from first sampling campaign that had PFC levels >LOQ). Some sites possibly affected by commercial/industrial releases.	Overall: n = 135, DF (frequency of quantitation) 2%, maximum = 52 ng/L *1st Sampling Campaign* n = 99, DF 5%, mean, median (maximum) = <1, <1 (4) ng/L *2nd Sampling Campaign* n = 36, results not reported (LOD = 1.3 ng/L, LOQ = 4 ng/L)
Eschauzier et al. (2012)	The Netherlands (Amsterdam)	Intake water from the Lek canal (n = 2) was collected in January and September 2010 to determine the behavior of PFAAs during the drinking water treatment processes. The Lek canal, a tributary of the river Rhine, is the source of drinking water for the city of Amsterdam and is downstream of an industrial point source in the German part of the Lower Rhine.	n = 2, DF[a] 100%, mean (range) = 0.6 (0.5–0.8) ng/L (LOQ = 0.24 ng/L)
Kovarova et al. (2012)	Czech Republic (Brno)	Seven locations in the Svitava and Svratka Rivers upstream and downstream of Brno, a city with highly developed chemical, engineering, textile, and food-processing industries. A sampler was installed at each site for 30 days twice a year (May and September 2008). Due to technical problems, samples were produced from only four of seven sites in May and from five of seven sites in September.	May: n = 4, DF[a] 100%, mean[a] (range) = 2,735 (240–5,700) ng/L September: n = 5, DF[a] 100%, mean[a] (range) = 2,690 (170–8,100) ng/L (LOD not reported)
Llorca et al. (2012)	Germany (Hesse), Spain (national)	Forty-eight surface river waters were sampled in 2010–2012 (24 from Spain and 24 from Germany).	Germany: n = 24, DF 0%

Study	Location	Site Details	Results
		Samples from Germany were collected from agriculturally or industrially influenced streams. Samples from Spain were collected from the Xúquer River Basin, Llobregat River Basin, and Ebro River Basin.	Spain: n = 24, DF 13%, mean, median (range) = 26, 20 (0.03–52) ng/L (MLOQ = 0.03 ng/L) *MLOQ reported above is from Table 3; Table 2 reports MLOD = 1.9 ng/L and MLOQ = 6.3 ng/L
Labadie and Chevreuil (2011)	France (Paris)	Samples collected weekly in January–May 2010 in an urban stretch of the River Seine at the Austerlitz Quay, downtown Paris during a flood cycle. The sampling station is under the influence of two major WWTPs and two major combined sewer overflow outfalls.	n = 16, DF 100%, mean, median (range) = 0.5, 0.5 (0.1–1.2) ng/L (LOQ = 0.17 ng/L)
Möller et al. (2010)	Germany (Rhine River watershed)	Raw freshwater samples collected in September–October 2008 along the River Rhine (stations 1–36) and major tributaries of the River Rhine (e.g., Rivers Neckar, Main, Rhur, stations 37–48). Along the River Rhine, samples were taken upstream and downstream of Leverkusen, where effluent of a WWTP treating industrial wastewater was discharged. All samples taken at a water depth ≤1 m.	n = 48, DF NR, authors noted that PFNA was quantified but results not provided (LOD = 0.014–1.60 ng/L for all PFAS)
Rostkowski et al. (2009)	Poland (national)	Inland surface water samples were collected at 12 locations in the southern part of Poland and 14 locations in the northern part of Poland in October and December 2004. Inland surface waters included rivers, lakes, and streams. The northern locations flowed through forested, agricultural, and rural areas; these areas are considered unpolluted with industrial chemicals. Some southern locations were near chemical industrial activities.	North: DF[a] 57%, range = <0.1–0.6 ng/L South: n = 11, DF[a] 36%, range = <0.1–0.6 ng/L (LOQ = 0.1–0.5 ng/L)
Barreca et al. (2020)	Italy (Lombardia Region)	Fifty-two surface water sampling stations (rivers and streams) throughout the region. Samples collected in 2018.	n = 286, DF[a] 6% (LOQ = 1 ng/L)
Loos et al. (2017)	Austria, Bulgaria, Croatia, Moldova, Romania, Serbia, Slovakia (Danube River and tributaries)	Samples were collected in August–September 2013 from 68 sites along a 2,581 km-stretch of the Danube River, with 14 of the sites in the mouths of tributaries or side arms. Three additional samples were also collected between the Iskar and Olt tributaries, the Olt River, and between the Siret and Prut tributaries. The investigated tributary rivers were the Morava (Austria/Slovakia), the Vah (Slovakia), the Drava	n = 71, DF 79%, mean, median (range) = 1.2, 1.1 (<LOQ–3.3) ng/L *Results <LOQ were replaced by zero (LOD = 0.29 ng/L, LOQ = 0.66 ng/L)

Study	Location	Site Details	Results
		(Croatia), the Tisa (Serbia), the Sava (Serbia), the Velika Morava (Serbia), the Timok (Serbia/Bulgaria), the Iskar (Bulgaria), the Olt (Romania), the Jantra (Bulgaria), the Russenski Lom (Bulgaria), the Arges (Romania), the Siret (Romania) and the Prut (Romania/Moldavia). Some sampling locations were downstream of major cities.	
Shafique et al. (2017)	Germany (River Elster; River Pleiße; River Saale; and River Elbe)	Surface water samples were collected from the River Elster, River Pleiße, River Saale, and River Elbe at the start of 2015.	Elster: n = 4, DF NR, mean = 0.96 ng/L Pleiße: n = 2, DF NR, mean = 1.65 ng/L Saale (Site A): n = 10, DF NR, mean = 6.57 ng/L Saale (Site B): n = 10, DF NR, mean = 9.89 ng/L Saale (Site C): n = 10, DF NR, mean = 0.83 ng/L Elbe: n = 2, DF NR, mean = 0.91 ng/L (MDL = 0.07 ng/L) *Values extracted from SI, which provides a more detailed breakdown of sites compared to that reported in the main text (where Elster and Pleiße sites were combined and Saale sites were combined)
Eriksson et al. (2013)	Denmark (Faroe Islands)	Grab samples collected in April–May 2012 from Lakes Leitisvatn, Havnardal, Kornvatn, and Á Mýranar.	Leitisvatn: n = 1, point = 0.16 ng/L Havnadal Lake: n = 1, point = 0.14 ng/L Kornvatn Lake: n = 1, point = 0.22 ng/L Á Mýranar: n = 1, point = 0.13 ng/L (LOD = 0.028 ng/L)
Jurado-Sánchez et al. (2013)	Spain (southeast)	Raw water samples were collected from a reservoir used as the source for tap water production and analyzed using a newly developed analytical method. Samples were collected in triplicate once a week in six different months at the intake of two different DWTPs. River water samples were also collected. Authors did not report sampling details or sampling year.	DWTP 1 intake: n = 3, DF[a] 0% DWTP 2 intake: n = 3, DF[a] 0% River water: n = NR, DF 0% (LOD = 0.1 ng/L)

Study	Location	Site Details	Results
Villaverde-de-Sáa et al. (2012)	Spain (Santiago de Compostela; Pontevedra)	Surface water samples were collected from Sar river in Santiago de Compostela on January 2011 and from Lérez river in Pontevedra on March 2011.	n = 3, DF 0% (LOD = 0.6 ng/L)
Ahrens et al. (2009a)	Germany (Hamburg; Laurenburg)	Nine samples collected from the river Elbe in Hamburg city (sites 16-18) and from Laurenburg to Hamburg (sites 19-24) in August 2006. Samples were collected at a water depth of 1 m. Dissolved and particulate phases were analyzed for each of the water samples.	Hamburg: Dissolved: n = 3, DF[a] 100%, mean (range) = 1.8 (1.7–2.0) ng/L Particulate: n = 3, DF[a] 67%, mean (range) = 0.040 (ND–0.088) ng/L Laurenburg to Hamburg: Dissolved: n = 6, DF[a] 100%, mean (range) = 1.7 (0.6–2.1) ng/L Particulate: n = 6, DF[a] 100%, mean (range) = 0.044 (0.003–0.074) ng/L (MDL = 0.045 ng/L for dissolved phase; 0.005 ng/L for particulate phase)
Ahrens et al. (2009b)	Germany (Elbe River)	Samples collected at 53 to 122 km (sites 1 to 9) upstream of estuary mouth of Elbe River in June 2007. *Only locations with conductivity <1.5 mS/cm were assumed to be freshwater and extracted	Site 1 (122 km): n = NR, DF NR, mean = 0.73 ng/L Site 2 (118 km): n = NR, DF NR, mean = 1.1 ng/L Site 3 (115 km): n = NR, DF NR, mean = 0.7 ng/L Site 4 (103 km): n = NR, DF NR, mean = 0.8 ng/L Site 5 (90 km): n = NR, DF NR, mean = 0.6 ng/L Site 6 (80 km): n = NR, DF NR, mean = 0.9 ng/L Site 7 (74 km): n = NR, DF NR, mean = 0.7 ng/L Site 8 (64 km): n = NR, DF NR, mean = 0.6 ng/L Site 9 (53 km): n = NR, DF NR, mean = 0.7 ng/L (MDL = 0.04 ng/L; MQL = 0.12 ng/L)
Ericson et al. (2008b)	Spain (Tarragona Province)	River water samples collected from the Ebro (at two points, Garcia and Mora), Francolí, and Cortiella Rivers in February 2007.	Ebro site 1: n = 1, point = 0.44 ng/L Ebro site 2: n = 1, point = 0.36 ng/L Francolí: n = 1, point = 0.64 ng/L

Study	Location	Site Details	Results
			Cortiella: n = 1, point = <0.42 ng/L (LOD = 0.42 ng/L)
Multiple Continents			
Pan et al. (2018)	United States (Delaware River)	Samples were collected from the Delaware River between September and December 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Trenton, Bristol, Philadelphia, Chester, Delaware, Smyrna, and Frederica.	n = 12, DF[a] 100%, mean, median (range) = 2.51, 2.36 (0.76–4.81) ng/L (MDL = 0.02 ng/L)
	United Kingdom (Thames River)	Samples were collected from the Thames River in October 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Oxford and London.	n = 6, DF[a] 100%, mean, median (range) = 1.18, 1.17 (0.77–1.71) ng/L (MDL = 0.02 ng/L)
	Germany and The Netherlands (Rhine River)	Samples were collected from the Rhine River in December 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities in Germany included Offenbach, Frankfurt, Goarshausen, Rheinbrohl, Bonn, Cologne, Leverkusen, Dormagen, Düsseldorf, Duisburg, Wesel, and Emmerich. Cities in The Netherlands included Arnhem, Lienden, Duurstede, Nijmegen, Wamel, and Zaltbommel.	n = 20, DF[a] 100%, mean, median (range) = 0.42, 0.39 (0.09–0.67) ng/L (MDL = 0.02 ng/L)
	Sweden (Mälaren Lake)	Samples were collected from Mälaren Lake in September 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Örebro and Stockholm.	n = 10, DF[a] 100%, mean, median (range) = 0.54, 0.54 (0.24–0.76) ng/L (MDL = 0.02 ng/L)

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; DWTP = drinking water treatment plant; ND = not detected; ng/L = nanogram per liter; PFAA = perfluoroalkyl acid; PFAS = per- and polyfluoroalkyl substances; NR = not reported; LOD = limit of detection; LOQ = limit of quantification; LCMRL = lowest concentration minimum reporting level; WWTP = wastewater treatment plant.

[a] The DF and/or mean was calculated using point data. Means were calculated only when DF = 100%.

C.2. RSC for PFNA, Literature Search and Screening Methodology

The EPA applies an RSC to the RfD when calculating an MCLG based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (USEPA, 2000b). The EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. The EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000b). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (USEPA, 2000b). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (USEPA, 2000b). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively-derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (USEPA, 2000b). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies (USEPA, 2000b).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of

20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (USEPA, 2000b). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

C.2.1. Literature Search and Screening

In 2020, the EPA conducted a literature search to evaluate evidence for pathways of human exposure to eight PFAS chemicals (PFOA, PFOS, PFBA, PFBS, PFDA, perfluorohexanoic acid (PFHxA), PFHxS, and PFNA) (Holder et al., 2023). This search was not date limited and spanned the information collected across the Web of Science (WOS), PubMed, and ToxNet/ToxLine (now ProQuest) databases. The results of the PFNA literature search of publicly available sources are available through the EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2633.

The 2,408 literature search results for PFNA were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and nonhuman (i.e., those not identified as human) studies (Holder et al., 2023). Studies identified as human studies were further categorized into seven major PFAS pathways (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) as well as an additional category for Human Exposure Measures. Nonhuman studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill. Only studies published between 2003 and 2020 were considered. Application of the SWIFT-Review tags identified 1,359 peer-reviewed papers matching these criteria for PFNA.

Holder et al. (2023) screened the 1,359 papers to identify studies reporting measured occurrence of PFNA in human matrices and media commonly related to human exposure (human blood/serum/urine, drinking water, food, food contact materials, consumer products, indoor dust, indoor and ambient air, and soil). For this synthesis, additional screening was conducted to identify studies relevant to surface water (freshwater only) and groundwater using a keyword[8] search for water terms.

Following the Population, Exposure, Comparator, and Outcome (PECO) criteria outlined in Table C-3, the title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree whether a study should be included or excluded, a third reviewer was consulted to make a final decision. The title and abstract screening of Holder et al. (2023) and of this synthesis resulted in 679 unique studies being tagged as relevant (i.e., having data on occurrence of PFNA in exposure media of interest)

[8] Keyword list: water, aquifer, direct water, freshwater, fresh water, groundwater, ground water, indirect water, lake, meltwater, melt water, natural water, overland flow, recreation water, recreational water, river, riverine water, riverwater, river water, springwater, spring water, stream, surface water, total water, water supply

that were further screened with full-text review using the same inclusion criteria. After additional review of the evidence collected by Holder et al. (2023), 98 studies originally identified for other PFAS also contained information relevant to PFNA. Based on full-text review, 171 studies were identified as having relevant, extractable data for PFNA from the United States, Canada, or Europe for environmental media, not including studies with only human biomonitoring data. Of these 171 studies, 156 were identified from (Holder et al., 2023), where primary data were extracted into a comprehensive evidence database. Parameters of interest included: sampling dates and locations, numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics. Fifteen of the 171 studies were identified in this synthesis as containing primary data on only surface water and/or groundwater.

The evidence database of Holder et al. (2023) additionally identified 18 studies for which the main article was not available for review. As part of this synthesis, 17 of the 18 studies could be retrieved. An additional three peer-reviewed references were identified through gray literature sources that were included to supplement the search results. The combined 20 studies underwent full-text screening using the inclusion criteria in Table C-3. Based on full-text review, five studies were identified as relevant.

Table C-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults and/or children in the general population and populations in the vicinity of PFAS point sources from the United States, Canada, or Europe
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater[a], human blood/serum/urine, indoor air, landfill, sediment, soil, surface water[a] (freshwater), wastewater/biosolids/sludge
Comparator	Not applicable
Outcome	Measured concentrations of PFNA (or measured emissions from food packaging and consumer products only)

Notes: PFNA = perfluorononanoic acid.
[a] Surface water and groundwater were not included as relevant media in Holder et al. (2023). Studies were re-screened for these two media in this synthesis.

Using the screening results from the evidence database and this synthesis, a total of 176 studies were identified as relevant. Forty-seven of these contained information relevant to the United States and were summarized for this effort.

C.2.2. Additional Screening

The EPA also searched the following publicly available gray literature sources for information related to relative exposure of PFNA for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- ATSDR's *Toxicological Profiles;*
- CDC's national reports on human exposures to environmental chemicals;
- EPA's CompTox Chemicals Dashboard;

- EPA's fish tissue studies;
- EPA's Toxics Release Inventory;
- Relevant documents submitted under the Toxic Substances Control Act and relevant reports from the EPA's Office of Chemical Safety and Pollution Prevention;
- U.S. Food and Drug Administration's (FDA's) *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada;
- National Oceanic and Atmospheric Administration's (NOAA's) National Centers for Coastal Ocean Science data collections;
- National Science Foundation direct and indirect food and/or certified drinking water additives;
- *Throwaway Packaging, Forever Chemicals: European wide survey of PFAS in disposable food packaging and tableware* (Straková et al., 2021);
- PubChem compound summaries;
- Relevant sources identified in the relative source contribution discussions (Section 5) of the EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA)/Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water; and*
- Additional sources, as needed.

The EPA has included available information from these gray literature sources for PFNA relevant to its uses, chemical and physical properties, and for occurrence in ambient or indoor air, foods (including fish and shellfish), soil, dust, and consumer products. The EPA has also included available information specific to PFNA below on any regulations that may restrict PFNA levels in media (e.g., water quality standards, air quality standards, food tolerance levels).

C.3. Summary of Potential Exposure Sources of PFNA Other than Water

C.3.1. Dietary Sources

C.3.1.1. Seafood

PFNA was detected in 108 of 157 fish tissue composite samples collected during the EPA's National Lake Fish Tissue Study, with a maximum concentration of 9.70 ng/g and a 50th percentile concentration of 0.32 ng/g (Stahl et al., 2014). It was detected in one of 162 fish tissue composite samples collected during the EPA's 2008–2009 National Rivers and Streams Assessment (NRSA) at a concentration of 2.48 ng/g (Stahl et al., 2014). More recently, PFNA was detected in 135 of 349 fish tissue composite samples at concentrations ranging from 0.100 ng/g to 1.910 ng/g in the EPA's 2013–2014 NRSA (USEPA, 2020a). PFNA was also detected in 119 of 152 fish tissue composite samples at concentrations ranging from 0.12 ng/g to 9.32 ng/g in the EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study (USEPA, 2021g). In 2001, PFNA was detected at mean concentrations of 1.0 ng/g, 0.57 ng/g, 2.8 ng/g, 2.9 ng/g, and 1.1 ng/g (wet weight) in whole body homogenates of lake trout collected from Lake Superior, Lake Michigan, Lake Huron, Lake Erie and Lake Ontario, respectively (Furdui et al., 2007 as cited in ATSDR, 2021 and NCBI, 2022). In addition, PFNA was detected in lake trout at concentrations of 0.70 ng/g for Lake Superior, 1.4 ng/g for Lake Huron, 2.6 ng/g for

eastern Lake Erie, and 0.90 ng/g for Lake Ontario; PFNA was also detected at a concentration of 1.2 ng/g in walleye collected from western Lake Erie (ATSDR, 2021; De Silva et al., 2011). PFNA was detected in mixtures of whole fish from the Missouri River, the Mississippi River, and the Ohio River at concentrations of 0.43 ng/g, 0.78 ng/g, and 1.03 ng/g, respectively (ATSDR, 2021; Ye et al., 2008). Concentrations of PFNA ranged from 0.01 ng/g to 0.73 ng/g in capelin whole body samples, <0.09 ng/g to 1.3 ng/g in cod muscle samples, and 0.05 ng/g to 8.0 ng/g in salmon muscle samples collected from the Hudson Bay region of northeast Canada in 1999 to 2003 (NCBI, 2022b; Kelly et al., 2009). PFNA was not included in NOAA's National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). Burkhard (2021) identified 79 studies reporting BAFs for PFNA and calculated a median (standard deviation) bioaccumulation factor (BAF) in muscle tissue/fillet of 144.54 ± 6.03 L/kg wet weight (reported as a logBAF of 2.16 ± 0.78 L/kg).

Among the peer-reviewed studies identified, there was considerable variability in sampling and analysis methodologies. Seafood products analyzed comprised a broad range of fish, crustaceans, and mollusks which were locally caught, farmed, obtained from local seafood markets, and/or obtained from large grocery chains. There was also considerable variability in sample preparation which might affect the interpretation and comparability of results. Some studies included tinned or prepared seafood while others focused only on unprepared or raw items; some studies composited many organisms into each analysis sample while others focused on single organism measurements; and some studies analyzed whole organisms while others measured only muscle tissue, potentially excluding fatty tissues likely to be higher in PFNA. Results from these studies are provided in detail in Table C-4.

Five U.S.-based studies were identified that evaluated PFNA levels in seafood (Young et al., 2022; Chiesa et al., 2019; Byrne et al., 2017; Young et al., 2013; Schecter et al., 2010). Four of these studies analyzed fish purchased from stores and fish markets. PFNA was detected infrequently in samples reported in Chiesa et al. (2019), Schecter et al. (2010) and Young et al. (2013): one of 10 samples of striped bass (1.4 ng/g) and in one of nine samples of shrimp (1.2 ng/g), but not in samples of crab meat, catfish, clams, cod, flounder, pangasius, pollock, tuna (including canned), salmon, scallops, tilapia, canned sardines, or frozen fish sticks. No other fish types were sampled in these three studies, and other than canned tuna and sardines, none were analyzed as prepared for eating. Seafood samples reported in Young et al. (2022) reported detectable PFNA in five out of the eight types of seafood evaluated. These included canned clams, canned tuna, cod, crab meat, and pollock (fish fillets and frozen fish sticks). No PFNA was detected in salmon, tilapia or shrimp. Seafood packaging was also evaluated for PFAS coatings, and it was determined the packaging did not contribute to any PFAS concentrations observed in the study.

One study evaluated fish samples collected directly from rivers and lakes (Byrne et al., 2017). As part of a study to assess exposure to PFNA and other PFAS among residents of two remote Alaska Native villages on St. Lawrence Island, Byrne et al. (2017) measured PFAS concentrations in stickleback and Alaska blackfish, resident fish used as sentinel species to detect accumulation of PFAS in the local environment. Stickleback were collected from three locations: Suqitughneq (Suqi) River watershed (n = 9 composite samples), Tapisaggak (Tapi) River (n = 2 composite samples), and Troutman Lake (n = 3 composite samples). Blackfish were collected from the Suqi River (n = 29) but were not found in the other water bodies. Authors reported that

the Suqi River watershed was upstream and downstream of a formerly used defense site and Tapi River was east of a military site, however at the start of the study none of the sites were known to be contaminated with PFAS. The sample dates were not reported. PFNA was not detected in the blackfish samples but was detected in 100%, 56%, and 50% of stickleback samples from Troutman Lake, Suqi River, and Tapi River, respectively, with authors noting that PFNA was the most frequently detected PFAS in stickleback. PFNA concentrations ranged between 2.72 ng/g and 4.13 ng/g ww at Troutman Lake, from below the limit of detection (LOD) to 1.52 ng/g ww at Suqi River, and <LOD–0.78 ng/g ww at Tapi River (LOD not reported; limit of quantitation (LOQ) = 0.5–1 ng/g ww). The authors reported that total PFAS levels were "exceptionally high" in Troutman Lake and hypothesized that stickleback were exposed to a local PFAS source and that contaminant may be leaching from village and military landfills.

The remaining four studies purchased seafood from stores and fish markets (Young et al., 2022; Chiesa et al., 2019; Young et al., 2013; Young et al., 2012; Schecter et al., 2010). Young et al. (2013) assessed fish and shellfish collected in 2010–2012 from retail markets across the continental United States. Retail markets in California, Florida, Illinois, Mississippi, New Jersey, New York, Tennessee, Texas, and Washington, D.C., were represented. Authors selected the 10 most consumed fish and shellfish in the United States that were farm raised, wild caught, or had unknown origin. Among the crab meat, shrimp, striped bass, catfish, clams, cod, flounder, pangasius, pollock, tuna, salmon, scallops, and tilapia, PFNA was only detected in one of nine samples of shrimp at a concentration of 1.2 ng/g and 1 of 10 samples of striped bass at a concentration of 1.4 ng/g. Young et al. (2022) evaluated fish and shellfish collected from retail markets in the Washington, D.C., metropolitan area, from March 2021 through May 2022. Some clam samples were also purchased online. Eight seafood products were selected that represented those in the top 10 types of seafood consumed in the United States. Seafood products were farm raised, wild caught, or of unknown origin. PFNA was detected in all clam (n = 10) and crab (n = 11) samples with concentrations ranging up to 796 ng/kg for clams and 350 ng/kg for crabs. Samples of cod (40%, n = 10), pollock (20%, n = 10) and canned/pouch tuna (30%, n = 10) also had detectable PFNA with concentrations of 45–103 ng/kg, 100–106 ng/kg and 44–77 ng/kg, respectively. Salmon, shrimp and tilapia did not have detectable levels of PFNA (MDL = 30–39 ng/kg). Schecter et al. (2010) evaluated PFNA and other PFAS in seafood collected from five Dallas, Texas, grocery stores in 2009. The origin or source of seafood was not described. Seafood included canned sardines in water, canned tuna, fresh catfish fillet, cod, frozen fish sticks, salmon, and tilapia (n = 1 composite sample for each seafood type). PFNA was not detected in any of the seafood samples. Finally, in a multicontinental study, Chiesa et al. (2019) collected salmon from a wholesale fish market in Milan, Italy; the sampling year was not reported. Wild-caught salmon samples originated from the United States (n = 7), Canada (n = 15), and Scotland (n = 2), while farmed salmon samples originated from Norway (n = 25) and Scotland (n = 17). Among the salmon that originated from the United States – Pacific Ocean (Food and Agriculture Organization Area (FAO) 67 and 77), two species – *Oncorhynchus kisutch* and *Oncorhynchus keta* – were analyzed, with PFNA not detected in either species (LOQ = 0.005 ng/g). PFNA was also not detected in wild-caught salmon from Canada and Scotland.

Results for all of the identified non-U.S. studies are presented in detail in Table C-4 and are summarized here. Among the non-U.S. studies, there were ten which provided PFNA measurements in seafood products identified as originating from the region of study. These

samples were wild caught, farmed, or purchased from local seafood markets and included seven species of fish caught off the coast of Iceland (Jörundsdóttir et al., 2014); three species of fish caught in lakes in Norway (Hansen et al., 2016); four fish species caught in Canadian Rivers (Bhavsar et al., 2014); rainbow trout collected from fish farms along the Swedish Baltic Sea coast (Johansson et al., 2014); shrimp, squid, mussels, and seven species of fish caught or farmed on the Greek coast (Vassiliadou et al., 2015); two species of fish originating from the West coast of Greenland (Carlsson et al., 2014); five species of fish collected from a river and lake in Germany (Hölzer et al., 2011); eight species of fish collected from commercially and recreationally important fishing areas across the Baltic Sea, a large freshwater Lake, and four fish farming facilities in Finland (Koponen et al., 2015); cod caught along the Polish Baltic Sea Coast (Falandysz et al., 2006); and two species of wild caught fish and one species of farmed fish from Faroe island area of Denmark (Eriksson et al., 2013). Two studies reported sampling in freshwater sources, one of which was likely to be contaminated with PFAS due to proximity to an airport with known AFFF usage (Hansen et al., 2016), and the other which had nearby industrial activities and previous monitoring results finding PFAS contamination (Bhavsar et al., 2014), leading the authors to expect elevated PFAS concentrations in fish captured from these sites. In both cases detectable levels of PFNA were measured in all fish sampled (Hansen et al., 2016; Bhavsar et al., 2014). The highest reported PFNA contents from Hansen et al. (2016) was 2.39 ng/g ww in brown trout muscle tissue, while the highest reported PFNA contents reported in Bhavsar et al. (2014) was 0.374 ng/g ww in fried Lake Trout. Among other European sites, there were often no organisms sampled with measurable concentrations of PFNA or it was present only in some organisms sampled.

Several studies from Europe provided PFNA measurements for seafood products purchased from supermarkets and other retailers. These samples were not identified as originating from the region of study and the product origin was often unknown or undisclosed. These included at least four fish species purchased in Germany (Hölzer et al., 2011); marine and freshwater fish purchased in Southern France (Yamada et al., 2014); fish purchased in the center region of France (Rivière et al., 2019); five species of fish purchased in Belgium, France, the Netherlands, and Portugal (Barbosa et al., 2018); fresh and processed fish samples purchased from major grocery store chains in Sweden (Gebbink et al., 2015; Vestergren et al., 2012); fish purchased in the Netherlands (Noorlander et al., 2011); and a variety of seafood products purchased in Spain (Domingo et al., 2012; Jogsten et al., 2009; Ericson et al., 2008a). Results from European market studies were similar to those of U.S. studies, with PFNA detected infrequently among the samples. However, several of the European seafood studies analyzed composites of all seafood products sampled rather than individual organisms, thus the results are less precise than in the U.S. studies.

Table C-4. Summary of PFNA Occurrence in Seafood

Study	Location and Source	Seafood Type	Results
United States			
Byrne et al. (2017)	United States (Alaska) Stickleback collected from three locations on St. Lawrence Island: Suqitughneq (Suqi) River watershed (upstream and downstream of a formerly used defense site), Tapisaggak (Tapi) River (located approximately 5 km east of military site), and Troutman Lake, a coastal lake situated adjacent to the village of Gambell. Alaska blackfish collected from the Suqi River but were absent from the other water bodies. Sampling year not reported. No sites were known to be contaminated with PFASs at the initiation of the study.	Stickleback and Alaska blackfish	**Stickleback:** **Troutman Lake: n = 3*, DF[a] 100%, mean[a] (range) = 3.43 (2.72–4.13) ng/g ww** **Suqi River: n = 9*, DF[a] 56%, range = <LOD–1.52 ng/g ww** **Tapi River: n = 2*, DF[a] 50%, range = <LOD–0.78 ng/g ww** Blackfish: n = 29, DF 0% (LOQ = 0.5–1 ng/g ww for all PFAS) *Number of composite samples, each composed of ~10 stickleback fish
Young et al. (2013)	United States (California; Illinois; Mississippi; Tennessee; Florida; New Jersey; New York; Texas; Washington, D.C.) Fish and shellfish collected from retail markets in 11 areas across the continental United States from 2010–2012. The fish and shellfish included farm raised, wild caught, and unknown origin, as well as freshwater fish, saltwater fish, and euryhaline fish. Crab meat, clams, cod, flounder, pangasius, salmon, scallops, and tilapia purchased from Washington, D.C. Shrimp purchased from Orlando, Florida; Memphis, Tennessee; and Nashville, Tennessee. Striped bass purchased from New York, New York and Cherry Hill, New Jersey. Catfish purchased from Indianola, Mississippi; Dallas, Texas; Tampa, Florida; and Orlando, Florida. Pollock purchased from Huntington Beach, California. Tuna purchased from Chicago, Illinois.	Crab, shrimp, striped bass, catfish, clams, cod, flounder, pangasius, pollock, tuna (can and pouch), salmon, scallops (bay and sea), tilapia	**Shrimp: n = 9, DF[a] 11%, range = ND–1.2* ng/g** **Striped bass: n = 10, DF[a] 10%, range = ND–1.4* ng/g** Crab meat: n = 1, DF 0% Catfish: n = 13, DF 0% Clams: n = 1, DF 0% Cod: n = 1, DF 0% Flounder: n = 1, DF 0% Pangasius: n = 1, DF 0% Pollock: n = 1, DF 0% Tuna: n = 3, DF 0% Salmon: n = 2, DF 0% Scallops: n = 2, DF 0% Tilapia: n = 1, DF 0% (MDL = 0.60 ng/g for all seafood) *This value was above the MDL but below the LOQ; LOQ is estimated as 3x the MDL
Schecter et al. (2010)	United States (Texas) Seafood samples from five different grocery stores in Dallas, Texas were collected in 2009. Ten individual samples were collected for each food type and combined to form composite samples. The origin/source of the food samples were not reported.	Salmon, canned tuna, fresh catfish fillet, tilapia, cod, canned sardines, frozen fish sticks	Salmon: n = 1, DF 0% Canned tuna: n = 1, DF 0% Fresh catfish fillet: n = 1, DF 0% Tilapia: n = 1, DF 0% Cod: n = 1, DF 0% Canned sardines: n = 1, DF 0% Frozen fish sticks: n = 1, DF 0% (LOD not reported for any seafood type)

Study	Location and Source	Seafood Type	Results
			*n reflects number of composite samples, each composed of ~10 individual samples
Canada			
Bhavsar et al. (2014)	Canada (Ontario) Recreationally caught fish from four rivers – Credit River, Thames River, Niagara River, Welland River – in summer and fall of 2010 and 2011. Chinook salmon were caught from Credit River, common carp from Thames River, lake trout from Niagara River, and walleye from Welland River. Elevated PFASs concentrations were expected in the fish based on nearby industrial activities or previous monitoring work conducted by the Ontario Ministry of Environment. Raw fish were analyzed, as well as cooked fish using three different cooking methods (baking, broiling, and frying).	Raw and cooked fish (chinook salmon, common carp, lake trout, walleye)	**Chinook salmon:** **Raw: n = 5, DF NR, mean = 0.067 ng/g ww** **Baked: n = 5, DF NR, mean = 0.086 ng/g ww** **Broiled: n = 5, DF NR, mean = 0.083 ng/g ww** **Fried: n = 5, mean = 0.078 ng/g ww** **Common carp:** **Raw: n = 5, DF NR, mean = 0.092 ng/g ww** **Baked: n = 5, DF NR, mean = 0.099 ng/g ww** **Broiled: n = 5, mean = 0.105 ng/g ww** **Fried: n = 5, mean = 0.101 ng/g ww** **Lake trout:** **Raw: n = 4, DF NR, mean = 0.298 ng/g ww** **Baked: n = 4, DF NR, mean = 0.370 ng/g ww** **Broiled: n = 4, mean = 0.358 ng/g ww** **Fried: n = 4, mean = 0.374 ng/g ww** **Walleye:** **Raw: n = 5, DF NR, mean = 0.063 ng/g ww** **Baked: n = 5, DF NR, mean = 0.079 ng/g ww** **Broiled: n = 5, mean = 0.074 ng/g ww** **Fried: n = 5, mean = 0.067 ng/g ww** (LOQ not reported)
Europe			
Hansen et al. (2016)	Norway (Evenes; Skånland) Fish were sampled from Lake Langvatnet, Lake Lavangsvatnet, River Tårstadelva, and the reference Lake Strandvatnet. A civilian airport (location also shared with the Air Station of the Royal Norwegian Air Force) is situated on a ridge between Lake Langvatnet and Lake Lavangsvatnet. These waters are affected by PFAS due to AFFF emissions from the airport.Lake Lavangsvatnet drains into the river Tårstadelva and Lake Strandvatnet is ~15 km away from the airport with no connection to the airport runoff. Samples of the dorsolateral muscle were taken from 10 salmon, 10 anadromous brown trout, 12 stationary brown trout, and 3 European flounder by local fishermen and by personnel from Sweco, an environmental consulting company. The samples were collected in August and September 2014.	Brown trout, European flounder, salmon	**Brown trout (stationary)** **Lake Langvatnet: n = 6, DF[a] 100%, mean[a] (range) = 1.05 (0.11–2.39) ng/g ww** **Lake Lavangsvatnet: n = 5, DF[a] 100%, mean[a] (range) = 0.7 (0.10–1.23) ng/g ww** **Lake Strandvatnet (reference): n = 1, point = 0.10 ng/g ww** **Brown trout (anadromous)** **Lake Lavangsvatnet: n = 3, DF[a] 100%, mean (range) = 0.06 (0.02–0.15) ng/g ww** **River Tårstadelva: n = 5, DF[a] 80%, range = <LOD–0.02 ng/g ww** **Lake Strandvatnet (reference): n = 2, DF[a] 100%, mean (range) = 0.007 (0.004–0.01) ng/g ww** **European flounder (catadromous)**

Study	Location and Source	Seafood Type	Results
			Lake Lavangsvatnet: n = 3, DF[a] 100%, mean[a] (range) = 0.153 (0.10–0.19) ng/g ww **Salmon (anadromous)** **Lake Lavangsvatnet: n = 5, DF[a] 60%, range = <LOD–0.07 ng/g ww** **River Tårstadelva: n = 5, DF[a] 60%, range = <LOD–0.02 ng/g ww** (LOD = 0.014–0.224 ng/g for all PFAS)
Hölzer et al. (2011)	Germany Germany Fish from Lake Möhne and River Möhne were caught by electric fishing or net fishing between June 2006 and October 2008. The River Möhne was contaminated with PFCs mainly by the application of polluted soil conditioner on agricultural lands between 2004 and 2006, which then drained into tributaries of the river. Fish samples for food monitoring were collected from retail trade, wholesale trade, supermarkets, and producers. Sampling year not provided.	Perch, pike, eel, cisco, and roach from Lake Möhne /River Möhne Eel, trout, pike/perch, and other from trade/markets	Lake Möhne: Perch: n = 15, DF 0% Pike: n = 6, DF 0% Eel: n = 5, DF 0% Cisco: n = 8, DF 0% Roach: n = 10, DF 0% Food monitoring: Eel: n = 2, DF 0% Trout: n = 73, DF 0% Pike/perch: n = 8, DF 0% Other: n = 34, DF 0% (LOD = 2.5 ng/g, LOQ = 5.1 ng/g)
Koponen et al. (2015)	Finland (Baltic Sea, Vanhankaupunginlahti bay, Lake Päijänne) A total of 296 individual fish samples were collected in 2009–2010 from five commercially and recreationally important fishing area across the Finnish coast of the Baltic Sea (Oulu, Pori, Turku, Hanko, and Kotka), Helsinki Vanhankaupunginlahti bay, a large freshwater Lake Päijänne, and four fish farming facilities. Most of the individual samples were pooled, each pool consisting of 2–10 individuals. Baltic herring, pike-perch, perch, burbot, whitefish, salmon, and vendace were collected from the Baltic sea; perch and pike-perch were collected from Helsinki Vanhankaupunginlahti bay; perch was collected from Lake Päijänne. Whitefish and rainbow trout were farmed fish. The selection of fish species was mainly based on the significance of fish in the Finnish diet.	Baltic herring, pike-perch, perch, burbot, whitefish, salmon, vendace, whitefish, rainbow trout	Baltic Sea: **Baltic herring: n = 58, DF NR, range = <0.21–2.7 ng/g** **Pike-perch: n = 30, DF NR, range = <0.28–0.35 ng/g** **Perch: n = 25, DF NR, range = <0.21–0.83 ng/g** **Burbot: n = 49, DF NR, range = <0.20–1.5 ng/g** **Whitefish: n = 27, DF NR, range = <0.26–0.63 ng/g** Salmon: n = 44, DF 0% (<0.39 ng/g) **Vendace: n = 20, DF[a] 100%, range = 0.35–0.36 ng/g** Vanhankaupunginlahti bay: **Pike-perch: n = 6, DF NR, range = <0.21–0.33 ng/g** **Perch: n = 7, DF NR, range = <0.23–0.24 ng/g** Lake Päijänne: Perch: n = 10, DF 0% (<0.18 ng/g) Farmed fish: Whitefish: n = 10, DF 0% (<0.37 ng/g)

Study	Location and Source	Seafood Type	Results
			Rainbow trout: n = 10, DF 0% (<0.35 ng/g) (LOQ = 0.18–0.39 ng/g)
Jörundsdóttir et al. (2014)	Iceland Samples were collected by the Icelandic Marine Research Institute in March 2011 during their biannual scientific survey. Cod and anglerfish were caught south-west of Iceland, blue whiting was caught south-east of Iceland, and lumpfish and pollock were caught north-west of Iceland, while ling, plaice, and lemon sole were caught west of Iceland. Each fish sample consisted of a pooled sample from the entire edible part from ten individuals of the same species.	Anglerfish, Atlantic cod, blue whiting, lemon sole, ling, lumpfish, plaice, pollock	Anglerfish (n = 1), Atlantic cod (n = 2), blue whiting (n = 2), lemon sole (n = 1), ling (n = 1), lumpfish (n = 4), plaice (n = 1), pollock (n = 1): DF 0% (LOD = 0.10 ng/g) *n represents number of composite samples
Yamada et al. (2014)	France Marine fish sampled were selected based on the fish consumption habits of the population of four areas – La Rochelle in Gironde-Charente Maritime Sud, Le Havre in Normandy-Baie de Seine, Lorient in South Brittany, and Toulon in Mediterranean-Var. Five primary samples of fish were bought from the fish market and/or supermarket in each region for each species in January–April 2005. Freshwater fish sampled were selected based on the individual dietary consumption analysis of anglers or their family members of the ICAR-PCB study. Freshwater fish were collected in six major French rivers with each river divided into three or four section in 2008–2009. Half of the samples were composite samples.	Freshwater fish, fresh or frozen marine fish	Results presented for lower bound and upper bound if LB value different from UB value **Fresh and frozen marine fish:** **Total LB: n = 95, DF NR, mean (range) = 0.09 (0–0.27) ng/g ww** **Total UB: n = 95, DF NR, mean (range) = 0.11 (0.04–0.27) ng/g ww** Anchovy: n = 1, LB–UB = 0–0.06 ng/g ww Monkfish: n = 4, LB–UB = 0.09–0.11 ng/g ww Catshark: n = 4, LB–UB = 0.06–0.08 ng/g ww Cod: n = 4, LB–UB = 0.15 ng/g ww Common dab: n = 4, LB–UB = 0.09–0.1 ng/g ww Orange roughy: n = 3, LB–UB = 0.19 ng/g ww Plaice/witch: n = 2, LB–UB = 0.16 ng/g ww Goatfish: n = 3, LB–UB = 0.15–0.19 ng/g ww Grenadier: n = 4, LB–UB = 0.05–0.07 ng/g ww Gurnard: n = 1, LB–UB =0–0.04 ng/g ww Haddock: n = 2, LB–UB = 0.17 ng/g ww Hake: n = 4, LB–UB = 0.05–0.06 ng/g ww

Study	Location and Source	Seafood Type	Results
			Halibut: n = 4, LB–UB = 0.03–0.06 ng/g ww John Dory: n = 2, LB–UB = 0.08–0.1 ng/g ww Ling: n = 4, LB–UB = 0.03–0.07 ng/g ww Mackerel: n = 4, LB–UB = 0.03–0.06 ng/g ww Pollack: n = 3, LB–UB = 0.19 ng/g ww Pout: n = 1, LB–UB = 0.12 ng/g ww Ray: n = 4, LB–UB = 0.13 ng/g ww Saithe: n = 4, LB–UB =0.04–0.06 ng/g ww Salmon: n = 4, LB–UB = 0.05–0.07 ng/g ww Sardine: n = 4, LB–UB = 0–0.07 ng/g ww Scorpionfish: n = 1, LB–UB = 0.12 ng/g ww Seabass: n = 4, LB–UB = 0.21–0.24 ng/g ww Sea bream: n = 4, LB–UB = 0.07–0.09 ng/g ww Sole: n = 4, LB–UB = 0.27 ng/g ww Swordfish: n = 4, LB–UB = 0–0.06 ng/g ww Tuna: n = 4, LB–UB = 0–0.04 ng/g ww Whiting: n = 4, LB–UB = 0.07–0.1 ng/g ww **Freshwater fish:** Barbel: n = 5, LB–UB = 1.19–1.21 ng/g ww Bleak: n = 9, LB–UB = 0.01–0.03 ng/g ww Brown trout: n = 31, LB–UB = 0.08–0.13 ng/g ww Chub: n = 9, LB–UB = 0.68–0.7 ng/g ww Common carp: n = 7, LB–UB = 0.03–0.1 ng/g ww Common roach: n = 67, LB–UB = 0.34–0.38 ng/g ww Minnow: n = 1, LB–UB = 0.19 ng/g ww European eel: n = 137, LB–UB =0.12–0.26 ng/g ww European perch: n = 9, LB–UB = 0.12–0.13 ng/g ww Freshwater bream: n = 34, LB–UB =0.11–0.13 ng/g ww Gudgeon: n = 5, LB–UB = 0.21–0.24 ng/g ww Northern pike: n = 8, LB–UB = 0.04–0.07 ng/g ww White bream: n = 22, LB–UB = 0.06–0.11 ng/g ww Thicklip grey mullet: n = 6, LB–UB = 0.15 ng/g ww Wels catfish: n = 14, LB–UB = 0.08–0.1 ng/g ww Western vairone: n = 1, LB–UB = 0–0.08 ng/g ww Pike-perch: n = 22, LB–UB = 0.03–0.08 ng/g ww

Study	Location and Source	Seafood Type	Results
			(LOD = 0.007–0.95 ng/g for PFAAs other than PFOA and PFOS) *Lower bound (LB) scenario defined as values <LOD replaced with 0 *Upper bound (UB) scenario defined as values <LOD replaced with LOD
Eriksson et al. (2013)	Denmark (Faroe Islands) Wild fish (cod and saithe) were sampled from the Faroe Shelf area; cod were sampled in October and August 2011, while saithe were sampled in April 2012. Three farmed salmon samples were collected from different fjords in Faroe Islands, sampling year not reported.	Farmed salmon, wild-caught cod, wild-caught saithe	Cod 1, n = 1, point = <0.035 ng/g Cod 2, n = 1, point = <0.035 ng/g Saithe 1, n = 1, point = <0.035 ng/g Saithe 2, n = 1, point = <0.035 ng/g Salmon 1, n = 1, point = <0.035 ng/g Salmon 2, n = 1, point = <0.035 ng/g Salmon 3, n = 1, point = <0.035 ng/g (LOD = 0.035 ng/g) *n represents number of pooled samples, each combining muscle tissue from five fish
Falandysz et al. (2006)	Poland Cod samples were collected from the Gulf of Gdañsk in the Baltic Sea (south coast of Poland) in February 2003.	Cod *(Gadus morhua)*	**n = 18, DF NR, mean, median (range) = 1.2, 1.1 (0.1–2.1) ng/mL** (LOD not reported) *Values reported for animal whole blood samples
Rivière et al. (2019)	France Samples collected between July 2011 and July 2012 in the center region of France. Food items were selected based on the results of a national consumption survey to obtain a representative and general view of children's (0–3 years old) food consumption. All analyzed samples were formed of 12 subsamples of the same food and of equal weight. The fish were cooked according to the practices reported in the survey of practices.	Fish (unspecified)	n = 1, DF 0% (LOD = 0.0002–3.7 ng/g fw for all PFAS) *n represents number of composite samples
Barbosa et al. (2018)	Belgium, France, The Netherlands, Portugal Fish were collected from different markets based on the assumption that the fish species were frequently consumed in European Union countries and the fish species contained high levels of contaminants of emerging concern. Sampling year not reported. The following fish species (origin, market country) were included: *P. platessa:* Channel, Belgium *M. australis:* South America, Portugal *M. capenis:* South Africa, Portugal *K. pelamis*: Azores, Portugal	Raw and steamed fish (*P. platessa, M. australis, M. capenis, K. pelamis*, and *M. edulis*)	*P. platessa*: n = 25, DF 0% *M. australis*: n = 25, DF 0% *M. capenis*: n = 25, DF 0% *K. pelamis*: n = 25, DF 0% *M. edulis*: The Netherlands: n = 50, DF 0% France: n = 50, DF 0% (LOD = <0.01 ng/g ww for all PFCs)

Study	Location and Source	Seafood Type	Results
	M. edulis: North Sea, The Netherlands; France, France		
Gebbink et al. (2015)	Sweden Food items were purchased at two major grocery store chains in four major Swedish cities in 1999 and 2005. In 2010, sampling was limited to Uppsala city since no systematic geographical differences in food contamination was observed in the two earlier market basket studies. The food items were selected based on Swedish food and production statistics and were not cooked before analysis. Homogenates of fish products (fresh and frozen fillets of fish, canned fish products, shellfish) were prepared for each collection year by mixing food items proportionally according to food consumption statistics. Results were not reported for the 2005 and 2010 fish product composite samples (only reported pooled with other food types).	Fish	**1999: n = 1, point = 0.07 ng/g** (MLOQ = 0.0003 ng/g) *n represents number of composite samples
Vassiliadou et al. (2015)	Greece Samples were obtained during the winter and early spring of 2011. Finfish, squids, and shrimps were purchased from the local fish market in Kallithea, Athens, while mussels were obtained from a mariculture farm within the Saronikos Gulf, Attika. Samples were analyzed raw as well as cooked in the ways favored in Greek cuisine (pan-fried in olive oil and/or grilled). Quadruplicate composite samples were created for each food type, each consisting of four to six items of raw or cooked fish or shellfish.	Anchovy, bogue, hake, picarel, sand smelt, sardine, striped mullet, mussel, shrimp, squid	**Striped mullet:** **Raw: n = 4, DF NR, mean = 0.60 ng/g ww** **Fried: n = 4, DF NR, mean = 0.57 ng/g ww** **Grilled: n = 4, DF NR, mean = 0.50 ng/g ww** **Shrimp:** **Raw: n = 4, DF NR, mean = 1.27 ng/g ww** **Fried: n = 4, DF NR, mean = 1.52 ng/g ww** Anchovy (raw, fried, grilled), bogue (raw, fried, grilled), hake (raw, fried, grilled), picarel (raw, fried), sand smelt (raw, fried), sardine (raw, fried, grilled), mussel (raw, fried), squid (raw, fried, grilled): n = 4, DF 0% (LOD = 0.42 ng/g; LOQ = 1.25 ng/g) *n represents number of composite samples
Carlsson et al. (2014)	Greenland (Nuuk) Seafood was purchased at the local fish market and grocery shops in June 2010. All items were originally caught in the vicinity of the Nuuk area and/or along the West coast of Greenland and represented the common food items consumed by the local Inuit population.	Salmon, halibut	Raw salmon fillet: n = 6, DF 0% Smoked salmon fillet: n = 6, DF 0% Smoked halibut fillet: n = 6, DF 0% (LOD = 0.014–0.224 ng/g for all PFAS)
Domingo et al. (2012)	Spain (Catalonia) Foods purchased from 4 shops/stores of each of the 12 representative cities of Catalonia (Barcelona,	Fish and seafood (sardine, tuna, anchovy, swordfish, salmon, hake, red mullet, sole,	**n = 2, DF NR, mean = 0.5 ng/g fw** (LOD not reported) *n represents number of composite samples

Study	Location and Source	Seafood Type	Results
	l'Hospitalet de Llobregat, Vilanova I la Geltrú, Matafr, Sabadell, Terrassa, Girona, Tarragona, Reus, Tortosa, Lleida and Manresa) in September 2011. Shops/stores included local markets, small stores, supermarkets, and big grocery stores. For each food item, two composite samples were prepared for analysis, where each composite sample consisted of 24 individual units. Only edible parts of each food item were included in the composites.	cuttlefish, clam, mussel, and shrimp)	
Vestergren et al. (2012)	Sweden (Malmoe, Gothenburg, Uppsala, Sundsvall) Purchasing locations of the two largest retail chains in Sweden were selected in each of four major Swedish cities. All purchases were made in spring/summer of 1999, 2005, and 2010. In 2010, the study was limited to the largest retail chains in Uppsala located in close vicinity to Stockholm. An equal amount of each food group from each of the four cities was combined into one sample pool to provide a representative sample for the Swedish urban population.	Fish products (fresh and frozen fillets of fish, canned fish products, shellfish)	**1999: n = 1, point = 0.090 ng/g** **2005: n = 1, point = 0.090 ng/g** **2010: n = 1, point = 0.072 ng/g** (MDL = 0.0025 ng/g; MQL = 0.0063 ng/g) *n represents number of composite samples
Noorlander et al. (2011)	The Netherlands Fish randomly purchased from several Dutch retail stores with nationwide coverage in November 2009. Fish samples were ground, homogenized, and pooled for analysis.	Fatty fish (herring, eel, mackerel, salmon), lean fish (cod, plaice, pollack, tuna), crustaceans (mussels, shrimp, crab)	**Fatty fish: n = 1, point = 0.005 ng/g** **Lean fish: n = 1, point = 0.077 ng/g** **Crustaceans: n = 1, point = 0.058 ng/g** (LOD not reported) *n represents number of composite samples
Jogsten et al. (2009)	Spain (Catalonia) Fish samples purchased from local markets, large supermarkets, and grocery stores from two different areas of Tarragona Province, Catalonia in January and February 2008. The cities of Tarragona and Reus were sampled in the northern area and L'Ametlla de Mar and Tortosa in the southern area. For each food item, two composite samples were analyzed (one composite for the northern area and one for the southern area). Each composite was formed of a minimum of six individual sub-samples of the same product.	Marinated salmon (homemade and packaged)	Homemade: n = 2, DF 0% Packaged: n = 2, DF 0% (LOD not reported) *n represents number of composite samples *Values of ND were replaced with 1/2×LOD
Ericson et al. (2008a)	Spain Food samples purchased from local markets, large supermarkets, and grocery stores within Tarragona County in July 2006. Food samples were randomly	White fish, seafood, tinned fish, blue fish	White fish: n = 2, DF 0% Seafood: n = 2, DF 0% Tinned fish: n = 2, DF 0% Blue fish: n = 2, DF 0%

Study	Location and Source	Seafood Type	Results
	purchased with origin source not specified. Each of the food samples were duplicated and combined to analyze a composite sample. Only the edible part of each food was included in the composite samples. Composite samples included the following: White fish: hake, whiting blue, sea bass, monkfish Seafood: mussel, shrimp Tinned fish: tuna, sardine, mussel Blue fish: salmon, sardine, and tuna		(LOD = 0.001–0.65 ng/g.fw) *n represents number of composite samples
Johansson et al. (2014)	Sweden Farmed rainbow trout (whole fish) were collected from fish farms along the Swedish Baltic Sea coast (brackish water). Only fish older than 12 months were sampled. Samples were collected annually from 1999 to 2010 within the Swedish National Food Agency's official food control program.	Rainbow trout	n = 36, DF 0% (MDL = 0.050 ng/g fw; MQL = 0.140 ng/g fw)
Multiple Continents			
Chiesa et al. (2019)	United States (Pacific Ocean) Wild-caught fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from USA-Pacific Ocean (Food and Agriculture Organization Area 67 and 77).	Wild-caught salmon (*Oncorhynchus kisutch* and *Oncorhynchus keta*)	*Oncorhynchus kisutch:* n = 5, DF 0% *Oncorhynchus keta*: n = 2, DF 0% (LOQ = 0.005 ng/g)
	Canada Wild-caught fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Canada (Food and Agriculture Organization Area 67).	Wild-caught salmon (*Oncorhynchus nerka)*	n = 15, DF 0% (LOQ = 0.005 ng/g)
	Norway Farmed fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Norway (Food and Agriculture Organization Area 27).	Farmed salmon (*Salmo salar)*	n = 25, DF 0% (LOQ = 0.005 ng/g)
	Scotland Wild-caught and farmed fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Scotland (Food and Agriculture Organization Area 27).	Wild-caught and farmed salmon (*Salmo salar)*	Wild-caught: n = 2, DF 0% Farmed: n = 17, DF 0% (LOQ = 0.005 ng/g)

Notes: DF = detection frequency; ww = wet weight, LOD = limit of detection; LOQ = limit of quantitation; MDL = method detection limit; ND = not detected.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

C.3.1.2. Other Food Types

PFNA was included in a suite of PFAS evaluated in FDA's 2019, 2021, and 2022 Total Diet Study Sampling (US FDA, 2022c, 2022b, 2022a, 2021b, 2021a, 2020b, 2020a); it was detected at concentrations of 233 ng/kg (0.233 ng/g) in baked cod and 50 ng/kg (0.050 ng/g) in frozen (oven-cooked) fish sticks or patties in 2021, but it was not detected in any of the other food samples tested. It should be noted that FDA indicated that the sample sizes used in the PFAS 2019, 2021, and 2022 Total Diet Study Sampling were limited and that the results should not be used to draw definitive conclusions about PFAS levels in the general food supply (US FDA, 2022c). PFNA was not detected in milk samples collected from a farm with groundwater known to be contaminated with PFAS; however, it was detected in produce (corn) collected from an area near a PFAS production plant in FDA studies of the potential PFAS exposure to the U.S. population (US FDA, 2021c, 2018). PFNA was detected in beef steak in the Canadian Total Diet studies from 1992 to 2004, but it was not detected in any of the other food samples tested (ATSDR, 2021; Tittlemier et al., 2007).

Several U.S. studies were identified that examined PFNA in breastmilk or food types other than breastmilk (Tipton et al., 2017; Blaine et al., 2014; Blaine et al., 2013; Young et al., 2012; Schecter et al., 2010; von Ehrenstein et al., 2009; Kuklenyik et al., 2004) (Table C-3). Few U.S. studies analyzed foods from any one origin – only two studies sampled from store- or market-bought meats, eggs, produce, and dairy, one studied wild alligator meat, two sampled from crops (produce and corn grain and stover) grown in biosolids-amended soils (and also control and municipal soils) as part of greenhouse and field studies, and two studied breastmilk.

Two studies purchased food items from stores and markets for evaluation (Young et al., 2012; Schecter et al., 2010). Schecter et al. (2010) assessed PFNA and other PFAS in food samples collected from five Dallas, Texas grocery stores in 2009. The origin or source of each food was not described. Food items included meat products (bacon, canned chili, chicken breast, ground beef, roast beef, ham, sausage, and turkey), dairy (butter, cheeses, frozen yogurt, ice cream, milk, and yogurt), eggs, and grains (cereal), fruits and vegetables (apples, potatoes), and fats/other (canola oil, margarine, olive oil, peanut butter). PFNA was not detected in any of the food samples. In Young et al. (2012), cow milk was purchased from retail markets across the continental United States representing 17 states; the sampling year was not reported. Cow milk samples included organic milk, vitamin D added milk, and ultra-pasteurized milk. PFNA was not detected in any of the 49 retail milk samples (method detection limit (MDL) = 0.28 ng/g).

One study investigated PFAS levels in wild meat (Tipton et al., 2017). Tipton et al. (2017) assessed alligator tail meat that was collected during the South Carolina recreational hunting season between September to October 2015. Tail meat samples were collected from four different public hunt units – Southern Coastal, Middle Coastal, Midlands, and Pee Dee. PFNA was detected in samples from all hunt units with the exception of the Midlands (n = 2), where PFNA was not detected. Median concentrations from Southern Coastal (n = 19), Middle Coastal (n = 17), and Pee Dee (n = 2) were 0.107 ng/g, 0.102 ng/g, and 0.117 ng/g wet mass, respectively.

Two studies by Blaine et al. (2014; 2013) evaluated PFNA in crops grown in greenhouse and field studies. In Blaine et al. (2014), PFAS levels were measured in celery root, pea fruit, and radish root grown in a greenhouse study with control (unamended) soil, industrially impacted soil, and municipal soil (n = 3–5). PFNA was detected in radish root and celery shoot from all three soils and pea fruit from only industrially impacted soil. Mean concentrations of PFNA in radish root for the control, industrially impacted, and municipal soil were 4.79 ng/g, 26.88 ng/g, and 5.99 ng/g, respectively. Mean concentrations of PFNA in celery shoot for the control, industrially impacted, and municipal soil were 1.89 ng/g, 13.81 ng/g, and 1.62 ng/g, respectively. The mean concentration of PFNA in pea fruit in the industrially impacted soil was 1.45 ng/g. Authors noted minor cross-contamination of the control soil due to the proximity of the unamended soil to biosolids-amended soil. In Blaine et al. (2013), authors studied the uptake of PFAS into edible crops in both field and greenhouse studies. In the field study, PFAS levels were measured in corn grain and corn stover grown with control (unamended), urban biosolids-amended, and rural biosolids-amended soil (n = 3–7). Mean PFNA concentrations were below the LOQ in both corn grain and corn stover grown in any field study plots (<0.10 ng/g for corn grain; <0.29 ng/g for corn stover). In the greenhouse study, lettuce and tomato plants were grown in control soil, industrially impacted soil, or municipal soil (n = 3–5). Mean PFNA concentrations were below the LOQ (2.96 ng/g) in any tomato plants but was detected in lettuce grown in industrially impacted soil and municipal soil at mean concentrations of 57.39 ng/g and 4.73 ng/g, respectively. PFNA was not detected above the LOQ (0.04 ng/g) in lettuce grown in control soil. Sampling year was not reported.

The remaining two studies evaluated the occurrence of PFNA in breastmilk (von Ehrenstein et al., 2009; Kuklenyik et al., 2004). von Ehrenstein et al. (2009) collected breastmilk samples between December 2004 and July 2005 from women between the ages of 18 and 38 at the time of recruitment as part of the pilot study Methods Advancement for Milk Analysis (MAMA). Women provided milk samples at two visits – the first visit was 2–7 weeks postpartum, and the second visit was 3–4 months postpartum. PFNA was not detected in any of the samples from the first visit (n = 18) or second visit (n = 20). Similarly, PFNA was below the LOD (1.0 ng/mL) in the samples reported by Kuklenyik et al. (2004). Kuklenyik et al. (2004) did not report information on the breastmilk donors or the sampling procedure as it was unavailable; PFNA was not detected in either of the two samples.

Results for all of the identified non-U.S. studies are presented in detail in Table C-5 and are summarized here. Among the European studies, many collected food samples of unknown origin from grocery stores (Scordo et al., 2020; Rivière et al., 2019; Sznajder-Katarzyńska et al., 2019; Sznajder-Katarzyńska et al., 2018; Papadopoulou et al., 2017; Surma et al., 2017; D'Hollander et al., 2015; Gebbink et al., 2015; Pérez et al., 2014; Herzke et al., 2013; Hlouskova et al., 2013; Domingo et al., 2012; Vestergren et al., 2012; Noorlander et al., 2011; Jogsten et al., 2009; Ericson et al., 2008a). A wide variety of items were analyzed including meats and seafood, dairy, fruits and vegetables, grains, pastries and other sweets, spices, sweeteners, and other beverages. Of these studies, five reported no detectable PFNA in any of the items sampled (Rivière et al., 2019; Sznajder-Katarzyńska et al., 2018; Surma et al., 2017; Jogsten et al., 2009; Ericson et al., 2008a). Among the studies that did report detectable PFNA in some food items, PFNA was found in all major food categories examined except spices, salts, and sweeteners (sugar and honey) though there was no apparent trend in specific food types with PFNA present in measurable quantities.

Three studies focused on PFNA in eggs. Ghelli et al. (2019) collected eggs from commercial laying hen farms in Italy. PFNA was detected at trace levels only in 5% of the 44 eggs sampled. Johansson et al. (2014) collected eggs from 20 to 25 commercial egg producers in Sweden every year between 1999-2010. Ten to 12 eggs were pooled for analysis and they reported detectable quantities of PFNA in 28% of 72 pooled samples, with a maximum observed value of 0.143 ng/g fw. Zafeiraki et al. (2016a) collected home and commercially produced eggs in Greece and the Netherlands. They reported detectable levels of PFNA in home produced eggs from both regions, with a maximum observed value of 2.0 ng/g. PFNA was not detected in any of the commercially produced eggs sampled.

Two studies focused on PFNA in milk and other dairy products. Johansson et al. (2014) collected milk from about 10 dairy farms in Sweden every year between 1999-2010. PFNA was not present at detectable levels in any of the samples. Still et al. (2013) collected commercially sold dairy product samples in Germany. PFNA was present at detectable levels in one of four milk samples, all three cheese samples and one butter sample, with a maximum observed value of 0.0094 ng/g. They reported non-detect results in all other products examined. Eriksson et al. (2013) identified PFNA concentrations in milk from two major dairy farms, with a maximum of 0.073 ng/g ww. They identified PFNA in 75% of four additional dairy samples from Faroe Island, but it was not detected in other samples of yogurt, créme fraiche, and potatoes. Vestergren et al. (2013) evaluated concentrations in milk and reported a mean of 0.0023 ng/mL. This study also evaluated concentrations in cow liver, blood, and muscle, finding the highest concentration in liver at a mean of 0,016 ng/g.

In addition, two non-U.S. studies focused on locally caught food items of importance to indigenous populations. Binnington et al. (2017) sampled whale blubber collected from two beluga whales captured off the West coast of Canada. PFNA was present at measurable concentrations in all solid samples (max = 0.1718 ng/g lipids) but was not detectable in rendered oil. Carlsson et al. (2014) assessed wild caught seal beef, narwhal, and whale beef in Greenland and found detectable concentrations of PFNA only in seal beef.

PFNA was also detected in some beverages. Stahl et al. (2014) measured PFNA in a selection of Hessian, Belgian, and Bavarian beers and found measurable concentrations in 14% of 93 samples. Eschauzier et al. (2013) measured PFNA in cola and brewed coffee samples collected from various locations in Amsterdam and found that PFNA was not present at measurable concentrations in any sample.

Of the thirteen non-U.S. studies that evaluated the occurrence of PFNA in breastmilk, five did not report detectable concentrations. These studies evaluated 13 women in Ontaria, Canada (Kubwabo et al., 2013); 10 women in Spain (Kärrman et al., 2010), 11 pooled samples obtained from 109 women in Ireland (Pratt et al., 2013), 61 women in France (Cariou et al., 2015), and 20 women in Spain (Llorca et al., 2010). However, the remaining European studies did report quantifiable concentrations of PFNA in breastmilk. Among these studies, detectable concentrations of PFNA were reported in 17% of samples measured from 12 individual Swedish women and 33% of nine composite samples composed of breastmilk from 25-90 Swedish women (Kärrman et al., 2007); 42.5% of breastmilk samples from 40 Belgian women (Croes et al., 2012); 6% of breastmilk samples from 67 French women (Motas Guzmàn et al., 2016); 100% of 31 composite samples composed of breastmilk from 5–116 Swedish women and 94% of samples measured from 46 individual Swedish women (Nyberg et al., 2018); 5% of samples

from 14 Spanish women (Beser et al., 2019); 69% of 16 samples from Irish women (Abdallah et al., 2020); 48% of samples from 50 women in the Czech Republic (Lankova et al., 2013); and 2% of samples from 48 French women (Antignac et al., 2013). Abdallah et al. (2020) reported the highest value for PFNA in breastmilk, at 0.1 ng/mL.

Several European studies focused on other sources of dietary exposures in children. Lankova et al. (2013) measured PFNA in infant formula samples purchased at Czech retail markets and reported a maximum observed value of 0.011 ng/g. Llorca et al. (2010) measured PFNA in baby cereals and infant formulas purchased from reatailers in Spain. They reported PFNA present in measureable quantities in all of the samples measured, with a maximum reported value of 0.1138 ng/g in baby cereal and 0.219 ng/g in infant formulas. Dellatte et al. (2013) measured PFNA in ready-to-eat meals collected from nursery and primary school canteens. PFNA was present at detectable levels in one of six samples, with a reported concentration of 0.0063 ng/g.

Table C-5. Summary of PFNA Occurrence in Other Food

Study	Location and Source	Food Types	PFNA Results
United States			
Schecter et al. (2010)	United States (Texas) Food samples from five different grocery stores in Dallas, Texas were collected in 2009. Ten individual samples were collected for each food type and combined to form composite samples. The origin/source of the food samples were not reported.	Dairy; fruits and vegetables; grains; meat; fats/other	Meat: Hamburger: n = 1, DF 0% Bacon: n = 1, DF 0% Sliced turkey: n = 1, DF 0% Sausages: n = 1, DF 0% Ham: n = 1, DF 0% Sliced chicken breast: n = 1, DF 0% Roast beef: n = 1, DF 0% Canned chili: n = 1, DF 0% Dairy and Eggs: Butter: n = 1, DF 0% American cheese: n = 1, DF 0% Other cheese: n = 1, DF 0% Whole milk: n = 1, DF 0% Ice cream: n = 1, DF 0% Frozen yogurt: n = 1, DF 0% Whole milk yogurt: n = 1, DF 0% Cream cheese: n = 1, DF 0% Eggs: n = 1, DF 0% Grains: Cereals: n = 1, DF 0% Fruits and Vegetables: Apples: n = 1, DF 0% Potatoes: n = 1, DF 0% Fats and Other: Olive oil: n = 1, DF 0% Canola oil: n = 1, DF 0% Margarine: n = 1, DF 0% Peanut butter: n = 1, DF 0% (LOD not reported for any food item) *n reflects number of composite samples, each composed of ~10 individual samples
Young et al. (2012)	United States (17 states) Retail cow's milk samples were all pasteurized whole milk, commercially available, and purchased at retail markets across the continental United States representing 17 states. Samples were organic milk,	Dairy	n = 49, DF 0% (MDL = 0.28 ng/g)

Study	Location and Source	Food Types	PFNA Results
	vitamin D added milk, or ultra-pasteurized milk. Sampling year not reported.		
Tipton et al. (2017)	United States (South Carolina) Alligator tail meat samples were collected from a local wild game meat processer during the South Carolina recreational hunt season between September to October 2015. Samples were from four different public hunt units—Southern Coastal, Middle Coast, Midlands, and Pee Dee.	Meat	**Alligator tail:** **Southern coastal: n = 19, DF[a] 74%, median (range) = 0.107 (<0.088–0.551) ng/g wet mass** **Middle coastal: n = 17, DF[a] 65%, median (range) = 0.102 (<0.073–0.553) ng/g wet mass** **Pee Dee: n = 2, DF[a] 100%, median (range) = 0.117 (0.100–0.135) ng/g wet mass** Midlands: n = 5, DF 0% (RL not reported)
Blaine et al. (2014)	United States (Midwest) Crops grown in in greenhouse study with control (unamended), industrially impacted soil, or municipal soil. Control soil had minor cross-contamination due to proximity to biosolids-amended fields. Industrially impacted soil was amended with industrially impacted biosolids, and municipal soil was amended with municipal biosolids for over 20 years. Crops grown in the greenhouse study were grown from seed in pots, which were randomly arranged within the greenhouse. Sampling year not reported.	Fruits and vegetables	**Radish root:** **Control: n = 3–5, DF NR, mean = 4.79 ng/g** **Industrially impacted; n = 3–5, DF NR, mean = 26.68 ng/g** **Municipal: n = 3–5, DF NR, mean = 5.99 ng/g** **Celery shoot:** **Control: n = 3–5, DF NR, mean = 1.89 ng/g** **Industrially impacted: n = 3–5, DF NR, mean = 13.81 ng/g** **Municipal: n = 3–5, DF NR, mean = 1.62 ng/g** Pea fruit: Control: n = 3–5, DF 0% **Industrially impacted: n = 3–5, DF NR, mean = 1.45 ng/g** Municipal: n = 3–5, DF 0% (LOQ = 0.07ng/g)
Blaine et al. (2013)	United States (Midwest) Crops grown in urban and rural full-scale field study with control (unamended) and biosolids-amended soil. Three agricultural fields were amended (0.5×, 1×, or 2×) with municipal biosolids. Urban biosolids (1× and 2×) were from a WWTP receiving both domestic and industrial waste. Rural biosolids (0.5×) were from a WWTP receiving domestic waste only. Control plots were proximal to the rural and urban amended corn grain and corn stover field sites; sampling year not provided.	Fruits and vegetables; grains	Field study: Corn grain: Urban nonamended: n = 3–7, DF NR, mean = <0.10 ng/g Urban 1×: n = 3–7, DF NR, mean = <0.10 ng/g Urban 2×: n = 3–7, DF NR, mean = <0.10 ng/g Rural nonamended: n = 3–7, DF NR, mean = <0.10 ng/g Rural 0.5×: n = 3–7, DF NR, mean = <0.10 ng/g Corn stover: Urban nonamended: n = 3–7, DF NR, mean = <0.29 ng/g

Study	Location and Source	Food Types	PFNA Results
	Crops grown in greenhouse study with control (nonamended) and biosolids-amended soil. Nonamended soil obtained from a field that received commercial fertilizers and had a similar cropping system as the nearby municipal soil site. Municipal soil was obtained from a reclamation site in Illinois where municipal biosolids were applied at reclamation rates for 20 years, reaching the cumulative biosolids application rate of 1,654 Mg/ha. Industrially impacted soil was created by mixing composted biosolids from a small municipal (but impacted by PFAA manufacturing) WWTP with control soil on a 10% mass basis. Sampling year not provided.		Urban 1×: n = 3–7, DF NR, mean = <0.29 ng/g Urban 2×: n = 3–7, DF NR, mean = <0.29 ng/g Rural nonamended: n = 3–7, DF NR, mean = <0.29 ng/g Rural 0.5×: n = 3–7, DF NR, mean = <0.29 ng/g (LOQ = 0.10 ng/g for corn grain; LOQ = 0.29 ng/g for corn stover) Greenhouse study: Lettuce: Nonamended: n = 3–5, DF NR, mean = <0.04 ng/g **Industrially impacted: n = 3–5, DF NR, mean = 57.39 ng/g** **Municipal: n = 3–5, DF NR, mean = 4.73 ng/g** Tomato: Nonamended: n = 3–5, DF NR, mean = <2.86 ng/g Industrially impacted: n = 3–5, DF NR, mean = <2.86 ng/g Municipal: n = 3–5, DF NR, mean = <2.86 ng/g (LOQ = 0.04 ng/g for lettuce; LOQ = 2.86 ng/g for tomato)
von Ehrenstein et al. (2009)	United States (North Carolina) As part of the Methods Advancement for Milk Analysis (MAMA) pilot study, 34 breastfeeding women aged 18 to 38 years at recruitment provided breastmilk samples at two visits. The first visit occurred 2–7 weeks postpartum, and the second visit occurred 3–4 months postpartum. Both visits were between December 2004 and July 2005.	Breastmilk	Visit #1: n = 18, DF 0% Visit #2: n = 20, DF 0% (LOQ = 0.30 ng/mL)
Kuklenyik et al. (2004)	United States (Georgia) Authors reported that no information was provided on the human milk donors or the sampling procedure.	Breastmilk	n = 2, DF 0% (LOD = 1.0 ng/mL)
Canada			
Binnington et al. (2017)	Canada (Tuktoyaktuk, Northwest Territory) Samples were collected from two beluga whales (HI-14-06 and HI-14-11) caught offshore of Tuktoyaktuk during summer 2014. The belugas	Meat	**Beluga whale blubber – muktuk** **Air dry: n = 4, DF NR, range of means = 0.0713–0.1288 ng/g lipids** **Hang dry: n = 4, DF NR, range of means = 0.1013–0.150 ng/g lipids**

Study	Location and Source	Food Types	PFNA Results
	were members of the Eastern Beaufort Sea population, with a home range including respective summering and wintering grounds in the Beaufort and Bering Seas. The selected individuals were harvested on the shore of nearby Hendrickson Island. Beluga whale blubber forms the basis for two distinct traditional food types: (i) muktuk designates food items composed of the outer layer of blubber and its attached skin and connective tissue, while (ii) uqsuq designates food items derived from the inner layers of blubber. Samples were analyzed in duplicate at each step in the preparation process.		**Boil drum: n = 4, DF NR, range of means = 0.0831–0.1268 ng/g lipids** **Boil pot: n = 4, DF NR, range of means = 0.0617–0.079 ng/g lipids** **Roast: n = 4, DF NR, range of means = 0.0813–0.1205 ng/g lipids** **Aged 2 days: n = 4, DF NR, range of means = <MDL–0.0914 ng/g lipids** **Aged 5 days: n = 4, DF NR, range of means = 0.0773–0.1004 ng/g lipids** **Beluga whale blubber – uqsuq** **Baseline: n = 4, DF NR, range of means = 0.0385–0.0769 ng/g lipids** **Aged 2 days: n = 4, DF NR, range of means = <MDL–0.1718 ng/g lipids** **Aged 5 days: n = 4, DF NR, range of means = 0.0582–0.1391 ng/g lipids** Oil: n = 4, DF 0% (MDL = 0.01 ng/mL)
Kubwabo et al. (2013)	Canada (Ontario) Breastmilk samples were collected in the Kingston region of Ontario in 2003–2004.	Breastmilk	n = 13, DF 0% (MDL = 0.062 ng/mL; LOQ = 0.208 ng/mL)
Europe			
Scordo et al. (2020)	Italy Commercially available strawberry and olive fruits were purchased in two Italian supermarkets in 2018.	Fruits	**Strawberries: n = 2, DF[a] 50%, range = <0.0013–0.047 ng/g dw** (MDL = 0.0013 ng/g; MQL = 0.0049 ng/g) **Olives: n = 2, DF[a] 100%, mean[a] (range) = 0.0385 (0.035–0.042) ng/g dw** (MDL = 0.0007 ng/g; MQL = 0.0026 ng/g)
Sznajder-Katarzyńska et al. (2019)	Poland Milk and milk products were purchased in Polish markets in 2017. Commercially available samples of each product were obtained from five different suppliers.	Dairy	**All dairy: n = 35, DF 45.7%, sum PFNA = 0.83 ng/g** **Milk: n = 5, DF[a] 80%, range = 0.04–0.09 ng/g** **Cottage cheese: n = 5, DF[a] 100%, range = 0.03–0.06 ng/g** **Natural yogurt: n = 5, DF[a] 60%, range = 0.07–0.10 ng/g** **Kefir/Bonny clabber: n = 5, DF[a] 20%, range = 0.07–0.07 ng/g**

Study	Location and Source	Food Types	PFNA Results
			Sour cream: n = 5, DF[a] 60%, range = 0.03–0.05 ng/g Camembert-type cheese (n = 5), butter (n = 5): DF 0% (LOD = 0.006 ng/g; LOQ = 0.019 ng/g) *Range reported for detected values
Rivière et al. (2019)	France Samples collected between July 2011 and July 2012 in the center region of France. Food items were selected based on the results of a national consumption survey to obtain a representative and general view of children's (0–3 years old) food consumption. All analyzed samples were formed of 12 subsamples of the same food and of equal weight. The products purchased were prepared in a way that reflected as closely as possible what is done in the home (preparation and cooking).	Meat; dairy; fruits and vegetables; fats/other	Infant-specific foods: Milk-based beverage (n = 8), cereals (n = 5), milk-based desserts (n = 6), growing-up milk (n = 9), soups and puree (n = 11), fruit puree (n = 4), vegetable-based ready-to-eat meal (n = 20), meat/fish-based ready-to-eat meal (n = 45), infant formula (n = 28), follow-on formula (n = 33): DF 0% Common foods: Non-alcoholic beverages (n = 1), dairy-based desserts (n = 1), milk (n = 1), mixed dishes (n = 1), ultra-fresh dairy products (n = 1), meat (n = 1), poultry and game (n = 1): DF 0% (LOD = 0.0002–3.7 ng/g for all PFAS) *n represents number of composite samples
Sznajder-Katarzyńska et al. (2018)	Poland Samples were purchased in Polish markets in 2017. Individual food items were selected among the most frequently consumed in Poland. Vegetables (potatoes, beetroots, carrots, white cabbage, tomatoes) and fruits (apples, cherries, strawberries) of Polish origin were bought in season when naturally ripe. Bananas, lemons, and oranges were bought after being imported to Poland. Five different samples of each fruit or vegetable were collected.	Fruits and vegetables	Apples, bananas, cherries, lemons, oranges, strawberries, beetroots, carrots, tomatoes, potatoes, and white cabbage: n = 5 for each, DF 0% (LOD = 0.007 ng/g; LOQ = 0.014 ng/g)
Surma et al. (2017)	Spain, Slovakia Spice samples were collected in powder form from Spain and Slovakia. Sampling year not reported.	Fats/other	Spain: Anise (n = 1), star anise (n = 1), white pepper (n = 1), fennel (n = 1), cardamom (n = 1), clove (n = 1), coriander (n = 1), nutmeg (n = 1), allspice (n = 1), cinnamon (n = 2), vanilla (n = 1), ginger (n = 1), peppermint (n = 1), parsley (n = 1), thyme (n = 1), laurel (n = 1), garlic (n = 1), cumin (n = 1), black

Study	Location and Source	Food Types	PFNA Results
			pepper (n = 1), mild hot pepper (n = 1), hot hot pepper (n = 1), oregano (n = 2): DF 0% Slovakia: Anise (n = 1), star anise (n = 1), white pepper (n = 1), fennel (n = 1), cardamom (n = 1), clove (n = 1), coriander (n = 1), nutmeg (n = 1), allspice (n = 1), cinnamon (n = 1), vanilla (n = 1), ginger (n = 1): DF 0% (LOD = 0.031g/g; LOQ = 0.093 ng/g)
Zafeiraki et al. (2016a)	Greece, The Netherlands Home and commercially-produced eggs were collected from different regions in the Netherlands and Greece in August 2013–August 2014. Home-produced eggs were voluntarily provided, and commercial eggs were purchased from supermarkets. The yolks of the same sample of eggs were pooled, homogenized, and then analyzed.	Fats/other	**Domestic eggs:** **The Netherlands: n = 73, DF 18%, median (range) = 0.9 (<0.5–2.0) ng/g** **Greece: n = 45, DF 20%, median (range) = 0.8 (<0.5–1) ng/g** Commercial eggs: The Netherlands: n = 22, DF 0% Greece: n = 31, DF 0% (LOD = 0.15 ng/g; LOQ = 0.5 ng/g) *Median calculated only on the concentrations above LOQ
Zafeiraki et al. (2016b)	The Netherlands Samples purchased from local markets and slaughterhouses in the Netherlands in 2014. Samples included liver samples of horse, sheep, bovine, pig, and chicken.	Meat	Horse: n = 19, DF 0% Sheep: n = 18, DF 0% Bovine: n = 22, DF 0% Pig: n = 20, DF 0% Chicken: n = 20, DF 0% (LOQ = 0.5 ng/g ww)
D'Hollander et al. (2015)	Czech Republic, Belgium, Norway, Italy The Czech Republic, Belgium, Norway, and Italy were selected to represent eastern, western, northern, and southern Europe. Sampling took place between spring and summer 2010 as part of the PERFOOD study. Individual items were randomly selected in three national retail stores covering different brands or countries of origin. Of each item, three to ten single samples were combined to create a pooled sample. Individual food items that were collected to create pooled samples were: Cereals: rice, wheat (white), oats, rye	Grains; fruits; fats/other	**Czech Republic:** Cereals: wheat (white), oats, rye: n = 1 each, point = <0.020 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.001 ng/g Fruits – berries: strawberries: n = 1, point = <0.001 ng/g Fruits – citrus fruit: oranges, tangerines: n = 1, point = <0.001 ng/g Fruits – pipe and stone fruit: **Apples: n = 1, point = 0.002 ng/g** **Pears: n = 1, points = 0.001 ng/g** Peaches: n = 1, point = <0.001 ng/g

Study	Location and Source	Food Types	PFNA Results
	Sweets: sugar (beet), sugar (cane), honey Fruits: berries – strawberries, citrus fruit – oranges, tangerines, lemons, grapefruits, pipe and stone fruit – apples, pears, peaches, plums, others and exotic fruit – melons, grape, bananas Miscellaneous: "rock" salt, "marine" salt		Fruits – others and exotic fruit: melons: n = 1, point = <0.004 ng/g Miscellaneous: rock salt: n = 1, point = <0.001 ng/g **Italy:** Cereals: Rice, maize: n = 1 each, point = <0.001 ng/g Wheat (white): n = 1, point = <0.020 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.001 ng/g Fruits – citrus fruit: lemons: n = 1, point = <0.001 ng/g Fruits – pipe and stone fruit: **Apples: n = 1, point = 0.016 ng/g** **Pears: n = 1, point = 0.002 ng/g** **Peaches: n = 1, point = 0.012 ng/g** Plums: n = 1, point = <0.001 ng/g Fruits – others and exotic fruit: Grapes: n = 1, point = <0.001 ng/g Bananas: n = 1, point = 0.003 ng/g Miscellaneous: marine salt: n = 1, point = <0.001 ng/g **Norway:** Cereals: wheat (white): n = 1, point = <0.020 ng/g Sweets: sugar (cane), honey: n = 1 each, point = <0.001 ng/g Fruits - berries: strawberries: n = 1, point = <0.001 ng/g Fruits – citrus fruit: Oranges: n = 1, point = <0.001 ng/g **Grapefruits: n = 1, point = 0.0248 ng/g** Fruits – pipe and stone fruit: apples, pears: n = 1 each, point = <0.001 ng/g **Fruits – others and exotic fruit: melons: n = 1, point = 0.0099 ng/g** Miscellaneous: rock salt: n = 1, point = <0.001 ng/g **Belgium:** Cereals: rice, wheat (white), wheat (dark), oats: n = 1 each, point = <0.001 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.001 ng/g

Study	Location and Source	Food Types	PFNA Results
			Fruits - berries: strawberries: n = 1, point = <0.001 ng/g Fruits – citrus fruit: oranges, lemons: n = 1 each, point = <0.001 ng/g Fruits – pipe and stone fruit: **Apples: n = 1, point = 0.205 ng/g** Pears: n = 1, point = <0.001 ng/g Plums: n = 1, point = <0.001 ng/g **Fruits – others and exotic fruit: grapes: n = 1, point = <0.007 ng/g** (LOD = 0.001 or 0.020 ng/g) *n represents number of composite samples
Gebbink et al. (2015)	Sweden Food items were purchased at two major grocery store chains in four major Swedish cities in 1999 and 2005. In 2010, sampling was limited to Uppsala city since no systematic geographical differences in food contamination was observed in the two earlier market basket studies. The food items were selected based on Swedish food and production statistics and were not cooked before analysis. The food items were divided into 12 groups and homogenates for each food group were prepared by mixing food items proportionally according to food consumption statistics. Results by food group were not reported for the 2005 and 2010 years. For all three sampling years, a homogenate was prepared by mixing proportional amounts of each food group according to consumption data for the respective year (includes fish samples).	Fruits and vegetables; meat; grains; fats/other	**1999:** **Dairy products: n = 1, point = 0.0005 ng/g** **Meat products: n = 1, point = 0.0067 ng/g** **Fats: n = 1, point = 0.0037 ng/g** **Pastries: n = 1, point = 0.0012 ng/g** **Egg: n = 1, point = 0.024 ng/g** **Fruit: n = 1, point = 0.0006 ng/g** **Soft drinks: n = 1, point = 0.0005 ng/g** Cereal products: n = 1, point = <0.0003 ng/g Vegetables: n = 1, point = <0.0003 ng/g Potatoes: n = 1, point = <0.0003 ng/g Sugar and sweets: n = 1, point = <0.0003 ng/g **Year pool: n = 12, point = 0.0077 ng/g** **2005:** **Year pool: n = 12, point = 0.016 ng/g** **2010:** **Year pool: n = 12, point = 0.015 ng/g** (MLOQ = 0.0003 ng/g) *n represents number of composite samples
Carlsson et al. (2014)	Greenland (Nuuk) Meat was purchased at the local fish market and grocery shops in June 2010. All items were originally caught in the vicinity of the Nuuk area and/or along the West coast of Greenland and represented the common food items consumed by the local Inuit population.	Meat	**Seal beef: n = 2, DF[a] 50%, range = <LOD–0.3 ng/g ww** Narwhal: n = 6, DF NR, median = <LOD Whale beef: n = 8, DF NR, median = <LOD (LOD = 0.014–0.224 ng/g for PFAS)

Study	Location and Source	Food Types	PFNA Results
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Between September 2011 and February 2013, samples were purchased from different supermarkets and retail stores in representative cites around the world, including cities in Serbia and Spain in Europe. Foodstuffs were grouped into the following categories: cereals; pulses and starchy roots; tree-nuts, oil crops, and vegetable oils; vegetables and fruits; meat and meat products; milk, animal fats, dairy products, and eggs; fish and seafood; and other such as candies and coffee.	Grains; fruits and vegetables; fats/other; meat; dairy; seafood	**Spain: n = 174, DF 13.3%, mean, median (range) = 1.175, 0.43 (ND–13) ng/g** **Serbia: n = 36, DF 10.3%, mean, median (range) = 0.208, 0.185 (ND–0.43) ng/g** (MLOD = 0.039–0.412 ng/g, depending on food item) *Results not reported for individual food categories
Stahl et al. (2014)	Belgium Samples of 83 Hessian beers were obtained from the Hessian control authority, 4 Bavarian beers were provided by the Bavarian Health and Food Safety Authority, and 6 Belgian beers were obtained from Belgian retail stores. Sampling year not provided.	Fats/other	**All beer: n = 93, DF (frequency of quantification) 14%, mean, median (maximum) = 0.00377, <LOQ (0.101) ng/mL** **German (Hesse): n = 83, DF (frequency of quantification) 12%, mean, median (maximum) = 0.00343, <LOQ (0.101) ng/mL** **German (Bavaria): n = 4, DF (frequency of quantification) 50%, mean, median (maximum) = 0.0073, 0.00628 (0.0167) ng/mL** **Belgian: n = 6, DF (frequency of quantification) 17%, mean, median (maximum) = 0.00611, <LOQ (0.0367) ng/mL** (LOQ = 0.005 ng/mL)
Eschauzier et al. (2013)	The Netherlands (Amsterdam) Brewed coffee samples (n = 12) from different coffee machines were collected from all over the city. Coffee beans from four of these locations were collected to manually brew coffee. Post-mixed cola was collected (n = 4) together with corresponding tap water and an additional three cola samples from different parts of town. Sampling was conducted between February and April 2011 at various locations (cafés, universities, and supermarkets).	Fats/other	Post-mixed cola: n = 6, DF NR, mean = <0.07 ng/L Brewed coffee from coffee machines: n = 12, DF NR, mean = <0.11 ng/L Manually brewed coffee: n = 4, DF NR, mean = <0.11 ng/L (LOQ = 0.07 ng/L for cola; 0.11 ng/L for coffee)
Herzke et al. (2013)	Belgium, Czech Republic, Italy, Norway The Czech Republic, Belgium, Norway, and Italy were selected to represent eastern, western, northern, and southern Europe. Sampling took place between spring 2010 and 2011 as part of the PERFOOD	Vegetables	Belgium: n = 21, DF 0% Czech Republic: n = 16, **DF NR, mean = 0.0028 ng/g** Italy: n = 15, DF 0%

Study	Location and Source	Food Types	PFNA Results
	study. Individual items were randomly selected in three national retail stores covering different brands or countries of origin. Of each item, three to ten single samples were combined to create one pooled sample per country. The following items were sampled: Root vegetables: carrots Bulb vegetables: onions Fruiting vegetables: tomatoes, courgettes, cucumbers, aubergine, peppers Brassica vegetables: cauliflower, cabbage, broccoli Leaf vegetables: lettuce and other salads, spinaches, chicory, pre-packed lettuce mix, pre-packed and minced frozen spinach Stem vegetables: asparagus, celery, fennel, cultivated mushrooms Starchy root tubers: potatoes, prepacked ready-to-cook pommes frites Legumes, beans, dried: peas, beans		Norway: n = 17, **DF NR, mean = 0.00226 ng/g** (MQL = 0.002–0.050 ng/g) *n represents number of composite samples *Values below the MQL were substituted with the MQL value
Hlouskova et al. (2013)	Belgium, Czech Republic, Italy, Norway Food products were randomly purchased in several nationwide supermarkets in four European regions during summer 2010. Within the sampling campaign, the collection of at least one food item per subcategory (meat, fish, hen eggs, milk and dairy products, and butter) in all four countries was acquired. Food items within each subcategory included the following: Meat: beef, canned pork meat, poultry, pork, pig/bovine liver, rabbit, and/or sheep/lamb Fish: farmed freshwater fish, farmed marine fish, and/or seafood) Hen eggs Milk and dairy products: ultra-high temperature whole cow milk, ultra-high temperature skimmed cow milk, cheese (yellow, Gouda/Edamer, etc.), and butter Samples were pooled within a respective food category but not across food groups.	Pooled milk/dairy products; meat; fish; hen eggs	**n = 50, DF 16%, mean, median (range) = 0.0295, 0.0253 (0.00503–0.0701) ng/g** (MQL = 0.005 ng/g for fish and seafood, meat, hen eggs, and cheese; 0.002 ng/mL for milk, and 0.025 ng/g for butter) *n represents number of pooled samples *Results not reported for individual food groups

Study	Location and Source	Food Types	PFNA Results
Domingo et al. (2012)	Spain (Catalonia) Foods purchased from 4 shops/stores of each of the 12 representative cities of Catalonia (Barcelona, l'Hospitalet de Llobregat, Vilanova I la Geltrú, Matarr, Sabadell, Terrassa, Girona, Tarragona, Reus, Tortosa, Lleida and Manresa) in September 2011. Shops/stores included local markets, small stores, supermarkets, and big grocery stores. Analyzed samples included 40 items: Meat and meat products: veal steak, loin of pork, chicken breast, steak of lamb, boiled ham, "Frankfurt"-type sausage, cured ham Vegetables and tubers: lettuce, tomato, potato, carrot Fresh fruits: apple, orange, banana Milk and dairy products: whole and semi-skimmed milk, yogurt, cheese I – low fat, cheese II – medium fat, cheese III – extra fat Cereals: French bread, pasta Pulses: lentils Industrial bakery: cookies Eggs: hen eggs Oils and fats: olive oil For each food item, two composite samples were prepared for analysis, where each composite sample consisted of 24 individual units. Only edible parts of each food item were included in the composites.	Meat; vegetables; grains; fruits; dairy; fats/other	**Dairy products: n = 2, DF NR, mean = 0.04 ng/g fw** Meat and meat products: n = 2, DF NR, mean = <0.079 ng/g fw Vegetables: n = 2, DF NR, mean = <0.37 ng/g fw Tubers: n = 2, DF NR, mean = <0.095 ng/g fw Fruits: n = 2, DF NR, mean = <0.096 ng/g fw Eggs: n = 2, DF NR, mean = <0.1 ng/g fw Milk: n = 2, DF NR, mean = <0.13 ng/g fw Cereals: n = 2, DF NR, mean = <0.033 ng/g fw Pulses: n = 2, DF NR, mean = <0.068 ng/g fw Oils: n = 2, DF NR, mean = <0.038 ng/g fw Industrial bakery: n = 2, DF NR, mean = <0.029 ng/g fw (LOD not reported) *n represents number of composite samples
Vestergren et al. (2012)	Sweden (Malmoe, Gothenburg, Uppsala, Sundsvall) Purchasing locations of the two largest retail chains in Sweden were selected in each of four major Swedish cities. All purchases were made in spring/summer of 1999, 2005, and 2010. In 2010, the study was limited to the largest retail chains in Uppsala located in close vicinity to Stockholm. An equal amount of each food group from each of the four cities was combined into one sample pool to provide a representative sample for the Swedish urban population.	Dairy; meat; grains; fruits; vegetables; fats/other	**Meat products:** **1999: n = 1, point = 0.0071 ng/g** **2005: n = 1, point = 0.0092 ng/g** 2010: n = 1, point = 0.0058 ng/g (estimated) (MDL = 0.0025 ng/g; MQL = 0.0062 ng/g) **Egg:** **1999: n = 1, point = 0.0022 ng/g** 2005: n = 1, point = 0.0056 ng/g (estimated) 2010: n = 1, point = <MDL (MDL = 0.0025 ng/g; MQL = 0.0062 ng/g) Fruits: 1999: n = 1, point = 0.0019 ng/g (estimated) 2005: n = 1, point = <MDL 2010: n = 1, point = <MDL

Study	Location and Source	Food Types	PFNA Results
			(MDL = 0.0013 ng/g; MQL = 0.0032 ng/g) Dairy products: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0034 ng/g; MQL = 0.0085 ng/g) Fats: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0030 ng/g; MQL = 0.0074 ng/g) Pastries: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0013 ng/g; MQL = 0.0031 ng/g) Cereal products: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0026 ng/g; MQL = 0.0063 ng/g) Vegetables: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0013 ng/g; MQL = 0.0031 ng/g) Potatoes: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0013 ng/g; MQL = 0.0032 ng/g) Sugar and sweets: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0013 ng/g; MQL = 0.0031 ng/g) Soft drinks, lemonade: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0006 ng/g; MQL = 0.0016 ng/g) *n represents number of composite samples *Paper reported that estimated concentrations are between MDL and MQL; however, there are some instances when the estimated concentrations are <MDL

Study	Location and Source	Food Types	PFNA Results
Noorlander et al. (2011)	The Netherlands Food products randomly purchased from several Dutch retail stores with nationwide coverage in November 2009. Food samples were ground, homogenized, and pooled for analysis. Food items within each subcategory included the following: Flour: whole wheat flour, flour Pork: sausage, slice of bacon, pork chop, bacon, minced meat rolled in bacon Eggs: chicken eggs Bakery products: cake, almond paste cake, biscuits, brown spiced biscuit, pie Vegetables/fruit: apple, orange, grape, banana, onion, carrot, beet, chicory or leek, tomato, cucumber, paprika, mushroom, cauliflower, broccoli, white cabbage, red cabbage, brussel sprout, spinach, endive, lettuce, French beans Cheese: gouda cheese, edammer cheese, cheese (>48% fat, less salt), cheese (>30% fat), brie cheese Beef: ground beef, beefburger, stewing steak, braising steak, minced steak Chicken/poultry: chicken leg, quarter chicken, chicken filet, chicken burger, collared chicken Butter: butter salt-free, salted, low-fat Milk: half cream milk Vegetable oil: margarine (solid/fluid), low-fat margarine, frying fat (vegetable), frying oil (vegetable), sunflower oil Industrial oil: low-fat margarine, frying fat (industrial), frying oil (industrial)	Meat; dairy; fruits and vegetables; grains; fats/other	**Butter: n = 1, point = 0.002 ng/g** **Cheese: n = 1, point = 0.007 ng/g** **Eggs: n = 1, point = 0.006 ng/g** **Pork: n = 1, point = 0.002 ng/g** **Beef: n = 1, point = 0.004 ng/g** **Chicken/poultry: n = 1, point = 0.001 ng/g** **Bakery products: n = 1, point = 0.001 ng/g** **Vegetables/fruit: n = 1, point = 0.001 ng/g** **Flour: n = 1, point = 0.015 ng/g** Milk: n = 1, <0.001 ng/g Vegetable oil: n = 1, <0.0001 ng/g Industrial oil: n = 1, <0.0003 ng/g (LOD not reported) *n represents number of composite samples
Jogsten et al. (2009)	Spain (Catalonia) Food samples purchased from local markets, large supermarkets, and grocery stores from two different areas of Tarragona Province, Catalonia in January and February 2008. The cities of Tarragona and Reus were sampled in the northern area and L'Ametlla de Mar and Tortosa in the southern area. For each food item, two composite samples were analyzed (one composite for the northern area and one for the southern area). Each composite was	Fruits and vegetables; meat; fats/other	Lettuce; raw, cooked, and fried meat (veal, pork, and chicken); fried chicken nuggets; black pudding; lamb liver; pate of pork liver; foie gras of duck; "Frankfurt" sausages; common salt: n = 2 for each food item, DF 0% (LOD not reported) *n represents number of composite samples *Values of ND were replaced with 1/2×LOD

Study	Location and Source	Food Types	PFNA Results
	formed of a minimum of six individual sub-samples of the same product.		
Ericson et al. (2008a)	Spain Food samples purchased from local markets, large supermarkets, and grocery stores within Tarragona County in July 2006. Food samples were randomly purchased with origin source not specified. Each of the food samples were duplicated and combined to analyze a composite sample. Composite samples included the following: Vegetables: lettuce, tomato, green bean, spinach Pulses: lentils, beans, chickpeas Cereals: rice, spaghetti, bread Pork: sausage, hot dogs, steak, hamburger, ham Chicken: breast, thighs, sausage Veal: steak, hamburger Lamb: steak Dairy products: three different kinds of cheese, yogurt, "petit-Swiss" creamy yogurt, cream caramel, custard Fruits: apple, orange, pear, banana Oil: olive oil, sunflower oil, corn oil Fats: margarine Eggs	Meat; dairy; fruits; vegetables; grains; fats/other	Vegetables (n = 2), pulses (n = 2), cereals (n = 2), pork (n = 2), chicken (n = 2), veal (n = 2), lamb (n = 2), eggs (n = 2), dairy products (n = 2), whole milk (n = 2), semi-skimmed milk (n = 2), fruits (n = 2), margarine (n = 2), oil (n = 2): DF 0% (LOD = 0.001–0.65 ng/g fw) *n represents number of composite samples
Papadopoulou et al. (2017)	Norway Participants of the A-TEAM project collected a duplicate portion of all consumed foods and drinks, prepared as for consumption, over two consecutive weekdays. Only the samples collected in the first day were analyzed. Sampling year not reported.	Solid foods: cereals and cereal products, dairy products (not milk), fish and seafood, meat and meat products, sugar and sugar products, fats and oils, vegetables and nuts, fruits, salty snacks, eggs, potatoes; liquid foods: coffee, tea and cocoa, milk, water, alcoholic beverages, soft drinks	**Solid foods:** **n = 61, DF 2%, median (range) = 0 (0–0.001) ng/g** (LOQ = not available) **Liquid foods:** **n = 61, DF 16%, median (range) = 0 (0–0.00057) ng/g** (LOQ = 0.00001 ng/g) *Concentrations <LOQ were replaced by 0
Dellatte et al. (2013)	Italy (Genoa, Brescia, Ferrara, Perugia, Portici) Ready-to-eat meals were collected at nursery and primary school canteens as they were delivered to children aged 3–10 years during the spring of 2011. One canteen was selected from each city, except for Genoa which was represented by two canteens because in one, the internal school regulation	Food composite meals	**Portici: n = 1, point = 0.0063 ng/g** Genoa (n = 2), Brescia (n = 1), Ferrara (n = 1), Perugia (n = 1): DF 0% (LOQ = 0.0060 ng/g) *n represents number of composite samples

Study	Location and Source	Food Types	PFNA Results
	forbade the use of anti-stick cookware. For each canteen, lunch meals related to five school days (from Monday to Friday) were weighed, pooled, and homogenized. Beverages were not included in the composites.		
Ghelli et al. (2019)	Italy Egg samples were collected from commercial laying hen farms in 2017. Sampling was based on geographical origin of the eggs and rearing system (e.g., organic, aviary system, battery cage and barn). A total of 132 eggs were collected and eggs were boiled. Four pools (containing three homogenized yolks) were created for each of the following groups (geographical origin, rearing system), for a total of 44 samples analyzed: Group A: Pavia, barn Group B: Verona, organic Group C: Forlì-Cesena, battery cage Group D: Bologna, barn Group E: Forlì-Cesena, battery cage Group F: Ravenna, aviary system Group G: Ravenna, aviary system Group H: Bologna, organic Group I: Romagna, battery cage Group L: Romagna, organic Group M: Romagna, barn	Fats/other	Group A: n = 4, DF 0% Group B: n = 4, DF 0% Group C: n = 4, DF[a] 25%, range = ND–traces Group D: n = 4, DF[a] 25%, range = ND–traces Group E: n = 4, DF 0% Group F: n = 4, DF 0% Group G: n = 4, DF 0% Group H: n = 4, DF 0% Group I: n = 4, DF 0% Group L: n = 4, DF 0% Group M: n = 4, DF 0% (LOD = 0.1 ng/g for all PFAS; LOQ = 0.25 ng/g for all PFAS) *Traces defined as value between LOD and LOQ
Johansson et al. (2014)	Sweden Eggs from 20 to 25 producers were collected each year from 1999 to 2010 within the Swedish National Food Agency's official food control program. Each sample consisted of a pool of 10–12 eggs from one producer. The pooled samples comprised eggs from both conventional and organic production. Information on the number of organic eggs sampled was not available. Fresh milk was sampled from the tanks of milk transport vehicles between 1999 and 2009 as part of the food control program. The tanks generally contained milk from ten dairy farms. In 2010, milk samples were taken from the milk storage tanks on individual dairy farms. Between 10–25 milk samples	Dairy; fats/other	**Hen's eggs:** **Total: n = 36, DF[a] 28%, range = ND–0.143 ng/g fw** **1999: n = 3, DF[a] 33%, range = <0.020–0.062 ng/g fw** **2000: n = 3, DF[a] 33%, range = <0.020–0.025 ng/g fw** **2001: n = 3, DF[a] 100%, mean[a] (range) = 0.052 (0.020–0.075) ng/g fw** **2002: n = 3, DF[a] 33%, range = <0.020–0.143 ng/g fw** **2003: n = 3, DF[a] 33%, range = <0.020–0.087 ng/g fw** **2004: n = 3, DF[a] 33%, range = <0.020–0.020 ng/g fw**

Study	Location and Source	Food Types	PFNA Results
	were collected each year. The milk samples were extracted in two different batches.		2005: n = 3, DF 0% 2006: n = 3, DF 0% **2007: n = 3, DF[a] 33%, range = <0.020–0.020 ng/g fw** 2008: n = 3, DF 0% 2009: n = 3, DF 0% **2010: n = 3, DF[a] 33%, range = <0.020–0.026 ng/g fw** (MDL = 0.020 ng/g fw; MQL = 0.097 ng/g fw) Cow's milk (1st batch): Total: n = 18, DF 0% (MDL = 0.0073 ng/g fw; MQL = 0.0156 ng/g fw) Cow's milk (2nd batch): Total: n = 18, DF 0% (MDL = 0.0073 ng/g fw; MQL = 0.0156 ng/g fw)
Eriksson et al. (2013)	Denmark (Faroe Islands) Locally produced food items sampled in 2011–2012. Packaged dairy products were supplied by Faroe Islands, Meginfelag Búnaðarmanna – dairy products included samples of milk, low fat (0.5%), semi-skimmed (1.5%), yoghurt with banana and pear (3.4% fat), low fat (0.9%) plain yoghurt, and créme fraiche (18% fat). Yoghurt with banana and pear was sampled from two production batches, and the low fat plain yoghurt and créme fraiche was sampled from one production batch. Potatoes were sampled from two different farms.	Dairy; fruits and vegetables	**Milk:** **Farmer (Innan Glyvur): n = 1, point = 0.061 ng/g ww** **Farmer (Havnardal): n = 1, point = 0.073 ng/g ww** **Diary (Faroe Island): n = 4, DF[a] 75%, range = <0.048–0.058 ng/g ww** Yogurt (n = 3), creme fraiche (n = 1), potato (n = 2): DF 0% (LOD = 0.0048 ng/L for milk; LOD = 0.0014 ng/g for dairy; LOD = 0.0017 ng/g for potato)
Still et al. (2013)	Germany Fourteen commercially available samples of various dairy products and raw milk were provided from a cooperating dairy.	Dairy	**Milk products:** Raw milk: n = 1, point = <0.0016 ng/g Fresh milk: n = 1, point = <0.0016 ng/g Fresh whole milk: n = 1, point = <0.0016 ng/g **UHT milk: n = 1, point = 0.0034 ng/g** Yogurt: Yogurt (0.1% fat): n = 1, point = <0.0016 ng/g Yogurt (3.8% fat): n = 1, point = <0.0016 ng/g **Cheese:** **Semihard cheese: n = 1, point = 0.0094 ng/g** **Semisoft cheese: n = 1, point = 0.0062 ng/g** **Soft cheese: n = 1, point = 0.0081 ng/g**

Study	Location and Source	Food Types	PFNA Results
			Other dairy products: Whey drink: n = 1, point = <0.0016 ng/g Butter milk: n = 1, point = <0.0016 ng/g Cream yogurt: n = 1, point = <0.0016 ng/g Cream: n = 1, point = <0.0016 ng/g **Butter: n = 1, point = 0.0047 ng/g (between LOQ and LOD)** (LOD = 0.0016 ng/g)
Vestergren et al. (2013)	Sweden (Kårsta) Study was conducted at a dairy cattle farm that was selected to represent a background contaminated agricultural area with no known point sources of PFAS in the proximity. The farm had no history of sewage sludge application to the pasture land. Milk samples were collected between November 2010 and April 2011 from a milk tank, where milk from the entire farm is stored after milking. Muscle, liver, and whole blood samples were obtained from five individual cows from the slaughterhouse on two different occasions (April and June 2011).	Meat; dairy	**Milk: n = 6, DF NR, mean = 0.0023 ng/mL** **Liver: n = 5, DF NR, mean = 0.016 ng/g** **Blood: n = 5, DF NR, mean = 0.014 ng/g** **Muscle: n = 5, DF NR, mean = 0.0045 ng/g** (MDL = 0.0012 ng/mL in milk; 0.0023 ng/g in liver; 0.0020 ng/g in blood; 0.0013 ng/g in muscle)
Falandysz et al. (2006)	Poland Eider duck samples were collected from the Gulf of Gdañsk in the Baltic Sea (south coast of Poland) in February 2003.	Meat	**n = 16, DF NR, mean, median (range) = 0.4, 0.32 (0.3–0.9) ng/mL** (LOD not reported) *Values reported for animal whole blood samples
Lankova et al. (2013)	Czech Republic Breastmilk samples were obtained from 50 women living in the Olomouc region from April to August 2010. The age of participating mothers ranged from 20 to 43 years. Six types of infant formula from the Czech retail market were also examined: one powdered formula for infants, two formulas for toddlers, one formula for babies with lactose intolerance, one formula for premature babies, and one soya-based formula for babies with non-milk diets. Sampling year not provided.	Fats/other; breastmilk	**Breastmilk:** **n = 50, DF (frequency of quantification) 48%, range = <0.006–0.015 ng/mL** (LOQ = 0.006 ng/mL) **Infant formula:** **n = 6, DF NR, maximum = 0.011 ng/g** (LOQ = 0.009 ng/g)
Llorca et al. (2010)	Spain (Barcelona)	Breastmilk; grains; fats/other	Breastmilk: n = 20, DF 0% (frequency of quantification) (MLOD = 0.0035 ng/mL; MLOQ = 0.0115 ng/mL)

Study	Location and Source	Food Types	PFNA Results
	Individual breastmilk samples were collected from 20 women in Barcelona city in 2008. All samples were collected within 40 days postpartum. Samples of powdered infant formula (three brands) and dry cereal baby food (two brands) were collected from retail stores (sample year not reported).		**Baby cereals:** **n = 2, DF[a] 100%, mean[a] (range) = 0.091 (0.044–0.138) ng/g** (MLOD = 0.017 ng/g; MLOQ = 0.0575 ng/g) **Milk infant formulas:** **n = 3, DF[a] 100%, mean[a] (range) = 0.166 (0.118–0.219) ng/g** (MLOD = 0.012 ng/g; MLOQ = 0.039 ng/g)
Abdallah et al. (2020)	Ireland (Dublin) Breastmilk samples obtained from mothers recruited from breastfeeding clinics at two Irish maternity hospitals. Mothers provided samples between 3 and 8 weeks postpartum. Mothers were up to 41 years of age, primiparas, in good health, and exclusively feeding one infant. Sampling year not reported.	Breastmilk	**n = 16, DF 69%, mean, median (range) = 0.026, 0.014 (<0.01–0.1) ng/mL** (LOQ = 0.01 ng/mL) *Values <LOQ assumed to equal DF × LOQ
Beser et al. (2019)	Spain (Valencian region) Breastmilk samples were collected from 14 Spanish women (aged 30–39 years) living in the Valencian region and recruited by the perinatology group of the Health Research Institute La Fe in Valencia. Milk samples were collected at different stages after birth during 2015.	Breastmilk	**n = 20, DF[a] 5%, mean, median (range) = 0.070, 0.070 (ND–0.070) ng/mL** (MDL = 0.008 ng/mL; LOQ = 0.066 ng/mL) *Median and mean values calculated from values above LOQ
Nyberg et al. (2018)	Sweden (Gothenburg, Stockholm) Breastmilk samples were collected between two weeks and three months after delivery from healthy native Swedish mothers, who were predominately non-smokers and primiparous. There were a total of 20 pooled samples analyzed from Stockholm (1972–2016), containing 9–116 individual samples per pool, and 11 pooled samples from Gothenburg (2007–2015), containing 5–11 individuals per pool. In addition, samples collected in 2012 (16 from Gothenburg and 20 from Stockholm) and in 2016 (10 from Stockholm) were analyzed individually.	Breastmilk	**Stockholm (pooled): n = 20, DF[a] 100%, mean[a] (range) = 0.018 (0.003–0.051) ng/mL** **Gothenburg (pooled): n = 11, DF[a] 100%, mean[a] (range) = 0.017 (0.011–0.024) ng/mL** **Stockholm (2012, individual): n = 20, DF[a] 100%, mean[a] (range) = 0.015 (0.003–0.030) ng/mL** **Gothenburg (2012, individual): n = 16, DF[a] 100%, mean[a] (range) = 0.017 (0.005–0.038) ng/mL** **Stockholm (2016, individual): n = 10, DF[a] 70%, range = <0.002–0.018 ng/mL** (MDL = 0.002 ng/mL)
Motas Guzmàn et al. (2016)	Spain (Murcia) Individual breastmilk samples were collected from 67 women in Portman Bay, Murcia, Spain in May 2014. The area was one of the most degraded zones	Breastmilk	**n = 67, DF 6%, mean, median (range) = 0.041, 0.040 (0.015–0.070) ng/mL** (LOD = 0.0002 ng/mL) *For calculations, values <LOD were considered equal to zero

Study	Location and Source	Food Types	PFNA Results
	of the Mediterranean Sea due to mining activities. Samples were collected a few weeks postpartum.		*Range reported only for detected values
Antignac et al. (2013)	France (Seine-Saint Denis, Ardèche, Isère, Loire, Savoie counties) Breastmilk samples collected from mothers participating in the ELFE pilot study. Sampling year not reported, though all mothers gave birth in October 2007. Mothers were contacted by phone one month after leaving the maternity and provided with instructions on breastmilk collection. Milk samples could be collected during several lactation sessions. On average, 15 aliquot samples of 10 mL were collected for each participant and pooled into one sample for analysis.	Breastmilk	**n = 48, DF 2%, range = <0.05–0.064 ng/mL** (LOD = 0.05 ng/mL)
Croes et al. (2012)	Belgium (Flanders) Breastfeeding mothers were recruited from 9 maternities in 24 rural communities in East and West Flanders and Flemish Brabant in May 2009 – June 2010. Breastmilk samples were collected between two and eight weeks after delivery and a subset was analyzed for perfluorinated compounds.	Breastmilk	**n = 40, DF 42.5%, median (10^{th}–90^{th} percentile) = <LOQ (<LOQ–0.02) ng/mL** (LOQ = 0.01 ng/mL) *For all calculations, values <LOQ were treated as ½ LOQ
Kärrman et al. (2007)	Sweden (Uppsala, Göteborg, Lund, Lycksele) Individual breastmilk samples from 12 women in Uppsala, Sweden were collected in 2004. Composite samples were created from breastmilk samples collected from 25–90 women each year between 1996 and 2004 and pooled into an annual composite sample. Donors originated from four regions in Sweden (Uppsala 1996 -2000, 2002; Göteborg 2001; Lund 2003; Lycksele 2003-2004). All samples were collected from primiparous women (19–41 years old) during the third week after delivery.	Breastmilk	**Individual samples:** **2004: n = 12, DF[a] 17%, mean, median (range) = 0.017, NA (<0.005–0.020) ng/mL** **Composite samples:** **1996: n = 1, point = 0.028 ng/mL** 1997: n = 1, point = <0.005 ng/mL 1998: n = 1, point = <0.005 ng/mL 1999: n = 1, point = <0.005 ng/mL **2000: n = 1, point = 0.019 ng/mL** 2001: n = 1, point = <0.005 ng/mL 2002: n = 1, point = <0.005 ng/mL 2003: n = 1, point = <0.005 ng/mL **2003–2004: n = 1, point = 0.020 ng/mL** *n represents number of composite samples (DL = 0.005 ng/mL)
Cariou et al. (2015)	France (Toulouse) Breastmilk samples obtained from female volunteers hospitalized between June 2010 and January 2013 for planned caesarean delivery. Samples were	Breastmilk	n = 61, DF 0% (LOD = 0.01–0.04 ng/mL; LOQ = 0.05 ng/mL)

Study	Location and Source	Food Types	PFNA Results
	collected between the fourth and fifth day after delivery.		
Pratt et al. (2013)	Ireland Pooled breastmilk samples were collected from 109 first-time mothers at four centers across Ireland. Sampling year not reported.	Breastmilk	n = 11, DF 0% (LOD = 0.5–5 ng/mL for all PFAS) *n represents number of pooled samples
Kärrman et al. (2010)	Spain (Catalonia) Breastmilk samples were collected from healthy primiparae mothers aged 30–39 years who lived in Tarragona County for at least the last five years. Babies were aged 41–60 days when milk samples were collected in 2007.	Breastmilk	n = 10, DF 0% (LOQ = 0.03 ng/mL)

Notes: DF = detection frequency; LOD = limit of detection; LOQ = limit of quantitation; 0.5×, 1×, or 2× = ½, 1, or 2 times the agronomic rate of biosolids application to meet nitrogen requirements of the crop; MDL = method detection limit; NR = not reported; PFAA = perfluoroalkyl acids; RL = reporting limit; WWTP = wastewater treatment plant.
Bold indicates detected levels of PFNA in food.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

C.3.2. Food Contact Materials

In 2011, FDA reached a voluntary agreement with industry to remove from the market certain PFAS grease-proofing agents used in fast food packaging. As such, the occurrence of PFNA in fast food packaging in the U.S. may be declining over time. The EPA identified two studies reporting PFNA in food contact materials (FCM) conducted in the U.S. Liu et al. (2014) analyzed the occurrence of PFAS in treated food contact paper and other consumer products purchased from local retailers and online stores in the United States between March 2007 and September 2011. All treated food contact paper was manufactured in the United States. PFNA was detected in 33% of samples (n = 9), with two of the detects below 10 ng/g and the third detect at 212 ng/g. Sinclair et al. (2007) sampled microwave popcorn bags purchased in 2005 in New York City that may have originated from international retailers. PFNA was detected in one of three brands of microwave popcorn bags, both before and after cooking.

Several peer-reviewed studies conducted in Europe also monitored for PFNA in food contact materials. PFNA was not detected in any FCMs including paper packaging, cardboard, coated bakery release papers for oven baking, paper filters for coffee, microwave popcorn bags, and an ice cream tub in two studies (Vavrouš et al., 2016; Moreta and Tena, 2013). Two additional studies had the majority of samples reported as <MDL and <LOD (Vestergren et al., 2015; Zafeiraki et al., 2014). Vestergren et al. (2015) measured levels of PFNA in a baking mold, paper plates, a baking cover, and a paper cup, with PFNA only being detected in one paper plate (0.022 µg/m^2) and one paper cup (0.030 µg/m^2). Zafeiraki et al. (2014) reported <LOD for beverage cups, an ice cream cup, fast food paper boxes, paper materials for baking, microwave bags (before and after cooking), and aluminum foil bags/wrappers, but reported a range of <LOD–4.97 ng/g for fast food wrappers. Kotthoff et al. (2015) analyzed both recent FCM samples and archived samples purchased before 2010. Recent samples ranged in concentrations from <LOQ to 1.0 ng/g and archived samples were reported in higher concentrations ranging from 68.5 ng/g to 478.2 ng/g. Schlummer et al. (2015) evaluated PTFE-coated pans and a ceramic surface pan under "overheating scenarios" (250–370°C) and found PFNA detected in all samples at a range of 1.60–869 ng/h for the PTFE-coated pans and 0.09–0.26 ng/h for the ceramic surface pan. This study also evaluated a sandwich maker and waffle iron under normal applications (185–221°C) with the sandwich maker having no PFNA detected, and the waffle maker being detected at a range of <LOD–0.40 ng/h. A study from Poland analyzed wrapping papers, breakfast bags, baking papers, and roasting bags from three different brands. They found PFNA in all three wrapping papers at a range of 0.04–0.11 pg/cm^2, all three breakfast bags at a range of 0.02–0.07 pg/cm^2, one baking paper sample at 0.02 pg/cm^2, and no roasting bag samples.

In an analysis performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology in Prague, Czech Republic, PFNA was not detected in 42 samples of disposable food packaging and tableware purchased from six different European countries between May and December 2020 (LOQ = 1.7 mg/kg) (Straková et al., 2021).

Table C-6. Summary of Studies Reporting the Occurrence of PFNA in Food Contact Materials

Study	Location	Site Details	Results
United States			
Liu et al. (2014)	United States (unspecified)	Treated food contact papers, including microwave cooking bags, were purchased between October 2007 and September 2011 from local retailers and online stores in the United States. All products originated from the United States. A total of nine samples were purchased.	N = 9, DF[a] 33%, range = BDL–212 ng/g (DL not reported)
Europe			
Vavrouš et al. (2016)	Czech Republic	Real samples of paper FCM (11 with direct food contact and 4 with indirect food contact) were acquired from a market. Samples included paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220°C (n = 3), and paper filters for coffee preparation (n = 3). Sampling year and country of origin for products not reported.	N = 15, DF 0% (LOQ = 0.0047 mg/kg)
Kotthoff et al. (2015)	Germany (Schmallenberg)	Thirty-three random samples of recent individual paper-based FCMs collected in the first until the third quarter of 2010 in Germany. Individual samples were bought from local retailers or collected by coworkers of the involved institutes. Sampled products spanned all quality levels from entry level to cutting edge products. The age of the samples ranged from a few years to decades. Country of origin not reported. "Archived" older samples of FCMs (baking paper purchased before 2010) were collected from the staff of the institutes. The age of these samples ranged from a few years to decade. Country of origin not reported.	Recent samples: n = 33, DF 24%, median (range) = <LOQ (<LOQ–1.0) ng/g Archived samples: n = 3, DF NR, median (range) = 284.9 (68.5–478.2) ng/g (LOQ = 0.5 ng/g) *Concentrations <LOQ were considered as zero *For recent samples, Table 1 reports n = 33, the Results text reports n = 36 according to the actual sampling plan, and Table 13 in Supplemental Appendix 3 reports n = 12
Schlummer et al. (2015)	Germany	Three PTFE coated pans (one low price product and two pans from the medium and upper quality range) and one pan with a ceramic surface were purchased from German stores in 2012. Producers of all four pans claimed their products were free of PFOA. Additionally, four electrically heated non-stick FCMs (three waffle irons and one sandwich maker) were acquired from households of members of the	Overheating scenario: PTFE-coated pans: n = 8, DF[a] 100%, mean[a] (range) = 221.7 (1.60–869) ng/h Ceramic surface pan: n = 2, DF[a] 100%, mean[a] (range) = 0.175 (0.09–0.26) ng/h (SDL = <0.8 ng/h) Application at normal temperatures: Sandwich maker: n = 3, DF[a] 0%

Study	Location	Site Details	Results
		Fraunhofer-Institut IVV. The authors estimated the institute-acquired items were purchased between pre-2000 to 2006. Two scenarios were tested: an "overheating scenario" for the pans (250–370°C) and a "normal application" scenario for 1 hour for all other food contact materials (185–221°C). Each product was tested 2–3 times.	Waffle irons: n = 9, DF[a] 22%, range = <LOD–0.40 ng/h (SDL = 0.1–0.5 ng/h)
Surma et al. (2015)	Poland	Three different brands of FCMs (A, B, C), including wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3), were obtained from typical, commercially available food contact products. Sampling year and country of origin for products not reported.	Wrapping paper: Brand A: n = 1, point = 0.11 pg/cm^2 Brand B: n = 1, point = 0.04 pg/cm^2 Brand C: n = 1, point = 0.06 pg/cm^2 Breakfast bag: Brand A: n = 1, point = 0.07 pg/cm^2 Brand B: n = 1, point = 0.03 pg/cm^2 Brand C: n = 1, point = 0.02 pg/cm^2 Baking paper: Brand A: n = 1, point = <LOQ Brand B: n = 1, point = <LOQ Brand C: n = 1, point = 0.02 pg/cm^2 Roasting bag: Brand A: n = 1, point = ND Brand B: n = 1, point = ND Brand C: n = 1, point = ND (LOD = 0.01 pg/cm^2; LOQ = 0.02 pg/cm^2)
Vestergren et al. (2015)	Norway (Tromsø, Trondheim)	Five samples of FCMs (one baking mold, two paper plates, one baking cover, and one paper cup) were purchased from major retail stores in November 2012. Sampling campaign designed to evaluate consumer products in product categories that were previously found to contain PFAS residuals and that were representative of products imported from China in large quantities. Individual products randomly selected without prior knowledge of surface treatment with PFAS. Year of manufacture not reported.	Baking mold: n = 1, point = <0.005 µg/m^2 Paper plates: n = 1, point = 0.022 µg/m^2 Baking cover: n = 1, point = <0.005 µg/m^2 Paper plates: n = 1, point = <0.005 µg/m^2 Paper cup: n = 1, point = 0.030 µg/m^2 (MDL = 0.005 µg/m^2)
Zafeiraki et al. (2014)	Greece	Forty-two samples of FCMs made of paper, paperboard, or aluminum foil were obtained randomly from retailers. Their exact composition was not stated and there was no information about perfluorochemicals used in their manufacturing process or not. Beverage and ice cream cups,	Beverage cups: n = 8, DF 0% Ice cream cup: n = 1, DF 0% Fast food paper boxes: n = 8, DF 0% Fast food wrappers: n = 6, DF NR, range = <LOD–4.97 ng/g

Study	Location	Site Details	Results
		wrappers, and paper boxes were collected in Athens from October to December 2012 from popular Greek fast food chain restaurants, coffee shops, and multiplex cinemas. Other FCMs (muffin cups, baking papers, and microwave popcorn and rice bags) were purchased from large supermarkets. All products except for microwave popcorn and rice bags were manufactured in Greece. Sampled packaging materials included unused items and used items (i.e., contained food products). A microwave popcorn bag was also analyzed before and after cooking.	Paper materials for baking: n = 2, DF 0% Microwave bags: n = 3, DF 0% Before cooking: n = 1, point = <LOD After cooking: n = 1, point = <LOD Aluminum foil bags/wrappers: n = 14, DF 0% (LOD = 0.42 ng/g; LOQ = 1.25 ng/g)
Moreta and Tena (2013)	Spain	Food-contact packings (microwave popcorn bag, ice cream tub, cardboard cup) were purchased from different local supermarkets between late 2011 and early 2012. Six microwave bags were purchased representing five different generic brands and one name brand; these corresponded to salty (n = 3), salty and buttered (n = 2), and sweet (n = 1) popcorn. The cardboard cup was made of printed cardboard and lined with a polymer layer. The ice cream tub was also made of printed cardboard and lined inside and outside with a polymer layer.	Popcorn bags: n = 6, DF 0% Cardboard cup: n = 1, DF 0% Ice cream tub: n = 1, DF 0% (LOD = 0.9 ng/g)

C.3.3. Consumer Products

Since the 1950's, PFNA has been used in industrial and consumer products, including fabric and carpet protective coatings, paper coatings, insecticide formulations, and surfactants (NCBI, 2022b). PFNA and other long-chain PFAS are found in aqueous film-forming foams, cosmetics, dental floss, floor polish, leather, food packaging materials, lithium batteries, ski wax, treated apparel, work apparel for medical staff, pilots, and firefighters, and in hair treatment products (NCBI, 2022b). Based on limited testing, PFNA has been detected in rinsates from fluorinated high-density polyethylene (HDPE) containers used by one pesticide product supplier (USEPA, 2022a). PFNA is not a registered pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and the EPA does not set a 40 CFR Part 180 pesticide tolerance in food and feed commodities for PFNA (US GPO, 2022). Maximum residue levels for PFNA were not found in the Global Maximum Residue Level Database (Bryant Christie Inc, 2022).

Two studies based in the U.S. were identified that analyzed PFNA concentrations in a range of consumer products, including children's nap mats, household carpet/fabric-care liquids, and textiles (Zheng et al., 2020; Liu et al., 2014) (Table C-7). Of these two studies, the consumer products evaluated are likely used by adults (e.g., floor waxes), can come into contact with both adults and children (e.g., treated upholstery), or the user was not specified (e.g., clothing). Zheng et al. (2020) determined the occurrence of ionic and neutral PFAS in the childcare environment (dust and nap mats). Samples of children's nap mats were collected from seven Seattle childcare centers (n = 26; 20 polyurethane foam, 6 vinyl cover samples). PFNA was detected in 36% of nap mat samples with a mean concentration of 0.19 ng/g. Half of the analyzed mats were purchased as new products and the other half were used. The authors reported that total PFAS levels in the new versus Used mats were not significantly different. Total PFAS levels in mat foam versus Mat covers were also similar. Based on these results, the authors suggested that indoor air was not the major source of PFAS in mats and that PFAS in mats could be associated with the manufacturing process. Liu et al. (2014) analyzed the occurrence of PFAS in consumer products (including pretreated carpeting, commercial carpet-care liquids, household carpet/fabric-care liquids, treated apparel, treated home textiles and upholstery, treated non-woven medical garments, treated floor waxes and stone-wood sealants, membranes for apparel, and thread-sealant tapes and pastes) purchased between March 2007 and September 2001 from local retailers and online stores in the United States. The consumer products originated from the United States, England, Vietnam, China, Thailand, El Salvador, Bangladesh, Dominican Republic, Malaysia, and Indonesia. PFNA was detected in 44% of nine pretreated carpeting samples (ranging from below the detection limit (BDL) to 236 ng/g); in 58% of 12 commercial carpet/fabric-care liquid samples (BDL–8,860 ng/g); in 15% of 13 household carpet/fabric-care liquid and foam samples (BDL–37.3 ng/g); in 60% of 15 treated apparel samples (BDL–235 ng/g); in 100% of six treated home textile and upholstery samples with a mean of 42.6 ng/g; in 56% of nine treated non-woven medical garment samples (BDL–334 ng/g); in 88% of eight treated floor wax and stone/wood sealant samples (BDL–2,740 ng/g); and in 75% of eight membranes for apparel samples (BDL–12.8 ng/g). PFNA was not detected in thread-sealant tapes and pastes (n = 6). Detection limits were not reported in the study.

Results for all of the identified non-U.S. studies are presented in detail in Table C-7 and are summarized here. One study from Germany did not detect PFNA in cleaners and wood glue, but among other consumer products such as nanosprays/impregnation sprays, gloves, and ski wax

found medians ranging from 2.8–10.7 ng/g (Kotthoff et al., 2015). Additional consumer products from this study found outdoor textiles, carpets, leather, and awing cloth median concentrations ranging between <LOQ–3.7 μg/m^2. Notably, ski wax had a maximum concentration of 678.0 ng/g, much larger than all other samples which fell within the range. Favreau et al. (2016) also evaluated concentrations in various consumer products in Switzerland, including cleaners, polishes, and "other" which all had detection frequencies of 0%. Impregnation products (n = 60) were the only type with detectable levels of PFNA (range of 100–1,900 ng/g; DF = 5%). This study also measured PFNA in firefighting foams, the results for which are presented in Table C-7. Three other studies investigated PFNA concentrations in firefighting foam, two of which reported detection frequencies of 0% (Dauchy et al., 2017; Laitinen et al., 2014), while Høisæter et al. (2019) reported PFNA concentrations to be 3,100 ng/L in a 1:100 diluted foam sample.

van der Veen et al. (2020) identified PFNA in 13 samples of outdoor water repellant clothing where samples were cut into two pieces with one piece receiving elevated UV radiation, humidity, and temperature for 300 hours and the other remaining untreated. The untreated samples ranged in concentrations from ND–1.1 μg/m^2 and the treated samples ranged in concentrations from ND–100 μg/m^2. Another study analyzed clothing samples, as well as furniture textiles and carpets, and found cotton/leather clothes ranging in concentration from <0.005–0.008 μg/m^2 (n = 4), however outdoor clothing was excluded (Vestergren et al., 2015). Random samples of furniture textiles ranged in concentrations from <0.005–0.097 μg/m^2 (n = 27) and carpets/mats ranged in concentrations from <0.005–0.077 μg/m^2 (n = 9).

Cosmetic products were evaluated for PFNA concentrations in samples that reported PFAS as an ingredient and those that did not (Schultes et al., 2018). Among the samples that did not report PFAS as an ingredient, including moisturizing cream, foundation, powder and eye shadow, and shaving cream, the detection frequency of PFNA was 0%. Among PFAS-containing samples (the same type of cosmetics as previously noted with the addition of an eye pencil), moisturizing cream, eye pencil, and shaving cream reported 0% detection frequency. However, foundation samples (n = 6) ranged in concentrations from <3.45–651 ng/g and powder and eye shadow samples (n = 10) ranged in concentrations from <3.45–47.2 ng/g. Schlummer et al. (2015) evaluated the concentration of PFNA in three types of PTFE coated products: electric irons (n = 9), iron sole plates (n = 3), and hair straighteners (n = 3) under "normal applications" (180–230°C). Electric irons had a range of <LOD–0.10 ng/h, iron sole plates had a range of <LOD–0.03 ng/h, and hair straighteners had a detection frequency of 0%.

Table C-7. Summary of PFNA Consumer Product Data

Study	Location	Site Details	Results
United States			
Zheng et al. (2020)	United States (Seattle, Washington)	Children's nap mat samples (n = 26, finely cut) from seven Seattle childcare centers, including polyurethane foam (n = 20) and vinyl cover (n = 6) samples. Sampling year not reported.	N = 26, DF 36%, mean, median (range) = 0.19, 0.11 (ND–0.65) ng/g (MDL = 0.08 ng/g)
Liu et al. (2014)	United States (unspecified)	Consumer products commonly used indoors were purchased between March 2007 and September 2011 from local retailers and online stores in the United States. The samples analyzed for PFCAs included pre-treated carpeting, commercial carpet/fabric-care liquids, household carpet/fabric-care liquids and foams, treated apparel, treated home textile and upholstery (i.e., mattress pads), treated non-woven medical garments, treated floor waxes and stone-wood sealants, membranes for apparel, and thread-sealant tapes and pastes. The products originated from the United States, England, Vietnam, China, Thailand, El Salvador, Bangladesh, Dominican Republic, Malaysia, and Indonesia.	Pre-treated carpeting: n = 9, DF[a] 44%, range = BDL–236 ng/g Commercial carpet/fabric-care liquids: n = 12, DF[a] 58%, range = BDL–8,860 ng/g Household carpet/fabric-care liquids and foams: n = 13, DF[a] 15%, range = BDL–37.3 ng/g Treated apparel: n = 15, DF[a] 60%, range = BDL–235 ng/g Treated home textile and upholstery: n = 6, DF[a] 100%, mean[a] (range) = 42.6 (3.80–213) ng/g Treated non-woven medical garments: n = 9, DF[a] 56%, range = BDL–334 ng/g Treated floor waxes and stone-wood sealants: n = 8, DF 88%, range = BDL–2,740 ng/g Membranes for apparel: n = 8, DF[a] 75%, range = BDL–12.8 ng/g Thread-sealant tapes and pastes: n = 6, DF[a] 0% (DL not reported)
Europe			
van der Veen et al. (2020)	Sweden (unspecified)	Samples of durable water repellent outdoor clothing collected from six suppliers from the outdoor textile industry in Sweden (one pair of outdoor trousers, six jackets, and six fabrics for outdoor clothes*). Each sample was cut into two pieces – one exposed to elevated UV radiation, humidity, and temperature for 300 hours (assumed lifespan of outdoor clothing) and one untreated (not aged). Sampling year not reported. Year of manufacturing not reported for nine of the 13 samples; the remaining four samples (samples 4–7)	Point values presented as before aging (n =1), after aging (n = 1) Sample 1 (outdoor trousers): ND, 1.3 µg/m^2 Sample 2 (fabric for jacket): 0.05 µg/m^2, 0.14 µg/m^2 Sample 3 (fabric for jacket): ND, 0.13 µg/m^2 Sample 4 (men's jacket): ND, ND Sample 5 (men's jacket): 0.08 µg/m^2, 12 µg/m^2

Study	Location	Site Details	Results
		reported a manufacturing year of 2012/2013. Country of origin not reported. *The breakdown of the 13 items of outdoor clothing is reported differently in Section 2.2 and Table 1 of the article. Section 2.2 reports one pair of outdoor trousers, seven jackets, four fabrics for outdoor clothes, and one outdoor overall. Table 1 shows one pair of outdoor trousers, six jackets, and six fabrics for outdoor clothes	Sample 6 (fabric for outdoor clothes): 0.29 µg/m^2, 100 µg/m^2 Sample 7 (children's jacket): ND, ND Sample 8 (jacket): 1.1 µg/m^2, 3.5 µg/m^2 Sample 9 (fabric for outdoor clothes): ND, 0.63 µg/m^2 Sample 10 (fabric for outdoor clothes): ND, 0.15 µg/m^2 Sample 11 (fabric for outdoor clothes): ND, 0.49 µg/m^2 Sample 12 (fabric for outdoor clothes): ND, 0.11 µg/m^2 Sample 13 (fabric for outdoor clothes): ND, ND (LOD = 0.02–0.1 µg/m^2 for ionic PFAS)
Schultes et al. (2018)	Sweden (unspecified)	Thirty-one cosmetic products from five product categories (moisturizing cream, foundation, eye pencil, powder and eye shadow, shaving foam) purchased from the Swedish market in 2016–2017. Cosmetic products were selected based on (i) the 2015 KEMI survey which reported the most frequently reported PFAS in cosmetic products and (ii) a database of ingredient lists compiled by the Swedish Society for Nature Conservation. Twenty-four products listing nine different PFAS as active ingredients were purchased. In addition, seven products which did not list PFAS in their ingredients were also purchased from the same stores as control samples. Year of manufacture and country of origin not reported.	Control: Moisturizing cream: n = 1, DF 0% Foundation: n = 3, DF 0% Powder and eye shadow: n = 2, DF 0% Shaving cream: n = 1, DF 0% PFAS-containing: Moisturizing cream: n = 6, DF 0% Foundation: n = 6, DF[a] 16%, range = <3.45–651 ng/g Eye pencil: n = 1, DF 0% Powder and eye shadow: n = 10, DF[a] 30%, range = <3.45–47.2 ng/g Shaving cream: n = 1, DF 0% (LOD = 3.45 ng/g)
Favreau et al. (2016)	Switzerland (national)	Liquid consumer products, including impregnation agents, cleansers, polishes, lubricants, miscellaneous items, and commercial AFFFs purchased in 2012 and 2013 from stores and supermarkets throughout Switzerland. Products were purchased from 82 different producers and were selected based on their susceptibility to contain PFAS according to previous screenings. Miscellaneous "other" products included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances. AFFFs were divided into two sets based on the sampling source. AFFF set 1 was derived from stock solution in	Impregnation products: n = 60, DF 5%, mean, median (range) = 800, ND (100–1,900) ng/g Cleansers: n = 24, DF 0% Polishes: n = 18, DF 0% Others: n = 23, DF 0% AFFF set 1: n = 27, DF 70%, mean, median (range) = 3,400, 200 (100–37,900) ng/g AFFF set 2: n = 35, DF 3%, mean, median (range) = 200, ND (200–200) ng/g

Study	Location	Site Details	Results
		fire installation of industrial sites storing chemicals and petroleum products and samples may be the result of multiple AFFF fillings over the years (1990–2010 was the last documented filling date). AFFF set 2 came from commercially available AFFFs between 2012 and 2013 from six producers.	(LOQ = 0.5 ng/mL) *Mean and range values only include samples where PFNA was detected *ND treated as 0 for median calculations
Kotthoff et al. (2015)	Germany (Schmallenberg)	Forty-nine random samples of consumer products collected in the first until the third quarter of 2010 in Germany, including outdoor textiles, carpets, cleaning agents, impregnating agents, leather samples, and ski waxes. Individual samples were bought from local retailers or collected by coworkers of the involved institutes or local clubs (e.g., ski waxes from local skiing club). Sampled products spanned all quality levels from entry level to cutting edge products. The age of the samples ranged from a few years to decades. Country of origin not reported.	Cleaner: n = 6, DF = 0% Wood glue: n = 1, DF = 0% Nanosprays and impregnation sprays: n = 3, DF = 56%, median (maximum) = 2.8 (8.0) ng/g Outdoor textiles: n = 3, DF = 67%, median (maximum) = 1.0 (8.3) µg/m^2 Carpet: n = 6, DF = 20%, median (maximum) = <LOQ (1.2) µg/m^2 Gloves: n = 3, DF = 100%, median (maximum) = 2.9 (5.7) ng/g Ski wax: n = 13, DF = 73%, median (maximum) = 10.7 (678.0) ng/g Leather: n = 13, DF = 92%, median (maximum) = 1.9 (1.9) µg/m^2 Awing cloth: n = 1, DF = 100%, median (maximum) = 3.7 (3.9) µg/m^2 (LOQ = 0.5 ng/g or 0.5 µg/m^2) *Concentrations <LOQ were considered as zero
Schlummer et al. (2015)	Germany	Three types of PTFE coated products (three electric irons, one iron sole plate, and one electric hair straightener) were acquired from households of members of the Fraunhofer-Institut IVV. The sampling year was not provided but assumed to be 2012 based on the collection of other food contact materials. The authors estimated the institute-acquired items were purchased between pre-2008 to 2013. The products were tested during a “normal application” scenario for 1 hour at 180–230°C. Each product was tested three times.	Electric iron: n = 9, DF[a] 44%, range = <LOD–0.10 ng/h Iron sole plate: n = 3, DF[a] 66%, range = <LOD–0.03 ng/h Hair straightener: n = 3, DF[a] 0% (SDL = 0.1–0.5 ng/h)

Study	Location	Site Details	Results
Vestergren et al. (2015)	Norway (Tromsø, Trondheim)	Samples of furniture textile (samples included baby-related items such as baby mattress, baby blanket, and baby bed cover), carpet, and clothing samples were purchased from three major retail stores during November 2012–February 2013. Sampling campaign designed to evaluate consumer products in product categories that were previously found to contain PFAS residuals and that were representative of products imported from China in large quantities. Individual products randomly selected without prior knowledge of surface treatment with PFAS. Outdoor clothing was excluded. Year of manufacture not reported.	Furniture textiles: n = 27, DF[a] = 15%, range = <0.005–0.097 µg/m^2 Carpets/mats: n = 9, DF[a] = 33%, range = <0.005–0.077 µg/m^2 Cotton/leather clothes: n = 4, DF[a] = 50%, range = <0.005–0.008 µg/m^2 (MDL = 0.005 µg/m^2)
Høisæter et al. (2019)	Norway (unspecified)	AFFF concentrate, containing PFOS as the main PFAS, from the same supplier as assumed to have been used historically at a firefighting training facility where AFFF containing PFOS was used extensively from the early 1990s until 2001 when it was phased out and replaced by fluorotelomer containing AFFF until 2011. Concentrations reported in 1:100 diluted AFFF. Number of samples not reported but assumed to be 1. Sampling year assumed to be 2016 based on when samples were collected for groundwater and soil.	1:100 diluted foam: n = 1, point = 3,100 ng/L Relative contribution to total PFAS = <0.1% (LOQ = 0.3 ng/L)
Dauchy et al. (2017)	France (unspecified)	Nine firefighting foam concentrates were provided by a professional user and included alcohol-resistant film-forming fluoroprotein foams (n = 5), alcohol-resistant aqueous film-forming foams (n = 2), film-forming fluoroprotein foams (n = 1), and fluorine-free foams (n = 1). These concentrates were manufactured after 2002 by four different manufacturers. Concentrate sampling year was not reported, though water sampling in the same study was conducted in November 2014.	N = 9, DF (frequency of quantification) 0% (LOQ = 5,000 µg/L)
Laitinen et al. (2014)	Finland (Oulu)	Sthamex 3% AFFF liquid, manufactured in Germany and available commercially in Finland, used by firefighters during training in the simulation of aircraft accidents. Samples collected in 2010.	N = NR, DF (frequency of quantification) 0% *Low concentrations of PFNA were detected, but were below LOQ (LOQ = 20 µg/mL)
Origin Unspecified			
Bečanová et al. (2016)	Not specified	One hundred twenty-six samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment (EEE), and plastics; includes	Household equipment: n = 55, DF[a] 2%, range = <MQL–9.85 ng/g

Study	Location	Site Details	Results
		children-related items such as teddy bear filling, teddy bear cover, and plush); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, I materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) purchased (for new materials) or collected from various sources (for older and used materials). Production year ranged from 1981 to 2010. Origin of production and location and year of purchase/collection not reported.	Building materials: n = 54, DF[a] 0% Car interior materials: n = 10, DF[a] 0% WEEE: n = 7, DF[a] 14%, range = <MQL–0.221 ng/g (IQL = 27 pg/mL; MQL = 0.16 ng/g)
Gremmel et al. (2016)	Not specified	Sixteen outdoor jackets (15 outdoor jackets and one working jacket) purchased during August 2011 to March 2012. Besides the working jacket and two other jackets (one arrived unpacked in shop and had been on sale for four weeks while the other had been on sale since February 2010), all other jackets were new and packed in a plastic shell. Jackets were selected considering factors such as origin of production (primarily Asia, with some origins not specified), price, market, and textile. Location of purchase and year of manufacture not reported.	J0: n = 2, DF NR, mean = <LOQ J1: n = 2, DF NR, mean = 0.14 ng/g J2: n = 2, DF NR, mean = 1.54 ng/g J3: n = 2, DF NR, mean = 0.33 ng/g J4: n = 2, DF NR, mean = 0.35 ng/g J5: n = 2, DF NR, mean = 0.22 ng/g J6: n = 2, DF NR, mean = 0.40 ng/g J7: n = 2, DF NR, mean = 0.33 ng/g J8: n = 2, DF NR, mean = 0.34 ng/g J9: n = 2, DF NR, mean = 1.17 ng/g J10: n = 2, DF NR, mean = 0.29 ng/g J11: n = 2, DF NR, mean = 6.87 ng/g J12: n = 2, DF NR, mean = 0.18 ng/g J13: n = 2, DF NR, mean = 0.47 ng/g J14: n = 2, DF NR, mean = 22.9 ng/g J15: n = 2, DF NR, mean = 62.9 ng/g (LOD = 0.05 ng/mL; LOQ = 0.01 µg/m^2)

Notes: BDL = below detection limit; DF = detection frequency; DL = detection limit; MDL = method detection limit; ND = not detected; PFCA = Perfluoroalkyl carboxylic acids.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

C.3.4. Indoor Dust

In a Wisconsin Department of Health Services study, Knobeloch et al. (2012) examined levels of 16 perfluoroalkyl chemicals in vacuum cleaner dust from 39 Wisconsin homes across 16 counties in March and April 2008 (Table C-8). Samples from these homes built between 1890 and 2005 were collected during a pilot study to assess residential exposure to persistent contaminants found in the Great Lakes Basin. PFNA was found in all samples at a median concentration of 12 ng/g. The number of rooms with synthetic, wall-to-wall carpeting and the square footage of the homes were both significantly positively correlated with dust concentrations of PFNA. Based on the results of this study, the authors suggested that perfluoroalkyl chemicals may be ubiquitous contaminants in U.S. homes. In an EPA study of 112 indoor dust samples collected from vacuum cleaner bags from homes and daycare centers in North Carolina and Ohio in 2000–2001 (EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study), samples were collected from 102 homes and 10 daycare centers in North Carolina (49 homes, 5 daycare centers) and Ohio (53 homes, 5 daycare centers) (Strynar and Lindstrom, 2008). Results were not reported separately for homes and daycares. Overall, PFNA was detected in 42.9% of all samples (n = 112) with mean and median concentrations of 22.1 ng/g and 7.99 ng/g, respectively. The authors concluded that the study measured perfluorinated compounds in house dust at levels that may represent an important pathway for human exposure.

Additional peer-reviewed studies based in the U.S. were identified that evaluated the occurrence of PFNA and other PFAS in dust of indoor environments, primarily in homes, as well as in schools, childcare facilities, offices, and vehicles (Zheng et al., 2020; Scher et al., 2019; Byrne et al., 2017; Karásková et al., 2016; Wu et al., 2014; Fraser et al., 2013; Kato et al., 2009) (Table C-8). For those studies with results stratified for U.S. homes, PFNA levels and detection frequencies were lowest in a study of remote Alaska Native villages (35% detection, median below 0.2 ng/g), while in other U.S. locations, PFNA was detected in at least 65% of samples (some studies reporting 100% detection) at widely varying mean and median levels across the studies (from approximately 4 ng/g to 70 ng/g). Few studies sampled childcare centers, vehicles, and offices, and none of the reviewed studies reported measurements in other microenvironments (e.g., public libraries, universities).

Several studies reported results from dust samples collected only from homes (Scher et al., 2019; Byrne et al., 2017; Wu et al., 2014), with one study sampling from locations near a PFAS production facility. Scher et al. (2019) evaluated indoor dust in 19 homes in Minnesota within a groundwater contamination area (GCA) in the vicinity of a former 3M PFAS production facility. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota PWS or a private well previously tested and shown to have detectable levels of PFOA or PFOS. In the house dust samples, collected from July to September 2010, the detection frequencies for PFNA were 68% and 95% for entryways to the yard and interior living spaces such as the family or living rooms, respectively (n = 19 each), with median concentrations of 9.7 ng/g and 26 ng/g, respectively. PFAS concentrations in both sampling locations were higher than corresponding soil concentrations, suggesting that interior sources were the main contributors to PFAS in house dust.

Byrne et al. (2017) assessed exposure to PFNA and other PFAS among residents of two remote Alaska Native villages on St. Lawrence Island. PFAS concentrations were measured in dust collected from the surfaces of floors and furniture of 49 homes on St. Lawrence Island during February–April of 2013 and 2014. Residents were asked not to sweep or dust for one week prior to sampling. The authors described the overall PFAS levels in dust samples as "on the lower end of those reported worldwide in other studies." PFNA was detected in 35% of all samples (n = 49) with a median value below the LOD (0.1 ng/g–0.2 ng/g). Wu et al. (2014) measured concentrations of five PFCs in residential dust in California in 2008–2009. Dust samples were collected from the carpet or area rug in the main living area of the home. Homes of parents with young children and homes with older adults were differentiated to characterize the relationship between serum concentrations of PFCs and several other factors, including PFC concentrations in residential dust. PFNA was detected in 65% of samples from households with young children in Northern California (n = 82), with mean and median concentrations of 67.4 ng/g and 9.70 ng/g, respectively. PFNA was detected in 72% of samples from households of older adults in central California (n = 42), with mean and median concentrations of 58.5 ng/g and 11.85 ng/g, respectively.

Apart from the information reported by Strynar and Lindstrom (2008), one other study included childcare centers in the locations sampled (Zheng et al., 2020). Zheng et al. (2020) collected dust samples from seven childcare centers in Seattle, Washington (n = 14) and one childcare facility in West Lafayette, Indiana (n = 6 across six rooms); the sampling year was not reported. The included childcare facilities consisted of several building types, including multiple classrooms, a former church, and a former home. Because centers were vacuumed and mopped daily, dust samples were obtained from elevated surfaces (shelving, tops of bookcases/storage cubbies) along with floor dust. PFNA was detected in all samples at mean and median concentrations of 3.2 ng/g and 1.7 ng/g, respectively.

One study evaluated PFNA levels in vehicles and offices, in addition to homes. Fraser et al. (2013) collected dust samples between January and March 2009 from three microenvironments of 31 individuals in Boston, Massachusetts (offices (n = 31), homes (n = 30), and vehicles with sufficient dust for analysis (n = 13)). Study participants worked in separate offices located across seven buildings, which were categorized as Building A (n = 6), Building B (n = 17), or Other (n = 8). Building A was a newly constructed (approximately one year prior to study initiation) building with new carpeting and new upholstered furniture in each office; Building B was a partially renovated (approximately one year prior to study initiation) building with new carpeting throughout hallways and in about 10% of offices. The other buildings had no known recent renovation occurred. Study offices were not vacuumed during the sampling week and participants were asked not to dust or vacuum their homes and vehicles for at least one week prior to home sampling. PFNA was detected in 94%, 67%, and 85% of office, home, and vehicle dust samples, respectively, with geometric mean concentrations of 63.0 ng/g, 10.9 ng/g, and 14.7 ng/g, respectively. Geometric mean PFNA concentrations were statistically significantly higher in offices compared to homes and vehicles. The study also observed that PFNA concentration in house dust was significantly predictive of PFNA serum concentration.

Two studies evaluated dust samples collected across multiple continents (Karásková et al., 2016; Kato et al., 2009). Karásková et al. (2016) examined PFAS levels in house dust collected between April and August 2013 from the living rooms and bedrooms of 14 homes in the United

States, 15 homes in Canada, and 12 homes in the Czech Republic (locations unspecified). PFNA was detected in all U.S. samples (n = 20) at mean and median concentrations of 10.9 ng/g and 3.9 ng/g, respectively. The authors reported PFNA concentrations were significantly higher in North America compared to the Czech Republic, which they indicated may suggest a faster shift from long-chain PFAS to their shorter-chain homologues in Europe than in North America. Overall, no significant differences in total PFAS concentrations were found between the bedroom and living room in the same household although significant relationships were found based on type of floors, number of residents, and age of the house. A second multicontinental study (Kato et al., 2009) measured PFC concentrations in 39 household dust samples collected in 2004 from homes in the United States (Atlanta, GA) (n = 10), United Kingdom (n = 9), Germany (n = 10), and Australia (n = 10). Across all 39 homes, PFNA was detected in 25.6% of samples with a median concentration below the LOQ (2.6 ng/g). The authors did not report stratified PFNA data by country.

Results for all of the identified non-U.S. studies are presented in detail in Table C-8 and are summarized here. One study conducted in Canada evaluated dust samples in homes by using the vacuum cleaners of participants and found PFNA detected in 69% of samples (n = 48) with a mean concentration of 0.71 ng/g (Makey et al., 2017). Studies conducted in Europe evaluated indoor dust concentrations from homes, schools, workplaces, and/or cars. One of these studies, Huber et al. (2011) from Norway, also sampled a storage room which contained chemicals and highly contaminated samples and found an elevated concentration at 43.4 ng/g when compared to other locations within the study. Other samples within the study include living rooms, carpets, sleeping rooms, sofas, and offices and concentrations ranged between 0.2–26.7 ng/g. Four studies sampled floor dust exclusively in homes with results ranging from not detected to 37 ng/g (de la Torre et al., 2019; Winkens et al., 2018; Padilla-Sánchez and Haug, 2016; Jogsten et al., 2012). Another study in Norway evaluated dust from elevated surfaces such as bookshelves and windowsills (n = 41) and reported a range of 3.9–92 ng/g, notably higher than other studies (Haug et al., 2011). One study from Sweden assessed 20 dust samples from elevated surfaces in preschools and reported a median and 95th percentile of 1.09 ng/g and 56.0 ng/g, respectively (Giovanoulis et al., 2019). Harrad et al. (2019) evaluated dust concentrations from living rooms, cars, and classrooms in Ireland ranging from <0.05 ng/g to 14 ng/g and, notably, from offices which had concentrations ranging from <0.05 ng/g to 120 ng/g. A study in Belgium evaluated randomly selected homes (n = 43) and offices (n = 10) and found dust samples containing median (95th percentile) values of 0.1 (2.1) ng/g dw and 0.4 (62) ng/g dw, respectively (D'Hollander et al., 2010).

Table C-8. Summary of PFNA Indoor Dust Data

Study	Location	Site Details	Results
United States			
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Nineteen homes in three cities within a GCA near former 3M PFAS production facility as well as from three homes in the Twin Cities Metro outside the GCA. Dust samples collected from an entryway to the yard and from an interior living space (e.g., family room, living room) in each home in July–September 2010. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. Results were not reported for homes outside the GCA.	Entryway: n = 19, DF 68%, median (range) = 9.7 (<RL–1,000) ng/g Living room: n = 19, DF 95%, median (range) = 26 (<RL–450) ng/g (RL = 5 ng/g)
Byrne et al. (2017)	United States (St. Lawrence Island, Alaska)	Dust samples collected from the surfaces of floors and furniture from 49 homes during February–April of 2013 and 2014. Participants were asked not to sweep or dust for one week prior to sampling.	n = 49, DF 35%, median (95th percentile) = <LOD (1.93) ng/g (MDL = 0.1–0.2 ng/g for all PFAS)
Wu et al. (2014)	United States (Central Valley area, California)	Distributions of PFC dust concentrations were determined for households with young children in Northern California (n = 82) and households of older adults in central California (n = 42). Dust samples were collected in 2008–2009 from the carpet or area rug in the main living area of the homes. Homes of parents with young children and homes with older adults were differentiated to characterize the relationship between serum concentrations of PFCs and PFC concentrations measured in residential dust.	Parents of young children: n = 82, DF 65%, mean, median (range) = 67.4, 9.70 (ND–1,910) ng/g Older adults: n = 42, DF 72%, mean, median (range) = 58.5, 11.85 (ND–883) ng/g (LOD = 0.10 ng/mL) *Data below LOQ replaced by LOD/√2
Knobeloch et al. (2012)	United States (Great Lakes Basin, Wisconsin)	Dust samples were collected by the Wisconsin Department of Health Services from 39 Wisconsin homes across 16 counties in March–April 2008. Vacuum bags were collected or bagless vacuums were emptied into sterilized glass jars. Homes were built between 1890 and 2005.	n = 39, DF 100%, median (range) = 12 (1.3–280) ng/g (RL = 1 ng/g)
Zheng et al. (2020)	United States (Seattle, Washington; West Lafayette, Indiana)	Seven childcare centers in Seattle (14 samples) and one center in Lafayette (6 samples); sampling year not reported. Since all centers were vacuumed and mopped daily, dust samples from elevated surfaces	n = 20; DF 100%, mean, median (range) = 3.2, 1.7 (0.11–13) ng/g (MDL = 0.08)

Study	Location	Site Details	Results
		(shelving, tops of bookcases/storage cubbies) were collected along with floor dust in the same sample.	
Strynar and Lindstrom (2008)	United States (North Carolina; Ohio)	Dust samples from vacuum cleaner bags were obtained in 2000–2001 during the EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study from North Carolina (49 homes, 5 daycare centers) and Ohio (53 homes, 5 daycare centers). Vacuum cleaner bags were only collected if available at each site.	n = 112; DF 42.9%, mean, median (maximum) = 22.1, 7.99 (263) ng/g (LOQ = 11.3 ng/g) *Values below the LOQ assigned a value of LOQ/√2
Fraser et al. (2013)	United States (Boston, Massachusetts)	Dust samples were collected in January–March 2009 from offices (n = 31), homes (n = 30), and vehicles (n = 13) of 31 individuals. Study participants worked in separate offices located across seven buildings, which were categorized into Building A, Building B, and Other. Six samples were collected from Building A, a newly constructed (approximately one year prior to study initiation) building with new carpeting and new upholstered furniture in each office. Seventeen samples were collected from Building B, a partially renovated (approximately one year prior to study initiation) building with new carpeting throughout hallways and in about 10% of offices. Eight samples were collected from the other five remaining buildings where no known recent renovation occurred. Study offices were not vacuumed during the sampling week and homes and vehicles were not vacuumed for at least one week prior to sampling. Entire accessible floor surface areas and tops of immovable furniture were vacuumed in offices and the main living area of homes. Entire surface areas of the front and back seats of vehicles were vacuumed. Number of home dust samples was reduced to 30 because 1 participant lived in a boarding house with no main living area. Sufficient mass of dust for analysis was available from only 13 vehicles.	Homes: n = 30, DF 67%, GM (range) = 10.9 (6.21–1,420 ng/g) Offices: n = 31, DF 94%, GM (range) = 63.0 (10.9–639) ng/g Vehicles: n = 13, DF 85%, GM (range) = 14.7 (4.95–101 ng/g) (LOQ = 5 ng/g) *GM calculated by replacing values <LOQ with LOQ/√2 *Range of detected values reported
Canada			
Makey et al. (2017)	Canada (Vancouver)	Dust samples were collected from a subset of Chemicals, Health, and Pregnancy (CHirP) Study participants' vacuum cleaners in 2007–2008; indoor air and serum samples were also collected. Vacuum cleaner dust was sampled by collecting whole vacuum	n = 48, DF 69%, GM = 0.71 ng/g (DL = 0.06 ng/g)

Study	Location	Site Details	Results
		cleaner bags or subsampling bagless or central vacuums.	
Europe			
de la Torre et al. (2019)	Spain (unspecified), Belgium (unspecified), Italy (unspecified)	Sixty-five homes belonging to the partners of Test-Achats (Belgium), Altroconsumo (Italy), and OCU Ediciones SA (Spain). Home occupants vacuumed the entire floor of their home from September 2016 to January 2017 and vacuum bags were collected	Total: n = 65, DF 46%, median (range) = 0.04 (ND–9.04) ng/g Spain: n = 21, DF 48%, median (range) = 0.04 (ND–5.70) ng/g Belgium: n = 22, DF 36%, median (range) = 0.04 (ND–9.04) ng/g Italy: n = 22, DF 55%, median (range) = 0.10 (ND–6.54) ng/g (LOQ = 0.06 ng/g) *Values below LOQ replaced with LOQ/(square root of 2)
Winkens et al. (2018)	Finland (Kuopio)	Sixty-three private households from the birth cohort study, LUKAS2. Floor dust samples collected in 2014/2015 from the children's bedroom (entire floor). Participants were instructed not to vacuum clean the room at least a week before sampling. For 55 rooms, dust samples were collected at the end of a 3-week air sampling period (indoor air results reported in a different study).	n = 63, DF 52.4%, mean, median (range) = 1.76, 1.05 (BDL–14.8) ng/g (MDL = 0.73 ng/g) *Values <MDL were treated as MDL/(square root of two)
Padilla-Sánchez and Haug (2016)	Norway (Oslo)	Homes of staff from the Norwegian Institute of Public Health. Dust samples collected from vacuum cleaner bags provided by staff. Sampling year not provided.	n = 7, DF[a] 71%, range = ND–3 ng/g (MDL = 0.028 ng/g; MQL = 0.094 ng/g)
Jogsten et al. (2012)	Spain (Catalonia)	Dust sampling was performed in December 2009 from ten households using household vacuum cleaner dust bags. Samples were collected out of convenience and may not be representative of the entire Catalan population.	n = 10, DF[a] 100%, mean[a] (range) = 6.77 (0.4–37) ng/g (LOD = 0.038 ng/g)
Haug et al. (2011)	Norway (Oslo)	Forty-one homes of breastfeeding mothers recruited for a study on exposure pathways. House dust samples collected between February and May 2008 on two consecutive days while the residence was in regular use. Samples taken from elevated surfaces such as bookshelves and window sills (deposited dust) and not from the floor.	n = 41, DF (frequency of quantification)[a] 61%, mean, median (range) = 29, 23 (3.9–92) ng/g (LOQ = 4.9–31 ng/g) *Concentrations that were not detected or <LOQ were replaced by the LOQ divided by the square root of two

Study	Location	Site Details	Results
Giovanoulis et al. (2019)	Sweden (Stockholm)	Twenty preschools that had been previously sampled in 2015 and then participated in the "chemical smart preschool" initiative to reduce the presence of hazardous chemicals in the indoor environment; 2015 results are reported elsewhere. Samples for this study were collected during January to February 2018. One settled dust sample was collected from elevated surfaces (50–250 cm above the floor) from different areas of a play room at each preschool.	n = 20, DF 55%, medi[an] (95th percentile) = 1.09 (56.0) ng/g (LOD = 0.5 ng/g) *Values <LOD were replaced with ½×LOD
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick)	Dust collected from homes (living rooms), offices, cars, and school classrooms; air samples also collected. Samples collected between August 2016 and January 2017. Sample numbers were split approximately equally from each of the three counties.	Homes: n = 32, DF 9.0%, mean, median (range) = <0.05, 0.52 (<0.05–14) ng/g Offices: n = 33, DF 34%, mean, median (range) = 8.6, <0.05 (<0.05–120) ng/g Cars: n = 31, DF 41%, mean, median (range) = 0.55, 0.05 (<0.05–3.1) ng/g Classrooms: n = 32, DF 6.0%, mean, median (range) = <0.05, <0.05 (<0.05–0.71) ng/g (LOD = 0.1 ng/g) *When analyte peaks are <LOD, concentrations were assumed to equal DF × LOD where DF is expressed as a fraction
Huber et al. (2011)	Norway (Tromsø)	Homes and workplaces sampled in winter 2007–2008. Home samples included seven different living rooms (L-1 to L-7), one sleeping room (S, related to L-3), one sofa (a stain repellant fabric, related to L-7), and one carpet (related to L-4). Workplace samples included an office and a storage room at Fram Center; old documents and chemicals and highly contaminated samples were stored in the storage room. Samples were taken from bookshelves, commodes, TVs, electrical heaters, picture frames, window sills and sun blinds. Dust from the floor was not sampled.	All homes: n = 7, DF NR, median = 7 ng/g Living room: n = 7, DF[a] 100%, mean, median (range) = 9.3, 7 (3.3–26.7) ng/g Carpet: n = 1, point = 0.2 ng/g Sleeping room: n = 1, point = 11.6 ng/g Sofa: n = 1, point = 2.4 ng/g Office: n = 1, point = 10.6 ng/g Storage room: n = 1, point = 43.4 ng/g (LOD on column = 0.001 ng; MDL = 0.25–4.92 ng'g)
D'Hollander et al. (2010)	Belgium (Flanders)	Forty-three randomly selected homes and ten randomly selected offices throughout Flanders. Samples collected using a vacuum from bare floor, possibly covered with carpet, in 2008. In homes, the living room, bedroom, kitchen, and working area were sampled.	Homes: n = 43, DF NR, median (95th percentile) = 0.1 (2.1) ng/g dw Offices: n = 10, DF NR, median (95th percentile) = 0.4 (62) ng/g dw (LOQ = 0.06 ng/g)

Study	Location	Site Details	Results
			*Concentrations <LOQ were replaced by DF × LOQ *For homes, both Table 3 and Section 2.1 reported n = 43; however, Table 2 reported n = 45
Multiple Continents			
Karásková et al. (2016)	United States (unspecified), Canada (unspecified), Czech Republic (unspecified)	Fifty-six dust samples from 14 homes in the United States, 15 homes in Canada, and 12 homes in the Czech Republic were collected between April and August 2013. Samples were collected in living rooms and bedrooms.	United States: n = 20, DF 100%, mean, median (range) = 10.9, 3.9 (1.1–62.9) ng/g Canada: n = 20, DF 95.0%, mean, median (range) = 19.4, 4.4 (<MQL–195) ng/g Czech Republic: n = 16, DF 50.0%, mean, median (range) = 3.0, <MQL (ND–11.0) ng/g (MDL = 0.27–1.33 ng/g; MQL = 0.72–3.48 ng/g; ranges represent lower bound and upper bound which were calculated by dividing the MDL/MQL by the biggest and smallest dust sample weight, respectively) *Mean calculated only from values >MQL *Median calculated by replacing values <MQL with √2/2*MQL
Kato et al. (2009)	United States (Atlanta, Georgia), Germany (unspecified), United Kingdom (unspecified), Australia (unspecified)	Thirty-nine household dust samples from the United States (n = 10), Germany (n = 10), United Kingdom (n = 9), and Australia (n = 10) collected in 2004 for method validation. Dust sampling procedures not described.	n = 39, DF 25.6%, median (range) = <LOQ (<LOQ–832) ng/g (LOQ = 2.6 ng/g)

Notes: CTEPP = Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants; GCA = groundwater contamination area; DF = detection frequency; RL = reporting limit; LOD = limit of detection; MDL = method detection limit; ND = not detected; LOQ = limit of quantitation; GM = geometric mean; MQL = method quantification limit; PFC = Perfluorochemicals

C.3.5. Air

Perfluoroalkyl chemicals have been released to air from wastewater treatment plants, waste incinerators, and landfills (Ahrens et al., 2011), though there is limited information on the detection levels or frequencies of PFNA in either indoor or ambient air. ATSDR (2021) notes perfluoroalkyl chemicals have been detected in air and they can be transported long distances via the atmosphere. For example, in a study performed from April 2007 to January 2009, PFNA was detected at an average concentration of 0.3 pg/m^3 in 8% of 141 atmospheric samples from Atlantic and Southern Oceans and coastal areas of the Baltic Sea (NCBI, 2022b; Dreyer et al., 2009). PFNA is not expected to be broken down directly by photolysis (NCBI, 2022b). PFNA can undergo hydroxylation in the atmosphere, with a (predicted average) atmospheric hydroxylation rate of 8.41×10^{-13} cm^3/molecule – second to a (derived) rate of 5.2×10^{-11} cm^3/molecule – second (with corresponding estimated half-life of 31 days for this reaction in air) (USEPA, 2022b; NCBI, 2022b). With a vapor pressure of 4.83×10^{-3} mm Hg at 20°C (extrapolated), 8.3×10^{-2} mm Hg at 25°C (estimated), 8.4 mm Hg at 99.63°C (measured), and a (measured) range of 4.80×10^{-3} mm Hg to 9.77×10^{-3} mm Hg, volatilization is not expected to be an important fate process for this chemical (USEPA, 2022b; NCBI, 2022b; ATSDR, 2021). The EPA's Toxics Release Inventory reported release data for PFNA in 2020, with a total onsite disposal, offsite disposal, and other releases concentration of 0 pounds from one facility (USEPA, 2022c). PFNA is not listed as a hazardous air pollutant (USEPA, 2022d).

C.3.5.1. Indoor Air

No studies from the U.S. reporting levels of PFNA in indoor air were identified from the primary or gray literature. However, the EPA identified studies from Canada and Europe that are summarized below and in Table C-9 (Harrad et al., 2019; Makey et al., 2017; Jogsten et al., 2012; Barber et al., 2007). All of these studies sampled from homes, while only one study also sampled from offices, cars, and classrooms and one study also sampled from a laboratory.

Two studies reported results from indoor air samples collected only from homes (Makey et al., 2017; Jogsten et al., 2012). In the Canadian study, Makey et al. (2017) collected indoor air, vacuum cleaner dust, and serum samples in 2007–2008 from the homes of women in the second trimester of pregnancy and analyzed the samples for levels of PFAAs. Participants were part of the Chemicals, Health, and Pregnancy (CHirP) Study. PFNA was detected in 42% of indoor air samples (n = 39), with a geometric mean of 1.5 pg/m^3. In Spain, Jogsten et al. (2012) sampled indoor air (n = 10) from selected homes in Catalonia in December 2009 and evaluated levels of 27 PFCs. PFNA was not detected (LOD = 3.1–280 pg/m^3 for all ionic PFAS).

The remaining two studies evaluated PFNA levels in offices, vehicles, and/or schools, in addition to homes. In Ireland, Harrad et al. (2019) collected air samples in homes (living rooms, n = 34), offices (n = 34), cars (n = 31), and school classrooms (n = 28) between August 2016 and January 2017. PFNA was detected in all four indoor microenvironments in 18%, 91%, 90%, and 93% of samples for homes, offices, cars, and classrooms, respectively. The mean (median) concentrations were 2.1 (1.7) pg/m^3 in homes, 3.7 (2.5) pg/m^3 in offices, 5.2 (2.1) pg/m^3 in cars, and 3.5 (2.5) pg/m^3 in classrooms. In Norway, neutral and ionic PFAS were analyzed in indoor air samples collected from three homes and one laboratory in Tromsø between April and June 2005 (Barber et al., 2007). The study detected PFNA in all four samples, with a mean concentration of 2.7 pg/m^3.

Table C-9. Summary of Studies Reporting the Occurrence of PFNA in Indoor Air

Study	Location	Site Details	Results
Canada			
Makey et al. (2017)	Canada (Vancouver)	Samples were collected from a subset of Chemicals, Health, and Pregnancy (CHirP) Study participants' homes in 2007–2008; dust and serum samples were also collected. Indoor air was sampled using passive samplers deployed in participants' bedrooms for four weeks.	n = 39, DF 42%, GM = 1.5 pg/m^3 (DL = 0.02 pg/m^3)
Europe			
Jogsten et al. (2012)	Spain (Catalonia)	Indoor air sampling was performed in December 2009 from ten households at approximately 1 m above the floor. Samples were collected out of convenience and may not be representative of the entire Catalan population. Both particulate and gas phases collected.	n = 10, DF 0% (LOD = 3.1–280 pg/m^3 for all ionic PFAS)
Harrad et al. (2019)	Ireland (Dublin, Galway, Limerick)	Air samples collected from homes (living rooms), offices, cars, and school classrooms; dust samples also collected. Samples collected between August 2016 and January 2017. Sample numbers were split approximately equally from each of the three counties. Gas or particulate phase not specified.	Homes: n = 34, DF 18%, mean, median (range) = 2.1, 1.7 (<0.3–13) pg/m^3 Offices: n = 34, DF 91%, mean, median (range) = 3.7, 2.5 (<0.3–18) pg/m^3 Cars: n = 31, DF 90%, mean, median (range) = 5.2, 2.1 (<0.3–24) pg/m^3 Classrooms: n = 28, DF 93%, mean, median (range) = 3.5, 2.5 (<0.3–15) pg/m^3 (LOD = 0.3 pg/m^3) *When analyte peaks are <LOD, concentrations were assumed to equal DF × LOD where DF is expressed as a fraction
Barber et al. (2007)	Norway (Tromsø)	Air samples taken from four indoor locations (three houses and one laboratory) in Tromsø in April–June 2005. PFNA was measured in the particulate phase.	n = 4, DF[a] 100%, mean (range) = 2.7 (0.9–4.7) pg/m^3 (MQL = 0.84 pg/m^3)

C.3.5.2. Ambient Air

A single U.S. study measured levels of PFNA in ambient air (Kim and Kannan, 2007). Kim and Kannan (2007) analyzed particle phase (n = 8) and gas phase (n = 8) concentrations of perfluorinated acids in ambient air samples collected in and around Albany, New York in May and July 2006 to examine the relative importance of certain media pathways to the contamination of urban lakes. PFNA was detected in all gas phase samples with mean and median concentrations of 0.21 pg/m^3 and 0.20 pg/m^3, respectively. PFNA was also detected in the particulate phase, but the detection frequency was not reported. Authors reported particulate phase mean and median concentrations of 0.13 pg/m^3 and below the LOQ (0.12 pg/m^3), respectively.

One Canadian PFNA study by Ahrens et al. (2011) analyzed the temporal ambient PFNA concentrations at two municipal solid waste (MSW) landfills and at one wastewater treatment plant. At the MSW landfills, they recorded a mean (range) concentration of 2.11 (0.97–3.24) pg/m^3 upwind of the site and 10.3 (4.82–15.8) pg/m^3 at the site. At the wastewater treatment plant, they measured the PFNA concentration within the facility in the primary clarifier, aeration tank, and secondary clarifier, finding mean concentrations between 2.97 and 3.62 pg/m^3. They also evaluated reference sites near to (<200m) and distant from (~600m) the facility, finding higher ambient PFNA concentrations at the near sites (mean of 1.64 pg/m^3) compared to the distant sites (single point estimate of 0.88 pg/m^3).

Among the European studies—conducted in Spain (Jogsten et al., 2012; Beser et al., 2011), Ireland (Harrad et al., 2019; Barber et al., 2007), Norway (Barber et al., 2007), and the United Kingdom (Barber et al., 2007)— each collected ambient (outdoor) air for PFNA measurements. Reported mean PFNA concentrations among these studies range from 0.08–3.8 pg/m^3. Beser et al. (2011) measured PFNA ($PM_{2.5}$-bound) in both residential and industrial areas (five sampling stations in total) of the Alicante Province in Spain in 2010, with mean concentrations ranging from 1.65–3.8 pg/m^3. Jogsten et al. (2012) did not detect PFNA in ten sites across Catalonia, Spain the year prior in 2009, however, they identified their limit of detection as 3.1 pg/m^3, which was higher than the mean PFNA concentration detected in four of the five sites in Spain evaluated by Beser et al. (2011). Harrad et al. (2020) compared PFNA concentrations both upwind and downwind of ten Irish MSW landfills in late 2018 and early 2019, finding comparable concentration ranges upwind (<0.08–0.31 pg/m^3) and downwind (0.08–0.52 pg/m^3). Barber et al. (2007) also detected ambient PFNA at detectable but not quantifiable levels (<3.3 pg/m^3 on average) in rural Mace Head, Ireland. United Kingdom samples (n = 15) from Hazelrigg and Manchester were reported in ranges of <0.06–0.9 pg/m^3 and two samples from Kjeller, Norway were reported at a range of 0.10–0.13 pg/m^3 (Barber et al., 2007).

Table C-10. Summary of Peer-Reviewed Studies Reporting the Occurrence of PFNA in Ambient Air

Study	Location	Site Details	Results
United States			
Kim and Kannan (2007)	United States (Albany, New York)	Roof of a lakehouse building located at Washington Park Lake in May and July 2006. Both particulate and gas phases collected.	Gas: n = 8, DF[a] 100%, mean, median (range) = 0.21, 0.20 (0.16–0.31) pg/m^3 Particle: n = 8, DF NR, mean, median (range) = 0.13, <LOQ (<LOQ–0.40) pg/m^3 (LOQ = 0.12 pg/m^3) *Non-detects were set to zero; values below the LOQ were set to ½ LOQ
Canada			
Ahrens et al. (2011)	Canada (Ontario)	Samples collected on and around one municipal WWTP for 63 days between July and September 2009. Samplers were placed at the primary clarifier, aeration tank, secondary clarifier, and at four reference sites (three near [within 200 m of the treatment tanks] and one distant [~600 m from the perimeter of the WWTP]). Samples also collected at two municipal solid waste landfills between June and August 2009 for 55 days. The two landfills were 60 km apart. Samplers were located upwind and onsite of the active zone of each landfill site and one field blank was collected at each site. Both sites collected landfill gas and the active area of the landfill was kept to a minimum by covering the waste with soil and a plastic film. The passive sampling configuration used resulted in the collection of mainly PFAS in the gas phase	WWTP: Reference sites (near): n = 3, DF[a] 100%, mean[a] (range) = 1.64 (1.10–2.11) pg/m^3 Primary clarifier: n = 2, DF[a] 100%, mean[a] (range) = 2.97 (1.10–4.84) pg/m^3 Aeration tank: n = 3, DF[a] 100%, mean[a] (range) = 3.63 (2.76–4.37) pg/m^3 Secondary clarifier: n = 2, DF[a] 100%, mean[a] (range) = 3.03 (2.24–3.81) pg/m^3 Reference site (distant): n = 1, point = 0.88 pg/m^3 Landfills: Upwind: n = 2, DF[a] 100%, mean[a] (range) = 2.11 (0.97–3.24) pg/m^3 On site: n = 2, DF[a] 100%, mean[a] (range) = 10.3 (4.82–15.8) pg/m^3 (MDL = 0.04–0.87 pg/m^3 for PFCAs, PFSAs, and PFOSA)
Europe			
Harrad et al. (2020)	Ireland (multiple cities)	Samples collected from ten municipal solid waste landfills upwind and downwind at each site between November 2018 and January 2019. Location of sampling sites based on wind direction data taken from the Irish Meteorological Service, with slight modification where necessary based on local information from site operators and ease of access. Sample sites were between 150 and 500 m of the center of the landfill. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction and demolition, and	Downwind: n = 10, DF[a] 100%, mean, median (range) = 0.23, 0.17 (0.08–0.52) pg/m^3 Upwind: n = 10, DF[a] 90%, mean, median (range) = 0.15, 0.13 (<0.08–0.31) pg/m^3 (LOD = 0.08 pg/m^3) *Non-detects replaced by ½ LOD

Study	Location	Site Details	Results
		biomedical waste. Gas or particulate phase not specified.	
Jogsten et al. (2012)	Spain (Catalonia)	Outdoor air sampling conducted in December 2009 for the purposes of comparison to indoor air and dust samples. Number of sites not specified but assumed to be ten because indoor air was sampled from ten homes. Samples were collected out of convenience and may not be representative of the entire Catalan population. Both particulate and gas phases collected.	n = 10; DF 0% (LOD = 3.1–280 pg/m^3 for all ionic PFAS)
Beser et al. (2011)	Spain (Alicante province)	Samples collected from April to July 2010 from five stations. Two stations were placed in Elche (one in a residential area and the other in an industrial area). The third station was placed in a residential area of Alicante City. The fourth station was in a rural area of Pinoso and the last station was in a residential area of Alcoy. Concentrations reported for PM2.5-bound PFNA.	Elche (residential): n = 11, DF[a] 55%, mean = 2.7 pg/m^3 Elche (industrial): n = 13, DF[a] 69%, mean = 3.8 pg/m^3 Alicante City: n = 11, DF[a] 36%, mean = 1.7 pg/m^3 Pinoso: n = 3, DF[a] 67%, mean = 1.65 pg/m^3 Alcoy: n = 3, DF[a] 100%, mean = 2.2 pg/m^3 (MQL = 1.4 pg/m^3) *Mean calculated from values >MQL
Barber et al. (2007)	United Kingdom (Hazelrigg, Manchester); Ireland (Mace Head); Norway (Kjeller)	Samples collected from four field sites in Europe: Hazelrigg (semirural) and Manchester (urban) were sampled in two sampling events in February–March 2005 and November 2005–January 2006; Mace Head (rural) was sampled in March 2006; and Kjeller (rural) was sampled in November–December 2005. PFNA was measured in the particulate phase.	Hazelrigg first sampling event: n = 2, DF NR, mean = <13.8 pg/m^3 (MQL = 13.9 pg/m^3) *The glass-fibre filters were analyzed in a batch of samples that showed contamination problems, so the high associated blank value used to calculate the MQL put most analytes <MQL. Hazelrigg second sampling event: n = 10, DF[a] = 90%, mean (range) = 0.9 (<0.06–1.7) pg/m^3 (MQL = 0.006 pg/m^3) Manchester first sampling event: n = 2, DF NR, mean = <26.6 pg/m^3 (MQL = 22.5 pg/m^3) Manchester second sampling event: n = 1, point = 0.8 pg/m^3 (MQL = 0.006 pg/m^3) Mace Head: n = 4, DF NR, mean = <3.3 pg/m^3

Study	Location	Site Details	Results
			(MQL = 3.32 pg/m^3) Kjeller: n = 2, DF[a] 100%, mean (range) = 0.12 (0.10–0.13) pg/m^3 (MQL = 0.10 pg/m^3) *Means calculated from values >MQL

C.3.6. Soil

The use and production of PFNA could result in its release to soils through various waste streams (NCBI, 2022b). When released to soil, based on its physico-chemical properties, PFNA is expected to have no mobility (NCBI, 2022b). PFNA has been measured in grass samples grown in soil containing PFNA and other PFAS near Decatur, Alabama (ATSDR, 2021; Yoo et al., 2011). In addition, PFNA has been found to accumulate in the roots of maize plants grown in soil containing PFNA and other PFAS (ATSDR, 2021; Krippner et al., 2014).

Seven U.S. studies were identified that evaluated the occurrence of PFNA and other PFAS in soil (Galloway et al., 2020; Nickerson et al., 2020; Zhu and Kannan, 2019; Eberle et al., 2017; Anderson et al., 2016; Venkatesan and Halden, 2014; Blaine et al., 2013) (Table C-11). Among these studies, three analyzed soils potentially impacted by past AFFF use. The PFNA detection frequencies varied widely (from less than 20% to over 90%) but mean concentrations tended to be below 5 ng/g. Few studies analyzed soils in the vicinity of fluoropolymer manufacturing facilities or by contaminated soil amendments. Other than control soils in two greenhouse and field studies and one reference site, the U.S. studies did not evaluate soils without amendments or without a nearby current or historical PFAS source.

Two studies analyzed soils in the vicinity of fluoropolymer manufacturing facilities (Galloway et al., 2020; Zhu and Kannan, 2019). Galloway et al. (2020) collected soil samples in December 2016 and March 2018 near a fluoropolymer production facility outside Parkersburg, West Virginia. The 2016 sampling included sites 4.0 km–48.1 km downwind to the north and northeast of the facility and the 2018 sampling included sites 1.3 km–45.4 km north of the facility. PFNA was detected in six of eight of the 2016 samples, however only one was above the LOQ with a concentration of 1.63 ng/g. PFNA was also detected in six of seven of the 2018 samples, however only one was above the LOQ with a concentration of 1.92 ng/g at a distance of 1.3 km. Both the 2016 and 2018 samples that were above the LOQ were reported at the site closest to the facility. In Zhu and Kannan (2019), authors studied PFAS concentrations in soil contaminated by a nearby fluoropolymer manufacturing facility in Little Hocking, Ohio, which had been manufacturing fluorochemicals for over five decades. The 45-acre well field located in a floodplain meadowland was divided into quadrants and surface soil samples were collected from multiple locations within each quadrant in October 2009. PFNA was detected in all 19 samples with mean and median concentrations of 2.7 ng/g and 2.5 ng/g, respectively.

Three studies analyzed soils potentially impacted by AFFF use (Nickerson et al., 2020; Eberle et al., 2017; Anderson et al., 2016). Anderson et al. (2016) assessed 40 sites across 10 active Air Force installations throughout the continental United States and Alaska between March and September 2014. Installations were included if there was known historic AFFF release in the period 1970–1990. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. The selected sites were not related to former fire training areas and were characterized according to volume of AFFF release – low, medium, and high. Across all sites, the PFNA detection frequency was 71.43% in 100 surface soil samples (median concentration of detects was 1.3 ng/g) and 14.42% in 112 subsurface soil samples (median concentration of detects was 1.5 ng/g). PFNA was detected more frequently at high-volume release sites (50.8% in 32 surface soil samples with mean concentration of 2.5 ng/g; 84.4% in 31 subsurface soil samples with mean concentration of

2.4 ng/g) than at low-volume sites (50.0% in 12 surface soil samples with mean concentration of 2.7 ng/g; 17.6% in 17 subsurface soil samples with mean concentration of 1.0 ng/g) and medium-volume sites (38.3% in 56 surface soil samples with mean concentration of 2.2 ng/g; 67.9% in 64 subsurface soil samples with mean concentration of 2.1 ng/g). Authors noted that given PFNA is not present in 3M AFFF formulations, there may be some degree of telomer-based AFFF contamination. Nickerson et al. (2020) developed a method to quantify anionic, cationic, and zwitterionic PFAS from AFFF-impacted soils. The method was applied to two soil cores collected from two different AFFF-impacted former fire training areas; the sampling year and geographic location were not provided. Eleven soil samples, corresponding to 11 depths ranging from 0.46 m to 15.1 m, were evaluated from Core E, and 12 soil samples, at depths ranging from 0.30 to 14.2 m, were evaluated from Core F. In Core E, PFNA was detected in 5 of 11 samples at depths both at the surface and further below ground with PFNA concentrations ranging from below the LOQ to 1.96 ng/g dw. In Core F, PFNA was detected in 5 of 12 samples at the five depths closest to the surface, with concentrations ranging from below the LOQ to 4.17 ng/g dw (LOQ not reported). Eberle et al. (2017) investigated the effects of an in situ chemical oxidation treatment for remediation of chlorinated volatile organic compounds and PFAAs co-contaminants. Soil samples were collected in 2012–2013 before and after a pilot scale field test at a former fire training site at Joint Base Langley-Eustis, Virginia. Monthly fire training activities were conducted at the site from 1968 to 1980 and irregular fire training activities continued until 1990. Impacted soil was excavated in 1982 but details were not provided. PFNA was detected in 1 of 5 pre-treatment samples and in 13 of 14 post-treatment samples. Of the available three paired pre- and post-treatment soil samples, PFNA was not detected pre-treatment in two pairings but detected post-treatment at 0.07 ng/g and 0.05 ng/g post-treatment. For the third pairing, PFNA was detected at 1.1 ng/g pre-treatment and below the LOQ (0.06 ng/g) post-treatment.

Of the remaining two studies conducted in the United States, Venkatesan and Halden (2014) conducted outdoor mesocosm studies to examine the fate of PFAS in biosolids-amended soil collected during 2005–2008. Biosolids were obtained from a wastewater treatment plant (WWTP) in Baltimore that primarily treated wastewater from domestic sources with only minor contribution (1.9%) from industry. The number of samples was not provided but PFNA was detected in the control (nonamended) soil at levels below 0.5 ng/g dw and in the biosolids-amended soil at a level not reported by the authors. In a field and greenhouse study, Blaine et al. (2013) studied the uptake of PFAS into edible crops grown in control and biosolids-amended soil. In the field study, urban biosolids were obtained from a WWTP receiving both domestic and industrial waste while rural solids were obtained from a WWTP receiving domestic waste only. PFNA was detected in soils from urban (mean = 0.20 ng/g, 0.28 n/g, and 0.40 ng/g in control, 1×[9] and 2× amended fields, respectively) and rural fields (mean = 0.06 ng/g and 0.75 ng/g in control and 0.5× amended fields, respectively). In the greenhouse study, three soils (nonamended control, industrially impacted, and municipal) were investigated. Industrially impacted soils contained composted biosolids from a small municipal WWTP that was impacted by PFAA manufacturing while municipal soils were obtained from a reclamation site in Illinois where municipal biosolids were applied for 20 years. PFNA was detected in all three soils at an average concentration of 0.30 ng/g, 20.15 ng/g, and 6.11 ng/g in control, industrially impacted,

[9] 0.5×, 1×, or 2× is defined as ½, 1, or 2 times the agronomic rate of biosolids application to meet nitrogen requirements of the crop.

and municipal soil, respectively. Authors noted that the trace levels of PFAS detected in the control soil may be due to minor cross-contamination from plowing, planting, or atmospheric deposition from the surrounding area where biosolids have been applied.

Results for all of the identified non-U.S. studies are presented in detail in Table C-11 and are summarized here. The EPA identified three Canadian studies, two were conducted at locations with prior AFFF use (Cabrerizo et al., 2018; Mejia-Avendaño et al., 2017). Cabrerizo et al. (2018) evaluated soil in two locations, one of which was relatively remote and largely did not have direct human contact and the other of which was previously used as a military training facility. The remote location (n = 19) had concentrations ranging from 0.0262 ng/g dw to 0.8749 ng/g dw and the historic military training location (n = 8) similarly had concentrations from 0.0836 ng/g dw to 0.7794 ng/g dw. Mejia-Avendaño et al. (2017) investigated soil samples at the site of the 2013 Lac-Mégantic train accident, where approximately 33,000 L of AFFF concentrates were used to put out fires. In 2013, 12 sample concentrations ranged from 0.138–19 ng/g dw and in 2015, two years after the incident, 11 sample concentrations ranged from 0.031–0.777 ng/g dw. In the third Canadian study, Dreyer et al. (2012) sampled bog peat cores in an undisturbed and well investigated location to determine historic atmospheric contamination. Estimated core segment dated back to 1912, with PFNA concentrations ranging between not detected and 0.412 ng/g.

Of the European studies, three were conducted at locations near firefighting facilities (Dauchy et al., 2019; Skaar et al., 2019; Hale et al., 2017). Dauchy et al. (2019) and Skaar et al. (2019) found varying results from not detected to 15 ng/g dw while Hale et al. (2017) reported a range of 2.8–41.3 ng/g for the 40% of samples that were detected. One study in Belgium (Groffen et al., 2019) evaluated soil samples at a perfluorochemical plant and at four sites increasing in distance from the plant. PFNA levels were detected at the plant ranging from not detected to 2.53 ng/g dw and ranging from not detected to 0.53 ng/g dw away from the plant in no discernable pattern. Harrad et al. (2020) investigated soil samples upwind and downwind from ten municipal solid waste landfills in Ireland. PFNA was found in all samples upwind of the landfills, ranging in concentrations from 0.0029–0.033 ng/g dw, and in 89% of samples downwind, ranging in concentrations from not detected to 0.0077 ng/g dw. A study in Norway (Grønnestad et al., 2019) found mean PFNA concentrations from a popular skiing location in Granåsen to be lower than that of the study's selected reference site in Jonsvatnet. Sammut et al. (2019) sampled soil from six random small urban fields in Malta and found concentrations ranging from 0.66–0.87 ng/g.

Table C-11. Summary of PFNA Data in Soil

Study	Location	Site Details	Results
United States			
Galloway et al. (2020)	United States (Parkersburg, West Virginia)	Soil samples collected near a fluoropolymer facility in two sampling trips in December 2016 and March 2018. The 2016 sampling trip included a collection radius 4.0–48.1 km downwind to the north and northeast of the facility. The 2018 sampling trip focused on samples collected to the north of the facility with a radius of 1.3–45.4 km.	2016 sampling: Drag Strip Road (4.0 km) = 1.63 ng/g Veto Lake (8.0 km) = <LOQ Veto Road (13.0 km) = ND Veto Road, dup. (13.0) = ND Strouds Run (15.3 km) = <LOQ Lookout Park (24.0 km) = <LOQ Archers Fork #1 (35.3 km) = <LOQ Archers Fork #2 (48.1 km) = <LOQ 2018 sampling: LHWA (1.3 km) = 1.92 ng/g Veto Lake (8.0 km) = <LOQ Veto Lake, dup. (8.0 km) = <LOQ Watertown (24.3 km) = ND Beverly (32.1 km) = <LOQ L. Olive Green Creek (39.9 km) = <LOQ Reinersville (45.4 km) = <LOQ (LOQ = 1 ng/g)
Zhu and Kannan (2019)	United States (Washington County, Ohio)	Surface soil (0–6 cm) samples collected in October 2009 from a 45-acre field located within a 1-mile radius of a fluoropolymer manufacturing facility in Little Hocking that had been manufacturing fluorochemicals for over five decades. The site was divided into quadrants and soil samples were collected from multiple locations within each quadrant.	n = 19, DF 100%, mean, median (range) = 2.7, 2.5 (1.6–6.3) ng/g dw (LOD = 0.1356 ng/g dw; LOQ = 0.452 ng/g dw)
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between 1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were not specifically controlled for in the site selection	Surface soil: Overall: n = 100, DF 71.43%, median (maximum) = 1.3 (23.0) ng/g *Breakdown by site:* Emergency Response (low-volume release): n = 12, DF 50.0%, mean (range) = 2.7 (1.5–4.1) ng/g Hangars and Buildings (medium-volume release): n = 56, DF 38.3%, mean (range) = 2.2 (0.21–12) ng/g

Study	Location	Site Details	Results
		process; active remedies had not been applied at any of the sites selected. Approximately ten samples were collected between March and September 2014 at each site for surface and subsurface soil; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases.	Testing and Maintenance (high-volume release): n = 32, DF 50.8%, mean (range) = 2.5 (0.24–23) ng/g (RL = 0.23 ng/g) Subsurface soil: Overall: n = 112, DF 14.42%, median (maximum) = 1.5 (6.49) ng/g *Breakdown by site:* Emergency Response (low-volume release): n = 17, DF 17.6%, mean (range) = 1.0 (0.5–1.5) ng/g Hangars and Buildings (medium-volume release): n = 64, DF 67.9%, mean (range) = 2.1 (0.21–12) ng/g Testing and Maintenance (high-volume release): n = 31, DF 84.4%, mean (range) = 2.4 (0.24–23) ng/g (RL = 0.24 ng/g) *Median calculated using quantified detections *Non-detects were substituted with ½ the reporting limit
Nickerson et al. (2020)	United States (unspecified)	Soil cores E and F from two different AFFF-impacted fire training areas; sampling year and geographic location not provided. Soil core E contained 11- 0.3 m increment samples from 0.3–15.2 m below ground surface and was collected in an area where the surficial soils were likely disturbed due to regrading and other soil redistribution activities. Soil core F contained 12- 0.61 m increment samples from 0–14.2 m below ground surface and was collected in an area where the surficial soils were highly permeable only within the upper 0.5 to 1 m, and the underlying impermeable clay layer exhibited a relatively high cation exchange capacity and organic carbon content.	Core E: 0.46 m = 1.96 ng/g dw 2.9 m = <LOQ 3.66 m = <LOQ 3.96 m = <LOQ 4.27 m = <LOQ 4.57 m = <LOQ 4.88 m = 0.22 ng/g dw 7.01 m = 0.26 ng/g dw 8.38 m = 0.73 ng/g dw 10.5 m = 1.09 ng/g dw 15.1 m = <LOQ Core F: 0.30 m = 0.70 ng/g dw

Study	Location	Site Details	Results
		The water table was relatively shallow (depth <3 m) at both sites.	1.22 m = 4.17 ng/g dw 1.83 m = 3.23 ng/g dw 2.44 m = 1.04 ng/g dw 3.05 m = 0.64 ng/g dw 4.11 m = <LOQ 7.62 m = <LOQ 8.84 m = <LOQ 9.45 m = <LOQ 10.5 m = <LOQ 11.9 m = <LOQ 14.2 m = <LOQ (LOQ not reported)
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Pilot testing area in former fire training area (Training Site 15) at Joint Base Langley-Eustis where monthly fire training activities were conducted from 1968 to 1980 in a zigzag pattern burn pit. Facility was abandoned in 1980 but irregular fire training activities using an above-ground germed burn pit continued until 1990. Impacted soil was removed in 1982 but additional details of the excavation are not well known. Soil samples collected for pre- (April and September 2012) and post- (December 2013) in situ chemical oxidation treatment using a peroxone activated persulfate (OxyZone) technology. Treatment was conducted in Test Cell 1 over 113 days (April–August 2013). Soil samples were collected adjacent to wells; wells outside Test Cell 1 were used as sentry wells. Well IDs for pre- and post-sampling were not provided but the following three pairings were assumed based on Table 2 in the paper: U-20 with SB-106; U-16 with SB-112; and I-1 with SB-109.	Pre-treatment: I-1 (1.2–4.3 m) = 1.1 ng/g I-2 (1.2–4.3 m) = ND U-12 (2.1 m) = ND U-16 (3.0 m) = ND U-20 (1.8 m) = ND (LOQ = 0.68–0.72 ng/g) Post-treatment: SB-101 (4.3 m) = 0.07 ng/g SB-105 (1.8 m) = 0.02 ng/g SB-106/U-20 (1.8 m) = 0.07 ng/g SB-106 (4.3 m) = 0.14 ng/g SB-107 (1.8 m) = 0.03 ng/g SB-107 (4.3 m) = 0.2 ng/g SB-108 (1.8 m) = 0.03 ng/g SB-108 (4.3 m) = 0.15 ng/g SB-109/I-1 (3 m) = <LOQ SB-111 (4.3 m) = 0.29 ng/g SB-112 (1.8 m) = 0.06 ng/g SB-112/U-16 (3 m) = 0.05 ng/g SB-114 (1.8 m) = 0.3 ng/g SB-114 (4.3 m) = 0.33 ng/g (LOQ = 0.06 ng/g)
Venkatesan and Halden (2014)	United States (Baltimore, Maryland)	Archived agricultural soil (nonamended) collected during 2005–2008 at a depth of 0–20 cm from the United States Department of Agriculture-Agricultural Research Service Beltsville Agricultural Research	Nonamended: n = NR, DF NR, authors noted PFNA concentration was between 0.1–0.5 ng/g dw Amended: n = NR, DF NR, authors noted the detected levels of PFNA, along with PFOA,

Study	Location	Site Details	Results
		Center; number of sampling sites and number of samples not provided. Biosolids-amended soil obtained by mixing biosolids and soil at a volumetric ratio of 1:2. Biosolids were from Back River WWTP in Baltimore, a full-scale activated sludge treatment plant. Raw wastewater was primarily from domestic sources with only minor contribution (1.9%) from industry.	PFNA, PFDA, and PFUnA in the control soil accounted for 0.3–3% of their initial levels in the amended soil mix (MDL = 0.08 ng/g)
Blaine et al. (2013)	United States (Midwest)	Urban and rural full-scale field study with control (nonamended) and biosolids-amended plots. Three agricultural fields were amended (0.5×, 1×, or 2×) with municipal biosolids. Urban biosolids (1× and 2×) were from a WWTP receiving both domestic and industrial waste. Rural biosolids (0.5×) were from a WWTP receiving domestic waste only. Control plots were proximal to the rural and urban amended corn grain and corn stover field sites; sampling year not provided. Greenhouse study with control (nonamended) and biosolids-amended soil. Nonamended soil obtained from a field that received commercial fertilizers and had a similar cropping system as the nearby municipal soil site. Municipal soil was obtained from a reclamation site in Illinois where municipal biosolids were applied at reclamation rates for 20 years, reaching the cumulative biosolids application rate of 1,654 Mg/ha. Industrially impacted soil was created by mixing composted biosolids from a small municipal (but impacted by PFAA manufacturing) WWTP with control soil on a 10% mass basis. Sampling year not provided.	Field study: Urban non-amended: n = 3–7, DF NR, mean = 0.20 ng/g Urban 1×: n = 3–7, DF NR, mean = 0.28 ng/g Urban 2×: n = 3–7, DF NR, mean = 0.40 ng/g Rural non-amended: n = 3–7, DF NR, mean = 0.06 ng/g Rural 0.5×: n = 3–7, DF NR, mean = 0.75 ng/g (LOQ not reported) Greenhouse study: Nonamended: n = 3–5, DF NR, mean = 0.30 ng/g Industrially impacted: n = 3–5, DF NR, mean = 20.15 ng/g Municipal: n = 3–5, DF NR, mean = 6.11 ng/g (LOQ not reported)
Canada			
Cabrerizo et al. (2018)	Canada (Melville and Cornwallis Islands)	Catchment areas of lakes in the Cape Bounty Arctic Watershed Observatory on southern Melville Island (West, East, and Headwater lakes) during summer (late July-early August) 2015 and 2016, representing an environment largely unimpacted by direct human activity; data for 19 sampling sites available (S6, S11–S28).	Melville Island lakes: n = 19, DF[a] 100%, mean[a] (range) = 0.3248 (0.0262– 0.8749) ng/g dw Cornwallis Island lakes: n = 8, DF[a] 100%, mean[a] (range) = 0.3333 (0.0836–0.7794) ng/g dw (LOD = 0.0001–0.018 ng/g for all PFAS)

Study	Location	Site Details	Results
		Catchment areas of lakes on Cornwallis Island (Resolute, North, Small, Meretta, 9 Mile, and Amituk lakes) near the community of Resolute Bay during summer (late July-early August) 2015 and 2016. Resolute Bay has a military and civilian airport which discharged its wastewaters into the upper area of the catchment until 1997, three old solid waste landfills 1.5–2 km west of the airport used until the mid-1990s, and Arctic research and military training facilities close to the airport that support activities such as vehicle use, firefighting, and construction/demolition; eight sampling sites (S29–S36).	
Mejia-Avendaño et al. (2017)	Canada (Lac-Mégantic, Quebec)	Site of July 2013 Lac-Mégantic train accident where 63 out of 72 train cars carrying 8 million liters of crude oil derailed and a major oil fire ignited. Seven types of AFFFs and approximately 33,000 L of AFFF concentrates were used. Samples were collected in July 2013 weeks after the accident from the western shores of Chaudière River, at the point where the oil and AFFF runoff reached the river, approximately 500 m from the edge of the derailment site; in July 2015 from the fire burn site and adjacent area in downtown Lac-Mégantic where the soil was continuously excavated for remediation (the site was the closest to the accident site among the areas open to sampling); and from a background, nonimpacted area next to Chaudière River, about 5 km from the accident site, on the east shore of the river and on the opposite side of the accident.	Background: n = 3, DF NR, mean = 0.212 ng/g dw 2013: n = 12 (from 12 sites), DF[a] 100%, mean[a] (range) = 4.41 (0.138–19) ng/g dw 2015: n = 11 (from 9 sites), DF[a] 100%, mean[a] (range) = 0.274 (0.031–0.777) ng/g dw (LOD = 0.02 ng/mL; LOQ = 0.05 ng/mL)
Dreyer et al. (2012)	Canada (Ottawa, Ontario)	Two ombrotrophic Mer Bleue bog peat core samples collected in October 2009 and cut into 5-cm segments (nine segments for the first core, eight segments for the second core); Mer Bleue selected because it is undisturbed and well investigated and is located in a meltwater channel of the postglacial Ottawa River. Peat cores sampled to determine their suitability for determining historic atmospheric contamination; contaminants present due to atmospheric deposition only. The year for each segment was estimated through dating of Mer Bleue peat cores collected in the same year for a different study.	First core (first parallel; second parallel): 2009: 0.041; 0.033 ng/g 2006: 0.143; 0.192 ng/g 2001: 0.229; 0.223 ng/g 1992: 0.259; 0.271 ng/g 1983: 0.241; 0.234 ng/g 1973: 0.203; 0.322 ng/g 1962: 0.263; 0.297 ng/g 1945: 0.148; 0.156 ng/g 1927: 0.069; 0.089 ng/g 1912: 0.044; 0.044 ng/g

Study	Location	Site Details	Results
			Second core (first parallel; second parallel): 2009: 0.052; 0.062 ng/g 2006: 0.193; 0.166 ng/g 2001: 0.162; 0.234 ng/g 1992: 0.320; 0.319 ng/g 1983: 0.396; 0.412 ng/g 1973: 0.206; 0.206 ng/g 1962: 0.082; 0.081 ng/g 1945: 0.160; 0.149 ng/g 1927: <MQL; <MDL (IDL = 0.003 ng/g; MQL = 0.019 ng/g; MDL = 0.048 ng/g) *Authors estimated the year for each core segment using cores for a different study that underwent dating
Europe			
Groffen et al. (2019)	Belgium (Antwerp)	3M perfluorochemical plant and four sites with increasing distance from plant were selected based on prior biomonitoring studies in the vicinity of the plant. The four sites are: Vlietbos (1 km SE from 3M), Rot-Middenvijver (2.3 km ESE from 3M), Burchtse Weel (3 km SE from 3M), and Fort 4 (11 km SE from 3M). Samples collected in June 2016.	Plant: n = 13, DF 69%, mean, median (range) = 0.83, 0.34 (<LOQ–2.53) ng/g dw 1 km from plant: n = 10, DF 30%, mean, median (range) = <LOQ, <LOQ (<LOQ–0.44) ng/g dw 2.3 km from plant: n = 10, DF 0% 3 km from plant: n = 10, DF 10%, mean, median (range) = <LOQ, <LOQ (<LOQ–0.38) ng/g dw 11 km from plant: n = 14, DF 29%, mean, median (range) = <LOQ, <LOQ (<LOQ–0.53) ng/g dw (LOQ = 0.26 ng/g dw)
Dauchy et al. (2019)	France (unspecified)	Site where fluorosurfactant-based foams have been used extensively. From 1969 to 1984, the site was an oil refinery, with the exact location of the firefighting training area, frequency of training sessions, and history of firefighting training activities unknown. From 1987 to date, it has been a large training area for firefighters. Samples collected in six areas from two sampling campaigns. First sampling campaign collected 30 soil cores between 2 m and 4 m in June 2015 from areas 1–5 (composite soil samples collected	Area 1: SC-71, SC-72, SC-73, SC-74, SC-75 = <2 ng/g dw (1–2 m) SC-76, SC-77, SC-78 = <2 ng/g dw (0–1 m) Area 2: SC-58 = <20, <20, <20, <20, <2, <20 ng/g dw (0–0.25, 0.5–0.75, 1–1.5, 2–2.5, 3–3.5, 3.5–4 m) SC-59 = <20, <4, <4, <2, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 3–3.5 m)

Study	Location	Site Details	Results
		every 25 cm in the topmost meter and then every 50 cm). Second sampling campaign collected 14 soil cores between 4 m and 15 m from areas 1–6 (thickness of composite soil samples ranged from 25 cm to 100 cm) in October 2016. Area 1 stored raw oil products when the oil refinery was operating; a preliminary survey showed hydrocarbon traces in the area, suggesting that an incident had occurred and that fluorinated surfactants could have been used. Area 2 is one of the main areas used for firefighting activities since 1987; training sessions held directly on the ground before 10-cm thick concrete slab was built in the 1990s. Area 3 was used for firefighting activities since 1987 and is situated on a 1-meter thick concrete slab on the foundations of the former oil refinery. Area 4 corresponds to the site's WWTP where sludge and sediment from a lagoon were stored directly on the ground; influents of the WWTP are highly contaminated by PFAS. Area 5 was used for firefighting training exercises by the former oil refinery. Area 6 is used for firefighting exercises with tank trucks.	SC-60 = <20, <20, <4, <4, <2, <2, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 2–2.5, 2.5–3 m) SC-61 = <20, <20, <4, <4, <20, <4, <4 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m) SC-62 = <20, <20, <4, <20, <4 ng/g dw (0–0.25, 0.5–0.75, 1–1.5, 2–2.5, 3.5–4 m) SC-63 = <20, <20, <4, <2, <2, <2, <2, <2, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5 m) SC-64 = <2, <2, <4, <4, <2, <4 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m) SC-65 = <20, <20, <20, <4, <4, <2, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 3–3.5, 3.5–4 m) SC-66 = <4, <4, <4, <2, <2, <2, <2, <2, <2, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5, 3.5–4 m) SC-58b = <10 ng/g dw (4–5, 5–6, 9–10, 14–15 m) SC-59b = <10, <10, 2, <10, <10 ng/g dw (3–4, 4–5, 6–7, 9–10, 14–15 m) SC-65b = <10 ng/g dw (4–5, 7–9, 9–11, 14–15 m) SC-67 = <20, <20, <10, <10 ng/g dw (0–1, 1.3–2, 2–3, 4–5 m) Area 3: SC-40 = <4 ng/g dw (0–1 m) SC-43 = <2 ng/g dw (1–2 m) SC-45 = <2 ng/g dw (0–1 m) SC-47 = <20 ng/g dw (0–1 m) SC-48 = <4 ng/g dw (0–1 m) SC-41 = <10 ng/g dw (0–0.25, 1–2 m) SC-42 = <10 ng/g dw (0–0.25, 1–2, 3–4 m) Area 4: SC-33 = <20 ng/g dw (0–1 m) SC-34 = <4 ng/g dw (1–2 m)

Study	Location	Site Details	Results
			SC-35 = <2 ng/g dw (0–1m) SC-36 = <4 ng/g dw (0.3–1 m) SC-37 = <20 ng/g dw (0.1–1.1 m) SC-37b = <10 ng/g dw (0–0.25, 1–1.5, 3–4 m) SC-38 = <10 ng/g dw (0.25–1, 2–3 m) Area 5: SC-10 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5 m) SC-11 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m) SC-12 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75 m) Area 6: SC-21 = 5, <10, <10, <10, <10 ng/g dw (0–0.25, 0.25–1, 2–3, 8–9, 13–15 m) SC-22 = 3, 2, <10, <20 ng/g dw (0–0.25, 0.25–1, 1–2, 3–4 m) SC-23 = 15, 3, <10, <10 ng/g dw (0–0.25, 0.25–1, 1–2, 3–4 m) SC-24 = 10, <10, 3 ng/g dw (0–0.25, 1–2, 3–4 m) SC-25 = 3, <10 ng/g dw (0–0.25, 1.5–2 m) SC-26 = <10 ng/g dw (2–3, 4–5 m) (LOQ = 2 ng/g dw)
Skaar et al. (2019)	Norway (Ny-Ålesund)	Samples collected in June 2016 in and around the international research facilities (Ny-Ålesund) near local firefighting training site. Background soil samples were collected at representative locations.	Background: n = 8, DF 0% Contaminated: n = 2, DF[a] 50%, range = <0.005–0.73 ng/g dw (IDL = 0.026 ng; LOD = 0.005 ng/g dw; LOQ = 0.01 ng/g dw) *Table 1 and Table S2 reported a total of nine samples across background and contaminated sites; however, Tables S11 and S13 report a total of ten samples (two contaminated sites from Table S11 and eight background sites from Table S13
Hale et al. (2017)	Norway (Gardermoen)	Samples collected in June 2015 from six locations around a firefighting training facility west of the Oslo airport site. Samples were taken at 0–1 m, 1–2 m, 2–3	n = 22, DF 40%, range = 2.8–41.3 ng/g (LOD = 1 ng/g)

Study	Location	Site Details	Results
		m, and 3 to groundwater table level (which was in all cases above 4 m). Facility was established in 1989 and AFFF was used extensively. AFFF containing PFOS was banned at the facility in 2007 and a complete ban on organofluorine AFFF was enforced in 2011. The soil is known to be contaminated with a range of perfluorinated compounds.	*Range reported for detects *The DF and range extracted are reported in the results (Section 3.1); however, Table S2 of the individual sample data show all concentrations ranging from <1.8 to <2.5 ng/g
Harrad et al. (2020)	Ireland (multiple cities)	Samples collected from ten municipal solid waste landfills upwind and downwind at each site between November 2018 and January 2019. At each upwind/downwind location, nine sub-samples of soil were taken in a "W" formation. Samples were collected from the same areas as air samples and were taken within the boundaries of the landfill operational facility. Soil used as capping on landfill cells was not sampled to ensure soil samples were not collected from soil placed after landfill operations ceased and that farming activities would not influence concentrations found. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction & demolition, and biomedical waste.	Downwind: n = 9, DF[a] 89%, mean, median (range) = 0.0045, 0.0043 (<0.001–0.0077) ng/g dw Upwind: n = 7, DF[a] 100%, mean, median (range) = 0.014, 0.006 (0.0029–0.033) ng/g dw (LOD = <0.001 ng/g dw) *Non-detects replaced by ½ LOD *Soil samples from three upwind locations and one downwind location destroyed in transit from field to laboratory
Grønnestad et al. (2019)	Norway (Granåsen, Jonsvatnet)	Upper layer soil samples (3–10 cm in depth) collected in June 2017 and 2018 from Granåsen (skiing area) and Jonsvatnet (reference site). Five samples per year were analyzed for each site. Located 10 km from Trondheim city center, Granåsen is the main arena for winter sports in Trondheim and hosts an annual ski jumping World Cup event and regional, national, and international competitions in cross-country skiing. Located 15 km away from Trondheim city center, Jonsvatnet is a natural forest area not used for ski-sports and is in the vicinity of an ecological farm next to Lake Jonsvatnet. The two study areas have similar vegetation.	Reference area: n = 10, DF 70%, mean (range) = 0.198 (<LOQ–0.928) ng/g dw Skiing area: n = 10, DF 90%, mean (range) = 0.179 (<LOQ–0.602) ng/g dw (LOQ = 0.056 ng/g dw)
Sammut et al. (2019)	Malta	Six surface soil samples (#10, 14, 15, 18, 20, and 22) collected between June and August 2015 from random small urban fields.	n = 6, DF[a] 100%, mean[a] (range) = 0.75 (0.66–0.87) ng/g (LOD = 0.50 ng/g; LOQ = 0.60 ng/g)

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; dw = dry weight; LOQ = limit of quantitation; LHWA = Little Hocking Water Association; LOD = limit of detection; MDL = method detection limit; 0.5×, 1×, or 2× = ½, 1, or 2 times the agronomic rate of biosolids application to meet nitrogen requirements of the crop; ND = not detected; NR = not reported; PFAA = perfluoroalkyl acids ; RL = reporting limit; WWTP = wastewater treatment plant.

C.3.7. Sediment

When released into water, based on its physico-chemical properties, PFNA is expected to adsorb to suspended solids and sediments (NCBI, 2022b). The EPA did not identify studies conducted in the U.S. that reported the occurrence of PFNA in sediment. Concentrations of PFNA in sediment samples collected from the Hudson Bay region of northeast Canada ranged from <0.06 ng/g to 0.14 ng/g (dry weight) (NCBI, 2022b; Kelly et al., 2009).

C.4. Recommended RSC

The EPA followed the Exposure Decision Tree approach to determine the RSC for PFNA (USEPA, 2000b). The EPA first identified three potential populations of concern (Box 1): pregnant women and their developing fetuses, lactating women, and women of childbearing age (see Section 2.3.2). However, limited information was available regarding specific exposure of these populations to PFNA in different environmental media. The EPA considered exposures in the general U.S. population as likely being applicable to these two populations. Second, the EPA identified several relevant PFNA exposures and pathways (Box 2), including dietary consumption, incidental oral consumption via exposure to dust, consumer products, and soil, dermal exposure via soil, consumer products, and dust, and respiration via ambient air. Several of these may be potentially significant exposure sources. Third, the EPA determined that there was not adequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, studies from Canada and Europe indicate that indoor air may be a significant source of exposure to PFNA. At the time of the literature search, the EPA was unable to identify studies assessing PFNA concentrations in indoor air samples from the U.S. and therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, based on the studies summarized in the sections above, there are significant known or potential uses/sources of PFNA other than drinking water (Box 6), though there is not information available on each source to make a characterization of exposure (Box 8A). For example, there are several studies from the U.S. indicating that PFNA may occur in dust sampled from various microenvironments (e.g., homes, offices, daycare centers, vehicles). However, the majority of studies sampled in only one location and few studies examined dust samples outside of the home (e.g., one study assessed PFNA occurrence in dust sampled from vehicles). Additionally, though several studies from around the U.S. measured PFNA concentrations in dust from houses, the detection frequencies in these studies varied widely (from 35% to 100%) and may be a result of uncertainties including home characteristics, behaviors of the residents, and the presence or absence of PFNA-containing materials or products (Haug et al., 2011). Therefore, it is not possible to determine whether dust can be considered a major or minor contributor to total PFNA exposure. Similarly, it is not possible to determine whether the other potentially significant exposure sources such as seafood and consumer products should be considered major or minor contributors to total PFNA exposure. Given these considerations, following recommendations of the Exposure Decision Tree (USEPA, 2000b), the EPA recommends an RSC of 20% (0.20) for PFNA.

Appendix D. PFHxS: Summary of Occurrence in Water and Detailed Relative Source Contribution

D.1. Occurrence in Water

The production of PFHxS and its use as a raw material or precursor for manufacturing PFAS-based products, as well as its previous use in firefighting foam and carpet treatment solutions, could result in its release to the aquatic environment through various waste streams (NCBI, 2022a). PFHxS has an estimated water solubility of 6,200 µg/L (6.2 mg/L) at 25°C and when released to surface water, it is not expected to adsorb to suspended solids and sediment (NCBI, 2022a). Volatilization from water surfaces is not expected to be an important fate process for PFHxS (NCBI, 2022a).

D.1.1. Groundwater

Several studies have evaluated the occurrence of PFHxS in groundwater in both the United States and Europe. PFHxS was detected in at least one groundwater sample site in each study in the U.S (Table D-1). Lindstrom et al. (2011) analyzed well water samples in Decatur, Alabama. The samples were collected in February 2009 from farms that had applied PFC-contaminated biosolids to local agricultural fields as a soil amendment for at least 12 years. PFHxS was detected in two wells at concentrations of 56.5 and 87.5 ng/L. In another study, median and maximum groundwater of 870 ng/L and 290,000 ng/L (0.870 µg/L and 290 µg/L), respectively, were detected at 10 U.S. military installations (Anderson et al., 2016). Three other studies of groundwater known to be impacted by nearby AFFF use similarly had PFHxS concentrations ranging from 36-120,000 ng/L and detection frequencies of 100% (Steele et al., 2018; Eberle et al., 2017; Moody et al., 2003).

Post et al. (2013) assessed raw water from public drinking water system intakes that were chosen to represent New Jersey geographically but were not necessarily associated with any known PFAS release. PFHxS was found in 2 of 18 systems at levels ≤10 ng/L. Appleman et al. (2014) evaluated groundwater contaminated by wastewater effluent discharge. At this site, detection frequency was 100%, but PFHxS levels did not exceed 11 ng/L. Procopio et al. (2017) collected groundwater from areas downstream of a manufacturer of PFAS-containing products but found minimal PFHxS in only 5% of samples, all of which ranged from non-detects to 5.5 ng/L. Boone et al. (2019) evaluated 17 PFAS in source and treated waters collected in 2010–2012. Of the three groundwater sources evaluated, PFHxS was detected in two out of three samples at levels of 1.88 and 44.8 ng/L. In the final U.S.-based study, Quiñones and Snyder (2009) examined levels of eight PFAS at two sites in Las Vegas Wash, Nevada that were highly impacted from treated wastewater. Samples were collected in 2008 as part of a study to assess both raw and treated water from utilities producing at least 75 megaliters of finished water per day. Mean PFHxS levels at the two sites were 6.8 and 5.6 ng/L at sites 1 (n = 7) and 2 (n = 8), respectively.

Of the studies conducted in Europe, 4 studies (Barreca et al., 2020; Sammut et al., 2019) were conducted in areas not associated with any known PFAS release. At these sites, the detection frequency of PFHxS was 0–40% with a maximum level of 32 ng/L. The remaining nine

European studies evaluated groundwater samples from sites with known or suspected PFAS releases associated with fluorochemical manufacturing (Boiteux et al., 2012; Loos et al., 2010) or AFFF use (Boiteux et al., 2017; Dauchy et al., 2012). Source categories for Gobelius et al. (2018) included fire training sites, but also included landfill/waste disposal sites, skiing areas, urban areas, and areas of unspecific industries. Of the sites with known sources of contamination, higher detection frequencies (up to 100%) and greater PFHxS levels (up to 3,470 ng/L) were reported at sites with AFFF use. Two related studies (Boiteux et al., 2017; Dauchy et al., 2012) sampled alluvial wells downstream of a fluorochemical manufacturing facility in France. Preliminary results showed low levels of PFHxS (up to 11 ng/L; (Dauchy et al., 2012)), but PFHxS was not detected in samples from the latter study (Boiteux et al., 2017).

Table D-1. Studies Reporting Occurrence of PFHxS in Groundwater

Study	Location	Site Details	Results
United States			
Procopio et al. (2017)	United States (New Jersey)	Groundwater from an industrial/business park located within the South Branch Metedeconk River watershed, where there was suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs. Samples were collected following the installation of 16 temporary monitoring wells by the NJ Geological and Water Survey or a contract driller during August 2013 (sampling event #7) and June–July 2014 (sampling event #8). Samples were taken from the upper 1.5 m (5 ft) of the water table from each well, except for one "profile well" in which samples were collected at three different depths (3.7–4.6, 6.7–7.6, and 10.7–11.6 m below grade; 12–15, 22–25, and 35–38 ft below grade, respectively).	n = 19, DF[a] 5%, range = <5–5.5 ng/L (Minimum RL = 5 ng/L)
Post et al. (2013)	United States (New Jersey)	Raw water collected from public drinking water system intakes in two sampling campaigns. In the first sampling campaign, samples from 18 drinking water systems were collected between August 2009 and February 2010 from 1 confined well (sunk into an aquifer located between two impermeable strata) and 17 unconfined wells in the upper unconfined aquifer; sites were chosen to represent NJ geographically and included 1 site with a nearby industrial facility that previously used large quantities of PFNA (site 5). In the second sampling campaign, samples from two drinking water systems (PWS-A and PWS-B) were collected in 2010–2013 from five unconfined wells. Groundwater at these two systems were known to be contaminated by PFOA.	1st sampling campaign: n = 18, DF 11%, range = ND–10 ng/L 2nd sampling campaign: PWS-A, WF1A: n = 5, DF 0% PWS-A, WF1B: n = 4, DF 0% PWS-A, WF2A: n = 9, DF[a]14%, range = ND–6 ng/L PWS-A, WF2B: n = 9, DF 0% PWS-B: n = 8, DF 0% (Minimum RL = 5 ng/L)
Lindstrom et al. (2011)	United States (Decatur, Alabama)	Thirteen samples collected in February 2009 from 13 wells located on farms with historical land application of PFC-contaminated biosolids to local agricultural fields between 1995 and 2008. Biosolids obtained from local municipal WWTP where sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers,	n = 13, DF (frequency of quantification)[a] 15%, range = <LOQ–87.5 ng/L (LOQ = 10 ng/L)

Study	Location	Site Details	Results
		fluorocarbon fibers, polymers, polymer films, and resins.	
Boone et al. (2019)	United States (unspecified)	Three groundwater sites used as source waters for three DWTPs, collected in 2010−2012; some locations with known or suspected sources of wastewater in the source water, but study did not differentiate which locations had known or suspected sources.	n = 3, DF[a] 67%, range = ND–44.8 ng/L (LCMRL = 0.034 ng/L)
Appleman et al. (2014)	United States (New Jersey)	Groundwater source water for five DWTPs, sampled November 2011 to September 2012. Majority of the utilities were selected because they were either known from previous monitoring or expected based on their source waters to contain detectable PFAS (i.e., impacted by upstream wastewater effluent discharge). Two sites were sampled twice and three sites were sampled only Once.	n = 7, DF[a] 100%, mean[a] (range) = 5.5 (0.48–11) ng/L (Method RL = 0.25 ng/L)
Quiñones and Snyder (2009)	United States (Nevada)	Samples collected in 2008 from two groundwater sites in Las Vegas Wash, Nevada that were highly impacted from treated wastewater.	Site 1: n = 7, DF NR, mean (maximum) = 6.8 (24) ng/L Site 2: n = 8, DF NR, mean (maximum) = 5.6 (13) ng/L (Method RL = 1.0 ng/L)
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between 1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were not specifically controlled for in the site selection process; active remedies had not been applied at any of the sites selected. Approximately ten samples were collected between March and September 2014 at each site; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases. Groundwater	Overall: n = 149, DF 94.93%, median (maximum) = 870 (290,000) ng/L *Breakdown by site group:* Emergency Response (low-volume release): n = 24, DF 87.5%, mean (range) = 20,100 (10–270,000) ng/L Hangars and Buildings (medium-volume release): n = 100, DF 90.6%, mean (range) = 71,400 (23–910,000) ng/L Testing and Maintenance (high-volume release): n = 25, DF 100.0%, mean (range) = 400 (390–420) ng/L (Median RL = 7 ng/L) *Minimum of detected values reported

Study	Location	Site Details	Results
		samples were collected from existing monitoring wells and temporary monitoring wells installed with direct push technology.	*Median calculated using quantified detections *Non-detects were substituted with ½ the reporting limit
Steele et al. (2018)	United States (Alaska)	Monthly samples collected from a military installation during July 2016–March 2017; six wells from around the installation were sampled each month, along with a seventh well that was only sampled in July 2016. PFAS contamination predominately from prior legacy AFFF use. Wells selected based on historical sample data indicating PFAS contamination.	Data for July, August, September, October, November, December, January, and February, respectively: Well A: 170, 140, 130, 180, 150, 96, 120, 100 ng/L Well B: 220, 360, 360, 370, 410, 300, 400, 370 ng/L Well D: 120 ng/L (for July only; no values provided for other months) Well E: 36, 39, 48, 240, 210, 94, 85, 77 ng/L Well F: 82, 110, 110, 240, 150, 110, 100, 110 ng/L DK: 460, 590, 590, 700, 700, 530, 690, 740 ng/L FG: 60, 69, 75, 61, 93, 64, 67, 59 ng/L (Minimum DL not reported)
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Pilot testing area in former fire training area (Training Site 15) at Joint Base Langley-Eustis where monthly fire training activities were conducted from 1968 to 1980 in a zigzag pattern burn pit. Facility was abandoned in 1980 but irregular fire training activities using an above-ground germed burn pit continued until 1990. Groundwater samples collected for screening/site characterization (April and December 2012), and for pre- (April 2013) and post- (October 2013 and February 2014) in situ chemical oxidation treatment using a peroxone activated persulfate (OxyZone) technology. Treatment was conducted in Test Cell 1 over 113 days (April through August 2013). Pre-treatment samples were collected from 14 wells screened in the deep zone, and 3 wells screened in the shallow zone. Post-treatment samples were collected from the same wells as the pre-treatment samples with an additional three wells (two shallow, one deep) sampled. Wells EC-1, EC-2, EC-3, EC-4, I-	Screening/site characterization: EC-1 (deep, sentry): 19,000 ng/L EC-2 (deep, sentry): 39,000 ng/L I-1 (deep):32,000 ng/L I-2 (deep, sentry): 57,000 ng/L I-4 (deep): 80,000 ng/L I-5 (shallow): 13,000 ng/L I-6 (shallow): 13,000 ng/L MW-2904 (deep): 9,400 ng/L U-16D (deep): 44,000 ng/L U-16S (shallow): 19,000 ng/L

Study	Location	Site Details	Results
		2, and I-3 were sentry wells to monitor the possible migration of oxidants and contaminants outside Test Cell 1. Limited data reported for post-treatment samples.	Pre-treatment: (values reported for two different laboratories: TA; CSM) EC-2 (deep, sentry): 40,000; 48,000 ng/L EC-3 (deep, sentry): 46,000; 59,400 ng/L I-1 (deep): 25,000; 24,900 ng/L I-2 (deep, sentry): 23,000; 20,400 ng/L I-4 (deep): 64,000; 66,400 ng/L Post-treatment: I-4 (deep), U-16D (deep), U-17D (deep), and U-20D (deep) showed a 56% reduction in PFHxS compared to pre-treatment values (LOQ = 1 ng/L)
Moody et al. (2003)	United States (Oscoda, Michigan)	Groundwater samples collected from ten wells during November 1998 and June 1999 from plume at Fire Training Area Two (FTA-02) at the former Wurtsmith Air Force Base; FTA-02 was used from 1952 to 1993 to train U.S. military personnel in firefighting procedures and included flooding a concrete pad with flammable liquids, igniting the fluids, and extinguishing the fire with firefighting agents including AFFF. Minimum of five years since active firefighting activity.	Well ID (distance from fire training pad): FT2 (17 m): n = 1, point = 120,000 ng/L FT3 (18 m): n = 1, point = 104,000 ng/L ML3 (114 m): n = 1, point = 70,000 ng/L ML8 (121 m): n = 1, point = 39,000 ng/L FT8 (183 m): n = 1, point = 30,000 ng/L FT9 (183 m): n = 1, point = 46,000 ng/L FT12 (305 m): n = 1, point = 23,000 ng/L FT14 (305 m): n = 1, point = 27,000 ng/L FT18 (518 m): n = 1, point = 33,000 ng/L FT17 (540 m): n = 1, point = 9,000 ng/L (LOD = 3,000 ng/L; LOQ = 13,000 ng/L) *Point value reported is from five replicate analyses of one sample
Europe			
Bach et al. (2017)	France (southern)	Samples were collected from alluvial wells that provide source water for two DWTPs. The two DWTPs are located on both sides of a river, ~15 km downstream from an industrial site where two facilities produce fluoropolymers; the industrial site	Alluvial wells for DWTP A: April 2013: n = 7, DF[a] 29%, range = <4–7 ng/L

Study	Location	Site Details	Results
		discharges its effluents at three points along a river. The alluvial wells are located along the river, with wells for the first DWTP (DWTP A) located on the left shore and alluvial wells for the second DWTP (DWTP B) located on the right shore, on an island formed by a backwater. Sample collection occurred in April, July, October, and December 2013.	July 2013: n = 7, DF[a] 43%, range = <4–8 ng/L October 2013: n = 7, DF[a] 43%, range = <4–8 ng/L December 2013: n = 7, DF[a] 57%, range = <4–6 ng/L Alluvial wells for DWTP B: April 2013: n = 8, DF[a] 50%, range = <4–7 ng/L July 2013: n = 8, DF[a] 63%, range = <4–8 ng/L October 2013: n = 7, DF[a] 57%, range = <4–8 ng/L December 2013: n = 8, DF[a] 63%, range = <4–6 ng/L (LOQ = 4 ng/L) *DF represents frequency of quantification
Boiteux et al. (2017)	France (northern)	Samples were collected in four sampling campaigns (May, July, October, and December 2013) from alluvial wells that provide source water for two DWTPs. The two DWTPs (A and B) are located downstream of an industrial WWTP that processes raw sewage from a facility that manufactures fluorotelomer-based products and side-chain-fluorinated polymers used in firefighting foams and stain repellents. DWTP A is located 15 km downstream from the WWTP and is supplied by five alluvial wells. DWTP B is located 20 km downstream of the WWTP and is supplied by four alluvial wells.	DWTP A: May 2013: n = 5, DF 0% July 2013: n = 5, DF 0% October 2013: n = 5, DF 0% December 2013: n = 5, DF 0% DWTP B: May 2013: n = 4, DF 0% July 2013: n = 4, DF 0% October 2013: n = 4, DF 0% December 2013: n = 4, DF 0% (LOQ = 4 ng/L) *DF represents frequency of quantification

Study	Location	Site Details	Results
Dauchy et al. (2012)	France (unspecified)	Raw water sampled in June 2010 from four monitoring wells at a fluoropolymer manufacturing plant (P13, P14, P15, P01). Groundwater flowed from well P14 to P01 and well P15 is nearest to the polyvinylidene fluoride production area. Raw water resources also collected from two DWTPs (five sampling sites – DWA-1, DWA-2, DWA-3, DWA-4, DWB-1); the first DWTP (DWA) is supplied by four alluvial wells, and the second DWTP (DWB) is supplied by one alluvial well. The two DWTPs are located on both sides of a river, 15 km downstream of fluorochemical manufacturing facility. The river receives wastewater from many domestic and industrial activities.	Fluoropolymer manufacturing plant: P13: n = NR, DF NR, 68 ng/L P14: n = NR, DF NR, 8 ng/L P15 and P01 not quantifiable due to dilution or matrix effects DWA: DWA-1: n = NR, DF NR, 11 ng/L DWA-2: n = NR, DF NR, 8 ng/L DWA-3: n = NR, DF NR, 4 ng/L DWA-4: n = NR, DF NR, <4 ng/L DWB: DWB-1: n = NR, DF NR, 5 ng/L (LOQ = 4 ng/L) *Study did not indicate whether concentrations reported were point values or means
Harrad et al. (2020)	Ireland (multiple cities)	Groundwater samples collected between November 2018 and January 2019 from ten municipal solid waste landfills at two sampling points down-gradient from the main body of each landfill. Each sampling point consisted of a borehole leading down to water reservoirs at a minimum depth of 5 m below ground level. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction and demolition, and biomedical waste.	n = 10, DF 20%, mean, median (range) = <0.1, <0.1 (<0.1–0.28) ng/L (LOD = <0.1 ng/L) *Non-detects replaced by ½ LOD
Gobelius et al. (2018)	Sweden (national)	Sampling conducted between May and August 2015, with the majority in July and a few samples in September and November 2015. Samples were collected in 21 regional counties by the County Administration Boards. Sampling locations selected based on potential vicinity of PFAS hot spots (i.e., fire training sites, unspecific industry, sewage treatment plant effluent, landfill/waste disposal, skiing, and urban areas) and/or importance as a drinking water source. Sample numbers varied for each county and sampling sites were spread unevenly across Sweden.	n = 161, DF[a] 37%, range = <0.15–80 ng/L (MDL = 0.15 ng/L)

Study	Location	Site Details	Results
Boiteux et al. (2012)	France (national)	Raw water from DWTPs distributed across 100 French departments to represent ~20% of the national water supply flow; samples collected during two sampling campaigns in July–September 2009 (first campaign) and June 2010 (second cam–aign - focused on sites from first sampling campaign that had PFC levels >LOQ). Some sites possibly affected by commercial/industrial releases.	Overall: n = 196, DF (frequency of quantification) 18%, maximum = 32 ng/L *1st Sampling Campaign* n = 163, DF 31%, mean, median (maximum) = 1, <1 (32) ng/L 2nd Sampling Campaign n = 33, results not reported (LOD = 1.3 ng/L, LOQ = 4 ng/L)
Loos et al. (2010)	23 European countries	Groundwater collected from 164 groundwater monitoring stations of participating European Union Member State laboratories during an 8-week window in Fall 2008. There were no strict selection criteria for the sampling sites such as “representative” or “contaminated”. Most monitoring stations were “official” monitoring stations also used for drinking water abstraction.	n = 164, DF 34.8%, mean, median (maximum) = 1, 0 (19) ng/L (LOD = 0.4 ng/L)
Dauchy et al. (2019)	France (unspecified)	Samples collected in two sampling campaigns in and around site where fluorosurfactant-based foams have been used extensively. From 1969 to 1984, the site was an oil refinery, with the exact location of the firefighting training area, frequency of training sessions, and history of firefighting training activities unknown. From 1987 to date, it has been a large training area for firefighters. First sampling campaign collected 13 samples from 9 monitoring wells and 4 springs in June 2015. Second sampling campaign collected from four monitoring wells in October 2016. Monitoring wells MW-1 to MW-5 were located upgradient from the firefighter training site around a landfill site. Monitoring well MW-11 and springs SW-A, SW-B, and SW-D located downgradient from the landfill or firefighter training site but not in the direction of groundwater flow. Monitoring wells MW-6 to MW-13 and spring SW-C were located downgradient from the firefighter training site in the direction of groundwater flow.	Upgradient: Monitoring wells: n = 5, DF[a] 40%, range = <4–42 ng/L Downgradient but not in the direction of groundwater flow: Monitoring wells: n = 1, point = 42 ng/L Spring water: n = 3, DF[a] 33%, range = <4–7 ng/L Downgradient in the direction of groundwater flow: Monitoring wells: n = 7, DF[a] 100%, mean[a] (range) = 660 (26–2,860) ng/L Spring water: n = 1, point = 122 ng/L (LOQ = 4 ng/L)
Høisæter et al. (2019)	Norway (unspecified)	Firefighting training site with an airport that extensively used AFFF containing PFOS since the early 1990s until 2001 when it was replaced by fluorotelomer containing AFFF. All PFAS containing	n = 19, DF NR, mean* = 2,900 ng/L (LOD/LOQ not reported) *Mean estimated from Figure 4b in the paper

Study	Location	Site Details	Results
		firefighting foams was banned at the airport in 2011. Groundwater samples collected in 2016 at five pumping wells installed down gradient of the site to intercept and pump and treat the plume spreading fr10irefightrefighting training site. A total of 19 sampling campaigns were performed.	
Dauchy et al. (2017)	France (unspecified)	Samples collected in the vicinity of three sites (A, C, D) where fluorosurfactant-based foams are or were being heavily used. Site A is an oil storage depot located in a river port. In June 1987, a large explosion occurred in the depot and the fire was extinguished by applying a large amount of fluorosurfactant-based foams. Two groundwater samples were collected in October 2014 and March 2015 from a monitoring well located in the center of the depot. The water table lies 2.5 – 3.5 m below the ground. Site C is a military airport, with the exact location of the training area, frequency of the training sessions, and history of the firefighting training activities unknown. The well supplying the DWTP was sampled in March 2015. Site D is a training center for firefighters. From 1969 to 1984, the site was an oil refinery. Starting in 1987, the site became a training area for firefighters, with exercises carried out directly on the soil. From the 1990s, some exercise areas were covered with concrete. In November 2014, groundwater samples were collected from five springs.	Site A: October 2014: n = 1, point = 139 ng/L March 2015: n = 1, point = 136 ng/L Site C: n = 1, point = 25 ng/L Site D: n = 5, DF[a] 20%, range = <4–132 ng/L (LOQ = 4 ng/L)
Filipovic et al. (2015)	Sweden (Stockholm)	Groundwater samples collected at the airfield and in the vicinity of the airport of the closed air force base F18 in Tullinge Riksten, 19 km south of Stockholm city center, where AFFFs were used. Samples collected in two sampling campaigns in December 2011 and May 2012. The air force base was formally demobilized in 1986 but continued to be used as an air force school for combat command and air surveillance until 1994. Of note, the air force base encountered numerous accidents and incidents during the transfer from propeller era to the jet engine era, including planes crashing upon takeoff and landing, fire incidents, accidental dispersion of jet engine starting	n = 16, DF 69%, range = <0.5–3,470 ng/L *Highest concentrations of 2,960 and 3,470 ng/L detected at sites G5 and G6 (MDL = <0.5 ng/L)

Study	Location	Site Details	Results
		fuel. The area was sold to a land developer in 1996 and is in the process of being transformed into a municipal area. Groundwater flow is directed from the military airfield towards Lake Tullingesjön. Sampling sites included locations under the main firefighting training facility (sites G5 and G6). Other groundwater sampling sites were not mapped to specific locations (note that soil samples were collected at the main firefighting training facility, intermediate soil depot, J34 Hawker Hunter site, old fire station, and soil depot).	
Gyllenhammar et al. (2015)	Sweden (Uppsala)	Three observation well sites (Tuna backar: n = 3 wells; Svartbäcken: n = 1 well, Librobäck: n = 2 wells;) were sampled from September 2012 to January 2013. Four DWTP production well sites (Storvad: n = 9 wells; Galgbacken: n = 1 well; Stadsträdgården and Kronåsen: n = 6 wells; Sunnersta: n = 5 wells) were sampled from July 2012 to February 2014. One private well (Klastorp) was sampled in September 2012. All wells located downstream of a military airport with firefighting training activities up to the year 2003. It is not known when the usage of AFFF started.	Observation wells: Tuna backar: n = 3, DF 100%, median = 690 ng/L Librobäck: n = 4, DF 0% Svartbäcken: n = 3, DF 100%, median = 250 ng/L Production wells: Storvad: n = 12, DF 0% Galgbacken: n = 7, DF 0% Stadsträdgården and Kronåsen: n = 103, DF 100%, median = 83 ng/L Sunnersta: n = 50, DF[a] 52%, median = 8 ng/L Private well: Klastorp: n = 1, point = 16 ng/L (MDL = 10 ng/L)
Wagner et al. (2013)	Germany (unspecified)	Groundwater samples collected downstream from a site contaminated by PFC-based AFFFs from firefighting activities. Sampling year not provided. Samples used to test out a new analytical protocol to determine trace levels of adsorbable organic fluorine.	n = 3, DF[a] 100%, mean (range) = 368 (230–510) ng/L (LOD = 50 ng/L F^-; LOQ = 150 ng/L F^-) *PFHxS concentrations were calculated using the fluorine concentrations reported in Table 4

Study	Location	Site Details	Results
Barreca et al. (2020)	Italy (Lombardia region)	Fifty-seven groundwater sampling stations throughout the region. Samples collected in 2018.	n = 130, DF 0% (LOQ = 1 ng/L)
Sammut et al. (2019)	Malta	Groundwater collected from ten boreholes at different areas on the island during November and December 2015 and January 2016. Collection sites were the most commonly used extraction sites by the Malta Water Services Corporation for water extraction as well as for sampling for water quality analysis.	n = 10, DF 70%, range = ND–2.22 ng/L (LOD = 0.02 ng/L; LOQ = 0.04 ng/L) *Of the ten samples, three were ND and three were <LOQ
Ciofi et al. (2018)	Italy (Tuscany)	Groundwater samples were collected at 12 locations. Sampling year was not reported. Each sample was collected from the phreatic layer with a mean depth between 10–75 m: GW-1: Siena, 10 m GW-2, GW-3, GW-4: Florence, 15 m GW-5: Prato, 75 m GW-6: Prato, 71 m GW-7: Prato, 70 m GW-8: Prato, 61 m GW-9: Florence, 17 m GW-10, GW-11, GW-12: Florence, 10 m	GW-1: n = 1, point = <0.014 ng/L GW-2: n = 1, point = <0.015 ng/L GW-3: n = 1, point = <0.016 ng/L GW-4: n = 1, point = <0.018 ng/L GW-5: n = 1, point = <0.015 ng/L GW-6: n = 1, point = 1.8 ng/L GW-7: n = 1, point = 1.0 ng/L GW-8: n = 1, point = <0.016 ng/L GW-9: n = 1, point = 1.8 ng/L GW-10: n = 1, point = <0.014 ng/L GW-11: n = 1, point = <0.013 ng/L GW-12: n = 1, point = 0.8 ng/L (MDL = 0.013–0.018 ng/L) *MDL varied by sample. MDL provided for 8 of 12 samples
Gellrich et al. (2013)	Germany (Hesse); France; Italy	Untreated water samples for preparation of mineral water included seven from Hesse, three from France, and four from Italy. The supplying waterworks obtain their untreated water either from Rhine river filtrate, a mixture of ground water and percolation water from the Rhine riverbed, drawn from wells 30–50 m deep or from wells in their closer vicinity. Sampling year not reported.	n = 14, DF (frequency of quantification) 0% (LOQ = 1 ng/L)
Llorca et al. (2012)	Spain (Barcelona)	Well water samples from two different sites were collected from the North of Barcelona metropolitan area in 2011.	n = 2, DF[a] 50%, range = <MLOQ–0.50 ng/L (MLOD = 0.27; MLOQ = 0.90 ng/L)

D.1.2. Surface Water

Overall, almost all U.S. studies reported PFHxS detected in at least one surface water sample site in each study. Three studies investigated surface water upstream and downstream of fluoropolymer facilities, with some sites also downstream of other potential PFAS sources (e.g., landfills, WWTP) (Galloway et al., 2020; Newsted et al., 2017; Newton et al., 2017). Galloway et al. (2020) assessed several rivers and tributaries along the Ohio River in three sampling trips in 2016. The sampling sites ranged from upstream, downstream, and north/northeast of a fluoropolymer facility and known PFAS containing landfills. In June 2016, samples were collected on a 188 km stretch of the Ohio River, from 130 km downstream to 58 km upstream of the facility, and tributaries that pass near known PFAS-containing landfills. In July 2016, samples were collected from lakes, rivers, and creeks to the north and northeast of the facility as far as 16 km downwind. The December 2016 trip expanded the collection radius to more than 48 km downwind to the north and northeast of the facility. PFHxS was detected in 96% of samples (n = 26) in June 2016, however all detects were below the LOQ (10 ng/L). Of the second sampling trip, PFHxS was detected at levels above the LOQ in three samples in July 2016 (n = 25), ranging from 64.5–79.0 ng/L. Finally, in December 2016, PFHxS was detected at levels above the LOQ in three samples ranging from 10.1–14.4 ng/L, detected but below the LOQ in 16 samples, and not detected in 21 samples. In Newsted et al. (2017), surface water samples were collected in August 2011 from a 3-mile section of the Upper Mississippi River: ten sampling reaches (three samples each) in an area between Ford Dam (between Minneapolis and St. Paul) and Hastings Dam (near Hastings) and which had been subject to 10–15 years of actions to reduce PFAS contamination from 3M Cottage Grove plant and other commercial/industrial entities. PFHxS was detected in one sample from reach 10, immediately downstream the 3M Cottage Grove facility outfall, at a concentration of 60.5 ng/L. PFHxS in all other samples was below the LOQ of 2.0 ng/L. Authors were not able to observe a clear and consistent time trend in water concentrations. Newton et al. (2017) investigated surface water upstream and downstream of facilities that manufactured or used fluorinated materials along the Tennessee River near Decatur, Alabama. Six sampling sites were located upstream of the manufacturing facilities and three sites were downstream. Among the upstream sites, three were also upstream of a WWTP. All samples were collected in October 2015. PFHxS was detected at one downstream site at a concentration of 39 ng/L; authors suggested elevated PFAS concentrations at downstream sites resulted from infiltration from groundwater or runoff from soil.

In five studies, sampling locations included surface waters potentially impacted by current and/or historic use of AFFFs (Genualdi et al., 2017; Anderson et al., 2016; Post et al., 2013; Nakayama et al., 2010; Nakayama et al., 2007). Genualdi et al. (2017) investigated a cranberry bog in Massachusetts approximately 10 miles from a military base with a history of AFFF usage. Bog water samples were collected in November 2016 and PFHxS was detected in all four samples (six total samples collected, two samples were lost due to evaporation) with a mean concentration of 10.98 ng/L. Authors concluded that given the presence and ratio of PFHxS and PFOS in the bog water samples, it was likely the surface water contamination was related to the previous AFFF usage. Anderson et al. (2016) assessed 40 sites across 10 active Air Force installations throughout the continental United States and Alaska between March and September 2014. Installations were included if there was known historic AFFF release in the period 1970–1990. The selected sites were not related to former fire training areas and were characterized according to volume of AFFF release—low (n = 2), medium (n = 32), and high (n = 2). PFHxS

was detected at both sites characterized to have high-volume AFFF releases, with mean concentration of 5,600 ng/L. Detection frequencies for sites with low- and medium-volume AFFF releases were 50.0% and 74.3%, respectively, and mean concentrations were 7.1 and 196,800 ng/L, respectively. Across all sites, the median concentration of detects was 710 ng/L. Post et al. (2013) evaluated raw surface water samples from 12 public drinking water system intakes collected between August 2009 and February 2010. Six rivers and six reservoirs, including two reservoirs in Atlantic County near a civil-military airport with possible AFFF use, were selected to represent New Jersey geographically. PFHxS was below the minimum RL (5 ng/L) in all six river samples. In reservoir samples, PFHxS was detected at two of six sites (n = 16) at concentrations of 44 and 46 ng/L. These two sites corresponded to the sites near the civil-military airport; given the presence of PFHxS and other PFCs, authors reported the contamination to be indicative of AFFF usage. Two studies from Nakayama et al. (2010; 2007) assessed surface water samples from the Upper Mississippi River, Missouri River, and Cape Fear River Basins. In Nakayama et al. (2010), a large-scale evaluation of the Upper Mississippi River Basin and portion of the Missouri River Basin was conducted to provide preliminary PFC data given the importance of the two basins in supplying drinking water. Between the two basins, 173 samples were collected across 88 sampling sites in March–August 2008 by several different agencies—Minnesota Pollution Agency, Wisconsin Department of Natural Resources, Illinois Environmental Protection Agency, and U.S. EPA Region 7 Water Quality Monitoring Team. Overall, the detection frequency of PFHxS was 89% with a median concentration of 0.71 ng/L. Authors reported higher PFC concentrations adjacent to chemical manufacturers, downstream of WWTPs receiving waste from those types of manufacturers, and near an airport with historic use of firefighting foams. In Nakayama et al. (2007), authors evaluated the performance of a new method for the collection and analysis of PFCs using samples collected from the Cape Fear River Basin, North Carolina during spring 2006. Authors noted possible sources of PFCs to the basin included firefighting foam from nearby air force bases and commercial/industrial facilities. One hundred surface water samples were taken from 80 sites selected to reflect water quality throughout the basin. PFHxS was detected in 98.7% of samples with mean and median concentrations of 7.29 and 5.66 ng/L, respectively. The highest concentrations were found in the middle reaches of the Cape Fear River and its two major tributaries.

Two studies examined surface water near or downstream of land application sites where PFC-contaminated WWTP effluent or biosolids were applied (Lasier et al., 2011; Lindstrom et al., 2011). In the first study, Lasier et al. (2011) sampled the Coosa River, Georgia during summer 2008; samples included two sites upstream (sites 1 and 2) and six sites downstream (sites 3–8) of a land application site, where treated effluent from a WWTP was sprayed. The WWTP processed effluents from multiple carpet manufacturers who were reported to use significant quantities of PFCs. Additionally, site 2 was downstream of a local airport, and site 4 was downstream of a manufacturing facility of latex and polyurethane back material—inputs for the carpet manufacturers. PFHxS was below the MDL (0.014 ng/g) at both of the upstream sites and at the most downstream sites (sites 7 and 8). Mean concentrations for sites 3–6 were 30, 31, 17, and 13 ng/L, respectively. Authors reported highest concentrations downstream of the land application and backing-material sites and then decreased concentrations increasingly downstream as a result of dilution. In the second study, Lindstrom et al. (2011) analyzed surface water samples from ponds and streams in Decatur, Alabama. The samples were collected in February 2009 from farms that had applied PFC-contaminated biosolids to local agricultural fields as a soil amendment for at least 12 years. The biosolids were obtained from a local municipal WWTP

where authors noted that sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers, fluorocarbon fibers, polymers, polymer films, and resins, although specific sources could not be characterized. PFHxS was detected in 22% of samples (n = 32), with levels ranging from below the LOQ (10 ng/L) to 218 ng/L.

Three studies evaluated surface water potentially impacted by wastewater (Boone et al., 2019; Subedi et al., 2015; Appleman et al., 2014). Boone et al. (2019) evaluated 17 PFAS in source and treated waters collected in 2010–2012. Authors attempted to select locations with known or suspected sources of wastewater in the source water, but ultimately the site selection was dependent upon the willingness of DWTPs to participate. The study did not differentiate which locations had known or suspected sources. Of the 22 surface water sources evaluated (16 river and 6 lake/reservoir), PFHxS was detected in 95% of samples (n = 22), ranging from not detected (LCMRL = 0.034 ng/L) to 19.7 ng/L. Subedi et al. (2015) collected 28 lake water samples from 3 sampling events in August–September 2012 and four sampling events in May–September 2013 from Skaneateles Lake. Sites were selected to be along the shoreline of homes that use an enhanced treatment unit for onsite wastewater treatment. Wastewater effluents were identified as a source of contamination to the lake. PFHxS was detected in 79% of samples with mean and median concentrations of 0.56 and 0.28 ng/L, respectively. Appleman et al. (2014) assessed source water from 11 utilities in Alaska, Alabama, Colorado, Nevada, New Jersey, Ohio, Oklahoma, and Wisconsin from August 2011 to May 2012, the majority of which were selected because they were either known from previous monitoring or expected to contain detectable PFAS because they were impacted by upstream wastewater effluent discharge. Authors evaluated the utilities and their effectiveness for removing PFAS. The study did not report an average concentration for PFHxS, but PFHxS was detected in 18 of 25 samples (from 8 of 11 utilities) with a maximum concentration of 13 ng/L.

In three studies, surface water samples were collected from locations with potential sources of PFAS that were not related to AFFF use (Procopio et al., 2017; Zhang et al., 2016; Sinclair et al., 2006). Procopio et al. (2017) evaluated samples collected between September 2011 and July 2014 from the Metedeconk River. Eight sampling events were conducted as part of a source trackdown study to identify potential sources of PFAS contamination after elevated PFOA levels were discovered at a raw surface water intake of the Brick Township Municipal Utilities Authority. In all 56 samples, PFHxS was below the minimum laboratory RL of 5 ng/L. Zhang et al. (2016) conducted analyses to determine major sources of surface water PFAS contamination. Freshwater sample collection sites included 22 sites in the state of Rhode Island (sampled June 2014) and 6 sites in the New York Metropolitan Area (sampled October 2014). Surface water sites were creeks and rivers in urban and rural locations. PFHxS was detected in 89% of samples (n = 28) ranging from below the limit of detection (LOD) to 35.022 ng/L. Authors identified potential PFAS sources at these sites to be metal coating plating; paint, coating, adhesive manufacturing; paper manufacturing; petroleum coal products manufacturing; printing activity; printing ink manufacturing; semiconductor manufacturing; sewage treatment; textile mills; waste management including landfills, and airports. PFHxS levels were below the LOD (0.06 ng/L) at three rural sites corresponding to a background site with no recorded upstream industrial facilities; a Pawcatuck River site 1 km upstream of a military, tactical, and performance synthetic and synthetic blend textiles manufacturer; and a Secret Lake-Oak Hill Brook site 2 km east of a legacy landfill site. Authors reported significantly higher concentrations in urban regions, with the highest being possibly attributed mainly to T.F. Green Airport near Mill Cove, Rhode Island.

In July 2004, Sinclair et al. (2006) collected 51 samples from nine major water bodies of New York to assess the distribution of PFAS. Water bodies included Lake Ontario, Niagara River, Lake Erie, Finger Lakes, Lake Onondaga, Lake Oneida, Erie Canal, Hudson River, and Lake Champlain. PFHxS was detected in 50 samples with median concentrations across all lakes ranged from 0.9 to 7.4 ng/L. The highest concentrations were detected at Lake Onondaga and Erie Canal with median concentrations of 7.4 and 2.6 ng/L, respectively. Authors noted that Lake Onondaga is a Superfund site, is influenced by several industrial sources located along the lake, and also receives effluent from the Metropolitan Syracuse sewage treatment plant. Based on the results of other PFAS detected, the authors also suggested that there may be greater industrial use of fluoropolymers and telomer-alcohol in the region, including the Erie Canal.

Of the remaining studies (Bradley et al., 2020; Pan et al., 2018; Boone et al., 2014; Quiñones and Snyder, 2009; Kim and Kannan, 2007), Bradley et al. (2020) analyzed samples of Lake Michigan untreated intake water as part of a study that also analyzed home tap water samples. Samples were collected in 2017 at four intake sites. PFHxS was detected in all seven samples with a mean concentration of 1.0 ng/L. Boone et al. (2014) developed and tested the accuracy and precision of an analytical method to determine PFCs in environmental and drinking waters. The authors analyzed PFCs in water samples collected from the surface of the Mississippi River at a low flow level (2.95 ft) in September 2010 and a high flow level (8.32 ft) in June 2009. Results were presented as means based on an average of primary and duplicate samples or an average of four replicates. PFHxS levels were 1.315 and 1.07 ng/L at low and high flow levels, respectively. In Quiñones and Snyder (2009), surface water samples from the Boulder Basin, Hoover Dam, and the lower Colorado River were collected in 2008, as part of a study to assess both raw and treated water from utilities producing at least 75 megaliters of finished water per day. PFC occurrence had not been previously determined or reported for these sites. Mean PFHxS levels at all sites were below the method RL (1.0 ng/L). Kim and Kannan (2007) sampled two urban lakes in Albany, New York during five sampling trips from February–November 2006. The lakes, Washington Park Lake are Rensselaer Lake, are located in downtown Albany and receive surface runoff from nearby roadways and residential areas during stormwater runoff. PFHxS was detected in Washington Park Lake (n = 6) at a mean and median concentration of 0.33 ng/L. PFHxS was detected in Rensselaer Lake (n = 5) at mean and median concentrations of 3.09 and 3.25 ng/L, respectively. Overall, PFHxS was detected in 81.8% of the 11 total samples. Finally, in a multicontinental study, Pan et al. (2018) assessed surface water samples from several countries including the United States (Delaware River), United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). Twelve samples were collected in September–December 2016 along the Delaware River that spanned seven cities—Trenton, Bristol, Philadelphia, Chester, Delaware, Smyrna, and Frederica. Authors noted that all sampling sites were along the main stream of the studied rivers and not proximate to known point sources of any fluorochemical facilities. PFHxS was detected in all samples from the Delaware River with a mean concentration of 1.68 ng/L and were similar to levels found in the Rhine River and Mälaren Lake.

Detailed results of the occurrence of PFHxS in European surface waters are presented in Table D-2. Nine studies conducted in Europe evaluated sites with no known point fluorochemical source (Barreca et al., 2020; Munoz et al., 2018; Pan et al., 2018; Shafique et al., 2017; Eriksson et al., 2013; Wagner et al., 2013; Ahrens et al., 2009b; Ahrens et al., 2009a; Ericson et al., 2008b). Pan et al. (2018) performed a study that included surface water sampling

sites in the United Kingdom (Thames River), Germany and the Netherlands (Rhine River), and Sweden (Mälaren Lake). None of the sites sampled were proximate to known sources of PFAS, but for all three water bodies, detection frequency for PFHxS was 100%. The highest PFHxS levels were detected in the Thames River (maximum = 11.3 ng/L), which was about 3 to 4 times greater than the maximum levels found in the other water bodies. For the remaining nine studies, most reported PFHxS levels were relatively low (≤7.8 ng/L) or were not detected at all. Eight studies in Europe evaluated urban areas possibly affected by industrial activities (Lorenzo et al., 2015; Zhao et al., 2015; Boiteux et al., 2012; Eschauzier et al., 2012; Kovarova et al., 2012; Rostkowski et al., 2009) or wastewater effluent discharges (Lorenzo et al., 2015; Labadie and Chevreuil, 2011; Möller et al., 2010). PFHxS occurrence in these studies varied, with some studies reported 0% detections and some reporting detectable levels in all samples. The remaining studies conducted in Europe evaluated surface water samples from sites with known or suspected PFAS releases associated with fluorochemical manufacturing (Bach et al., 2017; Boiteux et al., 2017; Gebbink et al., 2017; Valsecchi et al., 2015) or AFFF use (Mussabek et al., 2019; Gobelius et al., 2018; Dauchy et al., 2017; Filipovic et al., 2015). Of the four studies potentially impacted by nearby fluorochemical manufacturing sites, two conducted in France found no PFHxS (Bach et al., 2017; Boiteux et al., 2017). PFHxS levels were not detected or relatively low at most sampling locations of the remaining two studies (<3 ng/L) except for River Brenta in Italy with an approximately 10-fold higher maximum level (35.6 ng/L). This site is also impacted by nearby textile and tannery manufacturers (Valsecchi et al., 2015). Consistent with U.S.-based studies, the highest PFHxS levels were found at the AFFF-impacted sites (up to 7,550 ng/L).

Table D-2. Studies Reporting PFHxS Occurrence in Surface Water

Study	Location	Site Details	Results
United States			
Galloway et al. (2020)	United States (Ohio; West Virginia)	Rivers and tributaries near a fluoropolymer facility sampled throughout three trips on June, July, and December 2016. In June 2016, samples were collected on a 188 km stretch of the Ohio River, from 130 km downstream to 58 km upstream of the facility, and tributaries that pass near known PFAS-containing landfills. In July 2016, samples were collected from lakes, rivers, and creeks to the north and northeast of the facility as far as 16 km downwind. The December 2016 trip expanded the collection radius to more than 48 km downwind to the north and northeast of the facility.	June 2016: n = 26, DF[a] = 96%* *PFHxS was detected but below the LOQ in 25 samples, and ND in 1 sample July 2016: n = 25, DF[a] = 12%*, range = ND–79.0 ng/L December 2016: n = 40; DF[a] = 48%*, range[a] = <LOQ–14.4 ng/L *PFHxS was detected above the LOQ in 3 samples, detected but below the LOQ in 16 samples, and ND in 21 samples (LOQ = 10 ng/L)
Newsted et al. (2017)	United States (Minnesota)	Ten sampling reaches (three samples each) spanned 3 miles of the Mississippi River within Pool 2, an area between Ford Dam (between Minneapolis and St. Paul) and Hastings Dam (near Hastings) and subject to ongoing PFAS reduction efforts by the Minnesota Pollution Control Agency for 10–15 years. Surface water samples were collected in August 2011.	Upstream of 3M Cottage Grove facility: n = 27, DF 0% Downstream of 3M Cottage Grove facility: n = 3, DF[a] 33%, range= <LOQ–60.5 ng/L (LOQ = 2.0 ng/L)
Newton et al. (2017)	United States (Decatur, Alabama)	Samples collected in October 2015 from nine sites along the Tennessee River. Three sites were downstream of facilities that manufacture or use fluorinated materials and six sites were upstream. Among the upstream sites, three were also upstream of a WWTP.	Upstream: n = 6, DF 0% Downstream: n = 3, DF[a] 33%, range = <LOQ–39 ng/L (LOQ = 10 ng/L)
Genualdi et al. (2017)	United States (Massachusetts)	Cranberry bog with surface water contaminated with PFAS—likely due to proximity to a military base with a history of AFFF usage. The bog was located approximately 10 miles from the military base. Bog water samples were collected in November 2016.	n = 6, DF 100%, mean[a] (range) = 10.98 (8.1–14) ng/L (MDL= 15 ng/L) *2 sample extracts were not available for analysis due to sample loss from evaporation *Table 3 reports the MDL as 15 ng/uL but four of the six detected samples have values less than 15 ng/L
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between	Overall: n = 36, DF 88.00%, median (range) = 710 (ND–815,000) ng/L

Study	Location	Site Details	Results
		1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were not specifically controlled for in the site selection process; active remedies had not been applied at any of the sites selected. Approximately ten samples were collected between March and September 2014 at each site; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases. Surface water sample locations included engineered storm water channels, engineered AFFF ponds, and natural streams.	*Breakdown by site:* Emergency Response (low-volume release): n = 2, DF 50.0%, mean (range) = 7.1 (7.1–7.1) ng/L Hangars and Buildings (medium-volume release): n = 32, DF 74.3%, 196,800 (360–2,700,000) ng/L Testing and Maintenance (high-volume release): n = 2, DF 100.0%, mean (range) = 5,600 (4,400–6,700) ng/L (Median RL = 7 ng/L) *Median calculated using quantified detections *Non-detects were substituted with ½ the reporting limit
Post et al. (2013)	United States (New Jersey)	Raw water collected from 12 public drinking water system intakes between August 2009 and February 2010 from 6 rivers and 6 reservoirs. Sites were chosen to represent NJ geographically and included two reservoir sites near a civil-military airport with possible AFFF use.	Overall n = 12, DF 17%, range = ND–46 ng/L Rivers: n = 6, DF 0% Reservoirs: n = 6, DF 33%, range = <5–46 ng/L (RL = 5 ng/L)
Nakayama et al. (2010)	United States (Illinois; Iowa; Minnesota; Missouri; Wisconsin)	Eighty-eight sampling sites collected between March and August 2008 from tributaries and streams in the Upper Mississippi River Basin and a portion of the Missouri River Basin. Samples were collected by the Minnesota Pollution Agency, Wisconsin Department of Natural Resources, Illinois Environmental Protection Agency, and U.S. EPA Region 7 Water Quality Monitoring Team. Each agency selected sampling sites with the intention of providing preliminary PFC data to the individual regions. Sampling sites included locations adjacent to chemical manufacturers, downstream of WWTPs receiving waste from those types of manufacturers, and near an airport with historic use of firefighting foams.	n = 173, DF 89%, median (range) = 0.71 (ND–169) ng/L (LOD = 0.02 ng/L) *ND data points were substituted with LOD/sqrt(2) = 0.014 ng/L *The maximum concentration of 169 ng/L was collected from a waterway that potentially receives a run-off from a historical fire training site at the Duluth International Airport. Excluding this location, the maximum PFHxS level in surface water is 14.5 ng/L

Study	Location	Site Details	Results
Nakayama et al. (2007)	United States (North Carolina)	Eighty sampling sites in river basin during spring 2006. The sites were selected to reflect water quality throughout the basin. Possible sources of PFCs include use of firefighting foam from Fort Bragg and Pope Air Force, metal-plating facilities, textile, and paper production, and other industries.	n = 100, DF 98.7%, mean, GM, median (range) = 7.29, 5.73, 5.66 (<LOQ–35.1) ng/L (LOQ = 1 ng/L, LOD = 0.05 ng/L) *Values below the LOQ were excluded from the calculation of the mean and GM * Table 2 reports a maximum concentration of 35.1 ng/L but the text reports the highest PFHxS concentration was 26.4 ng/L at Little Rock
Lasier et al. (2011)	United States (Georgia)	Upstream (sites 1 and 2) and downstream (sites 3–8) of a land application site were sampled along the Conasauga, Oostanaula, and Coosa Rivers during summer 2008, where effluents from carpet manufacturers (suspected of producing wastewaters containing perfluorinated chemicals) are processed at a WWTP and the treated WWTP effluent is sprayed onto the site. Additionally, site 2 was downstream a local airport and site 4 was downstream of a manufacturing facility for latex and polyurethane backing material.	Upstream: Sites 1 and 2: DF 0% Downstream: Site 3: DF NR, mean = 30 ng/L Site 4: DF NR, mean = 31 ng/L Site 5: DF NR, mean = 17 ng/L Site 6: DF NR, mean = 13 ng/L Site 7: DF 0% Site 8: DF 0% (MDL = 0.014 ng/g; LOQ = 0.031 ng/g) *Half of the MDL was used when measured concentrations were below the MDL *Concentrations measured in triplicate samples
Lindstrom et al. (2011)	United States (Decatur, Alabama)	Samples collected in February 2009 from ponds and streams located on farms with historical land application of PFC-contaminated biosolids to local agricultural fields between 1995 and 2008. Biosolids obtained from local municipal WWTP where sources discharging to the WWTP included facilities involved in the production and use of fluoropolymers, fluorocarbon fibers, polymers, polymer films, and resins.	n = 32, DF (frequency of quantification)[a] 22%, range = <LOQ–218 ng/L (LOQ = 10 ng/L)
Boone et al. (2019)	United States (unspecified)	Twenty-two surface waters (16 rivers and 6 lakes/reservoirs) used as source waters for 22 DWTPs, collected in 2010−2012; some locations with known or suspected sources of wastewater in the source water, but study did not differentiate which locations had known or suspected sources.	n = 22, DF[a] 95%, range = ND–19.7 ng/L (LCMRL = 0.034 ng/L)

Study	Location	Site Details	Results
Subedi et al. (2015)	United States (New York)	Lake water along the shoreline of residences that use an enhanced treatment unit for onsite wastewater treatment; samples were collected ~40 ft from the lakeshore about 2 ft below surface. Sampling occurred August–September 2012 (three sampling events) and May–September 2013 (four sampling events). Wastewater effluents identified as source of contamination.	n = 28, DF[a] 79%, mean, median, (maximum) = 0.56, 0.28 (2.57) ng/L (LOQ = 0.2 ng/L) *Data points <LOQ were substituted with ½ LOQ and NDs were substituted with zero
Appleman et al. (2014)	United States (Wisconsin; Oklahoma; Alaska; Alabama; Colorado; Ohio; Nevada; New Jersey)	Surface water source water for 11 DWTPs, sampled from August 2011 to May 2012; majority of the utilities selected because they were either known from previous monitoring or expected based on their source waters to contain detectable PFAS (i.e., impacted by upstream wastewater effluent discharge). Each site was sampled between one and four times.	n = 25, DF[a] 72%, range = <0.25–13 ng/L (Method RL = 0.25 ng/L)
Procopio et al. (2017)	United States (New Jersey)	Surface water from the Metedeconk River, where there was suspected illicit discharge to soil and groundwater from a manufacturer of industrial fabrics, composites, and elastomers that use or produce products containing PFAAs. Majority of samples collected from a 4.0 km (2.5 mile) reach of the South Branch Metedeconk River, although samples from the North Branch were also collected in sampling event 1. Samples were collected in eight sampling events in September and December 2011; February, July, September, and December 2012; June and August 2013; and June and July 2014.	n = 56, DF 0% (Minimum RL = 5 ng/L)
Zhang et al. (2016)	United States (Rhode Island; New York)	River and creek samples from 22 sites in Rhode Island collected in June 2014 and from 6 sites in the NY Metropolitan Area collected in October 2014. Both urban and rural locations were sampled.	Overall n = 28, DF[a] 89%, range = <LOD–35.022 ng/L Rhode Island: n = 22, DF[a] 86%, range = ND–35.022 ng/L Urban: n = 10, DF[a] 100%, mean[a] (range) = 6.471 (0.864–35.022) ng/L Rural: n = 12, DF[a] 75%, range = <LOD–0.645 ng/L New York Metropolitan Area (all urban sites): n = 6, DF[a] 100%, mean[a] (range) = 3.019 (0.224–8.526) ng/L (LOD = 0.06 ng/L)

Study	Location	Site Details	Results
Sinclair et al. (2006)	United States (New York)	Nine major water bodies—Lake Ontario, Niagara River, Lake Erie, Finger Lakes, Lake Onondaga, Lake Oneida, Erie Canal, Hudson River, and Lake Champlain—were sampled in July 2004 to represent the major water bodies of New York State. Lake Onondaga is a Superfund site, is influenced by industrial sources along the lake, and receives WWTP effluent.	Lake Ontario: n = 13, DF 100%, median (range) = 1.4 (1.2–2.8) ng/L Niagara River: n = 3, DF 100%, median (range) = 1.2 (1.2–1.4) ng/L Lake Erie: n = 3, DF 100%, median (range) = 1.2 (1.2–1.6) ng/L Finger Lakes: n = 13, DF 100%, median (range) = 0.9 (0.7–1.3) ng/L Lake Onondaga: n = 3, DF 100%, median (range) = 7.4 (4.2–8.5) ng/L Lake Oneida: n = 1, point = 0.9 ng/L Erie Canal: n = 3, DF 100%, median (range) = 2.6 (2.5–5.6) ng/L Hudson River: n = 8, DF 100%, median (range) = 0.9 (0.7–1.6) ng/L Lake Champlain: n = 4, DF[a] 75%, median (range) = 1.3 (ND–1.6) ng/L (DL = 0.5 ng/L)
Bradley et al. (2020)	United States (Chicago, Illinois; East Chicago, Indiana)	Lake Michigan untreated intake water at four intake sites. Samples collected in July and November 2017 at the intakes of the Chicago North and Chicago South WTPs and in July and November 2017 at the intakes of the two East Chicago DwTPs.	n = 7, DF[a] 100%, mean[a] (range) = 1.0 (0.8–1.3) ng/L (LOQ = 0.120–0.580 ng/L) *Quantitative (≥LOQ) and semiquantitative (between LOQ and MDL) results treated as detections
Boone et al. (2014)	United States (New Orleans, Louisiana)	Surface samples from the Mississippi River collected in June 2009 when the river was at a high flow level (8.32 ft) and in September 2010 when the river was at a low flow level (2.95 ft).	Low flow (2.95 ft): mean based on a primary and duplicate sample = 1.315 ng/L High flow (8.32 ft): mean based on four replicates = 1.07 ng/L (DL = 0.016 ng/L; LCMRL = 0.034 ng/L)
Quiñones and Snyder (2009)	United States (Arizona; Nevada)	Samples collected in 2008 from three sites in Boulder Basin, one site in Hoover Dam, and two sites from the lower Colorado River. PFC occurrence had not been previously determined or reported for these sites.	n = 40, DF* 0% (Method RL = 1.0 ng/L) *Mean values at all sites were <Method RL; Figure 3 in the paper shows all quantifiable values were <Method RL
Kim and Kannan (2007)	United States (Albany, New York)	Samples collected from two urban lakes—Washington Park Lake and Rensselaer Lake—during five sampling	Total: n = 11, DF 81.8%, mean, median (range) = 1.58, 0.53 (<LOQ–4.05) ng/L

Study	Location	Site Details	Results
		trips from February–November 2006. Both lakes are located in downtown Albany and receive surface runoff from nearby roadways and residential areas during stormwater runoff.	Washington Park Lake: n = 6, DF NR, mean, median (range) = 0.33, 0.33 (<LOQ–0.53) ng/L Rensselaer Lake: n = 5, DF NR, mean, median (range) = 3.09, 3.25 (2.35–4.05) ng/L (LOQ = 0.25 ng/L) *Non-detects were set to zero; values below the LOQ were set to ½ LOQ
Canada			
Lescord et al. (2015)	Canada (Cornwallis Island, Nunavut)	Six lakes (Meretta, Resolute, Char, Small, North, and 9 Mile) located on Cornwallis Island and near the Inuit community of Resolute Bay were sampled weekly in July to August in 2010 and biweekly in July to August 2011. Two lakes (Meretta and Resolute) are approximately 0.5 km downstream from a local airport, where wastewater from the airport and military base was discharged with little treatment into the Meretta catchment from 1949 to 1998.	Meretta: n = 5, DF NR, mean = 30 ng/L Resolute: n = 5, DF NR, mean = 19.7 ng/L Char: n = 5, DF NR, mean = 0.12 ng/L Small: n = 5, DF NR, mean = 0.11 ng/L North: n = 5, DF NR, mean = 0.01 ng/L 9 Mile: n = 5, DF NR, mean = 0.02 ng/L (MDL = 0.0017 ng/L)
Yeung et al. (2017)	Canada (Ontario)	River water samples were collected from Mimico Creek and Rouge River in November 2014 and analyzed using two methods. Reference method used ultra performance liquid chromatograph and a newly developed method used ultra performance convergence chromatograph for separation.	Results presented as reference method, new method: Mimico: n = 1, point = 24, 20 ng/L Rouge: n = 1, point = <2, <5 ng/L (MLOQ for reference method not reported; MLOQ for new method reported in Table S3 as 200 ng/L but based on Table S7, this should likely be 2 ng/L) *Results in Table S7 are presented in ng/L but based on a comparison to Figure 4, Table S7 should be in ng/mL
Veillette et al. (2012)	Canada (Ellesmere Island, Nunavut)	Lake catchment area located on the northwest coast of the island. Surface water was collected from the center of the lake, the littoral zone (30 m from the delta), the delta, and lake inflow and outflow in July 2007, May 2008, and August 2008. Samples were collected at depths of 2 m (underneath the ice cover), 10 m (the bottom of the mixed layer), and 32 m (in the monimolimnion).	n = 11, DF[a] 100%, mean (range) = 0.009 (0.003–0.024) ng/L (MDL = 0.0007 ng/L)

Study	Location	Site Details	Results
Europe			
Bach et al. (2017)	France (southern)	Grab water samples were collected from six locations along the shore of a river in April, July, October, and December 2013. The river selected for the study receives effluent at three points along the river from an industrial site where two facilities produce fluoropolymers. The first facility has been active since the 1960s, with production including PTFE synthesis from the beginning of the 1960s to 1985 with PFOA as a processing aid; more recently, PVDF has been synthesized since the early 1970s with fluorotelomer sulfonic acid (6:2 FTSA) or PFNA as a processing aid. The second facility, established in 2002, produced fluoropolymers with PFOA as a processing aid until 2008 when it was replaced with PFHxA. Samples were collected starting ~1.3 km upstream from the industrial site and covered ~15 km of the river. Samples (point #1 to #6) were collected from upstream to downstream.	Upstream: Sampling point #1: n = 1, DF 0% for April, July, October, and December 2013 Downstream: Sampling points #2–6: n = 1, DF 0% for April, July, October, and December 2013 (LOQ = 4 ng/L) *DF represents frequency of quantification
Boiteux et al. (2017)	France (northern)	Grab water samples were collected from seven locations along a river in May, July, October, and December 2013. The river selected for the study receives wastewater from an industrial WWTP that treats raw sewage coming from a facility that manufactures fluorotelomer-based products and side-chain-fluorinated polymers used in firefighting foams and stain repellents. Samples were collected starting ~1.2 km upstream of the WWTP discharge and encompassed ~65 km of the river. Samples were collected from upstream to downstream.	Upstream: Sampling point #1: n = 1, DF 0% for May, July, October, and December 2013 Downstream: Sampling points #3, 4, 5, 7, 9, 11: n = 1, DF 0% for May, July, October, and December 2013 (LOQ = 4 ng/L) *DF represents frequency of quantification
Gebbink et al. (2017)	The Netherlands (Dordecht)	River water samples collected in October 2016 at sites downstream (R1−R13) and upstream (R14−R16) of the Dordrecht fluorochemical production plant. Samples (R17−R18) were also collected from different waterbodies at control sites.	Control sites: n = 2, DF[a] (frequency of quantification) 100%, mean[a] (range) = 1.85 (1.7–2.0) ng/L Upstream: n = 3, DF[a] (frequency of quantification) 100%, mean[a] (range) = 2.1 (2.0–2.2) ng/L Downstream: n = 13, DF[a] (frequency of quantification) 100%, mean[a] (range) = 2.0 (1.5–2.2) ng/L (MQL = 0.02 ng/L)

Study	Location	Site Details	Results
Valsecchi et al. (2015)	Italy (River Basins Po, Brenta, Adige, Tevere, and Arno)	Five river basins were sampled between 2008 and 2013. Two river basins (Po and Brenta) receive discharges from two chemical plants that produce fluorinated polymers and intermediates; two river basins (Tevere and Adige) are not impacted by relevant industrial activities; and one river basin (Arno) has textile and tannery districts located along parts of the river. In total, 20 rivers were sampled at their basin closure stations. Rivers Arno, Tevere, and Po were also sampled along the course of the river.	Po: n = 105, DF 0% Brenta: n = 5, DF[a] 20%, range = <LOD–35.6 ng/L Adige: n = 5, DF 0% Tevere: n = 7, DF 0% Arno: n = 19, DF 0% (LOD = 5 ng/L)
Skaar et al. (2019)	Norway (Ny-Ålesund; Lake Linnévatnet area)	Freshwater samples were collected from Ny-Ålesund (research facility) in June 2016 and from the Lake Linnévatnet area (background site) in March 2014 and from April to June 2015. Surface water in Ny-Ålesund was contaminated by a local firefighting training site. Lake Linnévatnet receives water from meltwater of the adjacent glaciers and has few potential pollution sources.	Ny-Ålesund: Background: n = 7, DF[a] 43%, range = <0.005–2.65 ng/L Contaminated: n = 3, DF[a] 100%, mean[a] (range) = 150.83 (30.36–307.51) ng/L Lake Linnévatnet: Background: n = 20, DF[a] 55%, range = <0.005–0.023 ng/L (LOD = 0.005 ng/L; LOQ = 0.006 ng/L) *Values reported for sum of branched and linear PFHxS isomers
Mussabek et al. (2019)	Sweden (Luleå)	Samples from a man-made lake and pond approximately 500 m southwest from a firefighting training facility at the Norrbotten Air Force Wing collected in October 2015. The training facility has been active since 1941 and has used PFAS-containing AFFFs in the last decades. The lake and pond lie above a groundwater reservoir with high permeable soil and were selected because they are isolated water bodies receiving PFAS contamination and can potentially impact groundwater.	Lake: n = 2, DF NR, mean = 570 ng/L Pond: n = 2, DF NR, mean = 500 ng/L (LOD = 0.5 ng/L)
Gobelius et al. (2018)	Sweden (national)	Sampling conducted between May and August 2015, with the majority in July and a few samples in September and November 2015. Samples were collected in 21 regional counties by the County Administration Boards. Sampling locations selected based on potential vicinity of PFAS hot spots (i.e., fire training sites, unspecific industry, sewage treatment plant effluent, landfill/waste disposal, skiing, and urban areas) and/or importance as a drinking water	n = 281, DF[a] 61%, range = <0.15–7,550 ng/L (MDL = 0.15 ng/L) *Two types of water (i.e., surface water and recipient water [surface water]) included

Study	Location	Site Details	Results
		source. Sample numbers varied for each county and sampling sites were spread unevenly across Sweden. Surface water samples collected approximately 10 cm below the water surface.	
Dauchy et al. (2017)	France (unspecified)	Samples collected in the vicinity of three sites (B, C, D) where fluorosurfactant-based foams are or were being heavily used. Site B is an international civilian airport built in 1974. The exact location of the training area, frequency of training sessions, and history of firefighting training activities are unknown. In November 2014, surface water samples were collected in the only river running alongside the airport. Downstream from the airport, this river joins two other rivers, which were also sampled. Site C is a military airport, with the exact location of the training area, frequency of the training sessions, and history of the firefighting training activities unknown. In April 2014, surface water samples were collected in several rivers surrounding the military base. Site D is a training center for firefighters. From 1969 to 1984, the site was an oil refinery. Starting in 1987, the site became a training area for firefighters, with exercises carried out directly on the soil. From the 1990s, some exercise areas were covered with concrete. In November 2014, two surface water samples were collected from the river receiving effluent from a WWTP at the site, one upstream and one downstream of the discharge pipe.	Site B: n = 5, DF[a] 100%, mean[a] (range) = 11.2 (5–18) ng/L Site C: n = 9, DF 0% Site D: n = 2, DF[a] 50%, range = <4–251 ng/L (LOQ = 4 ng/L)
Filipovic et al. (2015)	Sweden (Stockholm)	Fourteen lakes and ponds surrounding the closed air force base F18 in Tullinge Riksten, 19 km south of Stockholm city center, where AFFFs were used. Samples collected in two sampling campaigns in December 2011 and April 2012. The air force base was formally demobilized in 1986 but continued to be used as an air force school for combat command and air surveillance until 1994. Of note, the air force base encountered numerous accidents and incidents during the transfer from propeller era to the jet engine era, including planes crashing upon takeoff and landing, fire incidents, accidental dispersion of jet engine	n = 14, DF 64%, range = <0.5–25.1 ng/L (MDL = <0.5 ng/L)

Study	Location	Site Details	Results
		starting fuel. The area was sold to a land developer in 1996 and is in the process of being transformed into a municipal Area.	
Ciofi et al. (2018)	Italy (Tuscany)	Surface water samples were collected at 13 locations. Sampling year was not reported. SW-1: Arno river before the "Canale Maestro della Chiana" (Arezzo), receiving agricultural runoff and untreated urban wastewater SW-2: Arno river after the "Canale Maestro della Chiana" (Arezzo) SW-3: Arno river before entering in Florence SW-4: Arno river after the discharge of the Florence WWTP SW-5: Arno river after the confluence of the Bisenzio river SW-6: Arno river after the city of Empoli (Florence) SW-7: Arno river after receiving the WWTP effluent from the leather industrial district of Santa Croce (Pisa) SW-8: Arno river in the proximity of the mouth (Pisa) SW-9: Bisenzio river before the confluence with Arno river (Florence) SW-10: Serchio river in the proximity of the mouth (Lucca) SW-11: East area of the coastal lake "Massaciuccoli" (Lucca) SW-12: West area of the coastal lake "Massaciuccoli" (Lucca) SW-13: Central area of the artificial lake "Bilancino" (Florence)	SW-1: n = 1, point = <0.010 ng/L SW-2: n = 1, point = <0.010 ng/L SW-3: n = 1, point = <0.011 ng/L SW-4: n = 1, point = <0.010 ng/L SW-5: n = 1, point = <0.010 ng/L SW-6: n = 1, point = <0.026 ng/L SW-7: n = 1, point = <0.010 ng/L SW-8: n = 1, point = <0.010 ng/L SW-9: n = 1, point = <0.010 ng/L SW-10: n = 1, point = <0.010 ng/L SW-11: n = 1, point = <0.010 ng/L SW-12: n = 1, point = <0.026 ng/L SW-13: n = 1, point = <0.013 ng/L (MDL = 0.010–0.013 ng/L; MQL = 0.026 ng/L) *MDL/MQL varied by sample. MDL provided for 11 of 13 samples; MQL provided for 2 of 13 samples
Munoz et al. (2018)	France (Marnay-sur-Sein; Bougival; Triel-sur-Seine)	Surface water along the Seine River was collected during four sampling campaigns between September 2011 and December 2012, each conducted in a different season. For each campaign, two to four samples were collected over a one-month period. Three sampling sites were investigated: Marnay-sur-Sein, located 200 km upstream from Paris, was selected as a reference site, non-affected by the Greater Paris region; Bougival, situated 40 km downstream from Paris, was chosen to investigate the impact of Greater Paris on PFAS levels; Triel-sur-	n = 36, DF 100%, range = 0.28–7.8 ng/L (LOD = 0.02–0.3 ng/L for all PFAS) *PFHxS concentrations were not reported in text or table by site but relative abundance by site is available in Figure 1

Study	Location	Site Details	Results
		Seine, another 40 km further downstream, was selected to assess the global influence of the Paris urban area, including other inputs such as WWTps.	
Lorenzo et al. (2015)	Spain (Guadalquivir River Basin; Ebro River Basin)	Surface water was collected from the Guadalquivir River and its main tributaries and from the Ebro River and its main tributaries in October 2010. Guadalquivir sampling locations included downstream of WWTPs, near industrial areas, near a military camp, or through major cities; Ebro sampling locations included nearby ski resorts and downstream of WWTP and industrial areas.	Guadalquivir: n = 24, DF 13%, mean (range) = 4.1 (1.5–88.5) ng/L Ebro: n = 24, DF 13%, mean (range) = 0.5 (1.1–5.8) ng/L *Minimum reported is the lowest amount quantified *Mean was calculated with not detected concentrations as zeros (MQL = 0.004 ng/L)
Zhao et al. (2015)	Germany (Elbe River)	Four sampling campaigns conducted in February, April, August, and October 2011 to represent the four seasons. Freshwater samples (sites E619 to E689 with salinity <1 PSU) were collected at nine locations in the river Elbe. Some sampling sites were near Hamburg city and experienced occasional discharge of wastewater from industrial plants.	February: n = 8, DF[a] 87.5%, range = <0.08–0.96 ng/L April: n = 9, DF[a] 100%, mean[a] (range) = 0.46 (0.08–0.77) ng/L August: n = 9, DF[a] 100%, mean[a] (range) = 0.60 (0.42–1.0) ng/L October: n = 8, DF[a] 100%, mean[a] (range) = 0.38 (0.18–0.52) ng/L (MDL = 0.03 ng/L)
Boiteux et al. (2012)	France (national)	Raw water from rivers used as source water for DWTPs. Sites distributed across 100 French department to represent ~20% of the national water supply flow; samples collected during two sampling campaigns in July–September 2009 (first campaign) and June 2010 (second campaign – focused on sites from first sampling campaign that had PFC levels >LOQ). Some sites possibly affected by commercial/industrial releases.	Overall: n = 135, DF (frequency of quantification) 7%, maximum = 8 ng/L *1st Sampling Campaign* n = 99, DF 48%, mean, median (maximum) = 1, <1 (8) ng/L *2nd Sampling Campaign* n = 36, results not reported (LOD = 1.3 ng/L, LOQ = 4 ng/L)
Eschauzier et al. (2012)	The Netherlands (Amsterdam)	Intake water from the Lek canal (n = 2) was collected in January and September 2010 to determine the behavior of PFAAs during the drinking water treatment processes. The Lek canal, a tributary of the river Rhine, is the source of drinking water for the city of Amsterdam and is downstream of an industrial point source in the German part of the Lower Rhine.	n = 2, DF[a] 100%, mean (range) = 2.0 (1.9–2.2) ng/L (LOQ = 0.55 ng/L)

Study	Location	Site Details	Results
Kovarova et al. (2012)	Czech Republic (Brno)	Seven locations in the Svitava and Svratka Rivers upstream and downstream of Brno, a city with highly developed chemical, engineering, textile, and food-processing industries. A sampler was installed at each site for 30 days twice a year (May and September 2008). Due to technical problems, samples were produced from only four of seven sites in May and from five of seven sites in September.	n = 9, DF 0% (LOD not reported)
Llorca et al. (2012)	Germany (Hesse), Spain (national)	Forty-eight surface river waters were sampled in 2010–2011 (24 from Spain and 24 from Germany). Samples from Germany were collected from agriculturally or industrially influenced streams. Samples from Spain were collected from the Xúquer River Basin, Llobregat River Basin, and Ebro River Basin.	Germany: n = 24, DF 21%, mean, median (range) = 1.9, 0.5 (0.06–5.6) ng/L Spain: n = 24, DF 21%, mean, median (range) = 16, 5.8 (0.06–37) ng/L (MLOQ = 0.06 ng/L) *MLOQ reported above is from Table 3; Table 2 reports MLOD = 0.27 ng/L and MLOQ = 0.90 ng/L
Labadie and Chevreuil (2011)	France (Paris)	Samples collected weekly in January–May 2010 in an urban stretch of the River Seine at the Austerlitz Quay, downtown Paris during a flood cycle. The sampling station is under the influence of two major WWTPs and two major combined sewer overflow outfalls.	n = 16, DF 100%, mean, median (range) = 7.1, 6.8 (3.9–12.0) ng/L (LOQ = 0.15 ng/L)
Möller et al. (2010)	Germany (Rhine River watershed)	Raw freshwater samples collected in September–October 2008 along the River Rhine (stations 1–36) and major tributaries of the River Rhine (e.g., Rivers Neckar, Main, Rhur, stations 37–48). Along the River Rhine, samples were taken upstream and downstream of Leverkusen, where effluent of a WWTP treating industrial wastewater was discharged. All samples taken at a water depth ≤1 m.	Rhine upstream Leverkusen: n = 27, DF NR, mean (range) = 3.04 (<0.51–14.5) ng/L Rhine downstream Leverkusen: n = 9, DF 100%, mean (range) = 1.93 (1.66–2.44) ng/L River Ruhr: n = 3, DF NR, mean (range) = 0.18 (<0.51–0.53) River Moehne: n = 1, point = 1.03 ng/L Other tributaries: n = 8, DF NR, mean (range) = 1.41 (<0.51–2.93) ng/L (MDL = 0.51 ng/L)
Rostkowski et al. (2009)	Poland (national)	Inland surface water samples were collected at 12 locations in the southern part of Poland and 14 locations in the northern part of Poland in October and December 2004. Inland surface waters included rivers, lakes, and streams. The northern locations flowed through forested, agricultural, and rural areas; these	North: n = 14, DF[a] 79%, range = <0.02–2.8 ng/L South: n = 11, DF[a] 91%, range = <0.67–113 ng/L (LOQ = 0.1 ng/L)

Study	Location	Site Details	Results
		areas are considered unpolluted with industrial chemicals. Some southern locations were near chemical industrial activities.	
Barreca et al. (2020)	Italy (Lombardia Region)	Fifty-two surface water sampling stations (rivers and streams) throughout the region. Samples collected in 2018.	n = 286, DF[a] 4% (LOQ = 1 ng/L)
Shafique et al. (2017)	Germany (River Elster; River Pleiße; River Saale; and River Elbe)	Surface water samples were collected from the River Elster, River Pleiße, River Saale, and River Elbe at the start of 2015.	Elster: n = 4, DF NR, mean = 0.42 ng/L Pleiße: n = 2, DF NR, mean = 0.37 ng/L Saale (Site A): n = 10, DF NR, mean = 0.13 ng/L Saale (Site B): n = 10, DF NR, mean = 0.60 ng/L Saale (Site C): n = 10, DF NR, mean = 0.07 ng/L Elbe: n = 2, DF NR, mean = 0.61 ng/L (MDL = 0.11 ng/L) *Values extracted from SI, which provides a more detailed breakdown of sites compared to that reported in the main text (where Elster and Pleiße sites were combined and Saale sites were combined)
Eriksson et al. (2013)	Denmark (Faroe Islands)	Grab samples collected in April–May 2012 from Lakes Leitisvatn, Havnardal , Kornvatn, and Á Mýranar.	Leitisvatn: n = 1, point = <0.058 ng/L Havnardal Lake: n = 1, point = <0.024 ng/L Kornvatn Lake: n = 1, point = <0.027 ng/L Á Mýranar: n = 1, point = <0.024 ng/L (LOD = 0.035 ng/L)
Wagner et al. (2013)	Germany (Rhine River)	Surface water samples were collected from the Rhine River. Sampling year not provided. Samples used to test out a new analytical protocol to determine trace levels of adsorbable organic fluorine.	n = 2, DF[a] 100%, mean (range) = 2.4 (1.6–3.2) ng/L (LOD = 50 ng/L F^-; LOQ = 150 ng/L F^-) *PFHxS concentrations were calculated using the fluorine concentrations reported in Table 4
Ahrens et al. (2009a)	Germany (Hamburg; Laurenburg)	Nine samples collected from the river Elbe in Hamburg city (sites 16-18) and from Laurenburg to Hamburg (sites 19-24) in August 2006. Samples were collected at a water depth of 1 m. Dissolved and	Hamburg: Dissolved: n = 3, DF[a] 100%, mean (range) = 0.60 (0.56–0.67) ng/L Particulate: n = 3, DF 0%

Study	Location	Site Details	Results
		particulate phases were analyzed for each of the water samples.	Laurenburg to Hamburg: Dissolved: n = 6, DF[a] 100%, mean (range) = 0.36 (0.24–0.49) ng/L Particulate: n = 6, DF[a] 33%, mean (range) = 0.029 (ND–0.098) ng/L (MDL = 0.140 ng/L for dissolved phase; 0.045 ng/L for particulate phase)
Ahrens et al. (2009b)	Germany (Elbe River)	Samples collected at 53 to 122 km (sites 1 to 9) upstream of estuary mouth of Elbe River in June 2007. *Only locations with conductivity <1.5 mS/cm were assumed to be freshwater and extracted	Site 1 (122 km): n = NR, DF NR, mean = 0.85 ng/L Site 2 (118 km): n = NR, DF NR, mean = 0.9 ng/L Site 3 (115 km): n = NR, DF NR, mean = 1.0 ng/L Site 4 (103 km): n = NR, DF NR, mean = 1.2 ng/L Site 5 (90 km): n = NR, DF NR, mean = 0.8 ng/L Site 6 (80 km): n = NR, DF NR, mean = 1.3 ng/L Site 7 (74 km): n = NR, DF NR, mean = 0.9 ng/L Site 8 (64 km): n = NR, DF NR, mean = 1.1 ng/L Site 9 (53 km): n = NR, DF NR, mean = 0.9 ng/L (MDL = 0.17 ng/L; MQL = 0.57 ng/g)
Ericson et al. (2008b)	Spain (Tarragona Province)	River water samples collected from the Ebro (at two points, Garcia and Mora), Francolí, and Cortiella Rivers in February 2007.	Ebro site 1: n = 1, point = 0.40 ng/L Ebro site 2: n = 1, point = 0.43 ng/L Francolí: n = 1, point = 0.78 ng/L Cortiella: n = 1, point = <0.18 ng/L (LOD = 0.18 ng/L)
Multiple Continents			
Pan et al. (2018)	United States (Delaware River)	Samples were collected from the Delaware River between September and December 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Trenton,	n = 12, DF[a] 100%, mean, median (range) = 1.68, 1.72 (0.65–2.63) ng/L (MDL = 0.05 ng/L)

Study	Location	Site Details	Results
		Bristol, Philadelphia, Chester, Delaware, Smyrna, and Frederica.	
	United Kingdom (Thames River)	Samples were collected from the Thames River in October 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Oxford and London.	n = 6, DF[a] 100%, mean, median (range) = 7.14, 6.42 (4.96–11.3) ng/L (MDL = 0.05 ng/L)
	Germany and The Netherlands (Rhine River)	Samples were collected from the Rhine River in December 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities in Germany included Offenbach, Frankfurt, Goarshausen, Rheinbrohl, Bonn, Cologne, Leverkusen, Dormagen, Düsseldorf, Duisburg, Wesel, and Emmerich. Cities in The Netherlands included Arnhem, Lienden, Duurstede, Nijmegen, Wamel, and Zaltbommel.	n = 20, DF[a] 100%, mean, median (range) = 1.98, 2.03 (0.12–3.90) ng/L (MDL = 0.05 ng/L)
	Sweden (Mälaren Lake)	Samples were collected from Mälaren Lake in September 2016. Sampling sites were not proximate to known point sources of any fluorochemical facilities. Cities included Örebro and Stockholm.	n = 10, DF[a] 100%, mean, median (range) = 1.30, 0.97 (0.56–2.79) ng/L (MDL = 0.05 ng/L)

D.2. RSC for PFHxS, Literature Search and Screening Methodology

The EPA applies an RSC to the RfD when calculating an MCLG based on noncancer effects or for carcinogens that are known to act through a nonlinear mode of action to account for the fraction of an individual's total exposure allocated to drinking water (USEPA, 2000b). The EPA emphasizes that the purpose of the RSC is to ensure that the level of a chemical allowed by a criterion (e.g., the MCLG for drinking water) or multiple criteria, when combined with other identified sources of exposure (e.g., diet, ambient and indoor air) common to the population of concern, will not result in exposures that exceed the RfD. In other words, the RSC is the portion of total daily exposure equal to the RfD that is attributed to drinking water ingestion (directly or indirectly in beverages like coffee tea or soup, as well as from transfer to dietary items prepared with drinking water) relative to other exposure sources; the remainder of the exposure equal to the RfD is allocated to other potential exposure sources. For example, if for a particular chemical, drinking water were to represent half of total exposure and diet were to represent the other half, then the drinking water contribution (or RSC) would be 50%. The EPA considers any potentially significant exposure source when deriving the RSC.

The RSC is derived by applying the Exposure Decision Tree approach published in the EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA, 2000b). The Exposure Decision Tree approach allows flexibility in the RfD apportionment among sources of exposure and considers several characteristics of the contaminant of interest, including the adequacy of available exposure data, levels of the contaminant in relevant sources or media of exposure, and regulatory agendas (i.e., whether there are multiple health-based criteria or regulatory standards for the contaminant). The RSC is developed to reflect the exposure to the U.S. general population or a sensitive population within the U.S. general population and may be derived qualitatively or quantitatively, depending on the available data.

A quantitative RSC determination first requires "data for the chemical in question… representative of each source/medium of exposure and… relevant to the identified population(s)" (USEPA, 2000b). The term "data" in this context is defined as ambient sampling measurements in the media of exposure, not internal human biomonitoring metrics. More specifically, the data must adequately characterize exposure distributions including the central tendency and high-end exposure levels for each source and 95% confidence intervals for these terms (USEPA, 2000b). Frequently, an adequate level of detail is not available to support a quantitative RSC derivation. When adequate quantitative data are not available, the agency relies on the qualitative alternatives of the Exposure Decision Tree approach. A qualitatively-derived RSC is an estimate that incorporates data and policy considerations and thus, is sometimes referred to as a "default" RSC (USEPA, 2000b). Both the quantitative and qualitative approaches recommend a "ceiling" RSC of 80% and a "floor" RSC of 20% to account for uncertainties including unknown sources of exposure, changes to exposure characteristics over time, and data inadequacies (USEPA, 2000b).

In cases in which there is a lack of sufficient data describing environmental monitoring results and/or exposure intake, the Exposure Decision Tree approach results in a recommended RSC of

20%. In the case of MCLG development, this means that 20% of the exposure equal to the RfD is allocated to drinking water and the remaining 80% is reserved for other potential sources, such as diet, air, consumer products, etc. This 20% RSC value can be replaced if sufficient data are available to develop a scientifically defensible alternative value. If scientific data demonstrating that sources and routes of exposure other than drinking water are not anticipated for a specific pollutant, the RSC can be raised as high as 80% based on the available data, allowing the remaining 20% for other potential sources (USEPA, 2000b). Applying a lower RSC (e.g., 20%) is a more conservative approach to public health and results in a lower MCLG.

D.2.1. Literature Search and Screening

In 2020, the EPA conducted a literature search to evaluate evidence for pathways of human exposure to eight PFAS chemicals (PFOA, PFOS, PFBA, PFBS, PFDA, PFHxA, PFHxS, and PFNA) (Holder et al., 2023). This search was not date limited and spanned the information collected across the WOS, PubMed, and ToxNet/ToxLine (now ProQuest) databases. The results of the PFHxS literature search of publicly available sources are available through the EPA's Health & Environmental Resource Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2630.

The 950 literature search results for PFHxS were imported into SWIFT-Review (Sciome, LLC, Research Triangle Park, NC) and filtered through the Evidence Stream tags to identify human studies and nonhuman (i.e., those not identified as human) studies (Holder et al., 2023). Studies identified as human studies were further categorized into seven major PFAS pathways (Cleaning Products, Clothing, Environmental Media, Food Packaging, Home Products/Articles/Materials, Personal Care Products, and Specialty Products) as well as an additional category for Human Exposure Measures. Nonhuman studies were grouped into the same seven major PFAS pathway categories, except that the Environmental Media category did not include soil, wastewater, or landfill. Only studies published between 2003 and 2020 were considered. Application of the SWIFT-Review tags identified 654 peer-reviewed papers matching these criteria for PFHxS.

Holder et al. (2023) screened the 654 papers to identify studies reporting measured occurrence of PFHxS in human matrices and media commonly related to human exposure (human blood/serum/urine, drinking water, food, food contact materials, consumer products, indoor dust, indoor and ambient air, and soil). For this synthesis, additional screening was conducted to identify studies relevant to surface water (freshwater only) and groundwater using a keyword[10] search for water terms.

Following the PECO criteria outlined in Table D-3, the title and abstract of each study were independently screened for relevance by two screeners using *litstream*^{TM}. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion criteria. When two screeners did not agree whether a study should be included or excluded, a third reviewer was consulted to make a final decision. The title and abstract screening of Holder et al. (2023) and of this synthesis resulted in 494 unique studies being tagged as relevant (i.e., having data on occurrence of PFHxS in exposure media of interest) that were further screened with full-text

[10] Keyword list: water, aquifer, direct water, freshwater, fresh water, groundwater, ground water, indirect water, lake, meltwater, melt water, natural water, overland flow, recreation water, recreational water, river, riverine water, riverwater, river water, springwater, spring water, stream, surface water, total water, water supply

review using the same inclusion criteria. After additional review of the evidence collected by Holder et al. (2023), 109 studies originally identified for other PFAS also contained information relevant to PFHxS. Based on full-text review, 172 studies were identified as having relevant, extractable data for PFHxS from the United States, Canada, or Europe for environmental media, not including studies with only human biomonitoring data. Of these 172 studies, 161 were identified from Holder et al. (2023), where primary data were extracted into a comprehensive evidence database. Parameters of interest included: sampling dates and locations, numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics. Eleven of the 172 studies were identified in this synthesis as containing primary data on only surface water and/or groundwater.

Table D-3. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria

PECO Element	Inclusion Criteria
Population	Adults and/or children in the general population and populations in the vicinity of PFAS point sources from the United States, Canada, or Europe
Exposure	Primary data from peer-reviewed studies collected in any of the following media: ambient air, consumer products, drinking water, dust, food, food packaging, groundwater[a], human blood/serum/urine, indoor air, landfill, sediment, soil, surface water[a] (freshwater), wastewater/biosolids/sludge
Comparator	Not applicable
Outcome	Measured concentrations of PFHxS (or measured emissions from food packaging and consumer products only)

Notes: PFHxS = perfluorohexanesulfonic acid.
[a] Surface water and groundwater were not included as relevant media in Holder et al. (2023). Studies were re-screened for these two media in this synthesis.

The evidence database of Holder et al. (2023) additionally identified 18 studies for which the main article was not available for review. As part of this synthesis, 17 of the 18 studies could be retrieved. An additional three peer-reviewed references were identified through gray literature sources, described below, that were included to supplement the search results. The combined 20 studies underwent full-text screening using the inclusion criteria in Table D-3. Based on full-text review, five studies were identified as relevant.

Using the screening results from the evidence database and this synthesis, a total of 177 peer-reviewed studies were identified as relevant. Fifty of these contained information relevant to the United States.

D.2.2. Additional Screening

The EPA also searched the following publicly available gray literature sources for information related to relative exposure of PFHxS for all potentially relevant routes of exposure (oral, inhalation, dermal) and exposure pathways relevant to humans:

- ATSDR's *Toxicological Profiles*;
- CDC's national reports on human exposures to environmental chemicals;
- EPA's CompTox Chemicals Dashboard;

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- EPA's fish tissue studies;
- EPA's Toxics Release Inventory;
- Relevant documents submitted under the Toxic Substances Control Act and relevant reports from EPA's Office of Chemical Safety and Pollution Prevention;
- U.S. Food and Drug Administration's (FDA's) *Total Diet Studies* and other similar publications from FDA, U.S. Department of Agriculture, and Health Canada;
- NOAA's National Centers for Coastal Ocean Science data collections;
- National Science Foundation direct and indirect food and/or certified drinking water additives;
- *Throwaway Packaging, Forever Chemicals: European wide survey of PFAS in disposable food packaging and tableware* (Straková et al., 2021);
- PubChem compound summaries;
- Relevant sources identified in the relative source contribution discussions (Section 5) of EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA)/Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water*; and
- Additional sources, as needed.

The EPA has included available information from these gray literature sources for PFHxS relevant to its uses, chemical and physical properties, and for occurrence in ambient or indoor air, foods (including fish and shellfish), soil, dust, and consumer products. The EPA has included available information specific to PFHxS below on any regulations that may restrict PFHxS levels in media (e.g., water quality standards, air quality standards, food tolerance levels).

D.3. Summary of Potential Exposure Sources of PFHxS Other than Water

D.3.1. Dietary Sources

D.3.1.1. Seafood

PFHxS was detected in 71 of 157 fish tissue composite samples collected during the EPA's National Lake Fish Tissue Study, with a maximum concentration of 3.50 ng/g an[d] a 50th percentile concentration of <0.12 ng/g (Stahl et al., 2014). It was not detected in the 162 fish tissue composite samples collected during the EPA's 2008–2009 National Rivers and Streams Assessment (NRSA) (Stahl et al., 2014). More recently, PFHxS was detected in 32 of 349 fish tissue composite samples at concentrations ranging from 0.121 ng/g to 0.980 ng/g in the EPA's 2013–2014 NRSA (USEPA, 2020a). PFHxS was also detected in 1 of 152 fish tissue composite samples at a concentration of 0.96 ng/g in the EPA's 2015 Great Lakes Human Health Fish Fillet Tissue Study (USEPA, 2021g). PFHxS has been detected in a mixture of fish fillet samples collected from Mississippi River sites in Minnesota at a concentration of 0.47 ng/g (ATSDR, 2021; Delinsky et al., 2010). PFHxS has been detected in Irish pompano (*Diapterus auratus*), silver porgy (*Diplodus argenteus*), and gray snapper (*Lutjanus griseus*) from the St. Lucie Estuary in in NOAA's National Centers for Coastal Ocean Science, National Status and Trends Data (NOAA, 2022). Burkhard (2021) identified 47 studies reporting BAFs for PFHxS and

calculated a median (standard deviation) bioaccumulation factor (BAF) in muscle tissue/fillet of 19.95 ± 7.94 L/kg wet weight (reported as a logBAF of 1.30 ± 0.90 L/kg).

Five additional U.S. studies were identified that evaluated PFHxS levels in seafood (Young et al., 2022; Chiesa et al., 2019; Byrne et al., 2017; Young et al., 2013; Schecter et al., 2010) (Table D-4). One study evaluated fish samples collected directly from rivers and lakes (Byrne et al., 2017). As part of a study to assess exposure to PFHxS and other PFAS among residents of two remote Alaska Native villages on St. Lawrence Island, Byrne et al. (2017) measured PFAS concentrations in stickleback and Alaska blackfish, resident fish used as sentinel species to detect accumulation of PFAS in the local environment. Stickleback were collected from three locations – Suqitughneq (Suqi) River watershed (n = 9 composite samples), Tapisaggak (Tapi) River (n = 2 composite samples), and Troutman Lake (n = 3 composite samples). Blackfish were collected from the Suqi River (n = 29) but were not found in the other water bodies. Authors reported that the Suqi River watershed was upstream and downstream of a formerly used defense site and Tapi River was approximately 5 km east of a military site, however at the start of the study none of the sites were known to be contaminated with PFAS. The sample dates were not reported. PFHxS was not detected in any of the stickleback and blackfish samples, despite the authors noting that stickleback from Troutman Lake had "exceptionally high" total PFAS concentrations.

The remaining four studies purchased seafood from stores and fish markets (Young et al., 2022; Chiesa et al., 2019; Young et al., 2013; Schecter et al., 2010). Young et al. (2013) assessed fish and shellfish collected in 2010–2012 from retail markets across the continental United States. Retail markets in California, Florida, Illinois, Mississippi, New Jersey, New York, Tennessee, Texas, and Washington, D.C., were represented. Authors selected the 10 most consumed fish and shellfish in the United States that were farm raised, wild caught, or had unknown origin. Among the crab meat, shrimp, striped bass, catfish, clams, cod, flounder, pangasius, pollock, tuna, salmon, scallops, and tilapia, PFHxS was only detected in 1 of 10 samples of striped bass at a concentration of 0.66 ng/g. Young et al. (2022) evaluated fish and shellfish purchased from retail markets in the Washington. D.C. metropolitan area and online markets (clams only) from March 2021 through May 2022. Seafood samples represented 8 of the top 10 consumed fish and shellfish in the United States. Seafood samples were farm raised, wild caught, or of unknown origin, and location of harvest was provided when known. PFHxS was only detected in two seafood types, crab and clam meat. All samples of clam meat (n = 10) had detectable concentrations of PFHxS, ranging from 51 to 605 ng/kg. Only two samples of crabs (n = 11) had detectable levels of 112 and 242 ng/kg. Authors also analyzed food packaging for PFAS analytes and did not identify any packaging samples with detectable levels of PFAS. Schecter et al. (2010) evaluated PFHxS and other PFAS in seafood collected from five Dallas, Texas grocery stores in 2009. The origin or source of seafood was not described. Seafood included canned sardines in water, canned tuna, fresh catfish fillet, cod, frozen fish sticks, salmon, and tilapia (n = 1 composite sample for each seafood type). PFHxS was only detected in cod at a concentration of 0.07 ng/g ww. Finally, in a multicontinental study, Chiesa et al. (2019) collected salmon from a wholesale fish market in Milan, Italy; the sampling year was not reported. Wild-caught salmon samples originated from the United States (n = 7), Canada (n = 15), and Scotland (n = 2), while farmed salmon samples originated from Norway (n = 25) and Scotland (n = 17). Among the salmon that originated from the United States Pacific Ocean (FAO 67 and 77), two species – *Oncorhynchus kisutch* and *Oncorhynchus keta* – were analyzed, with PFHxS not

detected in either species (LOQ = 0.015 ng/g). PFHxS was also not detected in wild-caught salmon from Canada and Scotland.

In studies outside of the U.S., PFHxS was detected in multiple fish and shellfish species. Results for all of the identified non-U.S. studies are presented in detail in Table D-4and are summarized here. Approximately half of the studies including samples from outside the U.S. reported at least one sample with detectable levels of PFHxS. Bhavsar et al. (2014) reported detections of PFHxS in both cooked and raw samples of carp, lake trout, and walleye, but did not find detectable levels in raw or cooked Chinook salmon, all of which were caught recreationally from rivers in Ontario, Canada. PFHxS was also detected in several European studies that examined fresh-caught or farmed seafood, including market-bought samples (Hansen et al., 2016; Carlsson et al., 2014; Johansson et al., 2014; Yamada et al., 2014; Falandysz et al., 2006). The maximum PFHxS level detected in seafood was 2.22 ng/g ww in brown trout collected from Norway (Hansen et al., 2016). Though several studies reported on a variety of different species, many presented results for relatively small sample sizes ($n \leq 5$).

Table D-4. Summary of PFHxS Data in Seafood

Study	Location and Source	Seafood Type	Results
United States			
Byrne et al. (2017)	United States (Alaska) Stickleback collected from three locations on St. Lawrence Island: Suqitughneq (Suqi) River watershed (upstream and downstream of a formerly used defense site), Tapisaggak (Tapi) River (located approximately 5 km east of military site), and Troutman Lake, a coastal lake situated adjacent to the village of Gambell. Alaska blackfish collected from the Suqi River but were absent from the other water bodies. Sampling year not reported. No sites were known to be contaminated with PFASs at the initiation of the study.	Stickleback and Alaska blackfish	Stickleback: Troutman Lake: n = 3*; DF 0% Suqi River: n = 9*; DF 0% Tapi River: n = 2*; DF 0% Blackfish: n = 29; DF 0% (LOQ = 0.5–1 ng/g ww for all PFAS) *Number of composite samples, each composed of ~10 stickleback fish
Young et al. (2013)	United States (California; Illinois; Mississippi; Tennessee; Florida; New Jersey; New York; Texas; Washington, D.C.) Fish and shellfish collected from retail markets in 11 areas across the continental United States from 2010–2012. The fish and shellfish included farm raised, wild caught, and unknown origin, as well as freshwater fish, saltwater fish, and euryhaline fish. Crab meat, clams, cod, flounder, pangasius, salmon, scallops, and tilapia purchased from Washington, D.C. Shrimp purchased from Orlando, Florida; Memphis, Tennessee; and Nashville, Tennessee. Striped bass purchased from New York, New York and Cherry Hill, New Jersey. Catfish purchased from Indianola, Mississippi; Dallas, Texas; Tampa, Florida; and Orlando, Florida. Pollock purchased from Huntington Beach, California. Tuna purchased from Chicago, Illinois.	Crab, shrimp, striped bass, catfish, clams, cod, flounder, pangasius, pollock, tuna (can and pouch), salmon, scallops (bay and sea), tilapia	**Striped bass: n = 10, DF[a] 10%, range = ND–0.66* ng/g** Crab meat: n = 1, DF 0% Shrimp: n = 9, DF 0% Catfish: n = 13, DF 0% Clams: n = 1, DF 0% Cod: n = 1, DF 0% Flounder: n = 1, DF 0% Pangasius: n = 1, DF 0% Pollock: n = 1, DF 0% Tuna: n = 3, DF 0% Salmon: n = 2, DF 0% Scallops: n = 2, DF 0% Tilapia: n = 1, DF 0% (MDL = 0.55 ng/g for all seafood) *This value was above the MDL but below the LOQ; LOQ is estimated as 3x the MDL
Schecter et al. (2010)	United States (Texas) Seafood samples from five different grocery stores in Dallas, Texas were collected in 2009. Ten individual samples were collected for each food type and combined to form composite samples. The origin/source of the food samples were not reported.	Salmon, canned tuna, fresh catfish fillet, tilapia, cod, canned sardines, frozen fish sticks	**Cod: n = 1, point = 0.07 ng/g ww, LOD = NR** Salmon: n = 1, DF 0%, LOD = 0.07 ng/g ww Canned tuna: n = 1, DF 0%, LOD = 0.05 ng/g ww Fresh catfish fillet: n = 1, DF 0%, LOD = 0.06 ng/g ww Tilapia: n = 1, DF 0%, LOD = 0.04 ng/g ww Canned sardines: n = 1, DF 0%, LOD = 0.06 ng/g ww Frozen fish sticks: n = 1, DF 0%, LOD = 0.09 ng/g ww *Number of composite samples, each composed of ~10 individual samples

Study	Location and Source	Seafood Type	Results
Canada			
Bhavsar et al. (2014)	Canada (Ontario) Recreationally caught fish from four rivers – Credit River, Thames River, Niagara River, Welland River – in summer and fall of 2010 and 2011. Chinook salmon were caught from Credit River, common carp from Thames River, lake trout from Niagara River, and walleye from Welland River. Elevated PFASs concentrations were expected in the fish based on nearby industrial activities or previous monitoring work conducted by the Ontario Ministry of Environment. Raw fish were analyzed, as well as cooked fish using three different cooking methods (baking, broiling, and frying).	Raw and cooked fish (chinook salmon, common carp, lake trout, walleye)	Chinook salmon: Raw: n = 5, DF NR, mean = <0.006 ng/g ww Baked: n = 5, DF NR, mean = <0.006 ng/g ww Broiled: n = 5, DF NR, mean = <0.006 ng/g ww Fried: n = 5, DF NR, mean = <0.006 ng/g ww **Common carp:** **Raw: n = 5, DF NR, mean = 0.292 ng/g ww** **Baked: n = 5, DF NR, mean = 0.341 ng/g ww** **Broiled: n = 5, DF NR, mean = 0.291 ng/g ww** **Fried: n = 5, DF NR, mean = 0.359 ng/g ww** **Lake trout:** **Raw: n = 4, DF NR, mean = 0.258 ng/g ww** **Baked: n = 4, DF NR, mean = 0.248 ng/g ww** **Broiled: n = 4, DF NR, mean = 0.263 ng/g ww** **Broiled; n = 4, DF NR, mean = 0.245 ng/g ww** **Walleye:** **Raw: n = 5, DF NR, mean = 0.080 ng/g ww** **Baked: n = 5, DF NR, mean = 0.098 ng/g ww** **Broiled; n = 5, DF NR, mean = 0.088 ng/g ww** **Fried; n = 5, DF NR, mean = 0.083 ng/g ww** (LOQ not reported)
Europe			
Hansen et al. (2016)	Norway (Evenes; Skånland) Fish were sampled from Lake Langvatnet, Lake Lavangsvatnet, River Tårstadelva, and the reference Lake Strandvatnet. A civilian airport (location also shared with the Air Station of the Royal Norwegian Air Force) is situated on a ridge between Lake Langvatnet and Lake Lavangsvatnet. These waters are affected by PFAS due to AFFF emissions from the airport. Lake Lavangsvatnet drains into the river Tårstadelva and Lake Strandvatnet is ~15 km away from the airport with no connection to the airport runoff. Samples of the dorsolateral muscle were taken from 10 salmon, 10 anadromous brown trout, 12 stationary brown trout, and 3 European flounder by local fishermen and by personnel from Sweco, an environmental consulting company. The samples were collected in August and September 2014.	Brown trout, European flounder, salmon	**Brown trout (stationary):** **Lake Langvatnet: n = 6, DF[a] 83%, range = <LOD–0.15 ng/g ww** **Lake Lavangsvatnet: n = 5, DF[a] 100%, mean[a] (range) = 0.952 (0.21–2.22) ng/g ww** Lake Strandvatnet (reference): n = 1, point = <LOD **Brown trout (anadromous):** **Lake Lavangsvatnet: n = 3, DF[a] 67%, range = <LOD–0.86 ng/g ww** **River Tårstadelva: n = 5, DF[a] 60%, range = <LOD–0.01 ng/g ww** Lake Strandvatnet (reference): n = 2, DF 0% **European flounder (catadromous):** **Lake Lavangsvatnet: n = 3, DF[a] 100%, mean[a] (range) = 0.27 (0.10–0.53) ng/g ww** **Salmon (anadromous):** **Lake Lavangsvatnet: n = 5, DF[a] 60%, range = <LOD–0.03 ng/g ww**

Study	Location and Source	Seafood Type	Results
			River Tårstadelva: n = 5, DF[a] 20%, range = <LOD–0.01 ng/g ww (LOD = 0.014–0.224 ng/g for all PFAS)
Hölzer et al. (2011)	Germany Fish from Lake Möhne and River Möhne were caught by electric fishing or net fishing between June 2006 and October 2008. The River Möhne was contaminated with PFCs mainly by the application of polluted soil conditioner on agricultural lands between 2004 and 2006, which then drained into tributaries of the river. Fish samples for food monitoring were collected from retail trade, wholesale trade, supermarkets, and producers. Sampling year not provided.	Perch, pike, eel, cisco, and roach from Lake Möhne /River Möhne Eel, trout, pike/perch, and other from trade/markets	Lake Möhne: Perch: n = 15, DF 0% Pike: n = 6, DF 0% Eel: n = 5, DF 0% Cisco: n = 8, DF 0% Roach: n = 10, DF 0% Food monitoring: Eel: n = 2, DF 0% Trout: n = 73, DF 0% Pike/perch: n = 8, DF 0% Other: n = 34, DF 0% (LOD = 0.1 ng/g, LOQ = 0.2 ng/g)
Koponen et al. (2015)	Finland (Baltic Sea, Vanhankaupunginlahti bay, Lake Päijänne) A total of 296 individual fish samples were collected in 2009–2010 from five commercially and recreationally important fishing area across the Finnish coast of the Baltic Sea (Oulu, Pori, Turku, Hanko, and Kotka), Helsinki Vanhankaupunginlahti bay, a large freshwater Lake Päijänne, and four fish farming facilities. Most of the individual samples were pooled, each pool consisting of 2–10 individuals. Baltic herring, pike-perch, perch, burbot, whitefish, salmon, and vendace were collected from the Baltic sea; perch and pike-perch were collected from Helsinki Vanhankaupunginlahti bay; perch was collected from Lake Päijänne. Whitefish and rainbow trout were farmed fish. The selection of fish species was mainly based on the significance of fish in the Finnish diet.	Baltic herring, pike-perch, perch, burbot, whitefish, salmon, vendace, whitefish, rainbow trout	Baltic Sea: Baltic herring (n = 58), pike-perch (n = 30), perch (n = 25), burbot (n = 49), whitefish (n = 27), salmon (n = 44), vendace (n = 20): DF 0% Vanhankaupunginlahti bay: Pike-perch (n = 6), perch (n = 7): DF 0% Lake Päijänne: Perch (n = 10): DF 0% Farmed fish: Whitefish (n = 10), rainbow trout (n = 10): DF 0% (LOQ = 0.18–0.39 ng/g)
Jörundsdóttir et al. (2014)	Iceland Samples were collected by the Icelandic Marine Research Institute in March 2011 during their biannual scientific survey. Cod and anglerfish were caught south-west of Iceland, blue whiting was caught south-east of Iceland, and lumpfish and pollock were caught north-west of Iceland, while ling, plaice, and lemon sole were caught west of Iceland. Each fish sample consisted of a pooled sample from the entire edible part from ten individuals of the same species.	Anglerfish, Atlantic cod, blue whiting, lemon sole, ling, lumpfish, plaice, and pollock	Anglerfish (n = 1), Atlantic cod (n = 2), blue whiting (n = 2), lemon sole (n = 1), ling (n = 1), lumpfish (n = 4), plaice (n = 1), pollock (n = 1): DF 0% (LOD = 0.15 ng/g) *n represents number of composite samples

Study	Location and Source	Seafood Type	Results
Yamada et al. (2014)	France Marine fish sampled were selected based on the fish consumption habits of the population of four areas – La Rochelle in Gironde-Charente Maritime Sud, Le Havre in Normandy-Baie de Seine, Lorient in South Brittany, and Toulon in Mediterranean-Var. Five primary samples of fish were bought from the fish market and/or supermarket in each region for each species in January–April 2005. Freshwater fish sampled were selected based on the individual dietary consumption analysis of anglers or their family members of the ICAR-PCB study. Freshwater fish were collected in six major French rivers with each river divided into three or four section in 2008–2009. Half of the samples were composite samples.	Freshwater fish, fresh or frozen marine fish	Results presented for lower bound and upper bound if LB value different from UB value **Fresh and frozen marine fish:** **Total LB: n = 95, DF NR, mean (range) = 0.00 (0–0.03) ng/g ww** **Total UB: n = 95, DF NR, mean (range) = 0.03 (0.02–0.04) ng/g ww** Anchovy: n = 1, LB–UB = 0–0.03 ng/g ww Monkfish: n = 4, LB–UB = 0–0.02 ng/g ww Catshark: n = 4, LB–UB = 0.02–0.04 ng/g ww Cod: n = 4, LB–UB = 0–0.02 ng/g ww Common dab: n = 4, LB–UB = 0–0.02 ng/g ww Orange roughy: n = 3, LB–UB = 0–0.03 ng/g ww Plaice/witch: n = 2, LB–UB = 0–0.02 ng/g ww Goatfish: n = 3, LB–UB = 0–0.03 ng/g ww Grenadier: n = 4, LB–UB = 0–0.02 ng/g ww Gurnard: n = 1, LB–UB = 0.03 Haddock: n = 2, LB–UB = 0–0.02 ng/g ww Hake: n = 4, LB–UB = 0–0.02 ng/g ww Halibut: n = 4, LB–UB = 0–0.03 ng/g ww John dory: n = 2, LB–UB = 0.02–0.04 ng/g ww Ling: n = 4, LB–UB = 0–0.02 ng/g ww Mackerel: n = 4, LB–UB = 0–0.03 ng/g ww Pollack: n = 3, LB–UB = 0–0.02 ng/g ww Pout: n = 1, LB–UB = 0–0.03 ng/g ww Ray: n = 4, LB–UB = 0–0.02 ng/g ww Saithe: n = 4, LB–UB = 0–0.02 ng/g ww Salmon: n = 4, LB–UB = 0–0.03 ng/g ww Sardine: n = 4, LB–UB = 0–0.03 ng/g ww Scorpionfish: n = 1, LB–UB = 0–0.03 ng/g ww Seabass: n = 4, LB–UB = 0–0.03 ng/g ww Sea bream: n = 4, LB–UB = 0–0.03 ng/g ww Sole: n = 4, LB–UB = 0–0.02 ng/g ww Swordfish: n = 4, LB–UB = 0–0.03 ng/g ww Tuna: n = 4, LB–UB = 0–0.03 ng/g ww Whiting: n = 4, LB–UB = 0–0.02 ng/g ww **Freshwater fish:** Barbel: n = 5, LB–UB = 0.19–0.22 ng/g ww Bleak: n = 9, LB–UB = 0.02–0.41 ng/g ww Brown trout: n = 31, LB–UB = 0.06–0.17 ng/g ww Chub: n = 9, LB–UB = 0.05–0.16 ng/g ww Common carp: n = 7, LB–UB = 0.67–0.76 ng/g ww

Study	Location and Source	Seafood Type	Results
			Common roach: n = 67, LB–UB = 0.06–0.18 ng/g ww Minnow: n = 1, LB–UB = 0.4 ng/g ww European eel: n = 137, LB–UB = 0.67–0.77 ng/g ww European perch: n = 9, LB–UB = 0.11–0.2 ng/g ww Freshwater bream: n = 34, LB–UB = 0.1–0.24 ng/g ww Gudgeon: n = 5, LB–UB = 0.36–0.39 ng/g ww Northern pike: n = 8, LB–UB = 0.02–0.18 ng/g ww White bream: n = 22, LB–UB = 0.21–0.3 ng/g ww Thicklip grey mullet: n = 6, LB–UB = 0.01–0.16 ng/g ww Wels catfish: n = 14, LB–UB = 0.01–0.12 ng/g ww Western vairone: n = 1, LB–UB = 0–0.17 ng/g ww Pike-perch: n = 22, LB–UB = 0.06–0.16 ng/g ww (LOD = 0.007–0.95 ng/g for PFAAs other than PFOA and PFOS) *Lower bound (LB) scenario defined as values <LOD replaced with 0 *Upper bound (UB) scenario defined as values <LOD replaced with LOD
Eriksson et al. (2013)	Denmark (Faroe Islands) Wild fish (cod and saithe) were sampled from the Faroe Shelf area; cod were sampled in October and August 2011, while saithe were sampled in April 2012. Three farmed salmon samples were collected from different fjords in Faroe Islands, sampling year not reported.	Farmed salmon, wild-caught cod, wild-caught saithe	Cod 1, n = 1, DF 0% Cod 2, n = 1, DF 0% Saithe 1, n = 1, DF 0% Saithe 2, n = 1, DF 0% Salmon 1, n = 1, DF 0% Salmon 2, n = 1, DF 0% Salmon 3, n = 1, DF 0% (LOD = 0.018 ng/g) *n represents number of pooled samples, each combining muscle tissue from five fish
Falandysz et al. (2006)	Poland Cod samples were collected from the Gulf of Gdañsk in the Baltic Sea (south coast of Poland) in February 2003.	Cod *(Gadus morhua)*	**n = 18, DF NR, mean, median (range) = 0.1, 0.1 (0.05–0.8) ng/mL** (LOD not reported) *Values reported for animal whole blood samples
Rivière et al. (2019)	France Samples collected between July 2011 and July 2012 in the center region of France. Food items were selected based on the results of a national consumption survey to obtain a representative and general view of children's (0–3 years old) food consumption. All analyzed samples	Fish (unspecified)	n = 1, DF 0% (LOD = 0.0002–3.7 ng/g fw for all PFAS) *n represents number of composite samples

Study	Location and Source	Seafood Type	Results
	were formed of 12 subsamples of the same food and of equal weight. The fish were cooked according to the practices reported in the survey of practices.		
Sadia et al. (2020)	Sweden (Örebro) Three fish samples from different brands were purchased from a local supermarket in February 2019.	Fish	**n = 3, DF[a] 33%, range = ND–0.0055 ng/g ww** (LOD = 0.0011; LOQ = 0.0049 ng/g)
Barbosa et al. (2018)	Belgium, France, The Netherlands, Portugal Fish were collected from different markets based on the assumption that the fish species were frequently consumed in European Union countries and the fish species contained high levels of contaminants of emerging concern. Sampling year not reported. The following fish species (origin, market country) were included: *P. platessa:* Channel, Belgium *M. australis:* South America, Portugal *M. capenis:* South Africa, Portugal *K. pelamis*: Azores, Portugal *M. edulis*: North Sea, The Netherlands; France, France	Raw and steamed fish (*P. platessa, M. australis, M. capenis, K. pelamis*, and *M. edulis*)	*P. platessa*: n = 25, DF 0% *M. australis*: n = 25, DF 0% *M. capenis*: n = 25, DF 0% *K. pelamis*: n = 25, DF 0% *M. edulis*: The Netherlands: n = 50, DF 0% France: n = 50, DF 0% (LOD = <0.01 ng/g ww for all PFCs)
Gebbink et al. (2015)	Sweden Food items were purchased at two major grocery store chains in four major Swedish cities in 1999 and 2005. In 2010, sampling was limited to Uppsala city since no systematic geographical differences in food contamination was observed in the two earlier market basket studies. The food items were selected based on Swedish food and production statistics and were not cooked before analysis. Homogenates of fish products (fresh and frozen fillets of fish, canned fish products, shellfish) were prepared for each collection year by mixing food items proportionally according to food consumption statistics. Results were not reported for the 2005 and 2010 fish product composite samples (only reported pooled with other food types).	Fish	**1999: n = 1, point = 0.0069 ng/g** (MLOQ = 0.0001 ng/g) *n represents number of composite samples
Vassiliadou et al. (2015)	Greece Samples were obtained during the winter and early spring of 2011. Finfish, squids, and shrimps were purchased from the local fish market in Kallithea, Athens, while mussels were obtained from a mariculture farm within the Saronikos Gulf, Attika. Samples were analyzed raw as well as cooked in the ways favored in Greek cuisine (pan-fried in olive oil and/or grilled).	Anchovy, bogue, hake, picarel, sand smelt, sardine, striped mullet, mussel, shrimp, squid	Anchovy (raw, fried, grilled), bogue (raw, fried, grilled), hake (raw, fried, grilled), picarel (raw, fried), sand smelt (raw, fried), sardine (raw, fried, grilled), striped mullet (raw, fried, grilled), mussel (raw, fried), shrimp (raw, fried), and squid (raw, fried, grilled): n = 4 for each, DF 0% *n represents number of composite samples (LOD = 0.18 ng/g ww; LOQ = 0.54 ng/g ww)

Study	Location and Source	Seafood Type	Results
	Quadruplicate composite samples were created for each food type, each consisting of four to six items of raw or cooked fish or shellfish.		
Carlsson et al. (2014)	Greenland (Nuuk) Seafood was purchased at the local fish market and grocery shops in June 2010. All items were originally caught in the vicinity of the Nuuk area and/or along the West coast of Greenland and represented the common food items consumed by the local Inuit population.	Salmon, halibut	Raw salmon fillet: n = 6, DF 0% Smoked salmon fillet: n = 6, DF 0% Smoked halibut fillet: n = 6, DF 0% (LOD = 0.014–0.224 ng/g for all PFAS)
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Between September 2011 and February 2013, samples were purchased from different supermarkets and retail stores in representative cites around the world, including cities in Serbia and Spain in Europe.	Bivalves, whiting, cod, hake, salmon, herring, pangasius, trout, tuna	Spain: Bivalves (n = 28), whiting (n = 7), cod (n = 12), hake (n = 3), salmon (n = 9), herring (n = 22), pangasius (n = 9), trout (n = 19), tuna (n = 9): DF 0% Serbia: Canned tuna (n = 1), pangasius (n = 1), cod (n = 2), herring (n = 5), trout (n = 2): DF 0% (MLOD = 0.107 ng/g; MLOQ = 0.356 ng/g)
Domingo et al. (2012)	Spain (Catalonia) Foods purchased from 4 shops/stores of each of the 12 representative cities of Catalonia (Barcelona, l'Hospitalet de Llobregat, Vilanova I la Geltrú, Mataír, Sabadell, Terrassa, Girona, Tarragona, Reus, Tortosa, Lleida and Manresa) in September 2011. Shops/stores included local markets, small stores, supermarkets, and big grocery stores. For each food item, two composite samples were prepared for analysis, where each composite sample consisted of 24 individual units. Only edible parts of each food item were included in the composites.	Fish and seafood (sardine, tuna, anchovy, swordfish, salmon, hake, red mullet, sole, cuttlefish, clam, mussel, and shrimp)	**n = 2, DF NR, mean = 0.045 ng/g fw** (LOD not reported) *n represents number of composite samples
Vestergren et al. (2012)	Sweden (Malmoe, Gothenburg, Uppsala, Sundsvall) Purchasing locations of the two largest retail chains in Sweden were selected in each of four major Swedish cities. All purchases were made in spring/summer of 1999, 2005, and 2010. In 2010, the study was limited to the largest retail chains in Uppsala located in close vicinity to Stockholm. An equal amount of each food group from each of the four cities was combined into one sample pool to provide a representative sample for the Swedish urban population.	Fish products (fresh and frozen fillets of fish, canned fish products, shellfish)	**1999: n = 1, point = 0.0217 ng/g** **2005: n = 1, point = 0.0088 ng/g** **2010: n = 1, point = 0.0092 ng/g** (MDL = 0.0020 ng/g; MQL = 0.0059 ng/g) *n represents number of composite samples

Study	Location and Source	Seafood Type	Results
Noorlander et al. (2011)	The Netherlands Fish randomly purchased from several Dutch retail stores with nationwide coverage in November 2009. Fish samples were ground, homogenized, and pooled for analysis.	Fatty fish (herring, eel, mackerel, salmon), lean fish (cod, plaice, pollack, tuna), crustaceans (mussels, shrimp, crab)	**Fatty fish: n = 1, point = 0.009 ng/g** **Lean fish: n = 1, point = 0.023 ng/g** **Crustaceans: n = 1, point = 0.044 ng/g** (LOD not reported) *n represents number of composite samples
Jogsten et al. (2009)	Spain (Catalonia) Fish samples purchased from local markets, large supermarkets, and grocery stores from two different areas of Tarragona Province, Catalonia in January and February 2008. The cities of Tarragona and Reus were sampled in the northern area and L'Ametlla de Mar and Tortosa in the southern area. For each food item, two composite samples were analyzed (one composite for the northern area and one for the southern area). Each composite was formed of a minimum of six individual sub-samples of the same product.	Marinated salmon (homemade and packaged)	**Homemade: n = 2, DF NR, mean = 0.014 ng/g** Packaged: n = 2, DF NR, mean = <0.003 ng/g (LOD = 0.001 ng/g) *n represents number of composite samples *Values of ND were replaced with ½×LOD
Ericson et al. (2008a)	Spain Food samples purchased from local markets, large supermarkets, and grocery stores within Tarragona County in July 2006. Food samples were randomly purchased with origin source not specified. Each of the food samples were duplicated and combined to analyze a composite sample. Only the edible part of each food was included in the composite samples. Composite samples included the following: White fish: hake, whiting blue, sea bass, monkfish Seafood: mussel, shrimp Tinned fish: tuna, sardine, mussel Blue fish: salmon, sardine, and tuna	White fish, seafood, tinned fish, blue fish	White fish: n = 2, DF 0% Seafood: n = 2, DF 0% Tinned fish: n = 2, DF 0% Blue fish: n = 2, DF 0% (LOD = 0.001–0.65 ng/g fw) *n represents number of composite samples
Johansson et al. (2014)	Sweden Farmed rainbow trout (whole fish) were collected from fish farms along the Swedish Baltic Sea coast (brackish water). Only fish older than 12 months were sampled. Samples were collected annually from 1999 to 2010 within the Swedish National Food Agency's official food control program.	Rainbow trout	**Total: n = 36, DF[a] 58%, range = <0.011–0.04 ng/g fw** **1999: n = 10, DF[a] 90%, range = <0.011–0.034 ng/g fw** **2000: n = 3, DF[a] 100%, mean[a] (range) = 0.014 (0.012–0.017 ng/g fw** **2001: n = 4, DF[a] 75%, range = <0.011–0.040 ng/g fw** **2002: n = 1, point = 0.017 ng/g fw** 2003: n = 2, DF 0% **2004: n = 1, point = 0.020 ng/g fw**

Study	Location and Source	Seafood Type	Results
			2005: n = 4, DF[a] 25%, range = <0.011–0.020 ng/g fw **2006: n = 3, DF[a] 33%, range = <0.011–0.014 ng/g fw** **2007: n = 2, DF[a] 50%, range = <0.011–0.029 ng/g fw** 2008: n = 1, DF 0% **2009: n = 4, DF[a] 25%, range = <0.011–0.015 ng/g fw** 2010: n =1, DF 0% (MDL = 0.011 ng/g fw; MQL = 0.025 ng/g fw)
Multiple Continents			
Chiesa et al. (2019)	United States (Pacific Ocean) Wild-caught fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from USA-Pacific Ocean (Food and Agriculture Organization Area 67 and 77).	Wild-caught salmon (*Oncorhynchus kisutch* and *Oncorhynchus keta*)	*Oncorhynchus kisutch*: n = 5, DF 0% *Oncorhynchus keta*: n = 2, DF 0% (LOQ = 0.015 ng/g)
	Canada Wild-caught fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Canada (Food and Agriculture Organization Area 67).	Wild-caught salmon (*Oncorhynchus nerka*)	n = 15, DF 0% (LOQ = 0.015 ng/g)
	Norway Farmed fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Norway (Food and Agriculture Organization Area 27).	Farmed salmon (*Salmo salar*)	n = 25, DF 0% (LOQ = 0.015 ng/g)
	Scotland Wild-caught and farmed fish were collected at a wholesale fish market in Milan, Italy. Sampling year was not reported. The wild-caught salmon were from Scotland (Food and Agriculture Organization Area 27).	Wild-caught and farmed salmon (*Salmo salar*)	Wild-caught: n = 2, DF 0% Farmed: n = 17, DF 0% (LOQ = 0.015 ng/g)

Notes: DF = detection frequency; LOD = limit of detection; LOQ = limit of quantitation; MDL = method detection limit; ND = not detected; NR = not reported; ww = wet weight. Bold indicates detected levels of PFHxS in food.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

4.1.1.1 Other Food Sources

PFHxS was included in a suite of PFAS evaluated in FDA's 2019, 2021, and 2022 Total Diet Study Sampling (US FDA, 2022b, 2022a, 2021b, 2021a, 2020b, 2020a); however, it was not detected in any of the food samples tested. It should be noted that FDA indicated that the sample sizes used in the PFAS 2019, 2021, and 2022 Total Diet Study Sampling were limited and that the results should not be used to draw definitive conclusions about PFAS levels in the general food supply (US FDA, 2022c). PFHxS was detected in milk samples collected from a farm with groundwater known to be contaminated with PFAS; however, it was not detected in produce collected from an area near a PFAS production plant, in FDA studies of the potential exposure to the U.S. population to PFAS (US FDA, 2021c, 2018). PFHxS is not a registered pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and the EPA does not set a 40 CFR Part 180 pesticide tolerance in food and feed commodities for PFHxS (US GPO, 2022). Maximum residue levels for PFHxS were not found in the Global Maximum Residue Level Database (Bryant Christie Inc, 2022).

Several peer-reviewed studies were identified that examined PFHxS in food sources other than seafood, including cereals, dairy, fats/other (e.g., eggs, oils, and spices), fruits and vegetables, meat, and breastmilk (Table D-5). Few U.S. studies analyzed foods from any one origin – four sampled crops grown in areas with known or suspected PFAS contamination, including biosolids-amended soils, two sampled from crops as part of greenhouse and field studies, one studied wild-caught alligator meat. Only two studies sampled from store- or market-bought meats, eggs, produce, and dairy.

Scher et al. (2018) evaluated garden produce samples from homes in Minnesota within and outside of a GCA in the vicinity of a former 3M PFAS production facility. Twenty homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. A total of 279 produce samples (232 inside GCA, 47 outside GCA) were collected between May and October 2010. PFHxS was detected in 1% of the 232 produce samples from inside the GCA (one floret sample and one leaf sample). The authors suggested that the two detections were associated with PFAS present in irrigation water that had accumulated in produce. They also noted that accumulation of PFAS was particularly high in florets. Three homes that were outside the GCA served as a reference. No PFHxS was detected in produce samples from home gardens outside the GCA. Genualdi et al. (2017) analyzed PFAS contamination in a Massachusetts cranberry bog approximately 10 miles from a military base with a history of AFFF usage. Ten cranberry samples were taken directly from trucks transporting cranberries and 32 cranberry samples were collected directly from the bog water in November 2016. PFHxS was not detected in any samples (MDL = 0.79 ng/g).

Two studies purchased food items from stores and markets for evaluation (Young et al., 2012; Schecter et al., 2010). Schecter et al. (2010) assessed PFHxS and other PFAS in food samples collected from five Dallas, Texas grocery stores in 2009. The origin or source of each food was not described. Food items included meat products (bacon, canned chili, chicken breast, ground beef, roast beef, ham, sausage, and turkey), dairy (butter, cheeses, frozen yogurt, ice cream, milk, and yogurt), eggs, grains (cereal), fruits and vegetables (apples, potatoes), and fats/other (canola oil, margarine, olive oil, peanut butter). PFHxS was not detected in any of the food samples. In Young et al. (2012), cow milk was purchased from retail markets across the continental United

States representing 17 states; the sampling year was not reported. Cow milk samples included organic milk, vitamin D added milk, and ultra-pasteurized milk. PFHxS was not detected in any of the 49 retail milk samples (MDL = 0.15 ng/g).

One study investigated PFAS levels in wild meat (Tipton et al., 2017). Tipton et al. (2017) assessed alligator tail meat that was collected during the South Carolina recreational hunting season between September to October 2015. Tail meat samples were collected from four different public hunt units – Southern Coastal, Middle Coastal, Midlands, and Pee Dee. PFHxS was detected in all samples from all hunt units. Median concentrations from Southern Coastal (n = 19), Middle Coastal (n = 17), Midlands (n = 5), and Pee Dee (n = 2) were 0.087 ng/g, 0.099 ng/g, 0.0816 ng/g, and 0.093 ng/g wet mass, respectively.

Two studies by Blaine et al. (2014; 2013) evaluated PFHxS in crops grown in greenhouse and field studies. In Blaine et al. (2014), PFAS levels were measured in celery root, pea fruit, and radish root grown in a greenhouse with control (unamended) soil, industrially impacted soil, and municipal soil (n = 3–5). PFHxS was detected in radish root from all three soils, celery shoot from the industrially impacted and municipal soil, and pea fruit from only industrially impacted soil. Mean concentrations of PFHxS in radish root for the control, industrially impacted, and municipal soil were 3.81 ng/g, 2.84 ng/g, and 4.33 ng/g, respectively. Mean concentrations of PFHxS in celery shoot for the industrially impacted and municipal soil were 3.19 ng/g and 0.38 ng/g, respectively. The mean concentration of PFHxS in pea fruit in the industrially impacted soil was 0.24 ng/g. Authors noted minor cross-contamination of the control soil due to the proximity of the unamended soil to biosolids-amended soil. In Blaine et al. (2013), authors studied the uptake of PFAS into edible crops in both field and greenhouse studies. In the field study, PFAS levels were measured in corn grain and corn stover grown with control (unamended), urban biosolids-amended, and rural biosolids-amended soil (n = 3–7). Mean PFHxS concentrations were below the LOQ in both corn grain and corn stover grown in any field study plots (<0.04 ng/g for corn grain; <0.29 ng/g for corn stover). In the greenhouse study, lettuce and tomato plants were grown in control soil, industrially impacted soil, or municipal soil (n = 3–5). Mean PFHxS concentrations were below the LOQ for lettuce and tomato grown in the control soil and for tomato grown in municipal soil; however, mean PFHxS levels were 10.44 ng/g and 5.54 ng/g for lettuce grown in industrially impacted and municipal soils, respectively, and 0.76 ng/g for tomato grown in industrially impacted soil. Sampling year was not reported.

The remaining two U.S. studies evaluated the occurrence of PFHxS in breastmilk (von Ehrenstein et al., 2009; Kuklenyik et al., 2004). von Ehrenstein et al. (2009) collected breastmilk samples between December 2004 and July 2005 from women between the ages of 18 and 38 at the time of recruitment as part of the pilot study Methods Advancement for Milk Analysis (MAMA). Women provided milk samples at two visits – the first visit was 2–7 weeks postpartum, and the second visit was 3–4 months postpartum. PFHxS was not detected in any of the samples from the first visit (n = 18) or second visit (n = 20). Similarly, PFHxS was below the LOD (0.3 ng/mL) in the samples reported by Kuklenyik et al. (2004). Kuklenyik et al. (2004) did not report information on the breastmilk donors or the sampling procedure as it was unavailable; PFHxS was not detected in either of the two samples.

Of the studies conducted in Europe examining non-breastmilk and non-seafood food items, 15 found PFHxS in at least one food type. Results for all of the identified non-U.S. studies are

presented in detail in Table D-5 and are summarized here. Across the European studies, PFHxS was found in animal products such as meat, dairy, and eggs, as well as fruits and vegetables. Similarly to studies examining seafood, most studies reported on a variety of different food types, but the majority presented results for relatively small sample sizes ($n \leq 5$). Lastly, eight of the twelve studies conducted outside the U.S. examining PFHxS in breastmilk had detectable levels.

Table D-5. Summary of PFHxS Data in Other Food

Study	Location and Source	Food Types	Results
United States			
Scher et al. (2018)	United States (Minnesota) Home garden produce samples were collected between May and October 2010 from 20 homes in 3 cities within a GCA as well as 3 homes in the Twin Cities Metro outside the GCA. Homes within the GCA were near a former 3M PFAS production facility, had previous or ongoing PFAS contamination in drinking water, and were served by the Oakdale, Minnesota public water system or were formerly or currently using a private well previously tested and shown to have detectable levels of PFOA or PFOS. 279 produce samples (232 within GCA and 47 outside GCA) consisting of mature, edible portions of plants were analyzed. Plant part categories included floret, fruit, leaf, root, seed, and stem.	Fruits and vegetables	Within GCA: **All:** n = 232, DF 1%, median (range) = ND (ND–0.066) ng/g **Floret: n = 5, DF 20%, median (range) = ND (ND–0.066) ng/g** **Leaf: n = 35, DF 3%, median (range) = ND (ND–0.046) ng/g** Garden fruit (n = 98), yard fruit (n = 13), root (n = 29), seed (n = 29), and stem (n = 23): DF 0% Outside GCA: All: n = 47, DF 0% Floret (n = 1), garden fruit (n = 15), yard fruit (n = 4), leaf (n = 12), root (n = 5), seed (n = 5), and stem (n = 5): DF 0% (MDL = 0.003 to 0.029 ng/g depending on the analyte and type of produce)
Genualdi et al. (2017)	United States (Massachusetts) Samples from cranberry bog with surface water contaminated with PFAS—likely due to proximity to a military base with a history of AFFF usage. The bog was located approximately 10 miles from the military base. Ten cranberry samples taken directly from trucks transporting cranberries (five samples each from two trucks) and 32 cranberry samples taken directly from 12 sections of the bog water. Samples collected in November 2016.	Fruits	n = 42, DF 0% (MDL = 0.79 ng/g)
Schecter et al. (2010)	United States (Texas) Food samples from five different grocery stores in Dallas, Texas were collected in 2009. Ten individual samples were collected for each food type and combined to form composite samples. The origin/source of the food samples were not reported.	Dairy; fruits and vegetables; grains; meat; fats/other	Meat Hamburger: n = 1, DF 0%, LOD = 0.04 ng/g ww Bacon: n = 1, DF 0%, LOD = 0.05 ng/g ww

Study	Location and Source	Food Types	Results
			Sliced turkey: n = 1, DF 0%, LOD = 0.02 ng/g ww Sausages: n = 1, DF 0%, LOD = 0.04 ng/g ww Ham: n = 1, DF 0%, LOD = 0.02 ng/g ww Sliced chicken breast: n = 1, DF 0%, LOD = 0.02 ng/g ww Roast beef: n = 1, DF 0%, LOD = 0.02 ng/g ww Canned chili: n = 1, DF 0%, LOD = 0.01 ng/g ww Dairy and Eggs Butter: n = 1, DF 0%, LOD = 0.09 ng/g ww American cheese: n = 1, DF 0%, LOD = 0.04 ng/g ww Other cheese: n = 1, DF 0%, LOD = 0.04 ng/g ww Whole milk: n = 1, DF 0%, LOD = 0.02 ng/g ww Ice cream: n = 1, DF 0%, LOD = 0.03 ng/g ww Frozen yogurt: n = 1, DF 0%, LOD = 0.02 ng/g ww Whole milk yogurt: n = 1, DF 0%, LOD = 0.03 ng/g ww Cream cheese: n = 1, DF 0%, LOD = 0.02 ng/g ww Eggs: n = 1, DF 0%, LOD = 0.04 ng/g ww Grains Cereals: n = 1, DF 0%, LOD = 0.04 ng/g ww Fruits and Vegetables Apples: n = 1, DF 0%, LOD = 0.02 ng/g ww Potatoes: n = 1, DF 0%, LOD = 0.04 ng/g ww Fats/Other

Study	Location and Source	Food Types	Results
			Olive oil: n = 1, DF 0%, LOD = 0.3 ng/g ww Canola oil: n = 1, DF 0%, LOD = 0.5 ng/g ww Margarine: n = 1, DF 0%, LOD = 0.03 ng/g ww Peanut butter: n = 1, DF 0%, LOD = 0.03 ng/g ww *n reflects number of composite samples, each composed of ~10 individual samples
Young et al. (2012)	United States (17 states) Retail cow's milk samples were all pasteurized whole milk, commercially available, and purchased at retail markets across the continental United States representing 17 states. Samples were organic milk, vitamin D added milk, and ultra-pasteurized milk. Sampling year not reported.	Dairy	n = 49, DF 0%, (MDL = 0.15 ng/g)
Tipton et al. (2017)	United States (South Carolina) Alligator tail meat samples were collected from a local wild game meat processer during the South Carolina recreational hunt season between September to October 2015. Samples were from four different public hunt units—Southern Coastal, Middle Coast, Midlands, and Pee Dee.	Meat	**Alligator tail:** **Southern coastal: n = 19, DF[a] 100%, median (range) = 0.087 (0.051–0.252) ng/g wet mass** **Middle coastal: n = 17, DF[a] 100%, median (range) = 0.099 (0.063–0.272) ng/g wet mass** **Midlands: n = 5, DF[a] 100%, median (range) = 0.0816 (0.054–0.158) ng/g wet mass** **Pee Dee: n = 2, DF[a] 100%, median (range) = 0.093 (0.071–0.115) ng/g wet mass** (RL not reported)
Blaine et al. (2014)	United States (Midwest) Crops grown in in greenhouse study with control (unamended), industrially impacted soil, or municipal soil. Control soil had minor cross-contamination due to proximity to biosolids-amended fields. Industrially impacted soil was amended with industrially impacted biosolids, and municipal soil was amended with municipal biosolids for over 20 years. Crops grown in the greenhouse study were grown from seed in pots, which were randomly arranged within the greenhouse. Sampling year not reported.	Fruits and vegetables	**Radish root:** **Control: n = 3–5, DF NR, mean = 3.81 ng/g** **Industrially impacted; n = 3–5, DF NR, mean = 2.84 ng/g** **Municipal: n = 3–5, DF NR, mean = 4.33 ng/g** **Celery shoot:** Control: n = 3–5, DF 0% **Industrially impacted: n = 3–5, DF NR, mean = 3.19 ng/g**

Study	Location and Source	Food Types	Results
			Municipal: n = 3–5, DF NR, mean = 0.38 ng/g **Pea fruit:** Control: n = 3–5, DF 0% **Industrially impacted: n = 3–5, DF NR, mean = 0.24 ng/g** Municipal: n = 3–5, DF 0% (LOQ = 0.03 ng/g)
Blaine et al. (2013)	United States (Midwest) Crops grown in urban and rural full-scale field study with control (unamended) and biosolids-amended soil. Three agricultural fields were amended (0.5×, 1×, or 2×) with municipal biosolids. Urban biosolids (1× and 2×) were from a WWTP receiving both domestic and industrial waste. Rural biosolids (0.5×) were from a WWTP receiving domestic waste only. Control plots were proximal to the rural and urban amended corn grain and corn stover field sites; sampling year not provided. Crops grown in greenhouse study with control (nonamended) and biosolids-amended soil. Nonamended soil obtained from a field that received commercial fertilizers and had a similar cropping system as the nearby municipal soil site. Municipal soil was obtained from a reclamation site in Illinois where municipal biosolids were applied at reclamation rates for 20 years, reaching the cumulative biosolids application rate of 1,654 Mg/ha. Industrially impacted soil was created by mixing composted biosolids from a small municipal (but impacted by PFAA manufacturing) WWTP with control soil on a 10% mass basis. Sampling year not provided.	Fruits and vegetables; grains	Field study: Corn grain: Urban nonamended: n = 3–7, DF NR, mean = <0.04 ng/g Urban 1×: n = 3–7, DF NR, mean = <0.04 ng/g Urban 2×: n = 3–7, DF NR, mean = <0.04 ng/g Rural nonamended: n = 3–7, DF NR, mean = <0.04 ng/g Rural 0.5×: n = 3–7, DF NR, mean = <0.04 ng/g Corn stover: Urban nonamended: n = 3–7, DF NR, mean = <0.29 ng/g Urban 1×: n = 3–7, DF NR, mean = <0.29 ng/g Urban 2×: n = 3–7, DF NR, mean = <0.29 ng/g Rural nonamended: n = 3–7, DF NR, mean = <0.29 ng/g Rural 0.5×: n = 3–7, DF NR, mean = <0.29 ng/g (LOQ = 0.04 ng/g for corn grain; LOQ = 0.29 ng/g for corn stover) Greenhouse study: Lettuce:

Study	Location and Source	Food Types	Results
			Nonamended: n = 3–5, DF NR, mean = <0.01 ng/g **Industrially impacted: n = 3–5, DF NR, mean = 10.44 ng/g** **Municipal: n = 3–5, DF NR, mean = 5.54 ng/g** Tomato: Nonamended: n = 3–5, DF NR, mean = <0.03 ng/g **Industrially impacted: n = 3–5, DF NR, mean = 0.76 ng/g** Municipal: n = 3–5, DF NR, mean = <0.03 ng/g (LOQ = 0.01 ng/g for lettuce; LOQ = 0.03 ng/g for tomato)
von Ehrenstein et al. (2009)	United States (North Carolina) As part of the Methods Advancement for Milk Analysis (MAMA) pilot study, 34 breastfeeding women aged 18 to 38 years at recruitment provided breastmilk samples at two visits. The first visit occurred 2–7 weeks postpartum, and the second visit occurred 3–4 months postpartum. Both visits were between December 2004 and July 2005.	Breastmilk	Visit #1: n = 18, DF 0% Visit #2: n = 20, DF 0% (LOQ = 0.30 ng/mL)
Kuklenyik et al. (2004)	United States (Georgia) Authors reported that no information was provided on the human milk donors or the sampling procedure.	Breastmilk	n = 2, DF 0% (LOD = 0.3 ng/mL)
Canada			
Kubwabo et al. (2013)	Canada (Ontario) Breastmilk samples were collected in the Kingston region of Ontario in 2003–2004.	Breastmilk	n = 13, DF 0% (MDL = 0.125 ng/mL; LOQ = 0.416 ng/mL)
Europe			
Scordo et al. (2020)	Italy Commercially available strawberry and olive fruits were purchased in two Italian supermarkets in 2018.	Fruits	**Strawberries: n = 2, DF[a] 100%, mean[a] (range) = 0.469 (0.148–0.790) ng/g dw** (MDL = 0.010 ng/g; MQL = 0.037 ng/g)

Study	Location and Source	Food Types	Results
			Olives: n = 2, DF[a] 50%, range = <0.0024–0.022 ng/g dw (MDL = 0.0024 ng/g; MQL = 0.0086 ng/g)
Sadia et al. (2020)	Sweden (Örebro) Three samples of each food type (cow milk, butter, chicken meat, beef) of different brands were purchased from a local supermarket in February 2019.	Dairy; fats/other	**Milk: n = 3, DF[a] 100%, mean[a] (range) = 0.0021 (0.0014–0.0030) ng/g ww** **Butter: n = 3, DF[a] 100%, mean[a] (range) = 0.0082 (0.0026–0.018) ng/g ww** **Beef: n = 3, DF[a] 67%, range = ND–0.0044 ng/g ww** **Chicken: n = 3, DF[a] 100%, mean[a] (range) = 0.0035 (0.0033–0.0036) ng/g ww** (LOD = 0.0011; LOQ = 0.0049 ng/g)
Sznajder-Katarzyńska et al. (2019)	Poland Milk and milk products were purchased in Polish markets in 2017. Commercially available samples of each product were obtained from five different suppliers.	Dairy	**All dairy: n = 35, DF 48.6%, sum PFHxS = 0.47 ng/g** **Milk: n = 5, DF[a] 60%, range = 0.01–0.01 ng/g** **Cottage cheese: n = 5, DF[a] 100%, range = 0.04–0.05 ng/g** **Natural yogurt: n = 5, DF[a] 60%, range = 0.01–0.02 ng/g** **Kefir/Bonny clabber: n = 5, DF[a] 60%, range = 0.01–0.02 ng/g** **Butter: n = 5, DF[a] 60%, range = 0.02–0.03 ng/g** Sour cream (n = 5), camembert-type cheese (n = 5): DF 0% (LOD = 0.003 ng/g; LOQ = 0.010 ng/g) *Range reported for detected values
Rivière et al. (2019)	France Samples collected between July 2011 and July 2012 in the center region of France. Food items were selected based on the results of a national consumption survey to obtain a representative and general view of children's (0–3 years old) food consumption. All analyzed samples were formed of 12 subsamples of the same food and of equal weight. The products purchased were prepared in a way that reflected as closely as possible what is done in the home (preparation and cooking).	Meat; dairy; fruits and vegetables; fats/other	Infant-specific foods: Milk-based beverage (n = 8), cereals (n = 5), milk-based desserts (n = 6), growing-up milk (n = 9), soups and puree (n = 11), fruit puree (n = 4), vegetable-based ready-to-eat meal (n = 20), meat/fish-based ready-to-eat meal (n = 45), infant formula (n = 28), follow-on formula (n = 33): DF 0% Common foods:

Study	Location and Source	Food Types	Results
			Non-alcoholic beverages (n = 1), dairy-based desserts (n = 1), milk (n = 1), mixed dishes (n = 1), ultra-fresh dairy products (n = 1), meat (n = 1), poultry and game (n = 1): DF 0% (LOD = 0.0002–3.7 ng/g for all PFAS) *n represents number of composite samples
Sznajder-Katarzyńska et al. (2018)	Poland Samples were purchased in Polish markets in 2017. Individual food items were selected among the most frequently consumed in Poland. Vegetables (potatoes, beetroots, carrots, white cabbage, tomatoes) and fruits (apples, cherries, strawberries) of Polish origin were bought in season when naturally ripe. Bananas, lemons, and oranges were bought after being imported to Poland. Five different samples of each fruit or vegetable were collected.	Fruits and vegetables	Apples, bananas, cherries, lemons, oranges, strawberries, beetroots, carrots, tomatoes, potatoes, and white cabbage: n = 5 for each, DF 0% (LOD = 0.006 ng/g; LOQ = 0.017 ng/g)
Surma et al. (2017)	Spain, Slovakia Spice samples were collected in powder form from Spain and Slovakia. Sampling year not reported.	Fats/other	Spain: Anise (n = 1), star anise (n = 1), white pepper (n = 1), fennel (n = 1), cardamom (n = 1), clove (n = 1), coriander (n = 1), nutmeg (n = 1), allspice (n = 1), cinnamon (n = 2), vanilla (n = 1), ginger (n = 1), peppermint (n = 1), parsley (n = 1), thyme (n = 1), laurel (n = 1), garlic (n = 1), cumin (n = 1), black pepper (n = 1), mild hot pepper (n = 1), hot hot pepper (n = 1), oregano (n = 2): DF 0% Slovakia: Anise (n = 1), star anise (n = 1), white pepper (n = 1), fennel (n = 1), cardamom (n = 1), clove (n = 1), coriander (n = 1), nutmeg (n = 1), allspice (n = 1), cinnamon (n = 1), vanilla (n = 1), ginger (n = 1): DF 0% (LOD = 0.013 ng/g; LOQ = 0.039 ng/g)
Zafeiraki et al. (2016a)	Greece, The Netherlands Home and commercially-produced eggs were collected from different regions in the Netherlands and Greece in August 2013–August 2014. Home-produced eggs were voluntarily provided, and	Fats/other	**Domestic eggs:** **The Netherlands: n = 73, DF 7%, median (range) = 1.1 (<0.05–5.2) ng/g**

Study	Location and Source	Food Types	Results
	commercial eggs were purchased from supermarkets. The yolks of the same sample of eggs were pooled, homogenized, and then analyzed.		Greece: n = 45, DF 0%, median (range) = <0.5 (<0.5) ng/g Commercial eggs: The Netherlands: n = 22, DF 0% Greece: n = 31, DF 0% (LOD = 0.15 ng/g; LOQ = 0.5 ng/g) *Median calculated only on the concentrations above LOQ
Zafeiraki et al. (2016b)	The Netherlands Samples purchased from local markets and slaughterhouses in the Netherlands in 2014. Samples included liver samples of horse, sheep, bovine, pig, and chicken.	Meat	Horse: n = 19, DF 0% Sheep: n = 18, DF 0% Bovine: n = 22, DF 0% Pig: n = 20, DF 0% Chicken: n = 20, DF 0% (LOQ = 0.5 ng/g ww)
D'Hollander et al. (2015)	Czech Republic, Belgium, Norway, Italy The Czech Republic, Belgium, Norway, and Italy were selected to represent eastern, western, northern, and southern Europe. Sampling took place between spring and summer 2010 as part of the PERFOOD study. Individual items were randomly selected in three national retail stores covering different brands or countries of origin. Of each item, three to ten single samples were combined to create a pooled sample. The food items examined were: Cereals: rice, wheat (white), oats, rye Sweets: sugar (beet), sugar (cane), honey Fruits – berries: strawberries Fruits – citrus fruit: oranges, tangerines, lemons, grapefruits Fruits – pipe and stone fruit: apples, pears, peaches, plums Fruits – others and exotic fruit: melons, grape, bananas	Grain; fruits; fats/other	Czech Republic: Cereals: wheat (white), oats, rye: n = 1 each, point = <0.010 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.004 ng/g Fruits – berries: strawberries: n = 1, point = <0.004 ng/g Fruits – citrus fruit: oranges, tangerines: n = 1, point = <0.004 ng/g Fruits – pipe and stone fruit: apples, pears, peaches: n = 1, point = <0.004 ng/g Fruits – others and exotic fruit: melons: n = 1, point = <0.004 ng/g Miscellaneous: rock salt: n = 1, point = <0.004 ng/g **Italy:** Cereals:

Study	Location and Source	Food Types	Results
	Miscellaneous: “rock” salt, “marine” salt		Rice, maize: n = 1 each, point = <0.004 ng/g Wheat (white): n = 1, point = <0.010 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.004 ng/g Fruits – citrus fruit: lemons: n = 1, point = <0.004 ng/g Fruits – pipe and stone fruit: **Apples: n = 1, point = 0.015 ng/g** Pears: n = 1, point = <0.004 ng/g **Peaches: n = 1, point = 0.016 ng/g** Plums: n = 1, point = <0.004 ng/g Fruits – others and exotic fruit: Grapes: n = 1, point = <0.004 ng/g Bananas: n = 1, point = 0.008 ng/g Miscellaneous: marine salt: n = 1, point = <0.004 ng/g **Norway:** Cereals: wheat (white): n = 1, point = <0.010 ng/g Sweets: sugar (cane), honey: n = 1 each, point = <0.004 ng/g Fruits – berries: strawberries: n = 1, point = <0.004 ng/g Fruits – citrus fruit: Oranges: n = 1, point = <0.004 ng/g **Grapefruits: n = 1, point = 0.0189 ng/g** Fruits – pipe and stone fruit: apples, pears: n = 1 each, point = <0.004 ng/g

Study	Location and Source	Food Types	Results
			Fruits – others and exotic fruit: melons: n = 1, point = 0.0038 ng/g Miscellaneous: rock salt: n = 1, point = <0.004 ng/g **Belgium:** Cereals: rice, wheat (white), wheat (dark), oats: n = 1 each, point = <0.004 ng/g Sweets: sugar (beet), honey: n = 1 each, point = <0.004 ng/g **Fruits – berries: strawberries: n = 1, point = 0.123 ng/g** Fruits – citrus fruit: oranges, lemons: n = 1 each, point = <0.004 ng/g Fruits – pipe and stone fruit: **Apples: n = 1, point = 0.197 ng/g** Pears: n = 1, point = <0.004 ng/g Plums: n = 1, point = <0.004 ng/g Fruits – others and exotic fruit: grapes: n = 1, point = <0.004 ng/g (LOD = 0.004 or 0.010 ng/g) *n represents number of composite samples
Gebbink et al. (2015)	Sweden Food items were purchased at two major grocery store chains in four major Swedish cities in 1999 and 2005. In 2010, sampling was limited to Uppsala city since no systematic geographical differences in food contamination was observed in the two earlier market basket studies. The food items were selected based on Swedish food and production statistics and were not cooked before analysis. The food items were divided into 12 groups and homogenates for each food group were prepared by mixing food items proportionally according to food consumption	Fruits and vegetables; meat; grains; fats/other	**1999:** **Dairy products: n = 1, point = 0.0007 ng/g** **Meat products: n = 1, point = 0.0059 ng/g** **Egg: n = 1, point = 0.034 ng/g** **Potatoes: n = 1, point = 0.0002 ng/g** **Soft drinks: n = 1, mean = 0.0002 ng/g** Fats: n = 1, point = <0.0001 ng/g

Study	Location and Source	Food Types	Results
	statistics. Results by food group were not reported for the 2005 and 2010 years. For all three sampling years, a homogenate was prepared by mixing proportional amounts of each food group according to consumption data for the respective year (includes fish samples).		Pastries: n = 1, point = <0.0001 ng/g Cereal products: n = 1, point = <0.0001 ng/g Vegetables: n = 1, point = <0.0001 ng/g Fruit: n = 1, point = <0.0001 ng/g Sugar and sweets: n = 1, point = <0.0001 ng/g **Year pool: n = 12, point = 0.0022 ng/g** **2005:** **Year pool: n = 12, point = 0.0010 ng/g** **2010:** **Year pool: n = 12, point = 0.0007 ng/g** (MLOQ = 0.0001 ng/g) *n represents number of composite samples
Carlsson et al. (2014)	Greenland (Nuuk) Meat was purchased at the local fish market and grocery shops in June 2010. All items were originally caught in the vicinity of the Nuuk area and/or along the West coast of Greenland and represented the common food items consumed by the local Inuit population.	Meat	**Seal beef: n = 2, DF[a] 100%, range = 0.2–0.6 ng/g ww** Narwhal: n = 6, DF NR, median = <LOD Whale beef: n = 8, DF NR, median = <LOD (LOD = 0.014–0.224 ng/g for PFAS)
Pérez et al. (2014)	Serbia (Belgrade and Novi Sad), Spain (Barcelona, Girona, and Madrid) Between September 2011 and February 2013, samples were purchased from different supermarkets and retail stores in representative cites around the world, including cities in Serbia and Spain in Europe. Foodstuffs were grouped into the following categories: cereals; pulses and starchy roots; tree-nuts, oil crops, and vegetable oils; vegetables and fruits; meat and meat products; milk, animal fats, dairy products, and eggs; and other such as candies and coffee.	Grains; fruits and vegetables; fats/other; meat; dairy	Serbia: Cereals (n = 4); pulses and starchy roots (n = 1); tree-nuts, oil crops and vegetable oils (n = 3); vegetables and fruits (n = 6); meat and meat products (n = 3); milk, animal fats, and dairy products (n = 7); and coffee (n = 1): DF 0% Spain: Cereals (n = 5); pulses and starchy roots (n = 2); tree-nuts, oil crops and vegetable oils (n = 10); vegetables and fruits (n = 9); meat and meat products (n = 6); milk, animal fats, dairy products, and eggs (n = 22); and other such as candies or coffee (n = 2): DF 0%

Study	Location and Source	Food Types	Results
			(Cereals: MLOD, MLOQ = 0.100, 0.333 ng/g; Juice MLOD, MLOQ = 0.157, 0.522 ng/g; Milk: MLOD, MLOQ = 0.118, 0.393 ng/g; Olive oil: MLOD, MLOQ = 0.051, 0.161 ng/g; Meat: MLOD, MLOQ = 0.121, 0.365 ng/g) *Artichoke was reported as one of the five cereal samples in Spain – unclear if this was a typo/error
Eschauzier et al. (2013)	The Netherlands (Amsterdam) Brewed coffee samples (n = 12) from different coffee machines were collected from all over the city. Coffee beans from four of these locations were collected to manually brew coffee. Post-mixed cola was collected (n = 4) together with corresponding tap water and an additional three cola samples from different parts of town. Sampling was conducted between February and April 2011 at various locations (cafés, universities, and supermarkets).	Fats/other	Post-mixed cola: n = 6, DF NR, mean = <0.63 ng/L Brewed coffee from coffee machines: n = 12, analyte peak areas could not be quantified due to strong matrix effects Manually brewed coffee: n = 4, analyte peak areas could not be quantified due to strong matrix effects (LOQ = 0.63 ng/L for cola; 0.95 ng/L for coffee)
Herzke et al. (2013)	Belgium, Czech Republic, Italy, Norway The Czech Republic, Belgium, Norway, and Italy were selected to represent eastern, western, northern, and southern Europe. Sampling took place between spring 2010 and 2011 as part of the PERFOOD study. Individual items were randomly selected in three national retail stores covering different brands or countries of origin. Of each item, three to ten single samples were combined to create one pooled sample per country. The following items were sampled: Root vegetables: carrots Bulb vegetables: onions Fruiting vegetables: tomatoes, courgettes, cucumbers, aubergine, peppers Brassica vegetables: cauliflower, cabbage, broccoli	Vegetables	**Belgium: n = 21, DF NR, mean = 0.00032 ng/g fw** Czech Republic: n = 16, DF 0% Italy: n = 15, DF 0% Norway: n = 17, DF 0% (MQL = 0.002–0.050 ng/g) *n represents number of composite samples *Values below the MQL were substituted with the MQL value

Study	Location and Source	Food Types	Results
	Leaf vegetables: lettuce and other salads, spinaches, chicory, pre-packed lettuce mix, pre-packed and minced frozen spinach Stem vegetables: asparagus, celery, fennel, cultivated mushrooms Starchy root tubers: potatoes, prepacked ready-to-cook pommes frites Legumes, beans, dried: peas, beans		
Hlouskova et al. (2013)	Belgium, Czech Republic, Italy, Norway Food products were randomly purchased in several nationwide supermarkets in four European regions during summer 2010. Within the sampling campaign, the collection of at least one food item per subcategory (meat, fish, hen eggs, milk and dairy products, and butter) in all four countries was acquired. Food items within each subcategory included the following: Meat: beef, canned pork meat, poultry, pork, pig/bovine liver, rabbit, and/or sheep/lamb Fish: farmed freshwater fish, farmed marine fish, and/or seafood) Hen eggs Milk and dairy products: ultra-high temperature whole cow milk, ultra-high temperature skimmed cow milk, cheese (yellow, Gouda/Edamer, etc.), and butter Samples were pooled within a respective food category but not across food groups.	Pooled milk/dairy products, meat, fish, hen eggs	**n = 50, DF 7%, mean, median (range) = 0.0335, 0.0264 (0.00485–0.0763) ng/g** (MQL = 0.002 ng/g for fish and seafood, meat, hen eggs, and cheese; 0.001 ng/mL for milk, and 0.006 ng/g for butter) *n represents number of pooled samples *Results not reported for individual food groups
Domingo et al. (2012)	Spain (Catalonia) Foods purchased from 4 shops/stores of each of the 12 representative cities of Catalonia (Barcelona, l'Hospitalet de Llobregat, Vilanova I la Geltrú, Mataŕr, Sabadell, Terrassa, Girona, Tarragona, Reus, Tortosa, Lleida and Manresa) in September 2011. Shops/stores included local markets, small stores,	Meat; fruits and vegetables; dairy; grains; fats/other	**Meat and meat products: n = 2, DF NR, 0.0032 ng/g fw** **Vegetables: n = 2, DF NR, 0.0045 ng/g fw** Tubers: n = 2, DF NR, mean = <0.0019 ng/g fw Fruits: n = 2, DF NR, mean = <0.0019 ng/g fw Eggs: n = 2, DF NR, mean = <0.002 ng/g fw Milk: n = 2, DF NR, mean = <0.0026 ng/g fw

Study	Location and Source	Food Types	Results
	supermarkets, and big grocery stores. Analyzed samples included 40 items: Meat and meat products: veal steak, loin of pork, chicken breast, steak of lamb, boiled ham, "Frankfurt"-type sausage, cured ham Vegetables and tubers: lettuce, tomato, potato, carrot Fresh fruits: apple, orange, banana Milk and dairy products: whole and semi-skimmed milk, yogurt, cheese I – low fat, cheese II – medium fat, cheese III – extra fat Cereals: French bread, pasta Pulses: lentils Industrial bakery: cookies Eggs: hen eggs Oils and fats: olive oil For each food item, two composite samples were prepared for analysis, where each composite sample consisted of 24 individual units. Only edible parts of each food item were included in the composites.		Dairy products: n = 2, DF NR, mean = <0.0011 ng/g fw Cereals: n = 2, DF NR, mean = <0.0006 ng/g fw Pulses: n = 2, DF NR, mean = <0.0013 ng/g fw Oils: n = 2, DF NR, mean = <0.0007 ng/g fw Industrial bakery: n = 2, DF NR, mean = <0.00056 ng/g fw (LOD not reported) *n represents number of composite samples
Vestergren et al. (2012)	Sweden (Malmoe, Gothenburg, Uppsala, Sundsvall) Purchasing locations of the two largest retail chains in Sweden were selected in each of four major Swedish cities. All purchases were made in spring/summer of 1999, 2005, and 2010. In 2010, the study was limited to the largest retail chains in Uppsala located in close vicinity to Stockholm. An equal amount of each food group from each of the four cities was combined into one sample pool to provide a representative sample for the Swedish urban population.	Dairy; meat; grains; fruits and vegetables; fats/other	**Meat products:** **1999: n = 1, point = 0.0085 ng/g** **2005: n = 1, point = 0.0051 ng/g** **2010: n = 1, point = 0.0045 ng/g** (MDL = 0.0019 ng/g; MQL = 0.0058 ng/g) **Egg:** **1999: n = 1, point = 0.039 ng/g** 2005: n = 1, point = <MDL **2010: n = 1, point = 0.0025 ng/g**

Study	Location and Source	Food Types	Results
			(MDL = 0.0019 ng/g; MQL = 0.0058 ng/g) Dairy products: 1999: n = 1, point = <MDL 2005: n = 1, point = <MDL 2010: n = 1, point = 0.0010 ng/g (estimated) (MDL = 0.0027 ng/g; MQL = 0.0080 ng/g) Fats: 1999: n = 1, point = <MDL 2005: n = 1, point = 0.0009 ng/g (estimated) 2010: n = 1, point = <MDL (MDL = 0.0023 ng/g; MQL = 0.0070 ng/g) Pastries: 1999: n = 1, point = 0.0012 ng/g (estimated) 2005: n = 1, point = 0.0013 ng/g (estimated) 2010: n = 1, point = <MDL (MDL = 0.0010 ng/g; MQL = 0.0030 ng/g) Vegetables: 1999: n = 1, point = 0.0012 ng/g (estimated) 2005: n = 1, point = 0.0010 ng/g (estimated) 2010: n = 1, point = 0.0012 ng/g (estimated) (MDL = 0.0010 ng/g; MQL = 0.0029 ng/g) Sugar and sweets: 1999: n = 1, point = <MDL 2005: n = 1, point = 0.0013 ng/g (estimated) 2010: n = 1, point = 0.0015 ng/g (estimated)

Study	Location and Source	Food Types	Results
			(MDL = 0.0010 ng/g; MQL = 0.0030 ng/g) Soft drinks, lemonade: 1999: n = 1, point = 0.0007 ng/g (estimated) 2005: n = 1, point = <MDL 2010: n = 1, point = <MDL (MDL = 0.0005 ng/g; MQL = 0.0015 ng/g) Cereal products: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0020 ng/g; MQL = 0.0059 ng/g) Fruit: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0010 ng/g; MQL = 0.0030 ng/g) Potatoes: 1999, 2005, 2010: n = 1 each year, point = <MDL for each year (MDL = 0.0010 ng/g; MQL = 0.0030 ng/g) *n represents number of composite samples *Paper reported that estimated concentrations are between MDL and MQL; however, there are some instances when the estimated concentrations are <MDL

Study	Location and Source	Food Types	Results
Noorlander et al. (2011)	The Netherlands Food products randomly purchased from several Dutch retail stores with nationwide coverage in November 2009. Food samples were ground, homogenized, and pooled for analysis. Food items within each subcategory included the following: Flour: whole wheat flour, flour Pork: sausage, slice of bacon, pork chop, bacon, minced meat rolled in bacon Eggs: chicken eggs Bakery products: cake, almond paste cake, biscuits, brown spiced biscuit, pie Vegetables/fruit: apple, orange, grape, banana, onion, carrot, beet, chicory or leek, tomato, cucumber, paprika, mushroom, cauliflower, broccoli, white cabbage, red cabbage, brussel sprout, spinach, endive, lettuce, French beans Cheese: gouda cheese, edammer cheese, cheese (>48% fat, less salt), cheese (>30% fat), brie cheese Beef: ground beef, beefburger, stewing steak, braising steak, minced steak Chicken/poultry: chicken leg, quarter chicken, chicken filet, chicken burger, collared chicken Butter: butter salt-free, salted, low-fat Milk: half cream milk Vegetable oil: margarine (solid/fluid), low-fat margarine, frying fat (vegetable), frying oil (vegetable), sunflower oil Industrial oil: low-fat margarine, frying fat (industrial), frying oil (industrial)	Meat; dairy; fruits and vegetables; grains; fats/other	**Butter: n = 1, point = 0.016 ng/g** **Chicken/poultry: n = 1, point = 0.003 ng/g** **Bakery products: n = 1, point = 0.006 ng/g** **Flour: point = n = 1, 0.018 ng/g** **Industrial oil: n = 1, point = 0.007 ng/g** Cheese: n = 1, point = <0.025 ng/g Milk: n = 1, point = <0.002 ng/g Eggs: n = 1, point = <0.006 ng/g Pork: n = 1, point = <0.005 ng/g Beef: n = 1, point = <0.004 ng/g Vegetables/fruit: n = 1, point = <0.012 ng/g Vegetable oil: n = 1, point = <0.002 ng/g (LOD not reported) *n represents number of composite samples

Study	Location and Source	Food Types	Results
Jogsten et al. (2009)	Spain (Catalonia) Food samples purchased from local markets, large supermarkets, and grocery stores from two different areas of Tarragona Province, Catalonia in January and February 2008. The cities of Tarragona and Reus were sampled in the northern area and L'Ametlla de Mar and Tortosa in the southern area. For each food item, two composite samples were analyzed (one composite for the northern area and one for the southern area). Each composite was formed of a minimum of six individual sub-samples of the same product.	Fruits and vegetables; meat; fats/other	Raw veal: n = 2, DF NR, mean = <0.003 ng/g Grilled veal: n = 2, DF NR, mean = <0.001 ng/g Fried veal: n = 2, DF NR, mean = <0.003 ng/g Raw pork: n = 2, DF NR, mean = <0.001 ng/g Grilled pork: n = 2, DF NR, mean = <0.001 ng/g Fried pork: n = 2, DF NR, mean = <0.002 ng/g Raw chicken: n = 2, DF NR, mean = <0.001 ng/g Grilled chicken: n = 2, DF NR, mean = <0.001 ng/g Fried chicken: n = 2, DF NR, mean = <0.001 ng/g Fried chicken nuggets (packaged): n = 2, DF NR, mean = <0.005 ng/g Black pudding: n = 2, DF NR, mean = <0.008 ng/g Lamb liver: n = 2, DF NR, mean = <0.250 ng/g Pate of pork liver (packaged): n = 2, DF NR, mean = <0.088 ng/g Foie gras of duck (packaged): n = 2, DF NR, mean = <0.043 ng/g "Frankfurt" sausages (packaged): n = 2, DF NR, mean = <0.006 ng/g Lettuce: n = 2, DF NR, mean = <0.001 ng/g Lettuce (packaged): n = 2, DF NR, mean = <0.003 ng/g Common salt (packaged): n = 2, DF NR, mean = <0.010 ng/g (LOD = 0.001 ng/g) *n represents number of composite samples *Values of ND were replaced with ½×LOD
Ericson et al. (2008a)	Spain Food samples purchased from local markets, large supermarkets, and grocery stores within Tarragona County in July 2006. Food samples were randomly purchased with origin source not specified. Each of the food samples were duplicated and combined to analyze a composite sample. Composite samples included the following:	Meat; dairy; fruits and vegetables; grains; fats/other	Vegetables (n = 2), pulses (n = 2), cereals (n = 2), pork (n = 2), chicken (n = 2), veal (n = 2), lamb (n = 2), eggs (n = 2), dairy products (n = 2), whole milk (n = 2), semi-skimmed milk (n = 2), fruits (n = 2), margarine (n = 2), oil (n = 2): DF 0% (LOD = 0.001–0.65 ng/g fw) *n represents number of composite samples

Study	Location and Source	Food Types	Results
	Vegetables: lettuce, tomato, green bean, spinach Pulses: lentils, beans, chickpeas Cereals: rice, spaghetti, bread Pork: sausage, hot dogs, steak, hamburger, ham Chicken: breast, thighs, sausage Veal: steak, hamburger Lamb: steak Dairy products: three different kinds of cheese, yogurt, "petit-Swiss" creamy yogurt, cream caramel, custard Fruits: apple, orange, pear, banana Oil: olive oil, sunflower oil, corn oil Fats: margarine Eggs		
Papadopoulou et al. (2017)	Norway Participants of the A-TEAM project collected a duplicate portion of all consumed foods and drinks, prepared as for consumption, over two consecutive weekdays. Only the samples collected in the first day were analyzed. Sampling year not reported.	Solid foods: cereals and cereal products, dairy products (not milk), fish and seafood, meat and meat products, sugar and sugar products, fats and oils, vegetables and nuts, fruits, salty snacks, eggs, potatoes; liquid foods: coffee, tea and cocoa, milk, water, alcoholic beverages, soft drinks	**Solid foods:** **n = 61, DF 66%, median (range) = 0.00088 (0–0.1) ng/g** (LOQ = 0.00011 ng/g) **Liquid foods:** **n = 61, DF 8%, median (range) = 0 (0–0.002) ng/g** (LOQ = 0.00002 ng/g) *Concentrations <LOQ were replaced by 0
Dellatte et al. (2013)	Italy (Genoa, Brescia, Ferrara, Perugia, Portici) Ready-to-eat meals were collected at nursery and primary school canteens as they were delivered to children aged 3–10 years during the spring of 2011. One canteen was selected from each city, except for Genoa which was represented by two canteens because in one, the internal school regulation	Food composite meals	n = 6, DF 0% (LOQ = 0.006 ng/g) *n represents number of composite samples

Study	Location and Source	Food Types	Results
	forbade the use of anti-stick cookware. For each canteen, lunch meals related to five school days (from Monday to Friday) were weighed, pooled, and homogenized. Beverages were not included in the composites.		
Fromme et al. (2007)	Germany (Bavaria) Thirty-one participants provided 24-hour duplicate diet samples over seven consecutive days, including one weekend, in April–October 2005. One participant only provided samples over four consecutive days. All diet samples were a normal mixed diet; no participants were on a special diet or vegetarian.	Duplicate diet samples	n = 214, DF[a] 3%, range = 0.05–3.03 ng/g fw (LOD = 0.1 ng/g fw) *Values <LOD were assigned ½ LOD
Ghelli et al. (2019)	Italy Egg samples were collected from commercial laying hen farms in 2017. Sampling was based on geographical origin of the eggs and rearing system (e.g., organic, aviary system, battery cage and barn). A total of 132 eggs were collected and eggs were boiled. Four pools (containing three homogenized yolks) were created for each of the following groups (geographical origin, rearing system), for a total of 44 samples analyzed: Group A: Pavia, barn Group B: Verona, organic Group C: Forlì-Cesena, battery cage Group D: Bologna, barn Group E: Forlì-Cesena, battery cage Group F: Ravenna, aviary system Group G: Ravenna, aviary system Group H: Bologna, organic Group I: Romagna, battery cage Group L: Romagna, organic Group M: Romagna, barn	Fats/other	Group A: n = 4, Dfa 50%, range = ND–0.4 ng/g Group B: n = 4, Dfa 25%, range = ND–traces Group C: n = 4, DF 0% Group D: n = 4, DF 0% Group E: n = 4, DF 0% Group F: n = 4, DF 0% Group G: n = 4, DF 0% Group H: n = 4, DF 0% Group I: n = 4, Dfa 25%, range = ND–traces Group L: n = 4, DF 0% Group M: n = 4, DF 0% (LOD = 0.1 ng/g for all PFAS; LOQ = 0.25 ng/g for all PFAS) *Traces defined as value between LOD and LOQ
Johansson et al. (2014)	Sweden Eggs from 20 to 25 producers were collected each year from 1999 to 2010 within the Swedish National Food Agency's official food control program. Each	Dairy; fats/other	**Hen's eggs:**

Study	Location and Source	Food Types	Results
	sample consisted of a pool of 10–12 eggs from one producer. The pooled samples comprised eggs from both conventional and organic production. Information on the number of organic eggs sampled was not available. Fresh milk was sampled from the tanks of milk transport vehicles between 1999 and 2009 as part of the food control program. The tanks generally contained milk from ten dairy farms. In 2010, milk samples were taken from the milk storage tanks on individual dairy farms. Between 10–25 milk samples were collected each year. The milk samples were extracted in two different batches.		**Total: n = 36, DF[a] 61%, range = <0.010–0.128 ng/g fw** **1999: n = 3, DF[a] 100%, mean[a] (range) = 0.038 (0.019–0.072) ng/g fw** **2000: n = 3, DF[a] 100%, mean[a] (range) = 0.038 (0.016–0.069) ng/g fw** **2001: n = 3, DF[a] 100%, mean[a] (range) = 0.033 (0.015–0.051) ng/g fw** **2002: n = 3, DF[a] 67%, range = <0.010–0.128 ng/g fw** **2003: n = 3, DF[a] 67%, range = <0.010–0.054 ng/g fw** **2004: n = 3, DF[a] 33%, range = <0.010–0.011 ng/g fw** **2005: n = 3, DF[a] 100%, mean[a] (range) = 0.015 (0.011–0.018) ng/g fw** 2006: n = 3, DF 0% **2007: n = 3, DF[a] 33%, range = <0.010–0.020 ng/g fw** **2008: n = 3, DF[a] 33%, range = <0.010–0.011 ng/g fw** **2009: n = 3, DF[a] 33%, range = <0.010–0.012 ng/g fw** **2010: n = 3, DF[a] 67%, range = <0.010–0.020 ng/g fw** (MDL = 0.010 ng/g fw; MQL = 0.033 ng/g fw) **Cow's milk (1st batch):** **Total: n = 18, DF[a] 22%, range = <0.0010–0.0011 ng/g fw** 1999: n = 1, DF 0%

Study	Location and Source	Food Types	Results
			2000: n = 2, DF[a] 50%, range = <0.0010–0.0010 ng/g fw 2001: n = 2, DF 0% **2002: n = 2, DF[a] 50%, range = <0.0010–0.0011 ng/g fw** 2003: n = 1, DF 0% 2004: n = 2, DF 0% 2005: n = 1, DF 0% **2006: n = 1, point = 0.0010 ng/g fw** 2007: n = 1, DF 0% **2008: n = 2, DF[a] 50%, range = <0.0010–0.0011 ng/g fw** 2009: n = 1, DF 0% 2010: n = 2 DF 0% (MDL = 0.0010 ng/g fw; MQL = 0.0033 ng/g fw) **Cow's milk (2nd batch):** **Total: n = 18, DF 0%** (MDL = 0.0023 ng/g fw ; MQL = 0.0063 ng/g fw)
Eriksson et al. (2013)	Denmark (Faroe Islands) Locally produced food items sampled in 2011–2012. Packaged dairy products were supplied by Faroe Islands, Meginfelag Búnaðarmanna – dairy products included samples of milk, low fat (0.5%), semi-skimmed (1.5%), yoghurt with banana and pear (3.4% fat), low fat (0.9%) plain yoghurt, and créme fraiche (18% fat). Yoghurt with banana and pear was sampled from two production batches, and the low fat plain yoghurt and créme fraiche was sampled from one production batch. Potatoes were sampled from two different farms.	Dairy; fruits and vegetables	Milk (n = 6), yogurt (n = 3), crème fraiche (n = 1), potatoes (n = 2): DF 0% (LOD = 0.0058 ng/L for milk; LOD = 0.0017 ng/g for dairy; LOD = 0.0016 ng/g for potato)
Vestergren et al. (2013)	Sweden (Kårsta)	Meat; dairy	Dairy farm cow's milk: n = 6, DF 0%

Study	Location and Source	Food Types	Results
	Study was conducted at a dairy cattle farm that was selected to represent a background contaminated agricultural area with no known point sources of PFAS in the proximity. The farm had no history of sewage sludge application to the pasture land. Milk samples were collected between November 2010 and April 2011 from a milk tank, where milk from the entire farm is stored after milking. Muscle, liver, and whole blood samples were obtained from five individual cows from the slaughterhouse on two different occasions (April and June 2011).		Cow liver: n = 5, DF 0% Cow blood: n = 5, DF 0% Cow muscle: n = 5, DF 0% (MDL not reported)
Falandysz et al. (2006)	Poland Eider duck samples were collected from the Gulf of Gdañsk in the Baltic Sea (south coast of Poland) in February 2003.	Meat	**n = 16, DF NR, mean, median (range) = 1.1, 1.1 (0.4–2.9) ng/mL** (LOD not reported) *Values reported for animal whole blood samples
Lankova et al. (2013)	Czech Republic Breastmilk samples were obtained from 50 women living in the Olomouc region from April to August 2010. The age of participating mothers ranged from 20 to 43 years. Six types of infant formula from the Czech retail market were also examined: one powdered formula for infants, two formulas for toddlers, one formula for babies with lactose intolerance, one formula for premature babies, and one soya-based formula for babies with non-milk diets. Sampling year not provided.	Fats/other; breastmilk	**Breastmilk:** **n = 50, DF (frequency of quantification) 8%, range = <0.006–0.022 ng/mL** (LOQ = 0.006 ng/mL) Infant formula: n = 6, DF (frequency of quantification) 0% (LOQ = 0.005 ng/g)
Abdallah et al. (2020)	Ireland (Dublin) Breastmilk samples obtained from mothers recruited from breastfeeding clinics at two Irish maternity hospitals. Mothers provided samples between 3 and 8 weeks postpartum. Mothers were up to 41 years of age, primiparas, in good health, and exclusively feeding one infant. Sampling year not reported.	Breastmilk	**n = 16, DF 31%, mean, median (range) = <0.04, <0.04 (<0.04–0.087 ng/mL)** (LOQ = 0.04 ng/mL) *Values <LOQ assumed to equal DF × LOQ
Nyberg et al. (2018)	Sweden (Gothenburg, Stockholm) Breastmilk samples were collected between two weeks and three months after delivery from healthy	Breastmilk	**L-PFHxS:**

Study	Location and Source	Food Types	Results
	native Swedish mothers, who were predominately non-smokers and primiparous. There were a total of 20 pooled samples analyzed from Stockholm (1972–2016), containing 9–116 individual samples per pool, and 11 pooled samples from Gothenburg (2007–2015), containing 5–11 individuals per pool. In addition, samples collected in 2012 (16 from Gothenburg and 20 from Stockholm) and in 2016 (10 from Stockholm) were analyzed individually.		**Stockholm (pooled): n = 20, DF[a] 90%, range = <0.0008–0.021 ng/mL** **Gothenburg (pooled): n = 11, DF[a] 91%, range = <0.0008–0.012 ng/mL** **Stockholm (2012, individual): n = 20, DF[a] 95%, range = <0.0008–0.025 ng/mL** **Gothenburg (2012, individual): n = 16, DF[a] 94%, range = <0.0008–0.014 ng/mL** **Stockholm (2016, individual): n = 10, DF[a] 80%, range = <0.0008–0.013 ng/mL** **Br-PFHxS:** **Stockholm (pooled): n = 20, DF[a] 25%, range = <0.0008–0.0029 ng/mL** **Gothenburg (pooled): n = 11, DF[a] 18%, range = <0.0008–0.004 ng/mL** Stockholm (2012, individual): n = 20, DF 0% **Gothenburg (2012, individual): n = 16, DF[a] 6%, range = <0.0008–0.0012 ng/mL** **Stockholm (2016, individual): n = 10, DF[a] 30%, range = <0.0008–0.004 ng/mL** (MDL = 0.0008 ng/mL)
Cariou et al. (2015)	France (Toulouse) Breastmilk samples obtained from female volunteers hospitalized between June 2010 and January 2013 for planned caesarean delivery. Samples were collected between the fourth and fifth day after delivery.	Breastmilk	**n = 61, DF 15%, mean, median (range) = 0.026, <LOD (<LOD–0.217) ng/mL** (LOD = 0.01–0.03 ng/mL; LOQ = 0.03 ng/mL) * Individual LOD values and semi-quantified values (when below LOQ) taken into account for mean calculation
Antignac et al. (2013)	France (Seine-Saint Denis, Ardèche, Isère, Loire, Savoie counties) Breastmilk samples collected from mothers participating in the ELFE pilot study. Sampling year not reported, though all mothers gave birth in October 2007. Mothers were contacted by phone one month after leaving the maternity and provided	Breastmilk	**n = 48, DF 100%, mean, median (range) = 0.049, 0.050 (0.040–0.066) ng/mL** (LOD not reported)

Study	Location and Source	Food Types	Results
	with instructions on breastmilk collection. Milk samples could be collected during several lactation sessions. On average, 15 aliquot samples of 10 mL were collected for each participant and pooled into one sample for analysis.		
Croes et al. (2012)	Belgium (Flanders) Breastfeeding mothers were recruited from 9 maternities in 24 rural communities in East and West Flanders and Flemish Brabant in May 2009 – June 2010. Breastmilk samples were collected between two and eight weeks after delivery and a subset was analyzed for perfluorinated compounds.	Breastmilk	**n = 40, DF 20%, median (10^{th}–90^{th} percentile) = <LOQ (<LOQ–0.02) ng/mL** (LOQ = 0.01 ng/mL) *For all calculations, values <LOQ were treated as ½ LOQ
Sundström et al. (2011)	Sweden (Stockholm) Breastmilk samples were collected from healthy native Swedish mothers by the Mothers' Milk Center between the second and twelfth week after delivery. The majority of mothers (76%) were nursing their first infant. Samples were collected between 1972–2008 and pooled for each year.	Breastmilk	Overall: n = 684, DF NR, range = <0.005–0.028 ng/mL 1972: n = 75, DF NR, mean = <0.005 ng/mL 1976: n = 78, DF NR, mean = <0.005 ng/mL 1980: n = 116, DF NR, mean = 0.006 ng/mL 1984/85: n = 102, DF NR, mean = 0.006 ng/mL 1988: n = 20, DF NR, mean = 0.016 ng/mL 1990: n = 20, DF NR, mean = 0.010 ng/mL 1992: n = 20, DF NR, mean = 0.011 ng/mL 1994: n = 20, DF NR, mean = 0.015 ng/mL 1995: n = 20, DF NR, mean = 0.028 ng/mL 1996: n = 20, DF NR, mean = 0.016 ng/mL 1997: n = 20, DF NR, mean = 0.016 ng/mL 1998: n = 20, DF NR, mean = 0.028 ng/mL 1999: n = 20, DF NR, mean = 0.023 ng/mL 2000: n = 20, DF NR, mean = 0.024 ng/mL 2001: n = 20, DF NR, mean = 0.017 ng/mL 2002: n = 20, DF NR, mean = 0.027 ng/mL 2003: n = 15, DF NR, mean = 0.025 ng/mL 2004: n = 20, DF NR, mean = 0.017 ng/mL 2007: n = 20, DF NR, mean = 0.017 ng/mL 2008: n = 18, DF NR, mean = 0.014 ng/mL (LOQ = 0.005 ng/mL)

Study	Location and Source	Food Types	Results
			*n represents number of pooled samples
Kärrman et al. (2010)	Spain (Catalonia) Breastmilk samples were collected from healthy primiparae mothers aged 30–39 years who lived in Tarragona County for at least the last five years. Babies were aged 41–60 days when milk samples were collected in 2007.	Breastmilk	**n = 10, DF 100%, mean, median (range) = 0.04, 0.04 (0.02–0.11) ng/mL** (LOQ = 0.01 ng/mL)
Kärrman et al. (2007)	Sweden (Uppsala, Göteborg, Lund, Lycksele) Individual breastmilk samples from 12 women in Uppsala, Sweden were collected in 2004. Composite samples were created from breastmilk samples collected from 25–90 women each year between 1996 and 2004 and pooled into an annual composite sample. Donors originated from four regions in Sweden (Uppsala 1996 -2000, 2002; Göteborg 2001; Lund 2003; Lycksele 2003-2004). All samples were collected from primiparous women (19–41 years old) during the third week after delivery.	Breastmilk	**Individual samples:** **2004: n = 12, DF[a] 100%, mean, median (range) = 0.085, 0.070 (0.031–0.172) ng/mL** **Composite samples:** **1996: n = 1, point = 0.037 ng/mL** **1997: n = 1, point = 0.030 ng/mL** **1998: n = 1, point = 0.040 ng/mL** **1999: n = 1, point = 0.044 ng/mL** **2000: n = 1, point = 0.028 ng/mL** **2001: n = 1, point = 0.028 ng/mL** **2002: n = 1, point = 0.051 ng/mL** **2003: n = 1, point = 0.025 ng/mL** **2003–2004: n = 1, point = 0.016 ng/mL** *n represents number of composite samples (DL = 0.01 ng/mL)
Beser et al. (2019)	Spain (Valencian region) Breastmilk samples were collected from 14 Spanish women (aged 30–39 years) living in the Valencian region and recruited by the perinatology group of the Health Research Institute La Fe in Valencia. Milk samples were collected at different stages after birth during 2015.	Breastmilk	n = 20, DF[a] 30% *Six samples were >MDL but <MQL – these values were not reported (MDL = 0.004 ng/mL; LOQ = 0.133 ng/mL)
Pratt et al. (2013)	Ireland	Breastmilk	n = 11, DF 0% (LOD = 0.5–5 ng/mL for all PFAS)

Study	Location and Source	Food Types	Results
	Pooled breastmilk samples were collected from 109 first-time mothers at four centers across Ireland. Sampling year not reported.		*n represents number of pooled samples

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; GCA = groundwater contamination area; LOD = limit of detection; LOQ = limit of quantitation; MAMA = Methods Advancement for Milk Analysis; MDL = method detection limit; ND = not detected; NR = not reported; RL = reporting limit; ww = wet weight; WWTP = wastewater treatment plant.

Bold indicates detected levels of PFHxS in food.

[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

D.3.2. Food Contact Materials

No studies were identified that evaluated the occurrence of PFHxS in food packaging or food contact materials (FCMs) purchased in the United States. In an analysis performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology in Prague, Czech Republic, PFHxS was not detected in 42 samples of disposable food packaging and tableware purchased from six different European countries between May and December 2020 (LOQ = 1.7 mg/kg) (Straková et al., 2021). The five additional peer-reviewed European studies identified are summarized below and in Table D-6 (Vavrouš et al., 2016; Kotthoff et al., 2015; Surma et al., 2015; Vestergren et al., 2015; Zafeiraki et al., 2014). Three of these studies reported no detection of PFHxS in FCMs while two reported detectable levels of PFHxS in FCMs. Of these two studies, PFHxS was detected in 6% of paper-based FCM samples purchased recently in Germany (at the time of the study: 2010) and also was detected in samples purchased before 2010, but in both cases the median concentration was below the LOQ of 0.5 ng/g. For FCMs purchased in Poland, PFHxS was detected in one brand of cellulose wrapping paper (0.29 pg/cm^2) but was below the LOD (0.01 pg/cm^2) or below the LOQ (0.03 pg/cm^2) in other cellulose and polyether sulfone FCMs. Additional research is needed to evaluate PFHxS in FCMs purchased in U.S. and Canada and for FCMs with different countries of origin.

Two studies reported PFHxS in food contact materials (Kotthoff et al., 2015; Surma et al., 2015). Kotthoff et al. (2015) analyzed for PFSA and PFCA compounds in random samples of recent (purchased in 2010) individual paper-based FCMs (n = 33) from local retailers in Germany. Samples were purchased from local retailers or collected by co-workers of the institute in the first until third quarter of 2010. Baking paper purchased before 2010 (sample age ranging from a few years to decades) was collected from staff of the institutes and referred to as archived samples. PFHxS was detected in 6% of recent paper-based FCM samples with a median concentration below the LOQ (0.5 ng/g). PFHxS was also detected in archived FCM samples with a median concentration below the LOQ. In Surma et al. (2015), the authors measured levels of PFHxS in three different brands of FCMs that included wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3); sampling year was not reported. Items were obtained from typical, commercially available food contact products in Poland. Roasting bags were made of polyether sulfone; the remaining items were made of cellulose. PFHxS was detected in one brand of wrapping paper (brand B) at 0.29 pg/cm^2, but PFHxS was below the LOD (0.01 pg/cm^2) or below the LOQ (0.03 pg/cm^2) in all other FCMs. The authors reported that the highest content of perfluorinated compounds were reported for B brand FCM. They also reported that FCMs based on cellulose contained more PFCAs than PFASs; on the other hand, FCMs based on polyether sulfone contained more PFASs than PFCAs.

The remaining three studies did not detect PFHxS in FCMs (Vavrouš et al., 2016; Vestergren et al., 2015; Zafeiraki et al., 2014). Vavrouš et al. (2016) analyzed 15 samples of paper FCM (11 with direct food contact and 4 with indirect food contact) acquired from a market in the Czech Republic, including paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220°C (n = 3), and paper filters for coffee preparation (n = 3). PFHxS was below the LOQ (0.0030 mg/kg) in all samples. In Vestergren et al. (2015), the authors analyzed a random sample of FCMs collected in November 2012, including a baking mold (n = 1), baking cover (n = 1),

paper cup (n = 1), and paper plates (n = 2) that were purchased from major retail stores in Norway but were imported from China. PFHxS was below the MDL (0.01 $\mu g/m^2$) in all samples. Finally, Zafeiraki et al. (2014) analyzed 42 samples of FCMs made of paper, paperboard, or aluminum foil randomly obtained from retailers. All products except for microwave popcorn and rice bags were manufactured in Greece. Sampled packaging materials included unused items and used items (i.e., contained food products). Beverage and ice cream cups, wrappers, and paper boxes were collected in Athens from October to December 2012 from popular Greek fast food chain restaurants, coffee shops, and multiplex cinemas. Other FCMs (muffin cups, baking papers, and microwave popcorn and rice bags) were purchased from large supermarkets. PFHxS was below the LOD (0.18 ng/g) in all samples.

Table D-6. Studies Reporting PFHxS Occurrence in Food Contact Materials

Study	Location	Site Details	Results
Europe			
Kotthoff et al. (2015)	Germany (Schmallenberg)	Thirty-three random samples of recent individual paper-based FCMs collected in the first until the third quarter of 2010 in Germany. Individual samples were bought from local retailers or collected by coworkers of the involved institutes. Sampled products spanned all quality levels from entry level to cutting edge products. The age of the samples ranged from a few years to decades. Country of origin not reported. "Archived" older samples of FCMs (baking paper purchased before 2010) were collected from the staff of the institutes. The age of these samples ranged from a few years to decade. Country of origin not reported.	Recent samples: n = 33, DF 6%, median (range) = <LOQ (<LOQ–0.6) ng/g Archived samples: n = 3, DF NR, median (range) = <LOQ (<LOQ–0.6) ng/g (LOQ = 0.5 ng/g) *Concentrations <LOQ were considered as zero *For recent samples, Table 1 reports n = 33, the Results text reports n = 36 according to the actual sampling plan, and Table 13 in Supplemental Appendix 3 reports n = 12
Surma et al. (2015)	Poland	Three different brands of FCMs (A, B, C), including wrapping papers (n = 3), breakfast bags (n = 3), baking papers (n = 3), and roasting bags (n = 3), were obtained from typical, commercially available food contact products. Sampling year and country of origin for products not reported.	Wrapping paper: Brand A: n = 1, point = ND Brand B: n = 1, point = 0.29 pg/cm^2 Brand C: n = 1, point = ND Breakfast bag: Brand A: n = 1, point = <LOQ Brand B: n = 1, point = ND Brand C: n = 1, point = ND Baking paper: Brand A: n = 1, point = ND Brand B: n = 1, point = <LOQ Brand C: n = 1, point = <LOQ Roasting bag: Brand A: n = 1, point = ND Brand B: n = 1, point = ND Brand C: n = 1, point = ND (LOD = 0.01 pg/cm^2; LOQ = 0.03 pg/cm^2)
Vavrouš et al. (2016)	Czech Republic	Real samples of paper FCM (11 with direct food contact and 4 with indirect food contact) were acquired from a market. Samples included paper packages of wheat flour (n = 2), paper bags for bakery products (n = 2), sheets of paper for food packaging in food stores (n = 2), cardboard boxes for packaging of various foodstuffs (n = 3), coated bakery release papers for oven baking at temperatures up to 220°C (n	n = 15, DF 0% (LOQ = 0.0030 mg/kg)

Study	Location	Site Details	Results
		= 3), and paper filters for coffee preparation (n = 3). Sampling year and country of origin for products not reported.	
Vestergren et al. (2015)	Norway (Tromsø, Trondheim)	Five samples of FCMs (one baking mold, two paper plates, one baking cover, and one paper cup) were purchased from major retail stores in November 2012. Sampling campaign designed to evaluate consumer products in product categories that were previously found to contain PFAS residuals and that were representative of products imported from China in large quantities. Individual products randomly selected without prior knowledge of surface treatment with PFAS. Year of manufacture not reported.	n = 5, DF[a] 0% (MDL = 0.010 µg/m^2)
Zafeiraki et al. (2014)	Greece	Forty-two samples of FCMs made of paper, paperboard, or aluminum foil were obtained randomly from retailers. Their exact composition was not stated and there was no information about perfluorochemicals used in their manufacturing process or not. Beverage and ice cream cups, wrappers, and paper boxes were collected in Athens from October to December 2012 from popular Greek fast food chain restaurants, coffee shops, and multiplex cinemas. Other FCMs (muffin cups, baking papers, and microwave popcorn and rice bags) were purchased from large supermarkets. All products except for microwave popcorn and rice bags were manufactured in Greece. Sampled packaging materials included unused items and used items (i.e., contained food products). A microwave popcorn bag was also analyzed before and after cooking.	Beverage cups: n = 8, DF 0% Ice cream cup: n = 1, DF 0% Fast food paper boxes: n = 8, DF 0% Fast food wrappers: n = 6, DF 0% Paper materials for baking: n = 2, DF 0% Microwave bags: n = 3, DF 0% Before cooking: n = 1, point = <LOD After cooking: n = 1, point = <LOD Aluminum foil bags/wrappers: n = 14, DF 0% (LOD = 0.18 ng/g; LOQ = 0.54 ng/g)

D.3.3. Consumer Products

PFHxS has been used in laboratory applications and as a raw material or a precursor for the manufacture of PFAS/perfluoroalkyl sulfonate-based products, though production of PFHxS in the United States was phased out by its major manufacturer in 2002 (NCBI, 2022a; Sigma-Aldrich, 2014; Backe et al., 2013; Buck et al., 2011; OECD, 2011). PFHxS has also been used in firefighting foam and carpet treatment solutions, and it has been used as a stain and water repellant (NCBI, 2022a; Garcia and Harbison, 2015). PFHxS has been detected in aqueous film-forming foam, aftermarket carpet protection products, chipboards, leather, membranes for apparel, treated apparel, and photoprint ink and laser ink (NCBI, 2022a; Glüge et al., 2020; Norwegian Environment Agency, 2018; Bečanová et al., 2016; Kotthoff et al., 2015; Liu et al., 2014; Backe et al., 2013; Buck et al., 2011; Herzke et al., 2009).

Two U.S.-based studies were identified that analyzed PFHxS concentrations in a range of consumer products, including children's nap mats, household carpet/fabric-care liquids, and textiles (Zheng et al., 2020; Liu et al., 2014) (Table D-7). Few U.S. studies analyzed children's products, fabric treatments, treated fabrics, sealants, and similar products, and none of the U.S. studies reviewed sampled for PFAS in other household products and articles, such as cosmetics, cleaners, paints, upholstered furniture, etc. Of the U.S. studies, the majority of the consumer products evaluated are likely used by adults (e.g., floor waxes), can come into contact with both adults and children (e.g., treated upholstery), or the user was not specified (e.g., clothing).

Zheng et al. (2020) determined the occurrence of ionic and neutral PFAS in the childcare environment (dust and nap mats). Samples of children's nap mats were collected from seven Seattle childcare centers (n = 26; 20 polyurethane foam, 6 vinyl cover samples). PFHxS was detected in 73% of nap mat samples with a mean concentration of 0.32 ng/g. Half of the analyzed mats were purchased as new products and the other half were used. The authors reported that total PFAS levels in the new versus used mats were not significantly different. Total PFAS levels in mat foam versus mat covers were also similar. Based on these results, the authors suggested that indoor air was not the major source of PFAS in mats and that PFAS in mats could be associated with the manufacturing process.

Liu et al. (2014) analyzed the occurrence of PFAS in consumer products (including commercial carpet/fabric-care liquids, household carpet/fabric-care liquids, treated apparel, treated home textile and upholstery, treated floor waxes and stone-wood sealants, membranes for apparel, and thread-sealant tapes and pastes) purchased between March 2007 and September 2011 from local retailers and online stores in the United States. PFHxS was analyzed in a subset of these consumer products, originating from the United States, England, Dominican Republic, Vietnam, and China, and was detected in one out of two commercial carpet/fabric-care liquids samples at 194 ng/g, in two out of four household carpet/fabric-care liquids and foams samples at 88.8 ng/g and 155 ng/g, in one out of two treated children's apparel samples at 1.70 ng/g (in boy's uniform pants), in one out of two treated home textile and upholstery samples at 12.1 ng/g, in one apparel membrane sample at 7.10 ng/g, and in one out of two thread-sealant tapes and pastes samples at 60.3 ng/g. PFHxS was not detected in one treated floor wax and stone/wood sealant sample. Detection limits were not reported in the study.

Beesoon et al. (2012) detected PFHxS in all cleaning formulation-treated carpet samples (n = 9) from various rooms of a Canadian household whose members had previously been identified as having disproportionately high blood serum levels of PFHxS. The Scotchgard formulation-treated carpet samples had a mean (range) of 512 (12–2,880) ng/g of PFHxS, with a level of 17 ng/g detected in one untreated, uninstalled carpet sample taken from the same house.

Of the European studies, van der Veen et al. (2020) examined the effects of weathering on PFAS content in durable water repellent clothing collected from six suppliers in Sweden. Two pieces of each of the 13 fabrics were cut. One piece was exposed to elevated ultraviolet radiation, humidity, and temperature in an aging device for 300 hours (assumed lifespan of outdoor clothing); the other was not aged. Pieces of textile, aged and not aged, were analyzed for ionic PFAS (including PFHxS) and volatile PFAS. For 10 of 13 fabrics, PFHxS was not detected before or after aging. For one fabric, PFHxS was detected before and after aging, increasing from 0.11 to 0.68 $\mu g/m^2$. For one fabric, PFHxS was detected prior to aging at 0.89 $\mu g/m^2$ but was not detected afterward. For the remaining fabric, PFHxS was not detected prior to aging but was not analyzed after aging.

Kotthoff et al. (2015) analyzed 82 samples of consumer products collected in Germany, including outdoor textiles, carpets, cleaning agents, impregnating agents, leather samples, and ski waxes. Individual samples were bought from local retailers or collected by coworkers of the involved institutes or local clubs in Germany. The age of the samples ranged from a few years to decades. PFHxS was detected in 35% of ski wax samples (n = 13) up to 9.3 ng/g and in 96% of leather samples (n = 13) up to 10.1 $\mu g/m^2$. PFHxS was not detected in cleaning agents (n = 6), wood glue (n = 1), impregnating sprays (n = 3), outdoor textiles (n = 3), carpet (n = 6), gloves (n = 3), or awning cloth (n = 1). Favreau et al. (2016) analyzed the occurrence of 41 PFASs in a wide variety of liquid products (n = 194), including impregnating agents, lubricants, cleansers, polishes, AFFFs, and other industrial products purchased from stores and supermarkets in Switzerland. PFHxS was not detected in impregnation products (n = 60), cleansers (n = 24), or polishes (n = 18). PFHxS was detected in 4% of a miscellaneous category of products (n = 23) that included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances, with a maximum concentration of 1,700 ng/g.

The remaining two European studies did not detect PFHxS in the consumer products analyzed. Vestergren et al. (2015) analyzed furniture textile, carpet, and clothing samples (n = 40) purchased from major retail stores in Tromsø and Trondheim, Norway. All samples represented materials that had been imported from China. PFHxS was not detected in any of the samples. Schultes et al. (2018) determined levels of 39 PFAS in 31 cosmetic products collected in Sweden. The study found that 16 out of 31 samples contained measurable concentrations of at least one PFAS; however, PFHxS was not detected amongst the samples.

Of the two studies where the location of purchase was not specified, Gremmel et al. (2016) determined levels of 23 PFAS in 16 new outdoor jackets. PFHxS was detected in one jacket at a concentration of 0.01 ng/g. Bečanová et al. (2016) analyzed 126 samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment (EEE), and plastics); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, facade materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) for 15 target PFAS, including PFHxS. The condition (new versus used) and production year of the samples

varied; the production year ranged from 1981 to 2010. PFHxS was detected in 42%, 22%, 30%, and 14% of household equipment, building materials, car interior, and WEEE samples, respectively. The highest level was 24.5 ng/g found in a drywall sample.

Table D-7. Summary of PFHxS Consumer Product Data

Study	Location	Site Details	Results
United States			
Zheng et al. (2020)	United States (Seattle, Washington)	Children's nap mat samples (n = 26, finely cut) from seven Seattle childcare centers, including polyurethane foam (n = 20) and vinyl cover (n = 6) samples. Sampling year not reported.	n = 26, DF 73%, mean, median (range) = 0.32, 0.30 (<ND–0.73) ng/g (MDL = 0.05 ng/g)
Liu et al. (2014)	United States (unspecified)	Consumer products commonly used indoors were purchased between March 2007 and September 2011 from local retailers and online stores in the United States. A subset of samples were analyzed for PFSAs and included commercial carpet/fabric-care liquids, household carpet/fabric-care liquids and foams, treated apparel (i.e., one girl's uniform pants and one boy's uniform pants), treated home textile and upholstery (i.e., mattress pads), treated floor waxes and stone-wood sealants, membranes for apparel, and thread-sealant tapes and pastes. The subset of products originated from the United States, England, Dominican Republic, Vietnam, and China.	Commercial carpet/fabric-care liquids: n = 2, DF[a] = 50%, range = BDL–194 ng/g Household carpet/fabric-care liquids and foams: n = 4, DF[a] = 50%, range = BDL–155 ng/g Treated apparel: n = 2, DF[a] = 50%, range = BDL–1.70 ng/g Treated home textile and upholstery: n = 2, DF[a] = 50%, range = BDL–12.1 ng/g Treated floor waxes and stone-wood sealants: n = 1, DF 0% Membranes for apparel: n = 1, point = 7.10 ng/g Thread-sealant tapes and pastes: n = 2, DF[a] = 50%, range = BDL–60.3 ng/g (DL not reported)
Canada			
Beesoon et al. (2012)	Canada (Alberta)	Carpet samples (each approximately 5 cm by 2 cm) from the finished basement, family room, dining room, attic, and from the bedrooms of the parents and children currently living in a house in the Edmonton area built in 1989. Samples were collected in September 2008 prior to a major renovation where all carpets were removed. The carpets in all rooms were identical and had been installed at the same time. The house was identified after abnormally high serum levels of PFHxS were identified in a husband and wife that were enrolled as volunteer control participants in a clinical research project. Many rooms in the house had wall-to-wall carpeting installed on top of the heated floors. Since 1991, Scotchgard has been applied to the carpets in the main floor family room and occasionally to the dining	Main floor family room: n = 1, point = 2,880 ng/g Main floor dining room: n = 1, point = 890 ng/g Parents' bedroom: n = 1, point = 122 ng/g First son's bedroom: n = 1, point = 208 ng/g Daughter's bedroom: n = 1, point = 47.0 ng/g Third son's bedroom: n = 1, point = 250 ng/g Fourth son's bedroom: n = 1, point = 147 ng/g Attic: n = 1, point = 50.0 ng/g Basement: n = 1, point = 12.0 ng/g Untreated: n = 1, point = 17.0 ng/g

Study	Location	Site Details	Results
		room. The last application was in 2007, with six intermittent applications. The house did not have a fan-forced air circulation system and there was also no fresh-air intake to the house from the outdoors. Two additional pieces of the same carpet (untreated, uninstalled) stored in the basement for many years were also evaluated.	(LOD/LOQ not reported)
Europe			
van der Veen et al. (2020)	Sweden (unspecified)	Samples of durable water repellent outdoor clothing collected from six suppliers from the outdoor textile industry in Sweden (one pair of outdoor trousers, six jackets, and six fabrics for outdoor clothes*). Each sample was cut into two pieces – one exposed to elevated UV radiation, humidity, and temperature for 300 hours (assumed lifespan of outdoor clothing) and one untreated (not aged). Sampling year not reported. Year of manufacturing not reported for nine of the 13 samples; the remaining four samples (samples 4–7) reported a manufacturing year of 2012/2013. Country of origin not reported. *The breakdown of the 13 items of outdoor clothing is reported differently in Section 2.2and Table 1 of the article. Section 2.2 reports one pair of outdoor trousers, seven jackets, four fabrics for outdoor clothes, and one outdoor overall. Table 1 shows one pair of outdoor trousers, six jackets, and six fabrics for outdoor clothes.	Values presented as not aged, aged for L-PFHxS. Samples 1, 3, 4, 6–12 (1 outdoor trousers, 4 jackets, and 5 fabrics for outdoor clothes): n = 1 (for each sample), ND, ND Sample 2 (fabric for jacket): n = 1, 0.89 µg/m^2, ND Sample 5 (men's jacket): n = 1, 0.11, 0.68 µg/m^2 Sample 13 (fabric for outdoor clothes): n = 1, ND, NA (LOD = 0.02–0.1 µg/m^2 for ionic PFAS)
Schultes et al. (2018)	Sweden (unspecified)	Thirty-one cosmetic products from five product categories (moisturizing cream, foundation, eye pencil, powder and eye shadow, shaving foam) purchased from the Swedish market in 2016–2017. Cosmetic products were selected based on (i) the 2015 KEMI survey which reported the most frequently reported PFAS in cosmetic products and (ii) a database of ingredient lists compiled by the Swedish Society for Nature Conservation. Twenty-four products listing nine different PFAS as active ingredients were purchased. In addition, seven products which did not list PFAS in their ingredients were also purchased from the same stores as control	Control: n = 7, DF 0% PFAS-containing: n = 24, DF 0% (LOD = not reported)

Study	Location	Site Details	Results
		samples. Year of manufacture and country of origin not reported.	
Favreau et al. (2016)	Switzerland (national)	Liquid consumer products, including impregnation agents, cleansers, polishes, lubricants, miscellaneous items, and commercial AFFFs purchased in 2012 and 2013 from stores and supermarkets throughout Switzerland. Products were purchased from 82 different producers and were selected based on their susceptibility to contain PFAS according to previous screenings. Miscellaneous "other" products included foam-suppressing agents for the chromium industry, paints, ski wax, inks, and tanning substances. AFFFs were divided into two sets based on the sampling source. AFFF set 1 was derived from stock solution in fire installation of industrial sites storing chemicals and petroleum products and samples may be the result of multiple AFFF fillings over the years (1990–2010 was the last documented filling date). AFFF set 2 came from commercially available AFFFs between 2012 and 2013 from six producers. Results reported for L-PFHxS.	Impregnation products: n = 60, DF 0% Cleansers: n = 24, DF 0% Polishes: n = 18, DF 0% Others: n = 23, DF 4%, mean (range) = 1,700 (1,700–1,700) ng/g AFFF set 1: n = 27, DF 81%, mean, median (range) = 293,000, 89,500 (100–1,025,000) ng/g AFFF set 2: n = 35, DF 0% (LOQ = 0.5 ng/mL) *Mean and range values only include samples where L-PFHxS was detected *ND treated as 0 for median calculations
Kotthoff et al. (2015)	Germany (Schmallenberg)	Forty-nine random samples of consumer products collected in the first until the third quarter of 2010 in Germany, including outdoor textiles, carpets, cleaning agents, impregnating agents, leather samples, and ski waxes. Individual samples were bought from local retailers or collected by coworkers of the involved institutes or local clubs (e.g., ski waxes from local skiing club). Sampled products spanned all quality levels from entry level to cutting edge products. The age of the samples ranged from a few years to decades. Country of origin not reported.	Cleaner: n = 6, DF 0% Wood glue: n = 1, DF 0% Nanosprays and impregnation sprays: n = 3, DF 0% Outdoor textiles: n = 3, DF 0% Carpet: n = 6, DF 0% Gloves: n = 3, DF 0% Ski wax: n = 13, DF 35%, median (maximum) = <LOQ (9.3 ng/g) Leather: n = 13, DF 96%, median (maximum) = 10.1 (10.1 µg/m^2) Awning cloth: n = 1, DF 0% (LOQ = 0.5 ng/g or 0.5 µg/m^2) *Concentrations <LOQ were considered as zero
Vestergren et al. (2015)	Norway (Tromsø, Trondheim)	Samples of furniture textile (samples included baby-related items such as baby mattress, baby blanket, and baby bed cover), carpet, and clothing samples were	Furniture textiles: n = 27, DF[a] 0% Carpet: n = 9, DF[a] 0%

Study	Location	Site Details	Results
		purchased from three major retail stores during November 2012–February 2013. Sampling campaign designed to evaluate consumer products in product categories that were previously found to contain PFAS residuals and that were representative of products imported from China in large quantities. Individual products randomly selected without prior knowledge of surface treatment with PFAS. Outdoor clothing was excluded. Year of manufacture not reported.	Cotton/Leather clothing: n = 4, DF[a] 0% (MDL = 0.010 µg/m^2)
Origin Unspecified			
Bečanová et al. (2016)	Not specified	One hundred twenty-six samples of (1) household equipment (textiles, floor coverings, electrical and electronic equipment (EEE), and plastics; includes children-related items such as teddy bear filling, teddy bear cover, and plush); (2) building materials (oriented strand board, other composite wood and wood, insulation materials, mounting and sealant foam, facade materials, polystyrene, air conditioner components); (3) car interior materials; and (4) wastes of electrical and electronic equipment (WEEE) purchased (for new materials) or collected from various sources (for older and used materials). Production year ranged from 1981 to 2010. Origin of production and location and year of purchase/collection not reported.	Household equipment: n = 55, DF[a] 42%, range = <MQL–5.16 ng/g Building materials: n = 54, DF[a] 22%, range = <MQL–24.5 ng/g Car interior materials: n = 10, DF[a] 30%, range = <MQL–0.479 ng/g WEEE: n = 7, DF[a] 14%, range = <MQL–0.018 ng/g (IQL = 17 pg/mL; MQL = 0.11 ng/g)
Gremmel et al. (2016)	Not specified	Sixteen outdoor jackets (15 outdoor jackets and one working jacket) purchased during August 2011 to March 2012. Besides the working jacket and two other jackets (one arrived unpacked in shop and had been on sale for four weeks while the other had been on sale since February 2010), all other jackets were new and packed in a plastic shell. Jackets were selected considering factors such as origin of production (primarily Asia, with some origins not specified), price, market, and textile. Location of purchase and year of manufacture not reported.	Jackets J0–J2, J4–J6, J8–J11, J13–J15: n = 2 (for each jacket), DF 0% Jackets J3, J7: n = 2 (for each jacket), DF NR, mean = <LOQ Jacket J12: n = 2, DF NR, mean = 0.01 ng/g (LOD = 0.05 ng/mL; LOQ = 0.01 µg/m^2)

Notes: BDL = below detection limit; DF = detection frequency; DL = detection limit; MDL = method detection limit; ND = not detected.
[a] The DF and/or mean was not reported in the study and was calculated in this synthesis. Means were calculated only when DF = 100%.

D.3.4. Indoor Dust

Several studies from the U.S. and abroad were identified that evaluated the occurrence of PFHxS and other PFAS in dust of indoor environments, primarily in homes, as well as in schools, childcare facilities, offices, and vehicles. In a Wisconsin Department of Health Services study, Knobeloch et al. (2012) examined levels of 16 perfluoroalkyl chemicals in vacuum cleaner dust from 39 Wisconsin homes across 16 counties in March and April 2008 (Table D-8). Samples from these homes built between 1890 and 2005 were collected during a pilot study to assess residential exposure to persistent contaminants found in the Great Lakes Basin. PFHxS was found in all samples at a median concentration of 16 ng/g. Mean levels of PFHxS in dust were significantly higher in homes built between 1968 and 1995 (219 ng/g vs. 57 ng/g in homes constructed in other years). Based on the results of this study, the authors suggested that perfluoroalkyl chemicals may be ubiquitous contaminants in U.S. homes. In an EPA study of 112 indoor dust samples collected from vacuum cleaner bags from homes and daycare centers in North Carolina and Ohio in 2000–2001 (EPA's CTEPP study), samples were collected from 102 homes and 10 daycare centers in North Carolina (49 homes, 5 daycare centers) and Ohio (53 homes, 5 daycare centers) (Strynar and Lindstrom, 2008). Results were not reported separately for homes and daycares. Overall, PFHxS was detected in 77.7% of all samples (n = 112) at mean and median concentrations of 874 and 45.5 ng/g, respectively. The authors concluded that the study measured perfluorinated compounds in house dust at levels that may represent an important pathway for human exposure.

Additional peer-reviewed studies were identified that evaluated the occurrence of PFHxS and other PFAS in dust of indoor environments, primarily in homes, as well as in schools, childcare facilities, offices, and vehicles (Zheng et al., 2020; Scher et al., 2019; Byrne et al., 2017; Karásková et al., 2016; Wu et al., 2014; Fraser et al., 2013; Knobeloch et al., 2012; Kato et al., 2009) (Table D-8). For those studies with results stratified for U.S. homes, PFHxS levels and detection frequencies were lowest in a study of remote Alaska Native villages (27% detection, median below 0.2 ng/g), while in other U.S. locations, PFHxS was detected in at least 40% of samples (some studies reporting 100% detection) at widely varying mean and median levels across the studies (from on the order of 10 ng/g to on the order of 200 ng/g) with one study reporting the highest mean value (219 ng/g) from homes built between 1968 and 1995. The two studies also reporting home measurements from other countries differed in how PFHxS levels in the United States ranked relative to other countries, with one study ranking the U.S. highest and the other second lowest. Few studies sampled childcare centers, vehicles, and offices, and none of the reviewed studies reported measurements in other microenvironments (e.g., public libraries, universities).

Several studies reported results from dust samples collected only from homes (Scher et al., 2019; Byrne et al., 2017); Wu et al. (2014), with one study sampling from locations near a PFAS production facility. Scher et al. (2019) evaluated indoor dust in 19 homes in Minnesota within a GCA in the vicinity of a former 3M PFAS production facility. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. In the house dust samples, collected from July to September 2010, the detection frequencies for PFHxS were 68% and 84% for entryways to the yard and interior living spaces such as the family or living rooms, respectively (n = 19 each), with median concentrations

of 8.2 ng/g and 18 ng/g, respectively. PFAS concentrations in both sampling locations were higher than corresponding soil concentrations, suggesting that interior sources were the main contributors to PFAS in house dust.

Byrne et al. (2017) assessed exposure to PFHxS and other PFAS among residents of two remote Alaska Native villages on St. Lawrence Island. PFAS concentrations were measured in dust collected from the surfaces of floors and furniture of 49 homes on St. Lawrence Island during February–April of 2013 and 2014. Residents were asked not to sweep or dust for one week prior to sampling. The authors described the overall PFAS levels in dust samples as "on the lower end of those reported worldwide in other studies." PFHxS was found in 27% of all samples (n = 49) with a median value below the LOD (0.1 ng/g–0.2 ng/g). Wu et al. (2014) measured concentrations of five PFAS in residential dust in California in 2008–2009. Dust samples were collected from the carpet or area rug in the main living area of the home. Homes of parents with young children and homes with older adults were differentiated to characterize the relationship between serum concentrations of PFAS and several other factors, including PFAS concentrations in residential dust. PFHxS was detected in 51% of samples from households with young children in Northern California (n = 82), with mean and median concentrations of 142 ng/g and 5.30 ng/g, respectively. PFHxS was detected in 52% of samples from households of older adults in central California (n = 42), with mean and median concentrations of 55 ng/g and 5.55 ng/g, respectively.

Apart from the information reported by Strynar and Lindstrom (2008), one other study included childcare centers in the locations sampled (Zheng et al., 2020). Zheng et al. (2020) collected dust samples from seven childcare centers in Seattle, Washington (n = 14) and one childcare facility in West Lafayette, Indiana (n = 6 across six rooms); the sampling year was not reported. The included childcare facilities consisted of several building types, including multiple classrooms, a former church, and a former home. Because centers were vacuumed and mopped daily, dust samples were obtained from elevated surfaces (shelving, tops of bookcases/storage cubbies) along with floor dust. PFHxS was detected in 95% of samples at mean and median concentrations of 0.34 ng/g and 0.25 ng/g, respectively.

One study evaluated PFHxS levels in vehicles and offices, in addition to homes. Fraser et al. (2013) collected dust samples between January and March 2009 from three microenvironments of 31 individuals in Boston, Massachusetts (offices (n = 31), homes (n = 30), and vehicles with sufficient dust for analysis (n = 13)). Study participants worked in separate offices located across seven buildings, which were categorized as Building A (n = 6), Building B (n = 17), or Other (n = 8). Building A was a newly constructed (approximately one year prior to study initiation) building with new carpeting and new upholstered furniture in each office. Building B was a partially renovated (approximately one year prior to study initiation) building with new carpeting throughout hallways and in about 10% of offices. The Other buildings had no known recent renovation occurred. Study offices were not vacuumed during the sampling week and participants were asked not to dust or vacuum their homes and vehicles for at least one week prior to home sampling. Because PFHxS was detected in less than 50% of samples in all three microenvironments, geometric means were not reported. The detection frequencies for PFHxS were 23%, 40%, and 46% for offices, homes, and vehicles, respectively, with the range of detected values reported as 5.24 ng/g–18.5 ng/g, 6.05 ng/g–430 ng/g, and 5.22 ng/g–108 ng/g, respectively.

Two studies evaluated dust samples collected across multiple continents (Karásková et al., 2016; Kato et al., 2009). Karásková et al. (2016) examined PFAS levels in house dust collected between April and August 2013 from the living rooms and bedrooms of 14 homes in the United States, 15 homes in Canada, and 12 homes in the Czech Republic (locations unspecified). PFHxS was detected in all U.S. samples (n = 20) at mean and median concentrations of 13.8 ng/g and 8.7 ng/g, respectively. The authors reported significant differences between countries for PFHxS concentrations, with a trend of U.S. > Canada ~ Czech Republic and suggested that the differences may be explained by differences in the market, import history, and usage of these substances. Overall, no significant differences in total PFAS concentrations were found between the bedroom and living room in the same household although significant relationships were found based on type of floors, number of residents, and age of the house. A second multicontinental study (Kato et al., 2009) measured PFC concentrations in 39 household dust samples collected in 2004 from homes in the United States (Atlanta, GA) (n = 10), United Kingdom (n = 9), Germany (n = 10), and Australia (n = 10). Across all 39 homes, PFHxS was detected in 79.5% of samples with a median concentration of 185.5 ng/g. Authors presented the median and maximum PFHxS concentrations by country in a bar chart, which showed that PFHxS was detected in all countries. The median and maximum PFHxS concentrations for the 10 United States (Atlanta, GA) house dust samples were approximately 96.4 ng/g and 231.3 ng/g, respectively. The highest median was found in Australia, followed by the United Kingdom, the United States, and Germany in decreasing order; statistical significance on the comparison of median PFHxS concentrations by country was not reported.

In general, PFHxS concentrations in dust were higher in North America than Europe, with five studies in the United States or Canada reporting maximum concentrations >1,000 ng/g in homes and daycares (Wu et al., 2014; Beesoon et al., 2012; Knobeloch et al., 2012; Strynar and Lindstrom, 2008; Kubwabo et al., 2005) compared to one study from Europe for an office storage room (Huber et al., 2011). An additional study (Kato et al., 2009) measured PFHxS concentrations in both continents and reported higher maximum concentrations in the United States compared to Germany and the United Kingdom. One European study, conducted by Haug et al. (2011), indicated that house dust concentrations are likely influenced by a number of factors related to the building (e.g., size, age, floor space, flooring type, ventilation); the residents or occupants (e.g., number of people, housekeeping practices, consumer habits such as buying new or used products); and the presence and use of certain products (e.g., carpeting, carpet or furniture stain-protective coatings, waterproofing sprays, cleaning agents, kitchen utensils, clothing, shoes, cosmetics, insecticides, electronic devices). In addition, the extent and use of the items affects the distribution patterns of PFAS compounds in dust of these buildings. Results from the remaining studies conducted in Europe are presented in Table D-8.

Table D-8. Summary of PFHxS Indoor Dust Data

Study	Location	Site Details	Results
United States			
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Nineteen homes in three cities within a GCA near former 3M PFAS production facility as well as from three homes in the Twin Cities Metro outside the GCA. Dust samples collected from an entryway to the yard and from an interior living space (e.g., family room, living room) in each home in July–September 2010. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. Results were not reported for homes outside the GCA.	Entryway: n = 19, DF 68%, median (range) = 8.2 (<RL–94) ng/g Living room: n = 19, DF 84%, median (range) = 18 (<RL–790) ng/g (RL = 5 ng/g)
Byrne et al. (2017)	United States (St. Lawrence Island, Alaska)	Dust samples collected from the surfaces of floors and furniture from 49 homes during February–April of 2013 and 2014. Participants were asked not to sweep or dust for one week prior to sampling.	n = 49; DF 27%, median (95th percentile) = <LOD (3.13) ng/g (MDL = 0.1–0.2 ng/g for all PFAS)
Wu et al. (2014)	United States (Central Valley area, California)	Distributions of PFC dust concentrations were determined for households with young children in Northern California (n = 82) and households of older adults in central California (n = 42). Dust samples were collected in 2008–2009 from the carpet or area rug in the main living area of the homes. Homes of parents with young children and homes with older adults were differentiated to characterize the relationship between serum concentrations of PFCs and PFC concentrations measured in residential dust.	Parents of young children: n = 82, DF 51%, mean, median (range) = 142, 5.30 (ND–7,490) ng/g Older adults: n = 42, DF 52%, mean, median (range) = 55, 5.55 (ND–1,050) ng/g (LOD = 0.10 ng/ml) *Data below LOQ replaced by LOD/√2
Knobeloch et al. (2012)	United States (Great Lakes Basin, Wisconsin)	Dust samples were collected by the Wisconsin Department of Health Services from 39 Wisconsin homes across 16 counties in March–April 2008. Vacuum bags were collected or bagless vacuums were emptied into sterilized glass jars. Homes were built between 1890 and 2005.	n = 39, DF 100%, median (range) = 16 (2.1–1,000) ng/g (RL = 1 ng/g)
Zheng et al. (2020)	United States (Seattle, Washington; West Lafayette, Indiana)	Seven childcare centers in Seattle (14 samples) and one center in Lafayette (6 samples); sampling year not reported. Since all centers were vacuumed and mopped daily, dust samples from elevated surfaces (shelving, tops of bookcases/storage cubbies) were collected along with floor dust in the same sample.	n = 20; DF 95%, mean, median (range) = 0.34, 0.25 (<ND–0.89) ng/g (MDL = 0.05 ng/g)

Study	Location	Site Details	Results
Strynar and Lindstrom (2008)	United States (North Carolina; Ohio)	Dust samples from vacuum cleaner bags were obtained in 2000–2001 during EPA's Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study from North Carolina (49 homes, 5 daycare centers) and Ohio (53 homes, 5 daycare centers). Vacuum cleaner bags were only collected if available at each site.	n = 112, DF 77.7%, mean, median (maximum) = 874, 45.5 (35,700) ng/g (LOQ = 12.9 ng/g) *Values below the LOQ assigned a value of LOQ/√2
Fraser et al. (2013)	United States (Boston, Massachusetts)	Dust samples were collected between January–March 2009 from offices (n = 31), homes (n = 30), and vehicles (n = 13) of 31 individuals. Study participants worked in separate offices located across seven buildings, which were categorized into Building A, Building B, and Other. Six samples were collected from Building A, a newly constructed (approximately one year prior to study initiation) building with new carpeting and new upholstered furniture in each office. Seventeen samples were collected from Building B, a partially renovated (approximately one year prior to study initiation) building with new carpeting throughout hallways and in about 10% of offices. Eight samples were collected from the other five remaining buildings where no known recent renovation occurred. Study offices were not vacuumed during the sampling week and homes and vehicles were not vacuumed for at least one week prior to sampling. Entire accessible floor surface areas and tops of immovable furniture were vacuumed in offices and the main living area of homes. Entire surface areas of the front and back seats of vehicles were vacuumed. Number of home dust samples was reduced to 30 because 1 participant lived in a boarding house with no main living area. Sufficient mass of dust for analysis was available from only 13 vehicles.	Homes: n = 30, DF 40%, range = 6.05–430 ng/g Offices: n = 31, DF 23%, range = 5.24–18.5 ng/g Vehicles: n = 13, DF 46%, range = 5.22–108 ng/g (LOQ = 5 ng/g) *Range of detected values reported
Canada			
Beesoon et al. (2012)	Canada (Alberta)	Dust samples collected from the vacuum bag of the central vacuum system on the same day that carpets were sampled in a house built in 1989 in the Edmonton area. Samples were collected in September 2008 prior to a major renovation where all carpets were removed. The house was identified after abnormally high serum levels of PFHxS were	2008 sampling: n = 1, point = 2,780 ng/g 2012 sampling: n = 1, point = 253 ng/g (LOD/LOQ not reported) *The 2008 value is reported as 2,780 ng/g in the text but 2,900 ng/g in Figure 2

Study	Location	Site Details	Results
		identified in a husband and wife that were enrolled as volunteer control participants in a clinical research project. Many rooms in the house had wall-to-wall carpeting installed on top of the heated floors. Since 1991, Scotchgard has been applied to the carpets in the main floor family room and occasionally to the dining room. The last application was in 2007, with six intermittent applications. The house did not have a fan-forced air circulation system and there was also no fresh-air intake to the house from the outdoors. Vacuum dust sample also collected in January 2012.	
Kubwabo et al. (2005)	Canada (Ottawa)	Sixty-seven randomly selected homes with home selection described in a previous study. Samples collected in the winter of 2002–2003 from houses with varying age and percentage of surface area covered by carpet.	n = 67, DF 85%, mean, median (range) = 391.96, 23.1 (2.28–4,305) ng/g (MDL = 4.56 ng/g) *Non-detects and values <MDL were replaced with ½ × MDL
Europe			
de la Torre et al. (2019)	Spain (unspecified), Belgium (unspecified), Italy (unspecified)	Sixty-five homes belonging to the partners of Test-Achats (Belgium), Altroconsumo (Italy), and OCU Ediciones SA (Spain). Home occupants vacuumed the entire floor of their home from September 2016 to January 2017 and vacuum bags were collected.	Total: n = 65, DF 45%, median (range) = 0.13 (ND–11.3) ng/g Spain: n = 21, DF 76%, median (range) = 0.95 (ND–7.16) ng/g Belgium: n = 22, DF 23%, median (range) = 0.13 (ND–11.3) ng/g Italy: n = 22, DF 36%, median (range) = 0.13 (ND–3.62) ng/g (LOQ = 0.18 ng/g) *Values below LOQ replaced with LOQ/(square root of 2)
Winkens et al. (2018)	Finland (Kuopio)	Sixty-three private households from the birth cohort study, LUKAS2. Floor dust samples collected in 2014/2015 from the children's bedroom (entire floor). Participants were instructed not to vacuum clean the room at least a week before sampling. For 55 rooms, dust samples were collected at the end of a 3-week air sampling period (indoor air results reported in a different study).	n = 63, DF 33.3%, median (range) = 0.68 (BDL–37.0) ng/g (MDL = 0.47 ng/g) *Results reported for l-PFHxS *Values <MDL were treated as MDL/(square root of two)

Study	Location	Site Details	Results
Padilla-Sánchez and Haug (2016)	Norway (Oslo)	Homes of staff from the Norwegian Institute of Public Health. Dust samples collected from vacuum cleaner bags provided by staff. Sampling year not provided.	n = 7, DF[a] 71%, range = ND–1 ng/g (MDL = 0.012 ng/g; MQL = 0.040 ng/g)
Jogsten et al. (2012)	Spain (Catalonia)	Dust sampling was performed in December 2009 from ten households using household vacuum cleaner dust bags. Samples were collected out of convenience and may not be representative of the entire Catalan population.	n = 10, DF[a] 100%, mean[a] (range) = 1.07 (0.17–5.3) ng/g (LOD = 0.003 ng/g)
Haug et al. (2011)	Norway (Oslo)	Forty-one homes of breastfeeding mothers recruited for a study on exposure pathways. House dust samples collected between February and May 2008 on two consecutive days while the residence was in regular use. Samples taken from elevated surfaces such as bookshelves and window sills (deposited dust) and not from the floor.	n = 41, DF (frequency of quantification)[a] 54%, mean, median (range) = 8.4, 0.60 (0.21–142) ng/g (LOQ = 0.23–1.1 ng/g) *Concentrations that were not detected or <LOQ were replaced by the LOQ divided by the square root of two
Giovanoulis et al. (2019)	Sweden (Stockholm)	Twenty preschools that had been previously sampled in 2015 and then participated in the "chemical smart preschool" initiative to reduce the presence of hazardous chemicals in the indoor environment; 2015 results are reported elsewhere. Samples for this study were collected during January to February 2018. One settled dust sample was collected from elevated surfaces (50–250 cm above the floor) from different areas of a play room at each preschool.	n = 20, DF 0% (LOD = 0.5 ng/g) *Values <LOD were replaced with ½×LOD
Harrad et al. (2019)	Ireland (Dublin, Galway, and Limerick)	Dust collected from homes (living rooms), offices, cars, and school classrooms; air samples also collected. Samples collected between August 2016 and January 2017. Sample numbers were split approximately equally from each of the three counties.	Homes: n = 32, DF 47%, mean, median (range) = 1.4, <0.1 (<0.1–9.9) ng/g Offices: n = 33, DF 44%, mean, median (range) = 2.7, <0.1 (<0.1–57) ng/g Cars: n = 31, DF 47%, mean, median (range) = 6.2, <0.1 (<0.1–49) ng/g Classrooms: n = 32, DF 38%, mean, median (range) = 5.1, <0.1 (<0.1–120) ng/g (LOD = 0.1 ng/g) *When analyte peaks are <LOD, concentrations were assumed to equal DF × LOD where DF is expressed as a fraction.

Study	Location	Site Details	Results
Huber et al. (2011)	Norway (Tromsø)	Homes and workplaces sampled in winter 2007–2008. Home samples included seven different living rooms (L-1 to L-7), one sleeping room (S, related to L-3), one sofa (a stain repellant fabric, related to L-7), and one carpet (related to L-4). Workplace samples included an office and a storage room at Fram Center; old documents and chemicals and highly contaminated samples were stored in the storage room. Samples were taken from bookshelves, commodes, TVs, electrical heaters, picture frames, window sills and sun blinds. Dust from the floor was not sampled.	All homes: n = 7, DF NR, median = 1.4 ng/g Living room: n = 7, DF[a] 86%, mean, median (range) = 1.7, 1.4 (0.8–3.1) ng/g Carpet: n = 1, point = 0.5 ng/g Sleeping room: n = 1, point = 2.1 ng/g Sofa: n = 1, point = 1.7 ng/g Office: n = 1, point = 27.8 ng/g Storage room: n = 1, point = 1,814 ng/g (LOD on column = 0.006 ng; MDL = 0.1–1.85 ng/g)
D'Hollander et al. (2010)	Belgium (Flanders)	Forty-three randomly selected homes and ten randomly selected offices throughout Flanders. Samples collected using a vacuum from bare floor, possibly covered with carpet, in 2008. In homes, the living room, bedroom, kitchen, and working area were sampled.	Homes: n = 43, DF 40%, median (95th percentile) = 0.1 (9) ng/g dw Offices: n = 10, DF NR, median (95th percentile) = 0.2 (5.1) ng/g dw (LOQ = 0.1 ng/g) *Concentration <LOQ were replaced by DF × LOQ *For homes, both Table 3 and Section 2.1 reported n = 43; however, Table 2 reported n = 45
Multiple Continents			
Karásková et al. (2016)	United States (unspecified), Canada (unspecified), Czech Republic (unspecified)	Fifty-six dust samples from 14 homes in the United States, 15 homes in Canada, and 12 homes in the Czech Republic were collected between April and August 2013. Samples were collected in living rooms and bedrooms.	United States: n = 20, DF 100%, mean, median (range) = 13.8, 8.7 (1.4–84.4) ng/g Canada: n = 20, DF 90.0%, mean, median (range) = 3.1, 1.9 (ND–11.5) ng/g Czech Republic: n = 16, DF 93.8%, mean, median (range) = 2.8, 2.0 (<MQL–9.3) ng/g (MDL = 0.05–0.24 ng/g; MQL = 0.12–0.57 ng/g; ranges represent lower bound and upper bound which were calculated by dividing the MDL/MQL by the biggest and smallest dust sample weight, respectively) *Mean calculated only from values >MQL *Median calculated by replacing values <MQL with √2/2*MQL

Study	Location	Site Details	Results
Kato et al. (2009)	United States (Atlanta, Georgia), Germany (unspecified), United Kingdom (unspecified), Australia (unspecified)	Thirty-nine household dust samples from the United States (n = 10), Germany (n = 10), United Kingdom (n = 9), and Australia (n = 10) collected in 2004 for method validation. Dust sampling procedures not described.	n = 39, DF 79.5%, median (range) = 185.5 (<LOD–43,765) ng/g (LOQ = 2.6 ng/g)

Notes: DF = detection frequency; GCA = groundwater contamination area; LOD = limit of detection; LOQ = limit of quantitation; MDL = method detection limit; MQL = method quantification limit; ND = not detected; RL = reporting limit.

D.3.5. Air

Perfluoroalkyl chemicals have been released to air from wastewater treatment plants, waste incinerators, and landfills (Ahrens et al., 2011), though there is limited information on the detection levels or frequencies of PFHxS in either indoor or ambient air. NCBI (2022a) notes that PFHxS has been detected in particulates and in the vapor phase in air and it can be transported long distances via the atmosphere; it has been detected at low concentrations in areas as remote as the Arctic and ocean waters. For example, PFHxS was detected in particle-phase air samples collected in 2007 and 2008 from the Atlantic Ocean, Southern Ocean, and Baltic Sea (NCBI, 2022a; Dreyer et al., 2009). PFHxS is not expected to be broken down directly by photolysis (NCBI, 2022a). PFHxS in the particle-phase can be removed from the atmosphere through wet and dry deposition (NCBI, 2022a). PFHxS in the vapor phase can undergo hydroxylation in the atmosphere, with a (predicted average) atmospheric hydroxylation rate of 2.16×10^{-15} cm^3/molecule – second to a (derived) rate of 1.4×10^{-13} cm^3/molecule – second at 25°C (with corresponding estimated half-life of 115 days for this reaction in air) (USEPA, 2022a; NCBI, 2022a). With a vapor pressure of 0.0046 mm Hg at 25°C (estimated), 8.10×10^{-9} mm Hg (measured average), and 8.19×10^{-9} mm Hg (predicted average), volatilization is not expected to be an important fate process for this chemical (USEPA, 2022a; NCBI, 2022a). EPA's Toxics Release Inventory reported release data for PFHxS in 2020, with total onsite disposal, offsite disposal, and other releases concentrations of 1 pound at an individual facility and 122 pounds at a second facility (USEPA, 2022b). PFHxS is not listed as a hazardous air pollutant (USEPA, 2022c).

D.3.5.1. Indoor Air

No studies from the U.S. reporting levels of PFHxS in indoor air were identified from the primary or gray literature. However, several studies from Canada and Europe were identified and are discussed briefly below and presented in Table D-9 (Harrad et al., 2019; Beesoon et al., 2012; Goosey and Harrad, 2012; Jogsten et al., 2012; Barber et al., 2007). All of these studies sampled from homes, while two studies also sampled from offices, one study also sampled a laboratory, and only one study also sampled from cars and classrooms. In studies exclusively in homes, two studies did not detect PFHxS in indoor air while the remaining three studies had PFHxS detection frequencies ranging from 21 to 100%. In one of these studies, a Canadian household with wall-to-wall Scotchgard-treated carpets on top of heated floors (and whose residents had previously been found to have disproportionately high blood serum levels of PFHxS) found 27.4 pg/m^3 PFHxS in the family room and 426 pg/m^3 PFHxS in the basement (with no clear reason for the higher levels in the basement). Additional research is needed to evaluate PFHxS in indoor air from U.S. locations and from a variety of non-home microenvironments (offices, cars, classrooms, and laboratories) in Canada and Europe.

Jogsten et al. (2012) sampled indoor air (n = 10) from selected homes in Catalonia, Spain in December 2009 and evaluated levels of 27 PFCs, though PFHxS was not detected. The remaining studies evaluated PFHxS levels in offices, vehicles, and/or schools, in addition to homes (Harrad et al., 2019; Goosey and Harrad, 2012; Barber et al., 2007). In Ireland, Harrad et al. (2019) collected air samples in homes (living rooms, n = 34), offices (n = 34), cars (n = 31), and school classrooms (n = 28) between August 2016 and January 2017. PFHxS was detected in all four indoor environments in 21%, 44%, 23%, and 25% of samples for homes, offices, cars, and classrooms, respectively. However, the median concentrations were below the LOD for all

environments. Goosey and Harrad (2012) detected PFHxS with mean (median) concentrations of 36 (23) pg/m^3 in homes (n = 20) and 94 (84) pg/m^3 in offices (n = 12) sampled in the United Kingdom between September 2008 and March 2009. In addition, Goosey and Harrad (2012) also examined seasonal variation in these same five indoor microenvironments by sampling monthly between September 2008 and August 2009. The relative standard deviation for PFHxS was between 52–106%. The observed variation could not be attributed to changes in room contents or to seasonality. The study also measured ambient air concentrations in the same location and concluded that indoor air concentrations significantly exceeded ambient air concentrations; the authors suggested that indoor emissions may influence both indoor and outdoor PFAS levels. In Norway, neutral and ionic PFAS were analyzed in indoor air samples collected from three homes and one laboratory in Tromsø between in April and June 2005 (Barber et al., 2007). Results for 15 neutral PFAS and 16 ionic PFAS (including PFHxS) were presented but PFHxS was not detected (method quantification limit [MQL] = 4.09 pg/m^3; authors considered this a high MQL and likely due to much lower sampling volume).

Table D-9. Summary of PFHxS in Indoor Air

Study	Location	Site Details	Results
Canada			
Beesoon et al. (2012)	Canada (Alberta)	Indoor air samples collected from one home in the Edmonton area, built in 1989. The house was identified after abnormally high serum levels of PFHxS were identified in a husband and wife that were enrolled as volunteer control participants in a clinical research project. Many rooms in the house had wall-to-wall carpeting installed on top of the heated floors. Since 1991, Scotchgard has been applied to the carpets in the main floor family room and occasionally to the dining room. The last application was in 2007, with six intermittent applications. The house did not have a fan-forced air circulation system and there was also no fresh-air intake to the house from the outdoors. At the time of indoor air sampling in September 2008, renovations had begun in the basement. Analysis for PFHxS conducted on Fraction 1 (particulate phase) from the main floor family room and finished basement.	Main floor family room: n = 1, point = 27.4 pg/m^3 Basement: n = 1, point = 426 pg/m^3 (LOD/LOQ not reported)
Europe			
Jogsten et al. (2012)	Spain (Catalonia)	Indoor air sampling was performed in December 2009 from ten households at approximately 1 m above the floor. Samples were collected out of convenience and may not be representative of the entire Catalan population. Both particulate and gas phases collected.	n = 10, DF 0% (LOD = 3.1–280 pg/m^3 for all ionic PFAS)
Harrad et al. (2019)	Ireland (Dublin, Galway, Limerick)	Air samples collected from homes (living rooms), offices, cars, and school classrooms; dust samples also collected. Samples collected between August 2016 and January 2017. Sample numbers were split approximately equally from each of the three counties. Gas or particulate phase not specified.	Homes: n = 34, DF 21%, mean, median (range) = <0.4, <0.4 (<0.4–0.46) pg/m^3 Offices: n = 34, DF 44%, mean, median (range) = 0.40, <0.4 (<0.4–1.4) pg/m^3 Cars: n = 31, DF 23%, mean, median (range) = 0.15, <0.4 (<0.4–0.55) pg/m^3 Classrooms: n = 28, DF 25%, mean, median (range) = <0.4, <0.4 (<0.4–2.3) pg/m^3 (LOD = 0.4 pg/m^3) *When analyte peaks are <LOD, concentrations were assumed to equal DF × LOD where DF is expressed as a fraction.

Study	Location	Site Details	Results
Goosey and Harrad (2012)	United Kingdom (Birmingham)	Samples collected from homes and offices between September 2008 and March 2009. In addition, air samples were collected monthly from one office and four living rooms between September 2008 and August 2009. Gas or particulate phase not specified.	Homes: n = 20, DF[a] 90%, mean, median (range) = 36, 23 (<1.1–220) pg/m^3 Offices: n = 12, DF[a] 83%, mean, median (range) = 94, 84 (<1.1–330) pg/m^3 Seasonal Variations: concentrations reported for each month from Sept 2008 to Aug 2009 Home 1: 12, 100, 24, 21, 22, 17, 49, 35, 4, <1.1 pg/m^3 Home 2: 110, 49, 30, 7, 30, 9, 44, 50, 110, 44, 22 pg/m^3 Home 3: <1.1, 24, 11, 12, 49, 100, 9, 16, 84, 17, 19, <1.1 pg/m^3 Home 4: <1.1, 37, 44, 5, 27, 23, 47, 25, 3, 27, 22, 37 pg/m^3 Office 1: <1.1, 8, 30, 16, 50, 33, 12, 12, 18, 9, <1.1 pg/m^3 (DL = 1.1 pg/m^3) *Where concentration <DL, ½×DL was used for calculations
Barber et al. (2007)	Norway (Tromsø)	Air samples taken from four indoor locations (three houses and one laboratory) in Tromsø in April–June 2005. PFHxS was measured in the particulate phase.	n = 4, DF[a] 0%, mean = <4.1 pg/m^3 (MQL = 4.09 pg/m^3)

D.3.5.2. Ambient Air

The EPA identified a single study reporting ambient air concentrations of PFHxS from the U.S. Kim and Kannan (2007) analyzed particle phase (n = 8) and gas phase (n = 8) concentrations of perfluorinated acids in ambient air samples collected in and around Albany, New York, in May and July 2006 to examine the relative importance of certain media pathways to the contamination of urban lakes. PFHxS was detected in all gas phase samples with mean, and median concentrations of 0.31 pg/m^3 and 0.34 pg/m^3, respectively, but was not detected in the particle phase (LOQ = 0.12 pg/m^3).

Additional studies were identified that examined PFHxS in ambient air from sampling efforts conducted in Canada and Europe. These studies are summarized below and presented in Table D-10. In Canada, Ahrens et al. (2011) did not detect PFHxS in air around a WWTP and two landfill sites. In Europe, Barber et al. (2007) collected air samples from four field sites in the United Kingdom, Ireland, and Norway for analysis of neutral and ionic PFAS. PFHxS was detected in the ambient air particle phase at two sampling events in Manchester, UK at 0.1 and 1.0 pg/m^3, at one of two sampling events in Hazelrigg, UK at 0.04 pg/m^3, at one sampling event in Norway at 0.05 pg/m^3, and at one sampling event in Ireland at 0.07 pg/m^3. Goosey and Harrad (2012) collected ambient air samples from ten different locations within a 1.5 km radius of the University of Birmingham campus in the United Kingdom. PFHxS was detected with a mean (maximum) concentration of 7.0 (30) pg/m^3. Beser et al. (2011) detected PFHxS in 5% of ambient air particulate samples from five locations in Alicante province, Spain (3 residential, 1 rural, 1 industrial), with a mean (maximum) concentration of 8.7 (15.9) pg/m^3. The highest concentration observed was at the industrial site. Harrad et al. (2020) analyzed air samples near ten Irish municipal solid waste landfills in nonindustrial areas. PFHxS was detected in more than 20% of the samples, with mean (maximum) concentrations at downwind and upwind locations of 0.34 (0.79) pg/m^3 and 0.23 (0.81) pg/m^3, respectively. One European study (Jogsten et al., 2012) did not detect PFHxS in ambient air samples collected outside homes in Catalonia, Spain.

Table D-10. Summary of the Occurrence of PFHxS in Ambient Air

Study	Location	Site Details	Results
United States			
Kim and Kannan (2007)	United States (Albany, New York)	Roof of a lakehouse building located at Washington Park Lake in May and July 2006. Both particulate and gas phases collected.	Gas: n = 8, DF NR, mean, median (range) = 0.31, 0.34 (0.13–0.44) pg/m^3 Particle: n = 8, DF 0% (LOQ = 0.12 pg/m^3) *Non-detects were set to zero; values below the LOQ were set to ½ LOQ
Canada			
Ahrens et al. (2011)	Canada (Ontario)	Samples collected on and around one municipal WWTP for 63 days between July and September 2009. Samplers were placed at the primary clarifier, aeration tank, secondary clarifier, and at four reference sites (three near [within 200 m of the treatment tanks] and one distant [~600 m from the perimeter of the WWTP]). Samples also collected at two municipal solid waste landfills between June and August 2009 for 55 days. The two landfills were 60 km apart. Samplers were located upwind and onsite of the active zone of each landfill site and one field blank was collected at each site. Both sites collected landfill gas and the active area of the landfill was kept to a minimum by covering the waste with soil and a plastic film. The passive sampling configuration used resulted in the collection of mainly PFAS in the gas phase.	WWTP: Reference sites (near): n = 3, DF 0% Primary clarifier: n = 2, DF 0% Aeration tank: n = 3, DF 0% Secondary clarifier: n = 2, DF 0% Reference site (distant): n = 1, DF 0% Landfills: Upwind: n = 2, DF 0% On site: n = 2, DF 0% (MDL = 0.04–0.87 pg/m^3 for PFCAs, PFSAs, and PFOSA)
Europe			
Harrad et al. (2020)	Ireland (multiple cities)	Samples collected from ten municipal solid waste landfills upwind and downwind at each site between November 2018 and January 2019. Location of sampling sites based on wind direction data taken from the Irish Meteorological Service, with slight modification where necessary based on local information from site operators and ease of access. Sample sites were between 150 and 500 m of the center of the landfill. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction and demolition, and	Downwind: n = 10, DF[a] 60%, mean, median (range) = 0.34, 0.23 (<0.15–0.79) pg/m^3 Upwind: n = 10, DF[a] 40%, mean, median (range) = 0.23, 0.08 (<0.15–0.81) pg/m^3 (LOD = 0.15 pg/m^3) *Non-detects replaced by ½ LOD

Study	Location	Site Details	Results
		biomedical waste. Gas or particulate phase not specified.	
Goosey and Harrad (2012)	United Kingdom (Birmingham)	Ten different locations within a 1.5 km radius of the University of Birmingham campus. Samples collected in March 2009. In addition, air samples were collected monthly from three outdoor locations between September 2008 and August 2009 where two of the locations were among the ten sampled in March 2009 and the third location was Harwell, Oxfordshire in Southeast England, a semi-rural site. Gas or particulate phase not specified.	n = 10, DF[a] 50%, mean, median (range) = 7.0, 2.7 (<1.1–30) pg/m^3 (DL assumed to be 1.1 pg/m^3) Seasonal Variations: concentrations reported for each month from Sept 2008 to Aug 2009 Birmingham site 1: 12, 11, 3.8, 2.0, 420, <0.8, <0.8, <0.8, 1.3, 1.4 pg/m^3 Birmingham site 7: 1.5, 8.3, <0.8, 3.8, 1.9, 5.3, 5.6, <0.8, 12, <0.8, 20 pg/m^3 Harwell site: <0.8, 1.6, 4.9, <0.8, 1.3, <0.8, 1.0, <0.8, 4.9, 1.5, 20, 8.8 pg/m^3 (DL assumed to be 0.8 pg/m^3) *For concentration < DL, ½×DL was used for calculations
Jogsten et al. (2012)	Spain (Catalonia)	Outdoor air sampling conducted in December 2009 for the purposes of comparison to indoor air and dust samples. Number of sites not specified but assumed to be ten because indoor air was sampled from ten homes. Samples were collected out of convenience and may not be representative of the entire Catalan population. Both particulate and gas phases collected.	n = 10, DF 0% (LOD = 3.1–280 pg/m^3 for all ionic PFAS)
Beser et al. (2011)	Spain (Alicante province)	Samples collected from April to July 2010 from five stations. Two stations were placed in Elche (one in a residential area and the other in an industrial area). The third station was placed in a residential area of Alicante City. The fourth station was in a rural area of Pinoso and the last station was in a residential area of Alcoy. Concentrations reported for PM2.5-bound PFHxS.	Elche (residential): n = 11, DF[a] 0% Elche (industrial): n = 13, DF[a] 7.7%, mean = 15.9 pg/m^3 Alicante City: n = 11, DF[a] 0% Pinoso: n = 3, DF[a] 0% Alcoy: n = 3, DF[a] 33%, mean = 1.5 pg/m^3 (MQL = 1.4 pg/m^3) *Mean calculated from values >MQL
Barber et al. (2007)	United Kingdom (Hazelrigg, Manchester); Ireland (Mace Head); Norway (Kjeller)	Samples collected from four field sites in Europe: Hazelrigg (semirural) and Manchester (urban) were sampled in two sampling events in February–March 2005 and November 2005–January 2006; Mace Head (rural) was sampled in March 2006; and Kjeller (rural) was sampled in November–December 2005. PFHxS was measured in the particulate phase.	Hazelrigg first sampling event: n = 2, DF NR, mean = <5.9 pg/m^3 (MQL = 5.93 pg/m^3) Feb/Mar 2005 *The glass-fibre filters were analyzed in a batch of samples that showed contamination problems, so the high associated blank value

Study	Location	Site Details	Results
			used to calculate the MQL put most analytes <MQL. Hazelrigg second sampling event: n = 10, DF[a] = 100%, mean (range) = 0.04 (0.01–0.06) pg/m^3 (MQL = 0.002 pg/m^3) Manchester first sampling event: n = 2, DF[a] 100%, mean (range) = 1.0 (0.9–1.0) pg/m^3 (MQL = 0.45 pg/m^3) Manchester second sampling event: n = 1, point = 0.1 pg/m^3 (MQL = 0.002 pg/m^3) Mace Head: n = 4, DF[a] 100%, mean (range) = 0.07 (0.05–0.11) pg/m^3 (MQL = 0.004 pg/m^3) Kjeller: n = 2, DF[a] 100%, mean (range) = 0.05 (0.05) pg/m^3 (MQL = 0.01 pg/m^3) *Means calculated from values >MQL

D.3.6. Soil

The production of PFHxS and its use as a raw material or precursor for manufacturing PFAS-based products, as well as its previous use in firefighting foam and carpet treatment solutions, could result in its release to soils through various waste streams (NCBI, 2022a). When released to soil, PFHxS is expected to have very high mobility (NCBI, 2022a). The maximum concentration of PFHxS in soil samples collected from a PFAS production facility in Minnesota was 3,470 ng/g, with PFHxS detected in 90% of the samples collected (NCBI, 2022a; ATSDR, 2021; 3M, 2007). The maximum concentration of PFHxS in soil samples collected at a fire training area at a PFAS production facility in Minnesota was 62.2 ng/g, with PFHxS detected in 100% of samples (ATSDR, 2021; 3M, 2007). PFHxS was also detected in soil samples collected from a former sludge incorporation area at a PFAS production facility in Decatur, Alabama, with average levels ranging from 3.56 ng/g to 270 ng/g, with PFHxS detected in 86% of the samples collected (ATSDR, 2021; 3M, 2012). PFHxS has been found to accumulate in the roots of maize plants grown in soil containing PFHxS and other PFAS (ATSDR, 2021; Krippner et al., 2015).

Several additional peer-reviewed studies were identified that evaluated the occurrence of PFHxS and other PFAS in soil collected in the U.S. Two studies by Scher et al. (2019; 2018) evaluated soils collected in 2010 from the garden planting area of 20 homes in Minnesota within a GCA impacted by the former 3M PFAS production facility. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. Both studies reported similar median PFHxS levels of 0.08 ng/g and 0.057 ng/g (n = 20–34) from the 2019 and 2018 publications, respectively. Scher et al. (2018) also reported a median PFHxS concentration of 0.078 ng/g from six samples collected outside the GCA.

Three studies analyzed soils potentially impacted by AFFF use (Nickerson et al., 2020; Eberle et al., 2017; Anderson et al., 2016). Anderson et al. (2016) examined surface and subsurface soil from 40 sites across 10 active Air Force installations throughout the continental United States and Alaska between March and September 2014. Installations were included if there was known historic AFFF release in the period 1970–1990. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. The selected sites were not related to former fire training areas and were characterized according to volume of AFFF release – low, medium, and high. Across all sites, the PFHxS detection frequency was 76.92% in 100 surface soil samples (median concentration of detects was 5.7 ng/g) and 59.62% in 112 subsurface soil samples (median concentration of detects was 4.4 ng/g). PFHxS was detected more frequently at high-volume release sites (82.5% in 32 surface soil samples with mean concentration of 19.7 ng/g; 87.5% in 31 subsurface soil samples with mean concentration of 9.3 ng/g) than at low-volume sites (75.0% in 12 surface soil samples with mean concentration of 13.9 ng/g; 58.8% in 17 subsurface soil samples with mean concentration of 57.9 ng/g) and medium-volume sites (59.2% in 56 surface soil samples with mean concentration of 39.4 ng/g; 71.4% in 64 subsurface soil samples with mean concentration of 55.4 ng/g). Nickerson et al. (2020) developed a method to quantify anionic, cationic, and zwitterionic PFAS from AFFF-impacted soils. The method was applied to two soil cores collected from two different AFFF-impacted former fire training areas; the sampling year and geographic location were not provided. Eleven soil samples, corresponding to 11 depths ranging from 0.46 m to 15.1 m, were evaluated from Core E, and 12 soil samples, at depths ranging from

0.30 m to 14.2 m, were evaluated from Core F. PFHxS was detected at all depths analyzed for both cores, with concentrations ranging from 1.17 ng/g dw to 160.6 ng/g dw for Core E and 0.66 ng/g dw to 296.4 ng/g dw for Core F. Eberle et al. (2017) investigated the effects of an in situ chemical oxidation treatment for remediation of chlorinated volatile organic compounds and PFAAs co-contaminants. Soil samples were collected in 2012–2013 before and after a pilot scale field test at a former fire training site at Joint Base Langley-Eustis, Virginia. Monthly fire training activities were conducted at the site from 1968 to 1980 and irregular fire training activities continued until 1990. Impacted soil was excavated in 1982 but details were not provided. PFHxS was detected in 4 of 5 pre-treatment samples and in all 14 post-treatment samples. In the available three paired pre- and post-treatment soil samples, two pairings showed a decrease in PFHxS concentration after treatment, from 6.7 ng/g to 1.40 ng/g in one pairing and from 12 ng/g to 1.44 ng/g in the other pairing. In the third pairing, PFHxS was not detected pre-treatment and was found at 0.31 ng/g post-treatment.

Of the remaining two studies conducted in the United States, Venkatesan and Halden (2014) conducted outdoor mesocosm studies to examine the fate of PFAS in biosolids-amended soil collected during 2005–2008. Biosolids were obtained from a WWTP in Baltimore that primarily treated wastewater from domestic sources with only minor contribution (1.9%) from industry. PFHxS was not detected in the control (nonamended) soil and not consistently detected in the biosolids-amended soil (MDL = 0.03–0.14 ng/g dw). In a field and greenhouse study, Blaine et al. (2013) studied the uptake of PFAS into edible crops grown in control and biosolids-amended soil. In the field study, urban biosolids were obtained from a WWTP receiving both domestic and industrial waste while rural solids were obtained from a WWTP receiving domestic waste only. Mean PFHxS concentrations were below the LOQ (0.01 ng/g) in the urban control and biosolids-amended soils and in the rural control soil. In the rural biosolids-amended soil, the mean PFHxS concentration was 0.016 ng/g. In the greenhouse study, three soils (nonamended control, industrially impacted, and municipal) were investigated. Industrially impacted soils contained composted biosolids from a small municipal WWTP that was impacted by PFAA manufacturing while municipal soils were obtained from a reclamation site in Illinois where municipal biosolids were applied for 20 years. PFHxS was detected in all three soils at an average concentration of 0.44 ng/g, 1.38 ng/g, and 5.11 ng/g in control, industrially impacted, and municipal soil, respectively. Authors noted that the trace levels of PFAS detected in the control soil may be due to minor cross-contamination from plowing, planting, or atmospheric deposition from the surrounding area where biosolids have been applied.

Studies conducted in Europe and Canada were also identified and are summarized below and presented in Table D-11. Of the European studies, a study in Ireland (Harrad et al., 2020) examined soil samples collected upwind and downwind from ten municipal solid waste landfills and found PFHxS levels to be lower at downwind locations. Based on the overall study findings, however, the authors concluded there was no discernible impact of the landfills on concentrations of PFAS in soil surrounding these facilities. In the Maltese islands, Sammut et al. (2019) sampled soil from small urban fields and found PFHxS concentrations to range from non-detectable levels to 0.12 ng/g. Grønnestad et al. (2019) investigated soils from a skiing area in Norway to elucidate exposure routes of PFAS into the environment from ski products, such as ski waxes. PFHxS was below the limit of quantification in soil samples from both the Granåsen skiing area and the Jonsvatnet reference area. One study performed in Belgium (Groffen et al., 2019) evaluated soils collected at a 3M fluorochemical plant in Antwerp and at four sites located

at increasing distances from the plant. PFHxS levels were elevated at the plant site but not detected at other locations. Four studies were conducted at locations near firefighting training sites, which found varying results from non-detected levels to 2,344 ng/g dw (Dauchy et al., 2019; Skaar et al., 2019; Hale et al., 2017; Filipovic et al., 2015).

Table D-11. Summary of PFHxS Data in Soil

Study	Location	Site Details	Results
United States			
Scher et al. (2019)	United States (Twin Cities metropolitan region, Minnesota)	Area near former 3M PFAS production facility. Soil composite samples (200–500 g) collected in 2010 from the garden planting area of 20 homes in 3 cities within a GCA as well as from 3 homes in the Twin Cities Metro outside the GCA. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or a private well previously tested and shown to have detectable levels of PFOA or PFOS. Results were not reported for homes outside the GCA.	n = 20, DF 85%, median (90th percentile) = 0.08 (0.16) ng/g (RL = 0.0008–0.033 ng/g for all PFAS)
Scher et al. (2018)	United States (Twin Cities metropolitan region, Minnesota)	Area near former 3M PFAS production facility. Soil samples collected in 2010 at a sample depth of 0–6 inches from the garden planting area of 20 homes in 3 cities within a GCA as well as from 3 homes in the Twin Cities Metro outside the GCA. Homes within the GCA had previous or ongoing PFAS contamination in drinking water and were served by the Oakdale, Minnesota public water system or was formerly or currently using a private well previously tested and shown to have detectable levels of PFOA or PFOS. At 14 homes, soil samples were collected on two separate days.	Within GCA: n = 34, DF 71%, median (range) = 0.057 (ND–0.24) ng/g Outside GCA: n = 6, DF 100%, median (range) = 0.078 (0.028–0.11) ng/g (MDL = 0.008–0.033 ng/g for all PFAS) *Values below the method reporting limit but above the lowest calibration limit (estimated values) were included in all analyses as quantitative results *Values below the lowest calibration limit were replaced with ½ of the limit
Anderson et al. (2016)	United States (national)	Forty AFFF-impacted sites from ten active U.S. Air Force installations with historic AFFF release between 1970 and 1990 that were not related to former fire training areas. It is assumed that the measured PFAS profiles at these sites reflect the net effect of several decades of all applicable environmental processes. AFFF-impacted sites included emergency response locations, hangers and buildings, and testing and maintenance related to regular maintenance and equipment performance testing of emergency vehicles and performance testing of AFFF solution. Previous remedial activities for co-occurring contaminants were not specifically controlled for in the site selection process; active remedies had not been applied at any of the sites selected. Approximately ten samples were	Surface soil: Overall: n = 100, DF 76.92%, median (maximum) = 5.7 (1,300) ng/g *Breakdown by site:* Emergency Response (low-volume release): n = 12, DF 75.0%, mean (range) = 13.9 (0.38–64) ng/g Hangars and Buildings (medium-volume release): n = 56, DF 59.2%, mean (range) = 39.4 (0.34–1,300) ng/g Testing and Maintenance (high-volume release):

Study	Location	Site Details	Results
		collected between March and September 2014 at each site for surface and subsurface soil; sites were grouped according to volume of AFFF release—low-volume typically had a single AFFF release, medium-volume had one to five releases, and high-volume had multiple releases.	n = 32, DF 82.5%, mean (range) = 19.7 (0.29–180) ng/g (RL = 0.29 ng/g) Subsurface soil: Overall: n = 112, DF 59.62%, median (maximum) = 4.4 (520) ng/g *Breakdown by site:* Emergency Response (low-volume release): n = 17, DF 58.8%, mean (range) = 57.9 (0.37–520) ng/g Hangars and Buildings (medium-volume release): n = 64, DF 71.4%, mean (range) = 55.4 (0.35–1,300) ng/g Testing and Maintenance (high-volume release): n = 31, DF 87.5%, mean (range) = 9.3 (1.1–40) ng/g (RL = 0.31 ng/g) *Median calculated using quantified detections *Non-detects were substituted with ½ the reporting limit
Nickerson et al. (2020)	United States (unspecified)	Soil cores E and F from two different AFFF-impacted fire training areas; sampling year and geographic location not provided. Soil core E contained 11- 0.3 m increment samples from 0.3–15.2 m below ground surface and was collected in an area where the surficial soils were likely disturbed due to regrading and other soil redistribution activities. Soil core F contained 12- 0.61 m increment samples from 0–14.2 m below ground surface and was collected in an area where the surficial soils were highly permeable only within the upper 0.5 to 1 m, and the underlying impermeable clay layer exhibited a relatively high cation exchange capacity and organic carbon content. The water table was relatively shallow (depth <3 m) at both sites.	Core E: 0.46 m = 1.44 ng/g dw 2.9 m = 2.12 ng/g dw 3.66 m = 4.17 ng/g dw 3.96 m = 15.21 ng/g dw 4.27 m = 28.68 ng/g dw 4.57 m = 4.13 ng/g dw 4.88 m = 5.73 ng/g dw 7.01 m = 13.86 ng/g dw 8.38 m = 160.6 ng/g dw 10.5 m = 139.0 ng/g dw 15.1 m = 1.17 ng/g dw Core F: 0.30 m = 11.07 ng/g dw 1.22 m = 296.4 ng/g dw 1.83 m = 276.2 ng/g dw

Study	Location	Site Details	Results
			2.44 m = 106.2 ng/g dw 3.05 m = 42.69 ng/g dw 4.11 m = 28.78 ng/g dw 7.62 m = 14.19 ng/g dw 8.84 m = 6.26 ng/g dw 9.45 m = 3.25 ng/g dw 10.5 m = 0.66 ng/g dw 11.9 m = 3.06 ng/g dw 14.2 m = 7.96 ng/g dw (LOD/LOQ not reported)
Eberle et al. (2017)	United States (Joint Base Langley-Eustis, Virginia)	Pilot testing area in former fire training area (Training Site 15) at Joint Base Langley-Eustis where monthly fire training activities were conducted from 1968 to 1980 in a zigzag pattern burn pit. Facility was abandoned in 1980 but irregular fire training activities using an above-ground germed burn pit continued until 1990. Impacted soil was removed in 1982 but additional details of the excavation are not well known. Soil samples collected for pre- (April and September 2012) and post- (December 2013) in situ chemical oxidation treatment using a peroxone activated persulfate (OxyZone) technology. Treatment was conducted in Test Cell 1 over 113 days (April–August 2013). Soil samples were collected adjacent to wells; wells outside Test Cell 1 were used as sentry wells. Well IDs for pre- and post-sampling were not provided but the following three pairings were assumed based on Table 2 in the paper: U-20 with SB-106; U-16 with SB-112; and I-1 with SB-109.	Pre-treatment: I-1 (1.2–4.3 m) = 12 ng/g I-2 (1.2–4.3 m) = 83 ng/g U-12 (2.1 m) = 1.2 ng/g U-16 (3.0 m) = 6.7 ng/g U-20 (1.8 m) = ND (LOQ = 0.68–0.72 ng/g) Post-treatment: SB-101 (4.3 m) = 8.08 ng/g SB-105 (1.8 m) = 0.83 ng/g SB-106/U-20 (1.8 m) = 0.31 ng/g SB-106 (4.3 m) = 5.08 ng/g SB-107 (1.8 m) = 2.11 ng/g SB-107 (4.3 m) = 3.99 ng/g SB-108 (1.8 m) = 1.48 ng/g SB-108 (4.3 m) = 4.83 ng/g SB-109/I-1 (3 m) = 1.44 ng/g SB-111 (4.3 m) = 11.85 ng/g SB-112 (1.8 m) = 2.57 ng/g SB-112/U-16 (3 m) = 1.4 ng/g SB-114 (1.8 m) = 3.63 ng/g SB-114 (4.3 m) = 16.17 ng/g (LOQ = 0.12 ng/g)
Venkatesan and Halden (2014)	United States (Baltimore, Maryland)	Archived agricultural soil (nonamended) collected during 2005–2008 at a depth of 0–20 cm from the United States Department of Agriculture-Agricultural Research Service Beltsville Agricultural Research Center; number of sampling sites and number of samples not provided.	Nonamended: n = NR, DF 0% Amended: n = NR, authors reported that PFHxS was not consistently detected in biosolids-amended mesocosms (MDL = 0.03–0.14 ng/g dw for all PFAS)

Study	Location	Site Details	Results
		Biosolids-amended soil obtained by mixing biosolids and soil at a volumetric ratio of 1:2. Biosolids were from Back River WWTP in Baltimore, a full-scale activated sludge treatment plant. Raw wastewater was primarily from domestic sources with only minor contribution (1.9%) from industry.	
Blaine et al. (2013)	United States (Midwest)	Urban and rural full-scale field study with control (nonamended) and biosolids-amended plots. Three agricultural fields were amended (0.5×, 1×, or 2×) with municipal biosolids. Urban biosolids (1× and 2×) were from a WWTP receiving both domestic and industrial waste. Rural biosolids (0.5×) were from a WWTP receiving domestic waste only. Control plots were proximal to the rural and urban amended corn grain and corn stover field sites; sampling year not provided. Greenhouse study with control (nonamended) and biosolids-amended soil. Nonamended soil obtained from a field that received commercial fertilizers and had a similar cropping system as the nearby municipal soil site. Municipal soil was obtained from a reclamation site in Illinois where municipal biosolids were applied at reclamation rates for 20 years, reaching the cumulative biosolids application rate of 1,654 Mg/ha. Industrially impacted soil was created by mixing composted biosolids from a small municipal (but impacted by PFAA manufacturing) WWTP with control soil on a 10% mass basis. Sampling year not provided.	Field study: Urban non-amended: n = 3–7, DF NR, mean < 0.01 ng/g Urban 1×: n = 3–7, DF NR, mean < 0.01 ng/g Urban 2×: n = 3–7, DF NR, mean < 0.01 ng/g Rural non-amended: n = 3–7, DF NR, mean < 0.01 ng/g Rural 0.5×: n = 3–7, DF NR, mean = 0.16 ng/g (LOQ = 0.01 ng/g) Greenhouse study: Nonamended: n = 3–5, DF NR, mean = 0.44 ng/g Industrially impacted: n = 3–5, DF NR, mean = 1.38 ng/g Municipal: n = 3–5, DF NR, mean = 5.11 ng/g (LOQ not reported)
Canada			
Cabrerizo et al. (2018)	Canada (Melville and Cornwallis Islands)	Catchment areas of lakes in the Cape Bounty Arctic Watershed Observatory on southern Melville Island (West, East, and Headwater lakes) during summer (late July-early August) 2015 and 2016, representing an environment largely unimpacted by direct human activity; data for 19 sampling sites available (S6, S11–S28). Catchment areas of lakes on Cornwallis Island (Resolute, North, Small, Meretta, 9 Mile, and Amituk lakes) near the community of Resolute Bay during summer (late July-early August) 2015 and 2016.	Melville Island lakes: n = 19, DF[a] 95%, range = <LOD–0.0070 ng/g dw Cornwallis Island lakes: n = 8, DF[a] 100%, mean[a] (range) = 0.0267 (0.0003–0.1062) ng/g dw (LOD = 0.0001–0.018 ng/g for all PFAS)

Study	Location	Site Details	Results
		Resolute Bay has a military and civilian airport which discharged its wastewaters into the upper area of the catchment until 1997, three old solid waste landfills 1.5–2 km west of the airport used until the mid-1990s, and Arctic research and military training facilities close to the airport that support activities such as vehicle use, firefighting, and construction/demolition; eight sampling sites (S29–S36).	
Mejia-Avendaño et al. (2017)	Canada (Lac-Mégantic, Quebec)	Site of July 2013 Lac-Mégantic train accident where 63 out of 72 train cars carrying 8 million liters of crude oil derailed and a major oil fire ignited. Seven types of AFFFs and approximately 33,000 L of AFFF concentrates were used. Samples were collected in July 2013 weeks after the accident from the western shores of Chaudière River, at the point where the oil and AFFF runoff reached the river, approximately 500 m from the edge of the derailment site; in July 2015 from the fire burn site and adjacent area in downtown Lac-Mégantic where the soil was continuously excavated for remediation (the site was the closest to the accident site among the areas open to sampling); and from a background, nonimpacted area next to Chaudière River, about 5 km from the accident site, on the east shore of the river and on the opposite side of the accident.	Background: n = 3, DF NR, mean = 0.015 ng/g dw 2013: n = 12 (from 12 sites), DF[a] 92%, range = <LOD–0.747 ng/g dw 2015: n = 11 (from 9 sites), DF[a] 73%, range = <LOD–0.234 ng/g dw (LOD = 0.01 ng/mL; LOQ = 0.02 ng/mL)
Dreyer et al. (2012)	Canada (Ottawa, Ontario)	Two ombrotrophic Mer Bleue bog peat core samples collected in October 2009 and cut into 5-cm segments (nine segments for the first core, eight segments for the second core); Mer Bleue selected because it is undisturbed and well investigated and is located in a meltwater channel of the postglacial Ottawa River. Peat cores sampled to determine their suitability for determining historic atmospheric contamination; contaminants present due to atmospheric deposition only. The year for each segment was estimated through dating of Mer Bleue peat cores collected in the same year for a different study.	First core (first parallel; second parallel): 2009: 0.131; 0.040 ng/g 2006: 0.028; 0.033 ng/g 2001: 0.017; 0.005 ng/g 1992: 0.006; 0.010 ng/g 1983: 0.010; 0.012 ng/g 1973: 0.014; 0.029 ng/g 1962: 0.025; 0.021 ng/g 1945: 0.014; 0.022 ng/g 1927: 0.019; 0.015 ng/g 1912: 0.019; 0.017 ng/g Second core (first parallel; second parallel): 2009: ND; 0.157 ng/g 2006: 0.046 ng/g; ND 2001: 0.006 ng/g; ND 1992: ND; 0.014 ng/g

Study	Location	Site Details	Results
			1983: 0.010; 0.018 ng/g 1973: 0.011; 0.007 ng/g 1962: 0.017; 0.015 ng/g 1945: 0.012; 0.008 ng/g 1927: ND; ND (IDL = 0.0022 ng/g) *Authors estimated the year for each core segment using cores for a different study that underwent dating
Europe			
Groffen et al. (2019)	Belgium (Antwerp)	3M perfluorochemical plant and four sites with increasing distance from plant were selected based on prior biomonitoring studies in the vicinity of the plant. The four sites are: Vlietbos (1 km SE from 3M), Rot-Middenvijver (2.3 km ESE from 3M), Burchtse Weel (3 km SE from 3M), and Fort 4 (11 km SE from 3M). Samples collected in June 2016.	Plant: n = 13, DF 31%, mean, median (range) = 6.88, <LOQ (<LOQ–32) ng/g dw 1 km from plant: n = 10, DF 0% 2.3 km from plant: n = 10, DF 0% 3 km from plant: n = 10, DF 0% 11 km from plant: n = 14, DF 0% (LOQ = 4.91 ng/g dw)
Dauchy et al. (2019)	France (unspecified)	Site where fluorosurfactant-based foams have been used extensively. From 1969 to 1984, the site was an oil refinery, with the exact location of the firefighting training area, frequency of training sessions, and history of firefighting training activities unknown. From 1987 to date, it has been a large training area for firefighters. Samples collected in six areas from two sampling campaigns. First sampling campaign collected 30 soil cores between 2 m and 4 m in June 2015 from areas 1–5 (composite soil samples collected every 25 cm in the topmost meter and then every 50 cm). Second sampling campaign collected 14 soil cores between 4 m and 15 m from areas 1–6 (thickness of composite soil samples ranged from 25 cm to 100 cm) in October 2016. Area 1 stored raw oil products when the oil refinery was operating; a preliminary survey showed hydrocarbon traces in the area, suggesting that an incident had occurred and that fluorinated surfactants could have been used. Area 2 is one of the main areas used for firefighting activities since 1987; training sessions held directly on the ground before 10-cm	Area 1: SC-71, SC-72, SC-73, SC-74, SC-75 = <2 ng/g dw (1–2 m) SC-76, SC-77, SC-78 = <2 ng/g dw (0–1 m) Area 2: SC-58 = 2,344, 322, 277, 101, 95, 147 ng/g dw (0–0.25, 0.5–0.75, 1–1.5, 2–2.5, 3–3.5, 3.5–4 m) SC-59 = 79, 37, 113, 61, 25 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 3–3.5 m) SC-60 = 37, <20, 6, 5, 9, 6, <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 2–2.5, 2.5–3 m) SC-61 = 88, <20, 11, 13, <20, 30, 58 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m) SC-62 = 231, <20, 25, 59, 46 ng/g dw (0–0.25, 0.5–0.75, 1–1.5, 2–2.5, 3.5–4 m) SC-63 = 112, 54, 126, 53, 32, 12, 11, 10, 6 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5 m)

Study	Location	Site Details	Results
		thick concrete slab was built in the 1990s. Area 3 was used for firefighting activities since 1987 and is situated on a 1-meter thick concrete slab on the foundations of the former oil refinery. Area 4 corresponds to the site's WWTP where sludge and sediment from a lagoon were stored directly on the ground; influents of the WWTP are highly contaminated by PFAS. Area 5 was used for firefighting training exercises by the former oil refinery. Area 6 is used for firefighting exercises with tank trucks.	SC-64 = 6, 15, 29, 49, 14, 6, <4 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m) SC-65 = 12, 30, 25, <4, 17, 18, 21 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 3–3.5, 3.5–4 m) SC-66 = 8, <4, <4, 8, 3, 2, <2, <2, <2, 8 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5, 3.5–4 m) SC-58b = 17, 2, <10, 4 ng/g dw (4–5, 5–6, 9–10, 14–15 m) SC-59b = 14, 39, 34, 11, 63 ng/g dw (3–4, 4–5, 6–7, 9–10, 14–15 m) SC-65b = 3, 15, 5, 5 ng/g dw (4–5, 7–9, 9–11, 14–15 m) SC-67 = 4, 5, 5, 12 ng/g dw (0–1, 1.3–2, 2–3, 4–5 m) Area 3: SC-40 = <4 ng/g dw (0–1 m) SC-43 = <2 ng/g dw (1–2 m) SC-45 = <2 ng/g dw (0–1 m) SC-47 = 18 ng/g dw (0–1 m) SC-48 = <4 ng/g dw (0–1 m) SC-41 = 5, <10 ng/g dw (0–0.25, 1–2 m) SC-42 = <10 ng/g dw (0–0.25, 1–2, 3–4 m) Area 4: SC-33 = <20 ng/g dw (0–1 m) SC-34 = 13 ng/g dw (1–2 m) SC-35 = <2 ng/g dw (0–1m) SC-36 = <4 ng/g dw (0.3–1 m) SC-37 = 43 ng/g dw (0.1–1.1 m) SC-37b = 11, 6, <10 ng/g dw (0–0.25, 1–1.5, 3–4 m) SC-38 = 35, 2 ng/g dw (0.25–1, 2–3 m) Area 5: SC-10 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–3.5 m) SC-11 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75, 0.75–1, 1–1.5, 1.5–2, 2–2.5 m)

Study	Location	Site Details	Results
			SC-12 = <2 ng/g dw (0–0.25, 0.25–0.5, 0.5–0.75 m) Area 6: SC-21 = 148, 290, 273, 278, 98 ng/g dw (0–0.25, 0.25–1, 2–3, 8–9, 13–15 m) SC-22 = 8, 11, 6, 7 ng/g dw (0–0.25, 0.25–1, 1–2, 3–4 m) SC-23 = 59, 29, 29, 16 ng/g dw (0–0.25, 0.25–1, 1–2, 3–4 m) SC-24 = 85, 31, 43 ng/g dw (0–0.25, 1–2, 3–4 m) SC-25 = 7, 26 ng/g dw (0–0.25, 1.5–2 m) SC-26 = 4, 3 ng/g dw (2–3, 4–5 m) (LOQ = 2 ng/g dw)
Skaar et al. (2019)	Norway (Ny-Ålesund)	Samples collected in June 2016 in and around the international research facilities (Ny-Ålesund) near local firefighting training site. Background soil samples were collected at representative locations.	Background: n = 8, DF 0% Contaminated: n = 2, DF[a] 100%, mean[a] (range) = 29.42 (13.82–45.02) ng/g dw (IDL = 0.003 ng; LOD = 0.001 ng/g dw; LOQ = 0.002 ng/g dw) *Values reported for sum of branched and linear PFHxS isomers *Table 1 and Table S2 reported a total of nine samples across background and contaminated sites; however, Tables S11 and S13 report a total of ten samples (two contaminated sites from Table S11 and eight background sites from Table S13
Hale et al. (2017)	Norway (Gardermoen)	Samples collected in June 2015 from six locations around a firefighting training facility west of the Oslo airport site. Samples were taken at 0–1 m, 1–2 m, 2–3 m, and 3 to groundwater table level (which was in all cases above 4 m). Facility was established in 1989 and AFFF was used extensively. AFFF containing PFOS was banned at the facility in 2007 and a complete ban on organofluorine AFFF was enforced in 2011. The soil is known to be contaminated with a range of perfluorinated compounds.	n = 22 (across all depths), DF 36%, range = 3.0–25.3 ng/g (LOD = 1 ng/g) *Range reported for detects *The DF and range extracted are reported in the results (Section 3.1); however, Table S2 of the individual sample data show all concentrations ranging from <1.8 to <2.5 ng/g
Filipovic et al. (2015)	Sweden (Stockholm)	Five locations at a closed air force base F18 in Tullinge Riksten, 19 km south of Stockholm city	Intermediate soil depot: n = 10, DF 80%, range = <0.02–3.1 ng/g dw

Study	Location	Site Details	Results
		center, where AFFFs were used. Samples collected in two sampling campaigns in December 2011 and May 2012. The air force base was formally demobilized in 1986 but continued to be used as an air force school for combat command and air surveillance until 1994. Of note, the air force base encountered numerous accidents and incidents during the transfer from propeller era to the jet engine era, including planes crashing upon takeoff and landing, fire incidents, accidental dispersion of jet engine starting fuel. The area was sold to a land developer in 1996 and is in the process of being transformed into a municipal area. Of the five sites samples, the intermediate soil depot site (sampling depth 0–3 m) and the soil depot site (sampling depth 0–3 m) were assembled by the land developing corporation; the J34 Hawker Hunter site was the collision site of two J34 Hawker Hunter aircrafts in the 1960s, resulting in huge amounts of firefighting foam being dispersed (sampling depth 0.5–1.0 m); the main firefighting training facility was intensively used between 1970–1985 and sparsely used between 1985–1994 (sampling depth 0.5–1.0 m); and the old fire station was used to store AFFF stock solution (sampling depth 0.5–1.0 m).	J34 Hawker Hunter site: n = 5, DF 0% Main firefighting training facility: n = 15, DF 100%, mean[a] (range) = 3.9 (0.1–21.3) ng/g dw Old fire station: n = 5, DF 100%, mean[a] (range) = 3.7 (1.6–6) ng/g dw Soil depot: n = 10, DF 40%, range = <0.02–0.33 ng/g dw (MDL = <0.02–<0.1 ng/g dw)
Harrad et al. (2020)	Ireland (multiple cities)	Samples collected from ten municipal solid waste landfills upwind and downwind at each site between November 2018 and January 2019. At each upwind/downwind location, nine sub-samples of soil were taken in a "W" formation. Samples were collected from the same areas as air samples and were taken within the boundaries of the landfill operational facility. Soil used as capping on landfill cells was not sampled to ensure soil samples were not collected from soil placed after landfill operations ceased and that farming activities would not influence concentrations found. Waste accepted by the landfills included: municipal solid waste, industrial (non-hazardous) waste, construction & demolition, and biomedical waste.	Downwind: n = 9, DF[a] 11%, mean, median (range) = 0.00077, <0.001 (<0.001–0.0029) ng/g dw Upwind: n = 7, DF[a] 57%, mean, median (range) = 0.0018, 0.0023 (<0.001–0.0037) ng/g dw (LOD = <0.001 ng/g dw) *Non-detects replaced by ½ LOD *Soil samples from three upwind locations and one downwind location destroyed in transit from field to laboratory
Grønnestad et al. (2019)	Norway (Granåsen, Jonsvatnet)	Upper layer soil samples (3–10 cm in depth) collected in June 2017 and 2018 from Granåsen (skiing area)	Reference area: n = 10, DF 0% Skiing area: n = 10, DF 0%

Study	Location	Site Details	Results
		and Jonsvatnet (reference site). Five samples per year were analyzed for each site. Located 10 km from Trondheim city center, Granåsen is the main arena for winter sports in Trondheim and hosts an annual ski jumping World Cup event and regional, national, and international competitions in cross-country skiing. Located 15 km away from Trondheim city center, Jonsvatnet is a natural forest area not used for ski-sports and is in the vicinity of an ecological farm next to Lake Jonsvatnet. The two study areas have similar vegetation.	(LOQ = 0.032 ng/g dw) *For the reference area, Table S2 reported a DF = 10% but a range, mean, and standard deviation of <LOQ
Sammut et al. (2019)	Malta	Six surface soil samples (#10, 14, 15, 18, 20, and 22) collected between June and August 2015 from random small urban fields	n = 6, DF[a] 17%, range = ND–0.12 ng/g (LOD = 0.40 ng/g; LOQ = 0.50 ng/g) *The study reported two samples <LOQ, two samples ND, one sample <LOD, and one sample with a detectable value

Notes: AFFF = aqueous film-forming foam; DF = detection frequency; dw = dry weight; GCA = groundwater contamination area; LOD = limit of detection; LOQ = limit of quantitation; MDL = method detection limit; 0.5×, 1×, or 2× = ½, 1, or 2 times the agronomic rate of biosolids application to meet nitrogen requirements of the crop; m = meter; cm = centimeter; ND = not detected; NR = not reported; RL = reporting limit; WWTP = wastewater treatment plant.

[a] Venkatesan and Halden (2014) reported that PFHxS was not consistently detected in biosolids-amended mesocosms.

D.3.7. Sediment

When released into water, PFHxS is not expected to adsorb to suspended solids and sediments based on its physico-chemical properties (NCBI, 2022a). However, PFHxS was detected in 28% of sediment samples collected along the Mississippi River shoreline in the vicinity of a PFAS production facility in Minnesota, at a maximum concentration of 11.5 ng/g (NCBI, 2022a; ATSDR, 2021; 3M, 2007). PFHxS was also detected in 96% of sediment samples collected from two coves of the Mississippi River (East and West coves) located at the eastern and western ends of the PFAS production facility, at a maximum concentration of 126 ng/g (ATSDR, 2021; 3M, 2007). PFHxS was not detected in any Mississippi River transect sediment samples (collected at points crossing the river near the PFAS facility) (ATSDR, 2021; 3M, 2007). PFHxS has been detected in sediment core samples collected from three Canadian Arctic lakes in 2003 and 2005 at concentrations ranging from approximately 1 ng/g to 10 ng/g (NCBI, 2022a; Stock et al., 2007).

D.4. Recommended RSC

The EPA followed the Exposure Decision Tree approach to determine the RSC for PFHxS (USEPA, 2000b). The EPA first identified the U.S. general population as the population of concern (Box 1; see Section 2.4.2). Second, the EPA identified several relevant PFHxS exposures and pathways (Box 2), including dietary consumption, incidental oral consumption via exposure to dust, consumer products, and soil, dermal exposure via soil, consumer products, and dust, and respiration via ambient air. Several of these may be potentially significant exposure sources. Third, the EPA determined that there was not adequate quantitative data to describe the central tendencies and high-end estimates for all of the potentially significant sources (Box 3). For example, studies from Canada and Europe indicate that indoor air may be a significant source of exposure to PFHxS. At the time of the literature search, the EPA was unable to identify studies assessing PFHxS concentrations in indoor air samples from the U.S. and therefore, the agency does not have adequate quantitative data to describe the central tendency and high-end estimate of exposure for this potentially significant source in the U.S. population. However, the agency determined there were sufficient data, physical/chemical property information, fate and transport information, and/or generalized information available to characterize the likelihood of exposure to relevant sources (Box 4). Notably, based on the studies summarized in the sections above, there are significant known or potential uses/sources of PFHxS other than drinking water (Box 6), though there is not information available on each source to make a characterization of exposure (Box 8A). For example, there are several studies from the U.S. indicating that PFHxS may occur in multiple food products (e.g., eggs, seafood, meats, vegetables, fruit) and consumer products (e.g., building materials, clothing, furniture). However, the majority of studies examined very few samples (i.e., n=1-5) of each type of media. Therefore, it is not possible to determine which source, if any, can be considered major or minor contributors to total PFHxS exposure. Given these considerations, following recommendations of the Exposure Decision Tree (USEPA, 2000b), the EPA recommends an RSC of 20% (0.20) for PFHxS.