



Attachment 30



Perfluoroheptanoic acid induces Leydig cell hyperplasia but inhibits spermatogenesis in rats after pubertal exposure

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Highlights

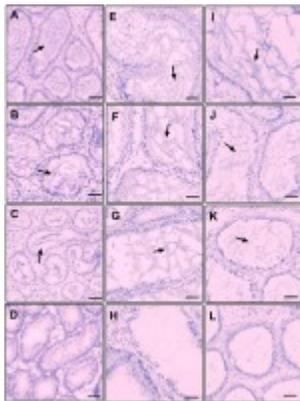
- PFHpA induces Leydig cell hyperplasia during puberty.
- PFHpA induces hypergonadotropic hypogonadism in rats.
- PFHpA down-regulates *Cyp11a1*, *Cyp17a1*, *Hsd3b1* expression in individual Leydig cells.
- PFHpA induces AKT1, AKT2, ERK1/2 and JNK phosphorylation.
- PFHpA increases BCL2 and decreases BAX in the testis.

Abstract

Perfluoroheptanoic acid (PFHpA) is a short-chain alternative to long-chain perfluoroalkyl substances, which have been reported to possess reproductive toxicity. However, it is unclear whether PFHpA affects Leydig cell development during puberty. The 35-day-old Sprague Dawley

male rats were exposed to PFHpA by gavage with 0 (corn oil), 10, 50, and 100 mg/kg/day for 21 days. PFHpA did not affect the body weight of rats, but it reduced testis weight, relative testis weight, and epididymis weight at 100 mg/kg. It significantly increased serum testosterone, luteinizing hormone, and follicle-stimulating hormone levels at a dose of 100 mg/kg without affecting serum estradiol levels. PFHpA suppressed sperm production at a dose of 100 mg/kg. PFHpA induced Leydig cell hyperplasia (increased number of CYP11A1-positive Leydig cells) at a dose of 100 mg/kg, but down-regulated the expression of *Cyp11a1*, *Hsd3b1*, and *Cyp17a1* in individual Leydig cell *pe se* and up-regulated the expression of *Fshr* in the Sertoli cell *pe se*. PFHpA did not affect the number of HSD11B1 (a biomarker for more mature Leydig cells) positive Leydig cells and SOX9 positive Sertoli cells. PFHpA increased BCL2, and the phosphorylation of AKT1, AKT2, ERK1/2, and JNK, but decreased BAX levels. However, it had no effect on SIRT1 and PGC-1 α levels. In conclusion, PFHpA induces Leydig cell hyperplasia due to the increase in the secretion of luteinizing hormone through negative feedback after down-regulating the expression of steroidogenic enzymes and inhibiting testosterone production in individual Leydig cells. This proliferation may be mediated by increasing BCL2 and phosphorylation of AKT, ERK1/2, and JNK, and decreasing BAX level.

Graphical abstract



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Keywords

Perfluoroheptanoic acid; PFHpA; Immature Leydig cells; Testosterone short-chain perfluoroalkyl substances

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Exposure Assessment Tools by Chemical Classes - Other Organics

Overview | Dioxins and PCBs | Flame Retardants | Hydrocarbons | PFCs | Phthalates | VOCs |
POPs | Tools | References

Overview



Organic compounds are generally defined as substances with structures based on carbon. Although some carbon-based substances are classified as inorganic, all substances classified as organic are carbon-based. Organic compounds comprise a highly variable group of ubiquitous substances with uses ranging from consumer products (e.g., ingredients in personal care products, building materials, food packaging, clothing, furniture) and drugs (e.g., Ibuprofen) to fuel components (e.g., hydrocarbons) and explosives (e.g., TNT). Many pesticides are also classified as organic – these are discussed in more detail in the Pesticides Module in the Chemical Classes Tool Set of EPA ExpoBox.

Organic compounds can be natural, synthetic, or semi-synthetic. The source and method by which organic compounds are produced can affect their properties and expected exposure scenarios. For example, many natural organic compounds, such as lipids, proteins, and carbohydrates, are plant-based and have medicinal or nutritional benefits that are exploited through direct exposure to these compounds.

On the other hand, some synthetic organic compounds are produced unintentionally, such as combustion byproducts like polycyclic aromatic hydrocarbons (PAHs) and dioxin released from industrial chlorinated processes, and exposures to these compounds might be largely indirect.

Organic compounds are often grouped according to structural similarities. Different “classes” of organic compounds refer to groups based on specific structural characteristics, including chemical bonds and functional groups. Similarities in chemical structure affect the properties of organic compounds, and thus their uses and human exposure considerations.

Importance of Physicochemical Characteristics

After contaminants are released to the environment, their transport, dispersion, and transformation are governed by certain physicochemical properties.

Physicochemical properties data can help determine whether a contaminant is likely to remain in place, partition to other media, or transform physically, chemically, or biologically after release.

Physicochemical properties data may be determined by measurements or estimated based on chemical structure.

Sources of physicochemical values include:

- EPA Aggregated Computational Toxicology Resource (ACToR) <<https://actor.epa.gov/actor/home.xhtml>>
- EPA Estimation Program Interface (EPI) Suite <<https://epa.gov/tsc-screening-tools/epi-suite/estimation-program-interface>>
- EPA High Production Volume Information System (HPVIS)
- EPA Superfund Chemical Data Matrix <<https://epa.gov/superfund/superfund-chemical-data-matrix-scdm>>
- National Library of Medicine (NLM) Hazardous Substances Data Bank (HSDB) EXIT
<<https://www.nlm.nih.gov/toxnet/index.html>>
- Organization for Economic Cooperation and Development (OECD) eChemPortal EXIT
<<https://www.echemportal.org/echemportal/>>

The environmental fate, transport, and exposure of organic compounds can be estimated based on models that take into account certain physicochemical properties that can help predict partitioning behavior in the environment. Some physicochemical properties are also important to consider when designing appropriate analytical methods for classes of organic compounds.

The behavior of organic compounds varies widely; however, some generalities can be made for subgroups of compounds. The behavior of organic compounds in the environment is affected by the physicochemical properties of the compound, including molecular weight and the presence of specific functional groups, such as alcohols, amines, carboxylic acids, and ketones.

These properties and characteristics often affect a compound's solubility, partitioning behavior, and reactivity in relatively predictable ways, allowing exposure assessors to easily conduct screening-level assessments to estimate which organic compounds might be of highest concern.

It is not the intent of this tool set to provide information relevant to all organic compounds. As mentioned above, organics comprise a highly variable group of substances with a large range of uses. Instead, this tool set focuses on tools for assessing exposures to five specific groups of organic compounds of concern, including:

- Dioxins, Furans, and PCBs
- Flame Retardants
- Hydrocarbons
- Perfluorinated Compounds (PFCs)
- Phthalates and Bisphenol A (BPA)

These compound groups are known to possess properties that increase the potential for human and ecological exposure and are addressed specifically in this tool set because of their relevance to developing policy and risk management efforts.

Many organic compounds identified above fall into a category known as persistent organic pollutants, or POPs, including PCBs, dioxins, furans, brominated flame retardants, and perfluorinated compounds (PFCs). These compounds have long half-lives in environmental media and are subject to long-range transport through the environment, meaning that exposure can occur long after the chemical is released and at locations far from the original source of the contaminant.

Many persistent organic compounds also have a tendency to accumulate in biota, meaning that significant secondary exposures can occur through ingestion of foods from higher levels of the food chain, like fish, meat, and dairy products. Information specific to POPs is also provided in this tool set.

Volatile Organic Compounds, or VOCs, are another category that overlaps with the other groups described in this tool set. Similar to POPs, VOCs are defined by a physicochemical property that imparts unique fate and transport and exposure considerations - high vapor pressure at room temperature. Because of their high volatility, VOCs are common air pollutants in both outdoor and indoor environments.

VOCs can be further categorized based on boiling point as very volatile organic compounds (VVOCs) and semi volatile organic compounds (SVOCs). Several substances including DDT, chlordane, and PCBs are classified as both SVOCs and POPs. Information specific to VOCs is also provided in this module.

PCB and dioxin resources can be found here:

Polychlorinated Biphenyls (PCBs) <<https://epa.gov/pcbs>>

Dioxin <<https://epa.gov/dioxin>>

Resources related to hydrocarbons and specifically PAHs are also available.

Technology Transfer Network - Polycyclic Organic Matter (POM) <<https://epa.gov/haps/health-effects-notebook-hazardous-air-pollutants>>

Toxic Substances Portal - Polycyclic Aromatic Hydrocarbons (PAHs) [EXIT](#)

National Biomonitoring Program - Polycyclic Aromatic Hydrocarbons (PAHs) [EXIT](#)

<https://www.cdc.gov/biomonitoring/pahs_factsheet.html>

Dioxins, Furans, and PCBs

- Physicochemical Properties
- Routes
- Media
- Exposed Populations
- Tools

Polychlorinated dibenzo dioxins (PCDDs) and furans (PCDFs) and polychlorinated biphenyls (PCBs) are examples of persistent organic pollutant (POPs).

Dioxins, furans, and PCBs share a similar base structure of two 6-carbon rings with variable chlorine substituents. The term "dioxin" can be somewhat ambiguous, however, because the term is used to describe various groups of chemicals as well as one chemical, in particular. "Dioxins" generally refer to a group of seven PCDDs, ten PCDFs, and twelve

"dioxin-like" PCBs, which are grouped together based on similar structural characteristics and mechanisms of action. The specific PCDD congener 2,3,7,8-TCDD is the most widely studied dioxin and is therefore often referred to simply as "dioxin".

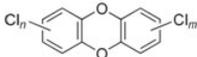
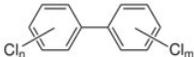
- Learn more at Dioxin <<https://epa.gov/dioxin>>

PCDDs and PCDFs are not commercially produced or manufactured chemicals; rather, they are released into the environment as a by-product of combustion (burning) or other industrial processes. PCBs, on the other hand, were manufactured until 1979 for use in numerous commercial and industrial applications. The flame-retardant and insulating properties of these compounds were exploited for use in transformers, capacitors, cable insulation and other electrical equipment, hydraulic systems, plastics, paints, and carbonless copy paper, among other applications. Before they were banned, PCBs were released into the environment during manufacture and use and are still released into the environment through poorly maintained hazardous waste sites or leaks from old equipment.

Physicochemical Properties

PCDDs, PCDFs, and PCBs are persistent, halogenated, aromatic hydrocarbons characterized by a basic structure of two joined, chlorinated, 6-carbon rings. PCBs have anywhere from 2 to 10 chlorine atoms bonded to the total 12 carbon atoms, resulting in 209 different congeners. PCDDs and PCDFs are different from PCBs in that the two carbon rings are joined together by oxygen atoms, creating a three - ring structure. Chlorine atoms bond to the remaining available carbons to create 75 distinct PCDD congeners and 135 PCDF congeners. Of the 75 PCDDs, 7 of them (including 2,3,7,8-TCDD) are especially toxic.

Ten of the 135 PCDF congeners and 12 of the 209 PCBs have "dioxin-like" properties. The number and configuration of the chlorine compounds play a large role in determining properties of each PCDD, PCDF, and PCB congener. For example, melting point and lipophilicity increase with increasing degree of chlorination and vapor pressure and water solubility decrease.

Examples of Organochlorine Structures: Dioxins, Furans, and PCBs		
PCDDs	PCDFs	PCBs
		

The table below provides a summary of key physicochemical factors that are likely to affect partitioning and fate of dioxins, furans, and PCBs in the environment. For chemical-specific values, consult the resources provided in the introduction to this module.

Property	Fate and Transport Implications
Dioxins and Furans	
Vapor pressure at 25°C (atm)	Low vapor pressure indicates that PCDDs and PCDFs will not readily volatilize from the pure organic state.

Henry's Law Constant	Henry's Law Constants indicate that volatilization of PCDDs and PCDFs from water to air might be a significant transfer mechanism during warmer temperatures, which could result in seasonal volatilization/deposition and long-range air transport.
Solubility in water (mg/L)	Low water solubility indicates that PCDDs and PCDFs will not readily dissolve in water.
Octanol-Water Partition Coefficient (log value)	Moderate-high log octanol-water partition coefficients indicate that PCDDs and PCDFs will preferentially partition to organic matter in soils and sediments, bioaccumulate, and biomagnify in aquatic ecosystems and possibly in terrestrial ecosystems and humans.
Octanol-Air Partition Coefficient (log value)	High log octanol-air partition coefficients indicate that PCDDs and PCDFs will preferentially partition to organic substances from air and bioaccumulate and biomagnify in terrestrial ecosystems and humans.
<p>Summary: Based on the physicochemical properties of PCDDs and PCDFs, these compounds are expected to accumulate in soils, sediments, and both aquatic and terrestrial biota. These substances might be driven to escape from water, which will lead to sorption to organic matter and volatilization to air, particularly during warmer weather. The expected seasonal volatilization/deposition cycle of these chemicals is expected to result in long-range transport in air and ultimate deposition at the poles.</p>	
PCBs	
Vapor pressure at 25°C (atm)	Low to moderate vapor pressure indicates that some PCBs will not readily volatilize from the pure organic state, while others are expected to be semi-volatile.
Henry's Law Constant	Henry's Law Constants indicate that volatilization of PCBs from water to air is expected to be a significant transfer mechanism which could result in seasonal volatilization/deposition and long-range air transport.
Solubility in water (mg/L)	Low water solubility indicates that PCBs will not readily dissolve in water.
Octanol-Water Partition Coefficient (log value)	Moderate log octanol-water partition coefficients indicate that PCBs will preferentially partition to organic matter in soils and sediments, bioaccumulate, and biomagnify in aquatic ecosystems and possibly in terrestrial ecosystems and humans.
Octanol-Air Partition Coefficient (log value)	Moderate-high log octanol-air partition coefficients indicate that PCDDs and PCDFs will preferentially partition to organic substances from air and bioaccumulate and biomagnify in terrestrial ecosystems and humans.

Summary: Based on the physicochemical properties of PCBs, these compounds are expected to accumulate in soils, sediments, and aquatic and terrestrial biota. These substances might be driven to escape from water and volatilize from the pure organic form, which could result in accumulation in air and long-range transport, particularly of semivolatile PCBs. The expected seasonal volatilization/deposition cycle of these compounds is expected to result in ultimate deposition at the poles.

Routes

Dioxins and PCBs are ubiquitous in the environment and partition readily to the fatty tissue of animals, resulting in accumulation in the food chain. Humans are primarily exposed to these compounds through ingestion of contaminated foods such as meat, dairy, fish, and shellfish. In utero exposure and exposure due to ingestion of breastmilk also occur and are exposure pathways of concern.

For PCBs that are semivolatile, inhalation exposure is also possible. However, the relatively low volatility of dioxins, furans, and some PCBs under typical environmental conditions suggests that direct inhalation might be a less significant route of exposure.

Note: The Routes Tool Set of EPA ExpoBox provides additional information and resources organized by route.

Media

Dioxins, furans, and PCBs are often found in sediment and soils. They are slow to degrade and tend to accumulate in living organisms, so can be found in biota and food, particularly dairy, meat, and fish products.

Media	Sources of Dioxins, Furans, and PCBs
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<p>Air</p>	<ul style="list-style-type: none"> • Anthropogenic releases of dioxins to air include a variety of industrial processes, especially those involving combustion/incineration and the production of bleached paper products. Chlorine bleaching processes used to whiten paper pulp, as well as uncontrolled burning of chlorine-bleached paper products, release dioxins. Uncontrolled burning of other types of solid waste and hospital waste can also release dioxins. • Incineration of PCB-containing waste (such as carbonless copy papers or electrical equipment) can release PCBs into the air. Leaks from landfilled electrical equipment can also result in volatilization of PCBs. • Certain older appliances and fixtures such as fluorescent lights, air heating/cooling units, and refrigerators found in homes, schools, offices, and other public buildings, as well as discarded in landfills, can contain PCBs in ballasts and transformers. The PCBs can leak and volatilize, contaminating indoor or outdoor air. • Natural sources of dioxin release include volcanic eruptions and fires. • PCBs are constantly recycled through the environment as a result of numerous natural physical processes. PCBs continuously re-enter air as a result of volatilization from water and soil (ATSDR, 2000).
<p>Water</p>	<ul style="list-style-type: none"> • A number of pesticide and herbicide manufacturing processes can release dioxins as a byproduct, and this release can result in contamination of surface waters. PCBs historically contaminated surface water through similar release following manufacturing processes, however, PCBs are no longer intentionally manufactured and are not a byproduct of other processes. • Production of bleached paper products can result in wastewater and pulp effluent that is contaminated with dioxins. These effluents can be released into surface water bodies, resulting in widespread contamination of surface and groundwater (ATSDR, 2000). • Production and recycling of carbonless copy papers can result in release of PCBs into wastewaters. Leaks from hydraulic fluids in factory machinery can also result in release of PCBs into wastewaters (ATSDR, 2000).

Soil	<ul style="list-style-type: none"> • PCBs and dioxins settle to soil and sediments, and can remain in these media for extended periods. Soil and sediment represent the largest sink of these compounds, compared to air or water. • Historically, dioxins and PCBs were used in pesticide products and applied directly to soils. Historic disposal of industrial wastes and paper mill sludge also resulted in soil and sediment contamination (ATSDR, 2000). • Sewage sludge used as fertilizers in farm settings can contain small levels of PCBs, resulting in contamination of soils.
Food	<ul style="list-style-type: none"> • Food, particularly fish, represents one an important source of PCB exposure for humans. Vegetables grown in PCB-contaminated soil might also contain high levels of PCBs.
Aquatic Biota	<ul style="list-style-type: none"> • PCBs released to water readily bioaccumulate and biomagnify in aquatic biota. Consumption of seafood from contaminated water bodies can result in exposure to PCBs.
Consumer Products	<ul style="list-style-type: none"> • PCBs are no longer manufactured in the US for use in consumer products, however, some older appliances found in homes or other buildings may contain PCBs in hydraulic fluids or lubricant oils, which could leak.

Note: The Media Tool Set of EPA ExpoBox provides additional information and resources organized by media.

Exposed Populations

Background exposure to ubiquitous dioxin and PCB mixtures results in body burdens of these compounds in all humans. The general population is further exposed to dioxins and PCBs through ingestion of contaminated food. Specific populations might experience increased exposures to the compounds due to lifestage, geographical location, or behavior.

These populations include developing fetuses, breast-feeding infants, children, human and ecological populations living in certain regions, and certain occupational workers.

- Long range atmospheric transport can result in higher environmental contamination levels in certain regions of the globe; people who live in these regions may have higher levels of exposure.
- The lipophilic nature of dioxins and PCBs contributes to the accumulation of these chemicals in breast milk. Infants who are breastfed may be exposed to the chemicals through ingestion.
- Individuals who consume high amounts of fish or aquatic mammals (i.e., seal, whale), may be exposed to dioxins and PCBs, particularly if the fish is sourced from contaminated waters. Fatty cuts of beef or other meats may also contain measureable dioxin or PCB contamination.
- Certain occupations may result in higher exposure levels to workers.

- PCB-waste is often labeled as industrial waste and treated as hazardous material. People who live near or work at hazardous waste landfills or hazardous waste incineration sites with PCBs may be at risk for higher exposure, especially if proper controls are not in place (ATSDR, 2000).

Note: The Lifestages and Populations Tool Set of EPA ExpoBox has addition information about particular population groups and lifestages.

Tools

- **Key:**  Information sources
-  Data sources
-  Models

Tool Type	Tools	Description
	Agency for Toxic Substances and Disease Registry (ATSDR). Case Studies in Environmental Medicine (CSEM): Substance-Specific Course Series for Health Professionals <small><https://www.atsdr.cdc.gov/csem/csem.html></small>	ATSDR has prepared a series of case studies to help health professionals in their evaluation of patients potentially exposed to hazardous substances. Courses have been developed for various inorganics and fibers—such as arsenic, asbestos, beryllium, cadmium, chromium, lead, and nitrate/nitrite—and volatile and semivolatile compounds, including benzene, toluene, tetrachloroethylene, trichloroethylene, PCBs, and PAHs.
	Agency for Toxic Substances and Disease Registry (ATSDR). (2000). Toxicological Profile for Polychlorinated Biphenyls (PCBs)	Toxicological Profiles contain a review of key studies and other data characterizing the exposure-related health effects and pertinent characteristics and processes that affect human exposures. Sections include other relevant information on releases to the environment, environmental fate, levels monitored in the environment, potential exposures, and analytical methods.
	California EPA Office of Scientific Affairs. (1994). Intermedia Transfer Factors for Contaminants Found at Hazardous Waste Sites: TCDD <small><https://dtsc.ca.gov/wp-content/uploads/sites/31/2018/01/tcdd.pdf></small>	This document was prepared to support the transport and transformation modules within the CalTox model for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD).

 	<p>Centers for Disease Control and Prevention (CDC). (2009). Fourth National Report on Human Exposure to Environmental Chemicals https://www.cdc.gov/exposurereport/</p>	<p>This report provides information on NHANES biomonitoring data (i.e., in human blood and urine) in the U.S. population. The 4th report includes new data from 2003-2004 as well as data from the 2nd and 3rd reports (from 1999-2000 and 2001-2002, respectively). Data are included for phthalates, PFCs, PAHs, PCBs, dioxins, VOCs, metals, flame retardants, and multiple pesticides among other substances.</p>
 	<p>Centers for Disease Control and Prevention (CDC). (2017). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables https://www.cdc.gov/exposurereport/</p>	<p>Updated Tables (March 2018) for the Fourth National Report on Human Exposure to Environmental Chemicals provide data for 346 chemicals (38 new and 176 updated since January 2017). These updated tables are represented in two separate volumes: Volume One contains data tables for chemicals measured in the general U.S. population, and Volume Two contains data tables for persistent organic pollutants and pesticides measured in pooled samples and adult cigarette smokers and nonsmokers.</p>
	<p>Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: All Chemicals https://www.cdc.gov/biomonitoring/chemical_factsheets.html</p>	<p>These fact sheets from the National Biomonitoring Program summarize exposure and health effects for various substances including: BPA, dioxins, PFCs, phthalates, PAHs, PBDEs and several metals including lead, mercury, arsenic, and cadmium among others.</p>
	<p>Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: Dioxins, Furans and Dioxin-Like Polychlorinated Biphenyls https://www.cdc.gov/biomonitoring/dioxinlikechemicals_factsheet.html</p>	<p>This resource provides a summary of exposure and health effects of dioxins, furans, and dioxin-like PCBs in the US. Includes links to summary information from other agencies (ATSDR, EPA, FDA, NIH).</p>
	<p>German Federal Environment Agency/Umweltbundesamt. Human Biomonitoring Commission (HBC) https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission-hbm-commission</p>	<p>HBC was established in 1992 as a joint effort between Germany's Federal Health Office and Federal Environment Agency. The Commission, composed of experts who are appointed every three years, seeks to clarify fundamental and practical issues related to human biomonitoring (HBM). Their efforts have included derivation of reference values (e.g., bisphenol A in urine; PCBs in human milk) and HBM values (e.g., cadmium, mercury, bisphenol A, PCB); monographs on individual substances; and position papers on various contaminants and analytical approaches (e.g., formaldehyde, PCBs in indoor air, creatinine correction approach, hair analyses).</p>

	<p>National Institute of Environmental and Health Sciences (NIEHS). Health & Education: Dioxins https://www.niehs.nih.gov/health/topics/agents/dioxins/</p>	<p>This website contains a series of pages and PDFs regarding NIEHS actions on dioxins and general information about dioxin human health effects.</p>
	<p>U.S. Department of Agriculture (USDA). Food Safety and Inspection Service: Production and Inspection Fact Sheets https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation</p>	<p>This resource is a series of fact sheets on dioxin in U.S. meat and poultry and includes information on dioxin in livestock, poultry, and eggs.</p>
	<p>U.S. EPA. (2013). America's Children and the Environment (3rd ed.) https://www.epa.gov/ace</p>	<p>This EPA report presents information on environmental stressors that can affect children's health. Information on biomonitoring for several organics, including PFCs, PBDEs, PCBs, phthalates, and BPA as well as lead and mercury are provided.</p>
	<p>U.S. EPA. Database of Sources of Environmental Releases of Dioxin-Like Compounds in the United States</p>	<p>This resource is a repository of specific CDD/CDF emissions data from all known sources in the United States. The database information can be analyzed to track emissions of CDD/CDF over time, compare specific profiles between and among source categories, and develop source specific emission factors that can then be used to develop emission estimates.</p>
	<p>U.S. EPA. Dioxin website https://www.epa.gov/dioxin</p>	<p>This EPA-wide dioxin portal provides summary information on dioxins and includes links to past EPA documents, including health assessments, workshops, studies, and surveys of sources.</p>
	<p>U.S. EPA. Exposure Model for Soil-Organic Fate and Transport (EMSOFT)</p>	<p>EMSOFT is used to explore how chemicals in the soil can vaporize and become an inhalation exposure risk in humans. The model can predict concentrations of soil contaminants, rates of transfer into the atmosphere, and potential ingestion and dermal contact risks. EMSOFT can also estimate average contaminant concentrations at a given soil depth over time.</p>
	<p>U.S. EPA. (2008). Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment https://www.epa.gov/risk/framework-application-toxicity-equivalence-methodology-polychlorinated-dioxins-furans-and</p>	<p>This document describes the toxicity equivalence methodology for assessing ecological risks associated with exposure to mixtures of PCDDs, PCDFs, and dioxin-like PCBs, in which risks are assessed based on potency estimates of dioxin-like chemicals relative to 2,3,7,8-TCDD. The Ecological Toxicity Equivalence Factor (Eco-TEF) framework is intended to be a guidance document only.</p>

	<p>U.S. EPA. (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories - Volume 2: Risk Assessment and Fish Consumption Limits</p> <p><https://www.epa.gov/sites/default/files/2015-06/documents/volume2.pdf></p>	<p>This document provides guidance on the development of risk-based fish meal consumption limits for 25 high-priority target analytes that include organochlorine and organophosphate pesticides, PAHs, PCBs, dioxins/furans, and some metals.</p>
	<p>U.S. EPA. National Study of Chemical Residues in Lake Fish Tissue <https://www.epa.gov/fish-tech/studies-fish-tissue-contamination></p>	<p>The National Lake Fish Tissue Study is a screening-level study conducted to estimate the national distribution of selected persistent, bioaccumulative, and toxic chemicals in fish tissue from lakes and reservoirs of the continental United States. It was conducted 2000–2003 and evaluated 268 chemicals in fish.</p>
	<p>U.S. EPA. (1996). PCBs: Cancer Dose-Response Assessment and Application to Environmental Mixtures</p>	<p>This archived EPA report describes cancer dose-response assessment for polychlorinated biphenyls (PCBs) and identifies the relevant characteristics that should be considered in evaluating human health risks from PCB mixtures.</p>
	<p>U.S. EPA. Site Evaluation for Dioxin at Superfund Sites <https://www.epa.gov/superfund/site-evaluation-dioxin-superfund-sites></p>	<p>This website provides links to documents relevant to site evaluation of dioxin at Superfund sites, including guidance documents related to dioxin sampling and analytical methods for dioxin. Guidance documents include: Guidance on Systematic Planning Using the Data Quality Objectives Process; Guidance on Choosing a Sampling Design for Environmental Data Collection for Use in Developing a Quality Assurance Project Plan; User Guide - Uniform Federal Policy Quality Assurance Project Plan Template For Soils Assessment of Dioxin Sites; and Template - Uniform Federal Policy Quality Assurance Project Plan For Soils Assessment of Dioxin Sites.</p>
	<p>Wisconsin Department of Health Services. Polychlorinated Biphenyls (PCBs) <https://www.dhs.wisconsin.gov/chemical/pcb.htm></p>	<p>This website provides information on exposure to PCBs including exposure from contaminated air, food, and drinking water.</p>
	<p>World Health Organization (WHO). (2010). Dioxins and their Effects on Human Health Fact Sheet <http://www.who.int/mediacentre/factsheets/fs225/en/></p>	<p>This fact sheet from WHO provides information on sources of dioxin, sensitive subgroups, and WHO activities related to dioxin.</p>

Flame Retardants

- Physicochemical Properties
- Routes

- Media
- Exposed Populations
- Tools

Flame retardants are chemicals or other manufactured materials that confer qualities that resist or inhibit the spread of fire. Although some flame-retardant organic compounds have been banned or voluntarily phased out by manufacturers due to health concerns (e.g., many polybrominated diphenyl ethers [PBDEs] and polychlorinated biphenyls [PCBs]), these compounds may persist in the environment.

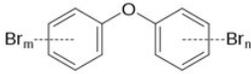
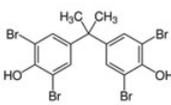
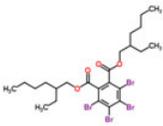
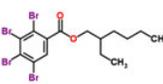
A wide range of organic chemicals are used in flame-retardant products, but most can be classified as halogenated (i.e., functionalized with bromine or chlorine), phosphorous-based, nitrogen-based, or as a combination of the aforementioned components. Two principal processes are used for incorporating flame-retardant materials into products: reactive and additive.

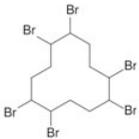
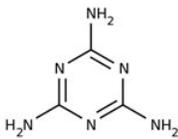
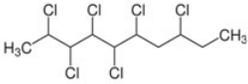
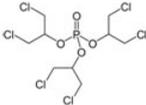
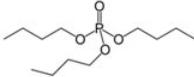
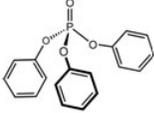
Whereas the reactive processes produce covalent bonds between the flame retardant and a polymer matrix that effectively lock most of the flame-retardant chemical in the product, the additive processes do not form such bonds and therefore increase the potential for the flame-retardant chemical to leach out of the product to which it is applied.

One of the principle mechanisms by which flame-retardant chemicals (particularly of the halogenated variety) confer flame resistance is through gas-phase radical quenching in which the flame-retardant material thermally degrades and releases chemical radicals that are highly reactive with oxygen, thereby reduces the amount of free oxygen available to supply the combustion process.

The result of this process is the release of some of the halogen atoms from the flame-retardant chemical and the transformation of the chemical from highly halogenated substances to a lower-halogenated congener.

Example structures selected from the range of traditional and emerging flame-retardant organic compounds are provided below to demonstrate the diversity of compounds in this group.

Example Flame Retardants	
Polybrominated Diphenyl Ether (PBDE) 	Tetrabromobisphenol A (TBBPA) 
Tetrabromophthalate (TBPH) 	Tetrabromobenzoate (TBB) 

Hexabromocyclododecane (HBCD)	Melamine
	
Chlorinated Paraffins	Tris (1,3-dichloro-2-propyl) Phosphate (Chlorinated Tris or TDCPP)
	
Tributyl Phosphate	Triphenyl Phosphate (TPP)
	

Physicochemical Properties

The table below provides a summary of key physicochemical factors that are likely to affect partitioning and fate of select flame retardants in the environment. For chemical-specific values, consult the resources provided in the Chemical Classes Overview.

Property	Fate and Transport Implications
Halogenated (brominated) Flame Retardants	
Vapor pressure at 25°C (atm)	Low vapor pressure indicates that BFRs will not readily volatilize from the pure organic state. Vapor pressure decreases with increased degree of halogenation.
Henry's Law Constant	The Henry's Law Constant of BFRs corroborates with the low vapor pressure, indicating low volatility.
Solubility in water (mg/L)	BFRs generally have low water solubility, meaning they are not likely to dissolve in surface water.

Octanol-Water Partition Coefficient (log value)	The high K_{ow} values of BFRs indicate high potential to bioaccumulate.
Octanol-Air Partition Coefficient (log value)	High log K_{oa} indicates strong propensity of the compounds to absorb to organic matter such as soil particles, dust, or vegetation rather than remain in air (U.S. EPA, 2010 < https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/956687 >).
Summary: Traditional brominated flame retardants are highly persistent in soil and sediment, and will sorb to organic matter in the water column or air (i.e., dust). These compounds have a high potential for bioaccumulation.	
Phosphorous-based Flame Retardants (PFRs)	
Vapor pressure at 25°C (atm)	Low vapor pressure indicates that PFRs will not readily volatilize from the pure organic state, where they exist as liquids. If aerosolized, PFRs are likely to exist in the vapor phase.
Henry's Law Constant	While vapor pressure indicates volatilization from dry soils is unlikely, the moderate Henry's Law constants of most PFRs indicate that volatilization from moist soil may occur for some PFRs.
Solubility in water (mg/L)	Relatively low water solubility of most PFRs indicates that they will not readily dissolve in water and have high soil adsorption potential.
Octanol-Water Partition Coefficient (log value)	While log K_{ow} values for PFRs vary greatly, most PFRs have positive log K_{ow} values, suggesting that these compounds will preferentially partition to organic matter in soils and sediments, and some could bioaccumulate and biomagnify in aquatic ecosystems and possibly in terrestrial ecosystems and humans.
Octanol-Air Partition Coefficient (log value)	While K_{oa} values have not been identified, vapor pressure indicates that transfers from organic media (i.e., soil) to air are possible.
Summary: Properties of the different PFRs vary greatly. PFRs are generally poorly soluble and lipophilic and will partition to soil, sediment, or organic matter rather than remain in water or air. However, most PFRs are less lipophilic than the brominated FRs and there is wide variation in Henry's Law Constants and vapor pressure which indicates that the PFRs may occur in any type of media (van der Veen and de Boer, 2012).	

Routes

Exposure to flame retardants occurs mainly through inhalation or ingestion of dust. Food and water contaminated with flame retardants is another source of exposure. Exposure from dermal contact with contaminated soil and dust may also occur.

Route	Potential Sources of Flame Retardant Exposure
Inhalation	<ul style="list-style-type: none"> <li data-bbox="375 226 1117 258">• Inhalation of household dust is a potential source of PBDE exposure. <li data-bbox="375 310 1360 415">• In certain occupational settings, such as electronic recycling facilities, dust containing high levels of flame retardants can also be present. In these environments, exposure could occur through inhalation.
Ingestion	<ul style="list-style-type: none"> <li data-bbox="375 472 1393 535">• Settled dust containing flame retardants and subsequent hand-to-mouth contact is a potential source of exposure. <li data-bbox="375 588 1401 693">• Ingestion of contaminated food or water is a potential source of exposure. PBDEs have been detected in meat, dairy products, and fish. Flame retardant chemicals have also been measured in human breast milk.
Dermal Contact	<ul style="list-style-type: none"> <li data-bbox="375 756 1393 819">• Exposure through dermal contact can occur from contaminated soil or water, or anthropogenic surfaces such as asphalt roads or coal tar surfaces.

Note: The Routes Tool Set of EPA ExpoBox provides additional information and resources organized by route.

Media

PBDEs have been measured in indoor and outdoor air, water and sediment, and soil. They have also been found in household dust. Flame retardants are widely used in consumer products, making indoor environments particularly important for this group of compounds.

Exposed Populations

Flame retardants are ubiquitous environmental contaminants, resulting in potential exposure across the general population. Certain populations may be at risk for higher exposures, for example:

- Children may be exposed to high levels of dust in homes due to the amount of time they spend on the floor and the higher level of hand-to-mouth contact. Additionally, many consumer products intended for children are intentionally treated with flame retardants, including pajamas, changing pads, and car seats.
- Workers involved with product manufacturing and product end-of-life processes (recycling, incineration, landfilling) may be exposed to flame retardant chemicals that are applied to numerous consumer products.
- Firefighters and other emergency personnel who may respond to home, business, or vehicular fires could be at risk for high exposures as flame retardants are released as materials succumb to fire.

Note: The Lifestages and Populations Tool Set of EPA ExpoBox includes additional information about particular population groups and lifestages.

Tools

- **Key:**  Information sources

-  Data sources
-  Models

Tool Type	Tools	Description
	Agency for Toxic Substances and Disease Registry (ATSDR). (2012). Toxicological Profile for Phosphate Ester Flame Retardants < https://www.atsdr.cdc.gov/toxprofiles/tp202.pdf >	This report characterizes the toxicological and adverse health information for phosphate ester flame retardants, including summary-level data on physical chemical properties, population exposure levels, and exposure routes.
	Agency for Toxic Substances and Disease Registry (ATSDR). (2004). Toxicological profile for Polybrominated Biphenyls (PBBs)	Toxicological Profiles contain a review of key studies and other data characterizing the exposure-related health effects and pertinent characteristics and processes that affect human exposures. Sections include other relevant information on releases to the environment, environmental fate, levels monitored in the environment, potential exposures, and analytical methods.
	California EPA Office of Environmental Health Hazard Assessment (OEHHA). (2008). Brominated and Chlorinated Organic Chemical Compounds Used as Flame Retardants < https://biomonitoring.ca.gov/downloads/brominated-and-chlorinated-organic-chemical-compounds-used-flame-retardants-0 >	This resource includes materials for the December 4-5, 2008 Meeting of the California Environmental Contaminant Biomonitoring Program (CECBP) Scientific Guidance Panel (SGP). A brief overview of several BFRs and CFRs is provided.
 	Centers for Disease Control and Prevention (CDC). (2009). Fourth National Report on Human Exposure to Environmental Chemicals < https://www.cdc.gov/exposurereport/ >	This report provides information on NHANES biomonitoring data (i.e., in human blood and urine) in the U.S. population. The 4th report includes new data from 2003-2004 as well as data from the 2nd and 3rd reports (from 1999-2000 and 2001-2002, respectively). Data are included for phthalates, PFCs, PAHs, PCBs, dioxins, VOCs, metals, flame retardants, and multiple pesticides among other substances.
 	Centers for Disease Control and Prevention (CDC). (2017). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables < https://www.cdc.gov/exposurereport/ >	Updated Tables (March 2018) for the Fourth National Report on Human Exposure to Environmental Chemicals provide data for 346 chemicals (38 new and 176 updated since January 2017). These updated tables are represented in two separate volumes: Volume One contains data tables for chemicals measured in the general U.S. population, and Volume Two contains data tables for persistent organic pollutants and pesticides measured in pooled samples and adult cigarette smokers and nonsmokers.

	<p>Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: All Chemicals</p> <p><https://www.cdc.gov/biomonitoring/chemical_factsheets.html></p>	<p>These fact sheets from the National Biomonitoring Program summarize exposure and health effects for various substances including: BPA, dioxins, PFCs, phthalates, PAHs, PBDEs and several metals including lead, mercury, arsenic, and cadmium among others.</p>
	<p>Environment Canada. Ecological Screening Assessment Report on Polybrominated Diphenyl Ethers (PBDEs)</p> <p><https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/publications/ecological-screening-assessment-report-polybrominated.html></p>	<p>This assessment report provides information on the use and sources, fate, exposure, and effects of PBDEs.</p>
	<p>European Food Safety Authority (EFSA). (2012). Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food</p> <p><http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2908/abstract></p>	<p>This opinion paper contains information on 17 “emerging” BFRs and 10 “novel” BFRs and identifies which of these substances are potential health concerns based on occurrence, exposure, and toxicity data.</p>
	<p>European Food Safety Authority (EFSA). (2011). Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food</p> <p><http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2156/abstract></p>	<p>This opinion paper contains information on PBDE occurrence, exposure, and toxicity.</p>
	<p>International Labor Organization (ILO) and World Health Organization (WHO). (2006). International Chemical Safety Card (ICSC): Melamine</p> <p><https://inchem.org/documents/icsc/icsc/eics1154.htm></p>	<p>This summary table for melamine contains classifications, physicochemical properties and general exposure information.</p>
	<p>Kimbrough, KL; Johnson, WE; Lauenstein, GG; Christensen, JD; Apeti, DA. (2009). An Assessment of Polybrominated Diphenyl Ethers (PBDEs) in Sediments and Bivalves of the U.S. Coastal Zone</p> <p><http://aquaticcommons.org/14877/1/pbdereport.pdf></p>	<p>This report is the first national assessment of PBDEs in the U.S. coastal zone developed by NOAA's National Status & Trends Program (NS&T).</p>
	<p>U.S. EPA. (2012). An Alternatives Assessment for the Flame Retardant Decabromodiphenyl Ether (DecaBDE)</p> <p><https://www.epa.gov/sites/production/files/2014-05/documents/decabde_final.pdf></p>	<p>This report provides hazard information (human toxicity, ecotoxicity and environmental fate) on flame retardants that were selected for evaluation as potentially functional alternatives to decaBDE.</p>

	<p>U.S. EPA. (2013). America's Children and the Environment (3rd ed.) <https://www.epa.gov/ace></p>	<p>This EPA report presents information on environmental stressors that can affect children's health. Information on biomonitoring for several organics, including PFCs, PBDEs, PCBs, phthalates, and BPA as well as lead and mercury are provided.</p>
	<p>U.S. EPA. (2005). Chemical Hazard Reviews Volume 1: Furniture Flame Retardancy Partnership: Environmental Profiles of Chemical Flame-Retardant Alternatives for Low-Density Polyurethane Foam <https://www.epa.gov/sites/production/files/2013-12/documents/ffr_foam_alternatives_vol1.pdf></p>	<p>This resource provides a description of the general characteristics of flame retardants, a general overview of exposure pathways and routes for flame retardants used in flexible polyurethane foam and the results of the assessments of 14 formulations of flame-retardant products most likely to replace commercially available pentabromodiphenyl ether (pentaBDE).</p>
	<p>U.S. EPA. (2005). Chemical Hazard Reviews Volume 2: Furniture Flame Retardancy Partnership: Environmental Profiles of Chemical Flame-Retardant Alternatives for Low-Density Polyurethane Foam <https://www.epa.gov/sites/production/files/2013-12/documents/ffr_foam_alternatives_vol2.pdf></p>	<p>This resource provides detailed hazard reviews of available information for each of the chemicals in the 14 flame-retardant formulations evaluated through the Furniture Flame Retardancy Partnership.</p>
	<p>U.S. EPA. (2013). Comprehensive Environmental Assessment Applied to Multiwalled Carbon Nanotube Flame-Retardant Coatings in Upholstery Textiles: A Case Study Presenting Priority Research Gaps for Future Risk Assessments (Final Report)</p>	<p>This case study report compares multiwalled carbon nanotubes (MWCNTs) and a non-nano-enabled product-decabromodiphenyl ether (decaBDE)-as flame-retardant coatings applied to upholstery textiles, and provides a characterization and comparison related the following: historical and current applications; product life cycle; fate, transformation, and transport in environmental media; exposure and dose; human health and ecological effects; and research needs.</p>
	<p>U.S. EPA. (2010). An Exposure Assessment of Polybrominated Diphenyl Ether</p>	<p>EPA's exposure assessment of polybrominated diphenyl ether (PBDE) provides a comprehensive assessment of the exposure of Americans to PBDEs. The report includes information on the production, use, lifecycle, and environmental fate of these persistent organic chemicals, as well as measured environmental levels and human exposure.</p>
	<p>U.S. EPA. (2015). Partnership to Evaluate Flame Retardants in Printed Circuit Boards (Final Report) <https://www.epa.gov/saferchoice/partnership-evaluate-flame-retardants-printed-circuit-boards-publications></p>	<p>This final report addresses the environmental and human health issues associated with the production, use, and disposal of FR4 Printed Circuit Boards using current and emerging flame retardant technologies.</p>

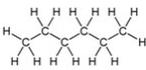
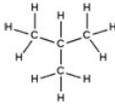
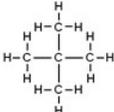
	<p>U.S. EPA. Polybrominated Diphenyl Ethers (PBDEs) Action Plan Summary</p> <p><https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/polybrominated-diphenyl-ethers-pbdes-action-plan></p>	<p>This website provides a summary of the status of EPA's action plan for considering the risks of PBDEs. This page includes links to previous and current actions by EPA towards rulemaking regarding PBDEs.</p>
	<p>World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). (2011). Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework</p> <p><https://europepmc.org/article/med/21466831></p>	<p>This website contains a description of the final WHO/IPCS Framework for Risk Assessment of Combined Exposures to Multiple Chemicals, which is a document that describes a framework for the assessment of combined risk from exposure to multiple chemicals. The frameworks for exposure and hazard assessments are presented as tiers—from simple (Tier 0) to more refined (Tier 3). This document is supplemented by two case studies that apply the frameworks—one case study on polybrominated diphenyl ethers (PBDEs) and the other on carbamates.</p>

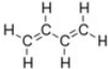
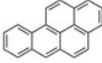
Hydrocarbons

- Physicochemical Properties
- Routes
- Media
- Exposed Populations
- Tools

Hydrocarbons contain only hydrogen and carbon atom in various configurations, including chains, branched chains, and rings. Most hydrocarbons fall into one of the five structural categories described in the table below.

Hydrocarbon Subclasses

Subclass	Description	Example Structures	
<i>n</i> -Alkanes	Saturated, unbranched, hydrocarbon chains	Methane, CH ₄ 	<i>n</i> -Hexane, C ₆ H ₁₄ 
Branched alkanes	Saturated, branched, hydrocarbon chains	Isobutane, C ₄ H ₁₀ 	Neopentane, C ₅ H ₁₂ 

Unsaturated and alicyclic hydrocarbons	Unsaturated hydrocarbon chains and hydrocarbon rings	1,3-Butadiene, C ₄ H ₆ 	Cyclohexane, C ₆ H ₁₂ 
Alkylated benzenes	Six-carbon, aromatic rings with hydrocarbon chain substituent groups	Toluene, C ₇ H ₈ 	Ethylbenzene, C ₈ H ₁₀ 
Polycyclic aromatic hydrocarbons and related compounds	Multiple, six-carbon aromatic rings, sometimes in combination with other hydrocarbon ring structures	Benzo(a)pyrene, C ₂₀ H ₁₂ 	Acenaphthene, C ₁₂ H ₁₀ 

Some hydrocarbons are derived from petroleum distillates; others (e.g., pine oil and turpentine) are derived from wood. Many hydrocarbons are used as fuel components, including methane, benzene, toluene, ethylbenzene, and xylenes. One specific group of hydrocarbons currently of interest to exposure assessors are the polycyclic aromatic hydrocarbons (PAHs), which contain rings of carbon atoms with alternating single and double bonds between carbons.

PAHs are found in coal tar and crude oil, and are sometimes used in medicines or the production of dyes, plastics, and pesticides (ATSDR, 1990).

Commercial production of PAHs is minimal; most PAHs found in the environment are created as a byproduct of incomplete combustion of fossil fuels or wood. PAHs can be created through burning of any organic material, including smoking cigarettes and grilling meat, vegetables, or other foods.

Some PAHs occur as byproducts of industrial processes such as metal production and refining. PAHs can also be released through natural processes such as volcanic eruptions or forest fires. As PAHs are accidentally produced and released rather than intentionally, they generally occur in mixtures (ATSDR, 1990).

Physicochemical Properties

In general, hydrocarbon rings without alternating single and double bonds and hydrocarbon chains are referred to as *alicyclic* or *aliphatic* hydrocarbons, respectively, and hydrocarbon rings with alternating single and double bonds are referred to as *aromatic* hydrocarbons. The number of double versus single bonds determines whether aliphatic hydrocarbons are "saturated" with respect to hydrogen or "unsaturated" due to the presence of double bonds.

Saturated hydrocarbons are more reactive than unsaturated hydrocarbons and are more easily broken down. The bond pattern in the ringed structure of PAHs, on the other hand, results in a chemical property called aromaticity, which confers stability in these compounds.

The table below provides a summary of key physicochemical factors that are likely to affect partitioning and fate of select aliphatic and aromatic hydrocarbons in the environment. For chemical-specific values, consult the resources provided in the introduction to this module.

Property	Fate and Transport Implications
Aliphatic Hydrocarbons	
Vapor pressure at 25°C (atm)	Vapor pressure of aliphatic hydrocarbons increases with decreasing chain length, suggesting that low-molecular-weight hydrocarbons will volatilize readily from the pure organic state, while high-molecular-weight compounds will exhibit low volatility.
Henry's Law Constant	H increases with increasing molecular size of the hydrocarbon, however most non-aromatic hydrocarbons have a Henry's Law Constant around 10 or greater. The high constant indicates a propensity to volatilize, implying that the compounds will readily move from water to air.
Solubility in water (mg/L)	As the number of carbons in the chain increase (i.e., molecular weight increases), solubility tends to decrease; branching also decreases solubility. Aliphatic hydrocarbons are less soluble than aromatic hydrocarbons of similar molecular weight. As hydrocarbons commonly occur in mixtures, solubility can vary greatly depending on the composition of the released hydrocarbon fraction.
Octanol-Water Partition Coefficient (log value)	The hydrophobicity of aliphatic hydrocarbons generally increases with increasing chain length; larger hydrocarbons have higher sorption potential and are more likely to bioaccumulate.
Octanol-Air Partition Coefficient (log value)	Like most other properties, K_{oa} also increases with increased molecular volume, implying that the high-molecular weight aliphatic hydrocarbons (longer chain length) are less likely to partition to air, and more likely to remain sorbed to soil, sediment, or biota than the shorter-chain hydrocarbons.
Summary: Lighter aliphatic hydrocarbons—with smaller chain lengths—have higher volatility and water solubility, and are less likely to sorb to particles or sediment than larger, longer-chain hydrocarbons. The behavior of a hydrocarbon mixture will therefore vary greatly depending on the mixture composition, and will change over time as the lighter constituents will more readily volatilize and migrate through soils, while heavier constituents will settle into soils, sorb to organic matter, and persist at the site of release.	
Aromatic Hydrocarbons	
Vapor pressure at 25°C (atm)	Vapor pressure is inversely related to PAH size. PAHs with more aromatic rings have higher vapor pressures and are more likely to sorb to particulate matter in air than smaller PAHs.

Henry's Law Constant	Henry's Law Constants decrease with increasing molecular weight of PAHs, suggesting that low-molecular weight PAHs volatilize readily from water to air whereas higher molecular weight PAHs will remain in water.
Solubility in water (mg/L)	PAHs have low water solubility, meaning they are primarily found sorbed to particles suspended in the water column or settled to sediment.
Octanol-Water Partition Coefficient (log value)	High K_{ow} values signify an affinity to move from water to lipid, suggesting potential for bioaccumulation and bioconcentration in aquatic systems.
Octanol-Air Partition Coefficient (log value)	High log K_{oa} values imply that PAHs in the atmosphere will sorb to particulates
Summary: While air release of PAHs is most likely, residence time in air is limited. PAHs are expected to partition to soil or sediment.	

Routes

Exposure to hydrocarbons occurs most commonly from unintentional ingestion or inhalation. Exposure from dermal contact can also occur.

Route	Potential Sources of Hydrocarbon Exposure
Inhalation	<ul style="list-style-type: none"> Inhalation of ambient air, particularly in urban environments with asphalt-paved roads and heavy vehicle traffic, can result in exposure to PAHs. Hydrocarbons can volatilize out of the asphalt material, are released in vehicle exhaust, and are a component of tire wear and brake lining particles. Environmental tobacco smoke is a large source of PAHs. Smokers or people who live or work in close proximity to smokers may be exposed to PAHs through inhalation.
Ingestion	<p>Ingestion of hydrocarbons occurs as the compounds are a common contaminant in processed foods, particularly foods that are grilled or smoked. PAHs can also occur in non-processed foods such as fruits and vegetables that are grown in environments with contaminated soil or air.</p> <ul style="list-style-type: none"> Some PAHs are bioaccumulative, which can result in higher contamination levels in some fish, meats, and milk products (both cow's milk and human breast milk). PAHs have been detected in some drinking water sources.

Dermal Contact	<p>Dermal exposure can occur through contact with contaminated soil or water, or anthropogenic surfaces such as asphalt roads or coal tar surfaces.</p> <ul style="list-style-type: none"> Exposure is common through use of multiple consumer products (e.g., gasoline and other fuels, furniture polish, household cleansers, and propellants).
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Note: The Routes Tool Set of EPA ExpoBox provides additional information and resources organized by route.

Media

Hydrocarbons are common contaminants in multiple environmental media, including air, water, and soil.

Media	Sources of Hydrocarbons
Air	<ul style="list-style-type: none"> Anthropogenic releases of hydrocarbons to air include residential burning of wood and open burning of organic matter (e.g., leaves, yard clippings), waste incineration, vehicle exhaust, cigarette smoke, and production of coal tar, coke, and asphalt. Accidental releases of petroleum hydrocarbons can occur through oil and gas spills, both large scale (such as the U.S. Gulf Oil spill in 2010) and small scale (drips from fuel pumps at automobile fill-up stations). Natural releases of hydrocarbons to air include forest fires and volcanic eruptions.
Water	<ul style="list-style-type: none"> Stormwater runoff in urban areas can contain hydrocarbons due to vehicle exhaust, asphalt paving, or rubber tire wearings. Runoff in industrial areas with wood treatment plants, petroleum refineries, or municipal waste treatment centers may contain hydrocarbons.
Soil	<p>The main source of hydrocarbons in soils is atmospheric deposition.</p> <ul style="list-style-type: none"> Contamination of soils along roadways occurs through deposition of emissions from vehicle exhaust, as well as emissions from the wearing of rubber tires and the asphalt. Soils may also become contaminated with hydrocarbons following activities such as disposal of sludge from wastewater treatment plants, landfilling, or application of compost or certain pesticides. Soils at or near sites where coal, gas, or wood was burned may also be contaminated.
Consumer Products	<p>Hydrocarbons are found in many consumer products (e.g., gasoline and other fuels, furniture polish, household cleansers, and propellants).</p>

Note: The Media Tool Set of EPA ExpoBox provides additional information and resources organized by media.

Exposed Populations

Hydrocarbons, especially PAHs, are ubiquitous in the environment and the general population can be exposed in both indoor and outdoor environments. Certain populations are at risk for higher exposures than the general population:

- Cigarette smokers or people who live or work in close proximity to smokers may be exposed to PAHs through inhalation of smoke.
- As PAHs are a common contaminant of vehicle exhaust, urban populations may be more highly exposed than rural populations; however, rural populations may have high exposures to diesel fuels used in farm equipment or trucks, which might be less efficient at combustion due to older age than some vehicles in urban areas.
- Certain occupations may result in high exposure to PAHs, including but not limited to: mechanics, construction workers involved with pavement, roads, or roofs, utility workers exposed to creosote, coal miners or those in the petroleum refinery business, steel foundry and aluminum workers.

Note: The Lifestages and Populations Tool Set of EPA ExpoBox has addition information about particular population groups and lifestages.

Tools

- **Key:**  Information sources
-  Data sources
-  Models

Tool Type	Tools	Description
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	<p>Agency for Toxic Substances and Disease Registry (ATSDR). Case Studies in Environmental Medicine (CSEM): Substance-Specific Course Series for Health Professionals <https://www.atsdr.cdc.gov/csem/csem.html></p>	<p>ATSDR has prepared a series of case studies to help health professionals in their evaluation of patients potentially exposed to hazardous substances. Courses have been developed for various inorganics and fibers—such as arsenic, asbestos, beryllium, cadmium, chromium, lead, and nitrate/nitrite—and volatile and semivolatile compounds, including benzene, toluene, tetrachloroethylene, trichloroethylene, PCBs, and PAHs.</p>
	<p>Agency for Toxic Substances and Disease Registry (ATSDR). Polycyclic Aromatic Hydrocarbons (PAHs): What are the Standards and Regulations for PAHs Exposure?</p>	<p>This ATSDR-supported online course module is focused on describing the OSHA permissible exposure levels and EPA maximum contamination level for PAHs.</p>

	<p>Agency for Toxic Substances and Disease Registry (ATSDR). (1995). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)</p> <p>https://www.atsdr.cdc.gov/toxprofiles/tp69.pdf</p>	<p>Toxicological Profiles contain a review of key studies and other data characterizing the exposure-related health effects and pertinent characteristics and processes that affect human exposures. Sections include other relevant information on releases to the environment, environmental fate, levels monitored in the environment, potential exposures, and analytical methods.</p>
	<p>Agency for Toxic Substances and Disease Registry (ATSDR). (1999). Toxicological Profile for Total Petroleum Hydrocarbons</p>	<p>This document characterizes the toxicological and adverse health information for total petroleum hydrocarbons, including summary-level data on population exposure levels and exposure routes.</p>



Centers for Disease Control and Prevention (CDC). (2009). Fourth National Report on Human Exposure to Environmental Chemicals <<https://www.cdc.gov/exposurereport/>>

This report provides information on NHANES biomonitoring data (i.e., in human blood and urine) in the U.S. population. The 4th report includes new data from 2003-2004 as well as data from the 2nd and 3rd reports (from 1999-2000 and 2001-2002, respectively). Data are included for phthalates, PFCs, PAHs, PCBs, dioxins, VOCs, metals, flame retardants, and multiple pesticides among other substances.

	<p>Centers for Disease Control and Prevention (CDC). (2017). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables https://www.cdc.gov/exposurereport/</p>	<p>Updated Tables (March 2018) for the Fourth National Report on Human Exposure to Environmental Chemicals provide data for 346 chemicals (38 new and 176 updated since January 2017). These updated tables are represented in two separate volumes: Volume One contains data tables for chemicals measured in the general U.S. population, and Volume Two contains data tables for persistent organic pollutants and pesticides measured in pooled samples and adult cigarette smokers and nonsmokers.</p>
	<p>Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: All Chemicals https://www.cdc.gov/biomonitoring/chemical_factsheets.html</p>	<p>These fact sheets from the National Biomonitoring Program summarize exposure and health effects for various substances including: BPA, dioxins, PFCs, phthalates, PAHs, PBDEs and several metals including lead, mercury, arsenic, and cadmium among others.</p>

	<p>Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: PAHs <https://www.cdc.gov/biomonitoring/pahs_factsheet.html></p>	<p>This resource provides a summary of exposure and health effects of PAHs in the US. It includes links to summary information from other agencies (ATSDR, EPA, USGS).</p>
	<p>European Commission Joint Research Centre. (2011). Polycyclic Aromatic Hydrocarbon (PAH) Factsheet, 4th Edition <http://dl.icdst.org/pdfs/files3/17c9950bdd34d63c4a5a13bf727e7e62.pdf></p>	<p>This document contains chemical structures, toxicity occurrence information, and data source links for analytical methods, calibration standards, and other resources.</p>
	<p>International Programme on Chemical Safety (IPCS). (2003). Environmental Health Criteria 229: Selected Nitro- And Nitrooxy- Polycyclic Aromatic Hydrocarbons <http://www.who.int/ipcs/publications/ehc/229_part_i.pdf></p>	<p>This IPCS document provides information on the relationship between exposure to environmental pollutants and human health and guidelines for setting exposure limits.</p>
	<p>U.S. Department of Commerce National Technical Information Service (NTIS). (1990). Compendium of Methods for the Determination of Air Pollutants in Indoor Air <https://www.arb.ca.gov/research/apr/reports/l3057a.pdf></p>	<p>This 1990 EPA publication provides information on monitoring and laboratory analysis techniques for indoor air pollutants including nitrogen dioxide, formaldehyde, PAHs, VOCs, carbon monoxide, and PM.</p>

	<p>U.S. EPA. (2010). Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures</p>	<p>This draft document provides a methodology for estimating cancer risk from exposure to PAH mixtures by summing doses of component PAHs after scaling the doses (with RPFs) using benzo[a]pyrene as the index PAH.</p>
	<p>U.S. EPA. (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories - Volume 2: Risk Assessment and Fish Consumption Limits https://www.epa.gov/sites/default/files/2015-06/documents/volume2.pdf</p>	<p>This document provides guidance on the development of risk-based fish meal consumption limits for 25 high-priority target analytes that include organochlorine and organophosphate pesticides, PAHs, PCBs, dioxins/furans, and some metals.</p>
	<p>U.S. EPA. Hydrocarbon Spill Screening Model (HSSM) https://www.epa.gov/water-research/hydrocarbon-spill-screening-model-hssm-dos</p>	<p>This model developed by EPA can be used to model subsurface releases of fuel hydrocarbons (e.g., leaking underground storage tanks).</p>

	<p>U.S. EPA. Mobile Source Observation Database (MSOD) <https://www.epa.gov/moves/mobile-source-observation-database-msod></p>	<p>The MSOD contains emission test data from in-use mobile air pollution sources such as cars, trucks, and engines from trucks and nonroad vehicles. Data are available from 1982 to present and are intended to be representative of in-use vehicle emissions in the United States. A reference guide is available for this database.</p>
	<p>U.S. EPA. MOtor Vehicle Emission Simulator (MOVES) Model and Other Mobile Source Emissions Models <https://www.epa.gov/moves></p>	<p>This website provides links to the MOtor Vehicle Emission Simulator (MOVES) and older models including the NONROAD model, National Mobile Inventory Model (NMIM), and MOBILE Model. MOVES is an emission modeling system that estimates emissions for mobile sources covering a broad range of pollutants. This tool allows users to perform multiple-scale analysis.</p>

	<p>U.S. EPA. OnSite: Modeling Subsurface Petroleum Hydrocarbon Transport https://www3.epa.gov/ceampubl/learn2model/</p>	<p>This resource provides access to a set of online tools for site assessment including calculators for formulas, models, unit conversion factors, and scientific demonstrations to assess the impacts from ground water contaminants.</p>
	<p>U.S. EPA. Overview of Greenhouse Gases: Methane Emissions https://www.epa.gov/ghgemissions/overview-greenhouse-gases#methane</p>	<p>This webpage provides a discussion of methane emissions by source and emission trends since 1990. Examples of methane reduction opportunities are also described.</p>
	<p>U.S. EPA. (1993). Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons</p>	<p>This provisional guidance provides an approach using weighted potency factors for PAHs. These estimates are recommended only for evaluation of risk from oral exposure and are proposed only for the assessment of potential carcinogenicity of PAHs.</p>

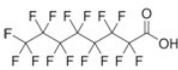
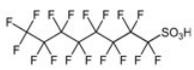
	<p>U.S. EPA. TANKS Model <https://www3.epa.gov/ttn/chief/software/tanks/index.html></p>	<p>TANKS is a Windows-based computer software program that estimates volatile organic compound (VOC) and hazardous air pollutant (HAP) emissions from fixed- and floating-roof storage tanks.</p>
	<p>UK Health Protection Agency (HPA). (2010). HPA Contaminated Land Information Sheet: Risk Assessment Approaches for Polycyclic Aromatic Hydrocarbons (PAHs) <http://webarchive.nationalarchives.gov.uk/20140714084352/http://www.hpa.org.uk/webc/hpawebfile/hpaweb_c/1287147342113></p>	<p>This document discusses the use of Toxic Equivalency Factor versus surrogate marker approach to conducting PAH cumulative risk assessment.</p>
	<p>World Health Organization (WHO). (2011). Polynuclear Aromatic Hydrocarbons (PAHs) FactSheet - 4th Edition <http://dl.icdst.org/pdfs/files3/17c9950bdd34d63c4a5a13bf727e7e62.pdf></p>	<p>This document provides summary-level information on chemical identify, physicochemical properties, and exposure characteristics for PAHs.</p>

Perfluorinated Compounds (PFCs)

- Physicochemical Properties
- Routes
- Media
- Exposed Populations
- Tools

Perfluorinated Compounds (PFCs) contain a carbon chain with fluorine atoms attached in the place of hydrogen atoms and one or more atom or functional groups attached to the end. These compounds are synthetic and used in a variety of manufacturing and industrial applications, including fire resistance and non-stick surfaces. A wide variety of consumer products contain varying levels of PFCs.

These chemicals have been used for many decades in products that resist or repel oil, grease, and water-based liquids. These include products advertised as stain resistant or non-stick, such as cookware, carpets, upholstered furniture, or other fabrics, as well as non-advertised products that have repellent qualities, such as microwave popcorn bags and food packaging.

Example PFC Structures		
8:2 Fluorotelomer Alcohol (8:2 FTOH)	Perfluorooctanoic acid (PFOA)	Perfluorooctanesulfonic acid (PFOS)
		

Physicochemical Properties

PFCs are generally resistant to further degradation in the environment or within biota (Olsen et al., 2007; Harada et al., 2004). The carbon-fluorine bonds that characterize PFCs are very strong and stable in air at high temperatures; are not readily degradable by strong acids, alkalis, or oxidizing agents; and generally do not undergo photolysis (Lau et al., 2007).

In general, the atmospheric lifespan of long-chain PFCs (LCPFCs) (i.e., 8 or more carbons) is on the order of days to weeks under most atmospheric conditions (Lau et al., 2007). The compounds tend to partition into other environmental media. The half-lives in soil of the C6 through C11 alkylates range from about 1 to 3 years and increase with increasing chain length (Washington et al., 2010). Based on the physicochemical characteristics of these compounds, the half-lives of LCPFCs and precursors in water also are expected to be very long.

The table below provides a summary of key physicochemical factors that are likely to affect partitioning and fate of select PFCs in the environment. For chemical-specific values, consult the resources provided in the Chemical Class Overview.

Property	Fate and Transport Implications
Vapor pressure at 25°C (atm)	Vapor pressure varies depending on the alkyl chain-length of the PFC. Low vapor pressure of some PFCs contributes to the phenomenon of Long-Range Atmospheric Transport (LRAT). PFCs remain in the vapor state for extended periods and move readily through the atmosphere, resulting in PFC contamination in areas far from the source of release.
Solubility in water (mg/L)	While PFCs are lipid soluble, they are also moderately water-soluble. Many PFCs are acids that will dissociate in freshwater, which increases their solubility. This allows them to remain in the water column.
Octanol-Water Partition Coefficient (log value)	PFCs are specifically designed to be hydrophobic and oleophobic, making it difficult to determine a K_{ow} value. However, biomonitoring studies have indicated a tendency to partition into organic fractions (biota), where they bind to proteins in blood serum rather than partition to fats. PFCs with longer alkyl chains are more likely to bioaccumulate and biomagnify than those with shorter chains.

Summary: PFCs are a unique type of compound in that they are both hydrophobic and oleophobic. They can be measured in the water column, but will also bioaccumulate in biota. They move readily out of products into the air, and can spread long distances through the atmosphere due to their low vapor pressure, meaning exposure can occur far from the source.

Routes

Ingestion is the primary pathway identified for human exposures to PFCs, but exposures from inhalation and dermal contact can also occur.

Route	Potential Sources of PFC Exposure
Inhalation	<ul style="list-style-type: none"> Inhalation of contaminated dust.
Ingestion	<ul style="list-style-type: none"> Because PFCs partition readily to aquatic systems and bioaccumulate, fish consumption represents a common exposure route and food consumption is an important exposure pathway for PFOS and PFOA (Egeghy and Lorber, 2011; Trudel et al., 2008), two of the major PFCs. PFCs can migrate from food packaging to food (with concentrations occurring in high-fat foods, such as microwave popcorn and fast food), resulting in unintentional ingestion. Traditional drinking water treatment techniques do not remove LCPFCs, so contamination of ground water or surface water, or both, can lead to contamination of drinking water. Dust ingestion is an important route of exposure, particularly because of the high number of household and consumer products that contain PFCs. Vapor-phase materials can sorb to dust or dust can be contaminated with PFCs from direct contact with consumer products containing PFCs, such as carpet or textiles. This pathway is of greatest concern for young children, who spend a high percentage of time on the floor and have high hand-to-mouth contact.
Dermal Contact	Although exposure from dermal contact to PFCs is not considered a dominant route of exposure, people could be exposed via uptake from carpets or other surfaces such as clothing.

Note: The Routes Tool Set of EPA ExpoBox provides additional information and resources organized by route.

Media

LCPFCs and precursors can be released to various environment media through multiple scenarios. PFCs can be released from manufacturing sites or from use of consumer or industrial products, and secondary releases at sites such as waste water treatment plants (WWTPs) and landfills are possible. These releases generally result in PFCs directly entering water or air, however, release to soils and sediment is also possible, particularly for secondary release scenarios such as application of biosolids to agricultural fields.

Media	Sources of PFCs
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Air	<ul style="list-style-type: none"> • Direct emissions from manufacturing facilities to ambient air have been measured (Davis et al., 2007). • Indoor air may become contaminated with PFCs due to off-gassing from consumer products containing PFCs.
Water and Sediment	<ul style="list-style-type: none"> • PFCs can be released into water and sediment through direct discharge of the chemicals into streams, rivers, and wastewater systems during manufacturing or other industrial processes (Nakayama et al., 2010; Boulanger et al., 2004; Saito et al., 2004). • Both PFOA and PFOS have been found to enter water from municipal and industrial WWTPs (Sinclair and Kannan, 2006).
Soil	<ul style="list-style-type: none"> • Soils can become contaminated with PFCs due to industrial releases and secondary releases.
Indoor Dust	<ul style="list-style-type: none"> • PFCs have also been measured in indoor dust in residential and commercial buildings. PFCs can be emitted from consumer and industrial product use.

Note: The Media Tool Set of EPA ExpoBox provides additional information and resources organized by media.

Exposed Populations

PFCs may be found in consumer products, household products, and food packaging, resulting in exposure to the general population. However, some populations may be at risk of higher exposure levels:

- PFCs are persistent in the environment and may be subject to long-range atmospheric transport, resulting in higher environmental contamination levels in certain regions. Therefore, people who live in certain regions, may be at higher risk of exposure.
- The lipophilic nature of PFCs may contribute to the accumulation of these chemicals in breast milk. Infants who are breastfed may be exposed to the chemicals through ingestion. Additionally, in utero exposure can occur due to transfer through cord blood. Infant food and formula have also been shown to contain PFCs, probably due to migration from food packaging.
- Because PFCs bioaccumulate, individuals who consume high amounts of fish or aquatic mammals (i.e., seal, whale), may be exposed to high levels of PFCs, particularly if the fish is sourced from contaminated waters. Fatty cuts of beef or other meats may also contain measureable PFC contamination.
- Residents who live near certain industrial sites may be at risk of exposure through contaminated drinking water, soil, or air.

Note: The Lifestages and Populations Tool Set of EPA ExpoBox has addition information about particular population groups and lifestages.

Tools

- **Key:**  Information sources
-  Data sources
-  Models

Tool Type	Tools	Description
 	Centers for Disease Control and Prevention (CDC). (2017). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables < https://www.cdc.gov/exposurereport/ >	Updated Tables (March 2018) for the Fourth National Report on Human Exposure to Environmental Chemicals provide data for 346 chemicals (38 new and 176 updated since January 2017). These updated tables are represented in two separate volumes: Volume One contains data tables for chemicals measured in the general U.S. population, and Volume Two contains data tables for persistent organic pollutants and pesticides measured in pooled samples and adult cigarette smokers and nonsmokers.
	Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: All Chemicals < https://www.cdc.gov/biomonitoring/chemical_factsheets.html >	These fact sheets from the National Biomonitoring Program summarize exposure and health effects for various substances including: BPA, dioxins, PFCs, phthalates, PAHs, PBDEs and several metals including lead, mercury, arsenic, and cadmium among others.
	Centers for Disease Control and Prevention (CDC). National Biomonitoring Program Fact Sheets: Per- and Polyfluorinated Substances (PFAS) < https://www.cdc.gov/biomonitoring/pfas_factsheet.html >	This CDC fact Sheet includes information on how people are exposed to PFASs, how PFASs effect human health, and levels of PFASs in U.S. population.
	Environment Canada. Ecological Screening Assessment Report: Perfluorooctane Sulfonate (PFOS), Salts, and Precursors < https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/publications/ecological-screening-report-sulfonate.html >	This assessment report provides information on the use and sources, fate, exposure, and effects of PFOS.
	Minnesota Department of Health. Perfluorochemicals (PFCs) < https://www.pca.state.mn.us/waste/perfluorochemicals-pfcs >	This website contains general information on PFCs and links to status reports for PFCs in Minnesota.

 	<p>Organisation for Economic Cooperation and Development (OECD). Risk Management of Chemicals: Perfluorooctane Sulfonate (PFOS) and Related Chemical Products</p> <p>http://www.oecd.org/env/ehs/risk-management/perfluorooctanesulfonatepfosandrelatedchemicalproducts.htm</p>	<p>This website provides information on a hazard assessment of PFOS and its salts including a link to the results of the survey the production and use of PFOS, PFOA, PFAS, PFCA, their related substances chemicals and products/mixtures containing these substances. The study results provide concentration data for PFOA.</p>
	<p>U.S. EPA. (2013). America's Children and the Environment (3rd ed.) https://www.epa.gov/ace</p>	<p>This EPA report presents information on environmental stressors that can affect children's health. Information on biomonitoring for several organics, including PFCs, PBDEs, PCBs, phthalates, and BPA as well as lead and mercury are provided.</p>
	<p>U.S. EPA. (2008). Climate Leaders Greenhouse Gas Inventory Protocol Core Module Guidance: Direct Fugitive Emissions from Refrigeration, Air Conditioning, Fire Suppression, and Industrial Gases https://www.epa.gov/climateleadership/center-corporate-climate-leadership-greenhouse-gas-inventory-guidance</p>	<p>This report provides guidance on estimating direct HFC and PFC emissions from the use of air refrigeration and air conditioning equipment. The guidance addresses household, commercial and industrial refrigeration and air conditioning, as well as fixed and portable fire suppression equipment.</p>
	<p>U.S. EPA. (2011). Global Anthropogenic Non-CO2 Greenhouse Gas Emissions: 1990-2030 https://www.epa.gov/sites/production/files/2016-08/documents/epa_global_nonco2_projections_dec2012.pdf</p>	<p>This 2012 report provides estimated projections to 2030 for emissions from more than twenty emissions sources. The gases included in this report are methane, nitrous oxide, and fluorinated greenhouse gases (hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride).</p>
	<p>U.S. EPA. Long-Chain Perfluorinated Chemicals (PFCs) Action Plan https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/long-chain-perfluorinated-chemicals-pfcs-action-plan</p>	<p>Long-Chain Perfluorinated Chemicals (PFCs) Action Plan is based on EPA's initial review of readily available use, exposure, and hazard information on PFCs. The document provides hazard, fate, and exposure characterization summaries and describes risk management considerations.</p>
	<p>U.S. EPA. Per- and Polyfluoroalkyl Substances (PFAS) Research https://www.epa.gov/chemical-research/research-and-polyfluoroalkyl-substances-pfas</p>	<p>This website describes an active research area at EPA to evaluate potential environmental and human health problems associated with release of PFASs into the environment. This research will help to provide the means to estimate human exposures to these materials or establish risks associated with these exposures.</p>

Phthalates and Bisphenol A (BPA)

- Physicochemical Properties

Attachment 32

Per- and Polyfluoroalkyl Substances (PFAS) Drinking Water Regulations Quick Reference Guide



MassDEP

Commonwealth of Massachusetts
Department of Environmental Protection

Overview of the Rule

Title	Per- and Polyfluoroalkyl Substances (PFAS) compliance requirements for Public Water Systems (PWS) - 310 CMR 22.07G
Purpose	Increase public health protection through the reduction of chemicals that have been linked to a variety of health risks, particularly for sensitive subgroups including pregnant women, nursing mothers and infants.
General Description	The amended Massachusetts Drinking Water Regulations establish a Maximum Contaminant Level (MCL) of 0.000020 milligrams per liter (mg/l) or 20 ng/l (also called parts per trillion or ppt) for the sum of six PFAS compounds (PFOS, PFOA, PFHxS, PFNA, PFHpA and PFDA), known as PFAS6. The regulations detail the sampling requirements and corrective actions that PWS must take when the MCL is exceeded, as well as the provisions for public education and notice of exceedances so that communities can be educated and proactive in protecting their drinking water quality.
Utilities Covered	The PFAS6 MCL applies to Community PWS and Non-transient, Non-community PWS. Transient Non-community PWS must collect a PFAS sample under the regulations and would be subject to a site-specific health assessment for elevated levels.

- This document provides a summary of MassDEP drinking water requirements; to ensure full compliance, please consult the regulations at 310 CMR 22.07G.

Public Health Benefits

Implementation of the PFAS regulations will result in:
<ul style="list-style-type: none"> • Monitoring for and identifying any elevated PFAS levels in public drinking water. • Corrective actions that reduce drinking water exposures to PFAS6 to below the levels that may cause a variety of health effects to sensitive subgroups, including developmental effects in fetuses and infants, effects on the liver, blood, immune system, thyroid, and may elevate the risk of certain cancers..

Critical Dates and Deadlines

October 2, 2020	MassDEP published its PFAS regulations establishing an MCL of 0.000020 milligrams per liter (mg/l) or 20 ng/l (also called parts per trillion or ppt) for the sum of PFAS6.
January 1, 2021	Large Community (COM) and Non-transient Non-community PWS (NTNC) (schools, workplaces, etc.) serving more than 50,000 people will begin regulatory compliance monitoring.
April 1, 2021	COM and NTNC PWS serving between 10,000 and 50,000 people will begin regulatory compliance monitoring.
October 1, 2021	Small COM and NTNC PWS serving 10,000 or fewer people will begin regulatory compliance monitoring.
September 30, 2022	Transient Non-community PWS (such as hotels and restaurants) must collect, analyze and report sampling results by this date.

Federal Drinking Water Standards

There are currently no federal PFAS drinking water standards. However, USEPA has a health advisory of 70 ppt for the sum of PFOA and PFOS.



What are the Major Provisions?	
Sampling Locations	
<ul style="list-style-type: none"> • PWS must sample at every entry point to the distribution system. • PWS that draw water from more than one source, where the sources are combined before distribution, must collect samples that are representative of all such combined sources after treatment during periods of normal operating conditions. • Consecutive PWS are exempt from conducting compliance monitoring for PFAS for the purchased portion of water when the PWS from which the water is obtained has conducted the required monitoring. 	
Initial Monitoring (First Year)	
<ul style="list-style-type: none"> • Four consecutive quarterly samples must be collected. • Each sample shall be collected in the first month of every quarter during initial monitoring. • The PWS may ask MassDEP to substitute previously conducted quarterly sampling. • If no PFAS is detected in the first two quarters of monitoring, the PWS may request to have MassDEP waive the third and fourth quarters of monitoring. 	
Routine Monitoring	
<ul style="list-style-type: none"> • If initial monitoring does not identify any PFAS a PWS may monitor during one year of each subsequent three-year Compliance Period. • PWS serving more than 3,300 individuals must collect two quarterly samples in that year. • PWS serving fewer than or equal to 3,300 individuals must collect one sample in that year. 	
Monitoring Waivers	
<ul style="list-style-type: none"> • After January 1, 2023, a PWS on routine monitoring may request a monitoring waiver from MassDEP. • Waivers cover a single three-year Compliance Period and must be renewed each Compliance Period. • Sampling under an approved waiver shall occur at least once during the first Compliance Period of each successive nine-year Compliance Cycle. 	
Confirmatory Sampling Requirements	
<ul style="list-style-type: none"> • Initial Monitoring: The first detection of PFAS during initial monitoring, not just the detection of PFAS6, triggers confirmation sampling. • Initial Monitoring: After first detection, subsequent PFAS6 detection greater than 10 ppt triggers confirmation sampling. • Routine Monitoring: Confirmatory sampling is required when PFAS6 is detected greater than 10 ppt during routine monitoring unless MassDEP determines that the location is Reliably and Consistently below the MCL. • The confirmatory sample must be collected as soon as possible after receipt of result requiring confirmation and no later than two weeks from receipt of such result (unless granted a MassDEP extension). • A detection is defined as any PFAS contaminant level greater than the lab's minimum reporting level (MRL). All certified labs must achieve an MRL of 2 ppt or lower for the six PFAS covered by the MCL. 	
Increased Monitoring if PFAS is detected	
Monthly monitoring	<ul style="list-style-type: none"> • If the average of a PFAS6 result and its associated confirmatory sample is greater than 10 ppt, the sampling location must be sampled monthly. • Monthly sampling continues until the source is shown to be Reliably and Consistently Below the MCL.



Quarterly monitoring	<ul style="list-style-type: none"> • A PWS that has installed PFAS treatment and is thereby Reliably and Consistently Below the MCL will be put on quarterly monitoring.
Annual monitoring	<ul style="list-style-type: none"> • If the initial monitoring is complete and PFAS is detected but PFAS6 is confirmed less than 10 ppt, the location must be sampled annually. • A PWS that is determined by MassDEP to be Reliably and Consistently Below the MCL without having to install PFAS treatment may be put on annual monitoring.

Public Education

- Any PWS where there has been a PFAS6 detection, and the average of such detection and an associated confirmatory sample exceeds the PFAS6 MCL, shall provide public education materials regarding the exceedance, as described by MassDEP. These should be provided as soon as possible, but within 30 days.
- Until the PWS obtains a monitoring result at or below the PFAS6 MCL at such locations, public education should be updated quarterly.

Compliance and Violations

- MCL compliance is calculated using the average of the monthly samples over a quarter.
- If any one sampling point location is in violation, then the PWS shall be considered in violation.
- If any sample result would cause the quarterly average to exceed the PFAS6 MCL, the PWS is immediately in violation and begins compliance actions.

Public Notice

- A violation of the MCL requires a Tier 2 Public Notice.
- Monitoring & testing procedure violations require Tier 3 Public Notice.

Seasonal System Provisions

If a PWS reactivates an existing source or opens a seasonal system after the applicable commencement date of this regulation, it shall commence initial monitoring of such locations within the first month of delivering water to the public.

MassDEP Technical Assistance and Grants

- Free testing is available until June 30, 2021 for PWS to sample drinking water for PFAS.
- The Commonwealth provided grant funding in October 2020 to assist PWS in the planning and design of treatment systems to remove PFAS. Another round of grant funding is anticipated.
- MassDEP has made PFAS-reducing drinking water projects a priority in the 2021 State Revolving Fund (SRF) Loan Program. PFAS mitigation projects may be eligible to receive an additional subsidy in the form of a 0% interest rate loan. The additional subsidy is contingent on the availability of funds and approval of the Massachusetts Clean Water Trust Board of Trustees. For more information: <https://www.mass.gov/doc/drinking-water-program-updates-2-13-2020/download>

Key Point for PWS to Remember

- All confirmed detections of PFAS6 > 20 ppt require public education.

For additional information on the PFAS6: Visit the MassDEP website at <https://www.mass.gov/info-details/per-and-polyfluoroalkyl-substances-pfas>; email the MassDEP Drinking Water Program at program.director-dwp@mass.gov; or call the MassDEP Drinking Water Program at 617-292-5770.

THIS DOCUMENT CONTAINS IMPORTANT INFORMATION FOR YOUR SYSTEM. HAVE SOMEONE TRANSLATE IT FOR YOU OR SPEAK WITH SOMEONE WHO UNDERSTANDS IT.

If you need this document translated, please contact MassDEP's Diversity Director, Michelle Waters-Ekanem, Diversity Director/Civil Rights: 617-292-5751 TTY# MassRelay Service 1-800-439-2370. You may also contact the Drinking Water Program at program.director-dwp@mass.gov.



Attachment 33

**HEALTH-BASED MAXIMUM CONTAMINANT LEVEL
SUPPORT DOCUMENT:
PERFLUORONONANOIC ACID (PFNA)**

**New Jersey Drinking Water Quality Institute
Health Effects Subcommittee
June 22, 2015**

Subcommittee Members:
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Table of Contents

ABSTRACT.....	1
INTRODUCTION	1
ENVIRONMENTAL SOURCES, FATE, AND OCCURRENCE	5
HUMAN BIOMONITORING.....	8
SOURCES OF HUMAN EXPOSURE.....	10
TOXICOKINETICS	11
HEALTH EFFECTS.....	17
<i>Human Studies</i>	17
<i>Animal Toxicology</i>	38
MODE OF ACTION.....	64
DEVELOPMENT OF HEALTH-BASED MCL.....	71
DISCUSSION OF UNCERTAINTIES	77
HEALTH-BASED MCL RECOMMENDATION.....	80
APPENDIX 1. Literature Search Criteria and Documentation	100
APPENDIX 2. Individual Study Tables for Epidemiologic Study of Human Health Effects and PFNA	101
APPENDIX 3. Individual Study Tables of Toxicological Studies of PFNA	147
APPENDIX 4. Detailed Benchmark Dose Modeling Results for 10% Increase in Maternal Liver Weight on GD 17 (Das et al., 2015)	169

List of Tables

Table 1. New Jersey versus national PFNA detections (≥ 20 ng/L) in public water supplies in initial UCMR3 results	7
Table 2. Geometric mean and 95% confidence interval and selected percentiles of PFOS, PFOA, PFHxS, and PFNA serum concentrations (ng/mL) for the U.S. population 12 years of age and older: Data from NHANES 2011-2012	9
Table 3. Half-lives of PFNA and PFOA in Male and Female Mice and Rats (days)	12
Table 4. Estimated half-lives of PFNA and PFOA in Humans (years)	13
Table 5. PFNA (ng/ml) in serum from 53 mother:infant pairs ^a	14
Table 6A. Summary of findings from epidemiologic studies of PFNA and serum lipids	19
Table 6B. Summary of findings from epidemiologic studies of PFNA and select metabolic effects.....	21
Table 6C. Summary of findings from epidemiologic studies of PFNA and immune system outcomes.....	22
Table 6D. Summary of findings from epidemiologic studies of PFNA and thyroid hormones and related outcomes. Page 1 of 2	23
Table 6E. Summary of findings from epidemiologic studies of PFNA and reproductive outcomes.	25
Table 6F. Summary of findings from epidemiologic studies of PFNA and neurobehavioral outcomes	27
Table 6G. Summary of findings from epidemiologic studies of PFNA and liver enzymes, bilirubin, and uric acid.....	28
Table 7A. Summary of decreased body weight after oral administration of PFNA	42
Table 7B. Summary of key endpoints for hepatic toxicity and carbohydrate/lipid metabolism after oral exposure to PFNA.....	43
Table 7C. Summary of key renal effects after oral administration of PFNA.....	45
Table 7D. Summary of key endpoints for immune system toxicity of PFNA after oral exposure	46
Table 7E. Summary of key endpoints for male reproductive system toxicity of PFNA after oral exposure...	47
Table 7F. Summary of key endpoints for reproductive/developmental effects of PFNA after oral exposure.	48
Table 8. Comparison of developmental delays in CD-1 mice from PFNA	59
Table 9. PFNA serum level NOAELs and LOAELs for increased liver weight in mice.....	72
Table 10. Benchmark Dose Modeling for 10% Increase in Liver Weight in Pregnant Mice from Das et al. (2015).....	78

Acronyms

BMD — Benchmark Dose Modeling
BMDL — Lower 95% Confidence Limit
CAR — Constitutive androstane receptor
DWQI — Drinking Water Quality Institute
ECHA — European Chemical Agency Risk Assessment Committee
FTOH — Fluorotelomer alcohol
GD — Gestational Day
GM — Geometric mean
IRIS — Integrated Risk Information System
KO — Knockout (PPAR-alpha null)
LD₅₀ — Lethal Dose
LOAEL — Lowest Observed Adverse Effect Level
MCL — Maximum Contaminant Level
NHANES — National Health and Nutrition Examination Survey
NJDEP — New Jersey Department of Environmental Protection
NJDOH — New Jersey Department of Health
NOAEL — No Observed Adverse Effect Level
PFC — Perfluorinated Chemical
PFHxS — Perfluorohexane sulfonate
PFOA — Perfluorooctanoic acid
PFOS — Perfluorooctane sulfonate
PFNA — Perfluorononanoic acid
PFUnDA — Perfluoroundecanoic acid
PND — Postnatal Day
POD — Point of Departure
PPAR --- Peroxisome proliferator activated receptor
PVDF — Polyvinylidene fluoride
PXR — Pregnane X receptor
RfD — Reference Dose
RSC — Relative Sources Contribution
SDWA — Safe Drinking Water Act
UCMR3 — Unregulated Contaminant Monitoring Rule 3
UFs — Uncertainty factors
USEPA — United States Environmental Protection Agency
WT — Wild Type

ABSTRACT

A Health-based Maximum Contaminant Level (Health-based MCL) for perfluorononanoic acid (PFNA, C9) was developed to protect for chronic (lifetime) drinking water exposure. The Health-Based MCL is based on a study of developmental effects in which pregnant mice were exposed to PFNA for 16 days. The Health-based MCL is further supported by data on effects in the offspring in the same study, and on increased liver weight and other effects in additional rodent studies from the same and other laboratories. Benchmark dose modeling was performed on PFNA levels in blood serum that caused increased maternal liver weight in the pregnant mice.

Appropriate uncertainty factors were applied to protect sensitive human subpopulations, account for differences between human and experimental animals, protect for chronic exposure, and account for the incomplete toxicology database for PFNA. Based on available toxicokinetic data from animal and humans, a ratio of 200:1 was used to estimate the increase in PFNA in human blood serum from ongoing exposure to a given concentration of PFNA in drinking water. A chemical specific Relative Source Contribution factor of 50% was developed based on the most recent NHANES data for the 95th percentile PFNA serum levels in the U.S. general population. Using this information, a Health-based MCL protective for chronic drinking water exposure of **13 ng/L (0.013 µg/L)** was derived.

INTRODUCTION

Development of Health-based MCLs by New Jersey Drinking Water Quality Institute

The New Jersey Drinking Water Quality Institute (DWQI), established by the 1984 amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A- 20, is charged with developing standards (Maximum Contaminant Levels; MCLs) for hazardous contaminants in drinking water and for recommending those standards to the New Jersey Department of Environmental Protection (NJDEP). The Health Effects Subcommittee (formerly “Lists and Levels Subcommittee”) of the DWQI is responsible for recommending health-based drinking water levels (Health-based MCLs) as part of the development of MCLs (NJDWQI, 1987; 1994; 2009).

Health-based MCLs are based on the goals specified in the 1984 Amendments to the NJ SDWA. For carcinogens, it is generally assumed that any level of exposure results in some level of cancer risk, and a one in one million (10^{-6}) risk level from lifetime exposure is specified in the statute. Health-based MCLs for carcinogens are thus set at levels that are not expected to result in cancer in more than one in one million persons ingesting the contaminant for a lifetime. For non-carcinogenic effects, it is generally assumed that exposure below a threshold level will not result in adverse effects. As specified in the statute, Health-based MCLs are set at levels which are not expected to result in in “any adverse physiological effects from ingestion” for a lifetime.

Other factors such as analytical quantitation limits and availability of treatment removal technology are also considered in the final MCL recommendation. For carcinogens, the 1984 Amendments to the NJ SDWA require that MCLs are set as close to the one in one million lifetime risk goal as possible “within the limits of medical, scientific and technological feasibility.” For non-carcinogens, MCLs are set as close to the goal of no adverse effects as possible “within the limits of practicability and feasibility.”

On March 21, 2014, the Commissioner Bob Martin of the NJDEP requested that DWQI recommend an MCL for perfluorononanoic acid (PFNA, C9).

To support the development of an MCL recommendation by the DWQI, the Health Effects Subcommittee has developed a Health-based Maximum Contaminant Level for PFNA. As specified in the Amendments to the NJ SDWA, this Health-based MCL is intended to be protective for chronic (lifetime) drinking water exposure.

Document development process

The Subcommittee conducted a literature search of the PubMed and Toxline databases for potentially relevant information. Additional references were identified through backward searching. In total, approximately 455 references were identified (Appendix 1). All of these references were screened by title, abstract and/or full text. Based on this screening, 169 references relevant to human health effects, human biomonitoring, animal toxicology, pharmacokinetics, and *in vitro* studies were designated for “further consideration” while 292 other references not relevant to these areas were excluded. Some references that were excluded as irrelevant to these topics were used to inform supporting sections of this assessment, such as the “Background Information” and “Environmental Sources, Fate, and Occurrence” sections.

In May 2014, the DWQI posted a request for public input regarding data or technical information about the toxicology, epidemiology, toxicokinetics, or other health effects topics related to PFNA that should be considered in developing an MCL. The Health Effects Subcommittee received and considered comments relevant to these topics that were submitted by the public

NJDEP has recently developed a draft Interim Specific Ground Water criterion for PFNA. Like Health-based MCLs, NJ Interim Specific Ground Water Criteria are intended to be protective for chronic (lifetime) drinking water exposure. The Health Effects Subcommittee reviewed the Draft Technical Support Document for the Interim Specific Ground Water Criterion for PFNA (NJDEP, 2014). Some sections of the Health-based MCL Support Document were developed *de novo* by the Health Effects Subcommittee, while other sections are based on updates of relevant information from the Draft Technical Support Document. In 2014, NJDEP solicited public comments on the Draft Technical Support Document for the Interim Specific Ground Water Criterion for PFNA. The Health Effects Subcommittee reviewed these comments and NJDEP’s draft responses.

In April 2015, the DWQI posted the Draft Health-based MCL Support Document for public comment. All comments relevant to the Support Document were reviewed by the Health Effects Subcommittee. Revisions, where appropriate, are incorporated into this updated version.

BACKGROUND INFORMATION

Perfluorinated chemicals (PFCs) are a class of anthropogenic chemicals with structures consisting of a totally fluorinated carbon chain of varying length and a charged functional group, such as carboxylic or sulfonic acid (Lindstrom et al., 2011). The eight carbon compounds, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) have been the most intensively studied PFCs in the past. Recent research has focused on a wider range of PFCs including PFNA, the nine carbon perfluorinated carboxylic acid, which is the subject of this document. Because PFOA and PFNA are closely related and share similar properties, some of the discussions to follow include comparisons with information on PFOA as a part of the basis for evaluation of PFNA.

Physical and Chemical Properties (ATSDR, 2009; ALS Environmental, 2014)

Chemical Name:	Perfluorononanoic acid
Synonyms:	PFNA, C9
CAS Number:	375-95-1
Chemical Formula:	C ₉ HF ₁₇ O ₂
Chemical Structure:	CF ₃ (CF ₂) ₇ COOH
Molecular Weight:	464.08
Physical State:	white crystalline powder
Melting Point:	65-68 °C
Boiling Point:	218 °C at 740 mm Hg
Vapor Pressure:	No data
Water Solubility	9.5 g/L at 25 °C
Log octanol/water partition coefficient:	Not applicable
Taste Threshold (water):	No data
Odor Threshold (water):	No data
Odor Threshold (air):	No data

PFNA is a fully fluorinated carboxylic acid. Because carbon-fluorine bonds are among the strongest found in organic chemistry, PFNA and other PFCs are extremely stable and resistant to chemical reactions (Post et al., 2013).

PFNA and other PFCs contain a long perfluorocarbon tail that is both hydrophobic and oleophobic (repels both water and oil) and a charged functional group that is hydrophilic. Because they form a separate layer when mixed with hydrocarbons and water, measurement of the octanol:water partition coefficient is not practical (Prevedouros et al., 2006).

PFNA is manufactured as its ammonium salt, ammonium perfluorononanoate (APFN), which dissociates in water. At the pH range found in drinking water (6.5-8.5), PFNA is present almost totally in the non-volatile anionic form, the perfluorononanoate anion (Goss, 2008; Rayne and Forest, 2010).

Production and Use

The production, industrial uses, and environmental fate of PFNA were reviewed by Prevedouros et al. (2006). Production of PFNA began in 1975; it was made primarily in Japan. It is produced as the linear isomer of its ammonium salt (ammonium perfluorononanoate, APFN). It was estimated that 10% of the APFN that was produced was released to air and water at the production facility, resulting in global emissions of 70,000 to 200,000 kg PFNA between 1975 and 2004.

PFNA is the primary component of Surflon S-111 (CAS # 72968-3-88), a commercial mixture of linear perfluorinated carboxylic acids. Prevedouros et al. (2006) report the composition of Surflon S-111 by weight as PFNA, 74%; perfluoroundecanoic acid (PFUnDA, C11), 20%; perfluorotridecanoic acid (C13), 5%; PFOA (C8), 0.78%; perfluorodecanoic acid (C10), 0.37%; and perfluorododecanoic acid (C12), 0.1%.

The primary historic use of PFNA was as a processing aid in the emulsion process used to make

fluoropolymers, mainly polyvinylidene fluoride (PVDF), similar to the use of PFOA as a processing aid in the production of polytetrafluoroethylene (PTFE). PFNA is used to solubilize the monomer, vinylidene fluoride, used to make PVDF (Prevedouros et al., 2006). Prevedouros et al. (2006) lists the 2002 production capacities of major producers of PVDF by the emulsion process which uses PFNA/Surflon S-111. The two highest capacity facilities using the emulsion process in 2002 were located in Calvert City, KY (8.4×10^6 kg/yr) and Thorofare (West Deptford), NJ (7.7×10^6 kg/yr), with lower capacity sites in France and Japan.

PVDF is resistant to high temperatures and is chemically non-reactive. Uses of PVDF include: in tanks, valves, pipes, and other components which come into contact with reactive chemicals; as insulation for wire and printed circuit boards; as a coating in pressure and thermal optic sensors; as a binder for electrodes on lithium ion batteries; in artificial membranes used for biomedical applications, for monofilament fishing lines; and in architectural coatings (TOEFCO, 2014). PFNA is not an intended component of PVDF and is present only at trace levels (100-200 ppm) in the PVDF fluoropolymer used in commercial and industrial products that is produced with PFNA (Prevedouros et al., 2006).

It is estimated that 60% of the PFNA used in PVDF manufacturing worldwide was released to the environment, resulting in global emissions of 400,000 to 1,400,000 kg from 1975-2004 (Prevedouros et al., 2006). Data provided to NJDEP about PFC use at the PVDF manufacturing facility located in Thorofare (West Deptford), NJ indicate that 86.6% of the 125,069 kg of the Surflon S-111 PFC mixture (primarily PFNA) used between 1991-2010 was released to the environment (air and water) (Roux Associates Inc., 2013). The environmental fate of PFNA is discussed below.

The manufacture and use of PFOA, PFNA, and other long-chain perfluorinated carboxylates is currently being phased out by eight major manufacturers through a voluntary stewardship agreement with USEPA, with the intent to reduce global facility emissions and product content of these chemicals by 95% by 2010, and with the ultimate goal of eliminating emissions and product content by 2015 (USEPA, 2010, 2012a). The manufacturer of PVDF at the facility located in Thorofare is a participant in the voluntary stewardship agreement. However, other manufacturers of long-chain PFCs that are not participants in the voluntary stewardship agreement continue to manufacture these compounds, in the U.S. and particularly overseas (USEPA, 2009; Lindstrom et al., 2011). It is not known if PFNA is produced by manufacturers that are not part of the voluntary stewardship agreement with USEPA.

Data provided to NJDEP show that Surflon S-111, the PFC mixture consisting primarily of PFNA, was not used in 2011-2012 at the PVDF manufacturing facility located in Thorofare, NJ (Roux Associates Inc., 2013). In 2010, only 171 kg were used, compared to 6,341-8,467 kg/year in each of the previous 10 years.

Evaluations by other government agencies

No health-based guidance values or standards have been developed for PFNA by U.S. federal agencies including USEPA, U.S. states, or other nations.

The European Chemical Agency (ECHA) Risk Assessment Committee finalized its harmonized classification and labeling opinion (CLH) for PFNA in September 2014 (ECHA, 2014). The ECHA classifications are related to hazard identification and

qualitative weight of evidence for various endpoints and do not include dose-response, quantitative risk assessment, or criteria development. ECHA concluded that PFNA is a presumed human reproductive toxicant for damage to the unborn child; a suspected human reproductive toxicant for fertility effects; a suspected human carcinogen; causes specific target organ toxicity to liver, thymus, and spleen after prolonged or repeated exposure; and causes harm to the breast-fed child through effects on or via lactation. These conclusions are based on data on PFNA itself, as well as supporting information from PFOA, to which it is closely related. The background document for the ECHA classification of PFNA was prepared by the Swedish Environmental Agency (Swedish Environmental Agency, 2013).

ENVIRONMENTAL SOURCES, FATE, AND OCCURRENCE

Because of the extreme stability of their carbon–fluorine bonds, PFCs are extremely persistent in the environment. PFCs are highly water-soluble in comparison with other well-studied persistent and bioaccumulative organic pollutants which have much lower water solubilities, such as polychlorinated dioxins and PCBs (Post et al., 2013). Although the production and use of PFNA is being phased out by major U.S. manufacturers, environmental contamination and human exposure to PFNA are anticipated to continue for the foreseeable future due to its persistence, formation from precursor compounds (discussed below), and the potential for continued production by other manufacturers in the U.S. and/or overseas (USEPA, 2009; Lindstrom et al., 2011).

PFCs including PFNA are found in environmental media, including wildlife, in worldwide locations including remote polar regions. In addition to release from industrial facilities where it is made or used, an additional possible source of PFNA in the environment is its formation under some conditions from precursor compounds such as fluorotelomer alcohols (FTOH), used industrially and in consumer products (Butt et al., 2010; Buck et al., 2011).

The fluorotelomer alcohol 8:2 FTOH [$\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{OH}$] is converted to some extent to both PFNA and PFOA through non-biological chemical reactions in the atmosphere (Ellis et al., 2004) and through metabolic reactions in soil bacteria, under some conditions, and in fish (Butt et al., 2014).

Polyfluoroalkyl phosphoric acid diesters such as diPAPs 8:2 (larger molecules found in grease proof food contact papers, wastewater treatment plant sludge, and paper fibers from paper mills; D'eon et al., 2009) release FTOH that can degrade to PFCs. Fluoroacrylate polymers, used in commercial products, may also degrade in soil to release FTOH (Russell et al., 2008; Washington et al., 2009). Since PFNA and other PFCs do not degrade appreciably, environmental PFC levels could be increased by even a small rate of conversion of the precursors to the terminal PFC product.

Two major pathways have been proposed for long-range transport of PFCs such as PFNA to remote locations worldwide (Lau et al., 2007; Butt et al., 2010). The relative contribution of each of these pathways is not known. The first pathway involves the atmospheric transport of volatile precursors, such as FTOH, followed by oxidation to PFCs (e.g. PFOA and PFNA) which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of perfluorinated carboxylates such as PFOA and PFNA in their anionic forms to remote locations by currents on the ocean's surface.

Attachment 34



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MUNICIPAL BUILDING
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MAYOR

Denice DiCarlo

TOWNSHIP COMMITTEE

James Mehaffey
 Megan Kerr
 Adam Reid
 Jim Robinson

Township Committee meeting dates are the first and third Wednesday of every month. Special meetings are advertised according to law.

Lee Ann DeHart
 Township Administrator
 Mike Kwasizur, C.F.O.

West Deptford Township prohibits discrimination on the basis of race, color, creed, sex, national origin, age, religion, veteran's status, marital status or handicap. We are committed to a program of affirmative action in compliance with Title IX.



Annual Drinking Water Quality Report 2021 (2020 Data)

West Deptford Township
 PWSID# NJ0820001



West Deptford Township's goal is to provide you with water that meets or surpasses all the standards for safe drinking water.

These health and safety standards are set by the United States Environmental Protection Agency (EPA) and the New Jersey Department of Environmental Protection (NJDEP). We're at work 24 hours a day, 365 days a year to provide you and your family with top quality water. We regularly test water samples to be sure that your water meets the safety standards. All the test results are on file with the NJDEP, the agency that monitors and regulates drinking water quality in our state. Both the EPA and the NJDEP require water suppliers to send a Consumer Confidence Report (CCR) to customers on an annual basis.

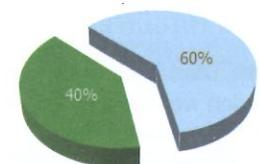
This CCR provides important information about your drinking water. It shows how your drinking water measured up to government standards during 2020. Please read it carefully and feel free to call West Deptford Township at 856.845.4004 or the EPA Safe Drinking Water Hotline at 800.426.4791 with any questions. If you have specific questions about water as it relates to your personal health we suggest that you contact your health care provider.

Where does your water come from?

West Deptford Township obtains our water from a blend of sources. The Township maintains five active wells drilled between 200 and 400 feet in the underground source of water called the Potomac-Raritan-Magothy (PRM) Aquifer. The Township controls the property around these wells and restricts any activity that could contaminate them. All of our water is treated at one of five treatment facilities located at or near the wells.

Water is also obtained from NJ American Water Company who maintain groundwater sources in the PRM Aquifer and a surface water treatment plant located in Delran, NJ. The source for this system is the Delaware River.

Water Sources



■ West Deptford ■ NJ American



How do drinking water sources become polluted?

In order to ensure that tap water is safe to drink, EPA prescribes regulations which limit the amount of certain contaminants in water provided by public water systems. Food and Drug Administration regulations establish limits for contaminants in bottled water which must provide the same protection for public health.

Drinking water, including bottled water, may reasonably be expected to contain at least small amounts of some contaminants. The presence of contaminants does not necessarily indicate that water poses a health risk. More information about contaminants and potential health effects can be obtained by calling the **EPA's Safe Drinking Water Hotline (800-426-4791)**.

The sources of drinking water (both tap water and bottled water) include rivers, lakes, streams, ponds, reservoirs, springs, and wells. As water travels over the surface of the land or through the ground, it dissolves naturally occurring minerals and, in some cases, radioactive material, and can pick up substances resulting from the presence of animals or from human activity.

Contaminants that may be present in source water include:

Microbial contaminants, such as viruses and bacteria, which may come from sewage treatment plants, septic systems, agricultural livestock operations, and wildlife.

Inorganic contaminants, such as salts and metals, which can be naturally-occurring or result from urban stormwater runoff, industrial or domestic wastewater discharges, oil and gas production, mining, or farming.

Pesticides and herbicides, which may come from a variety of sources such as agriculture, urban stormwater runoff, and residential uses.

Organic chemical contaminants, including synthetic and volatile organic chemicals, which are byproducts of industrial processes and petroleum production, and can also come from gas stations, urban stormwater runoff, and septic systems.

Radioactive contaminants, which can be naturally occurring or

Source Water Assessments

The NJDEP has completed and issued the Source Water Assessment Report and Summary for this public water system, which is available at <http://www.state.nj.us/dep/swap> or by contacting the NJDEP's Bureau of Safe Drinking Water at **609-292-5550**.

The source water assessment table for West Deptford Township is provided below. The table provides the number of wells that have either a high (H), medium (M), or low (L) susceptibility rating for each of eight contaminant categories.

If a water system is rated highly susceptible for a contaminant category, it does not mean a customer is or will be consuming contaminated drinking water. The rating reflects the potential for contamination of source water, not the existence of contamination. Public water systems are required to monitor for regulated contaminants and to install treatment if any contaminants are detected at frequencies and concentrations above allowable levels. As a result of the assessments, the DEP may change existing monitoring schedules based upon susceptibility ratings.

degreasers, and gasoline components. Examples include benzene, methyl tertiary butyl ether (MTBE), and vinyl chloride.

Inorganics: Mineral-based compounds that are both naturally occurring and man-made. Examples include arsenic, asbestos, copper, lead, and nitrate.

Radon: Colorless, odorless, cancer-causing gas that occurs naturally in the environment. For more information go to <http://www.nj.gov/dep/rpp/radon/index.htm> or call 800-648-0394.

Disinfection Byproduct Precursors: A common source is naturally occurring organic matter in surface water. Disinfection byproducts are formed when the disinfectants used to kill pathogens (usually chlorine) react with dissolved organic material (leaves, etc.) in surface water.

Pathogens: Disease-causing organisms such as bacteria and viruses. Common sources are animal and human fecal wastes.

Nutrients: Compounds, minerals and elements (both naturally occurring and man-made) that aid plant growth. Examples include nitrogen and phosphorus.

Pesticides: Man-made chemicals used to control pests, weeds and fungus. Common sources include land application and manufacturing of pesticides. Examples include herbicides such as atrazine, and insecticides such as chlorodane.

Radionuclides: Radioactive substances that are both naturally occurring and man-made. Examples include radium and uranium.

Volatile Organic Compounds: Man-made chemicals used as solvents,

Sources	Pathogens			Nutrients			Pesticides			Volatile-Organic Compounds			Inorganics			Radio nuclides			Radon			Disinfection Byproduct Precursors		
	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L
6 Wells	0	0	6	0	0	6	0	0	6	1	0	5	0	6	0	1	4	1	0	1	5	0	6	0

Waived Requirements

The Safe Drinking Water Act regulations allow monitoring waivers to reduce or eliminate the monitoring requirements for asbestos, volatile organic chemicals, and synthetic organic chemicals. Our system has been granted a waiver for asbestos.

Contact Information

Please contact West Deptford Township at 856-845-4004 regarding the content of this report. We encourage public participation at our regular meeting which is held every first and third Wednesday of each month. Meetings are located at the Municipal Building, 400 Crown Point Road, West Deptford, NJ.

This water quality report can be found at https://www.westdeptford.com/pdf/WDT_Water_Quality_Report_2020.pdf



Information on Perfluoroalkyl Substances (PFAS)

Q: What are Perfluoroalkyl Compounds (PFAS)?

A: PFAS are a large group of synthetic fluorinated organic compounds that contain at least one fully fluorinated carbon atom and are widely used in manufacturing everyday products to make them more resistant to stains, grease and water. PFAS are used in non-stick cookware, stain resistant carpets and waterproof clothing.

Q: How are people exposed to PFAS?

A: PFAS are used in manufacturing processes, so they are not usually present in high concentrations in most consumer products. PFAS are environmentally persistent and recalcitrant towards nature degradation. They would accumulate in fish and other animals that humans consume. Some PFAS are used in insecticides, firefighting foam and aftermarket carpet treatment.

Therefore PFAS could be ingested or inhaled from products that use the chemicals and from environmental release of the chemicals.

Q: Are PFAS in drinking water a concern?

A: The Environmental Protection Agency (EPA) and New Jersey Department of Environmental Protection (NJDEP) are regulatory agencies that are currently evaluating the health effects of PFC in the environment and in humans. The science is still evolving around this class of compounds.

Q: Are there any New Jersey regulatory standards for PFAS?

A: NJDEP has approved a MCL of 13 parts per trillion for PFNA, as of September 2018. Additional standards for PFOS (13 ppt) and PFOA (14 ppt) were issued in 2020.

Q: What about West Deptford Township residents who rely upon potable private wells for their drinking water?

A: West Deptford Township has worked in collaboration with the NJDEP and Solvay to identify and sample private wells used for drinking water. If the well results were above NJDEP drinking water standards for PFCs, local treatment systems known as Point of Entry Treatment (POET) water system were installed to remove the PFAS from the water prior to use. West Deptford Township entered into a contract with the NJDEP in 2015 to connect West Deptford residents with private potable wells, which have tested positive for the presence of PFAS, to the West Deptford Public Water Supply System. Since that time, many private wells have been connected to the Township water system. Some of the funding of this program is coming from the NJDEP. Solvay has also reimbursed the Township for some water connections.

Q: Where can I obtain additional information on the subject of PFAS?

A: There are numerous studies and informational resources on this subject on the internet and links exist on the EPA and NJDEP websites as well. If you don't have access to the internet at your home or on your phone, stop by the West Deptford Public Library, they are a great resource for our community.

Q: Are there any concerns with West Deptford's drinking water supply with respect to PFAS?

A: The West Deptford Township public water supply remains in compliance with EPA guidelines, NJDEP regulations, and all public safety requirements. West Deptford continues to provide safe drinking water to our residents. The Township obtains source water samples from all six (6) of the township supply wells, the interconnection with the New Jersey American System, and the distribution system on a quarterly basis. We continue to closely monitor the results of this testing and take precautionary actions to safeguard the public water supply.

The Township voluntarily removed Well #3 and Well #8 from service following PFNA exceedances in January 2014 and October 2015, respectively. Both wells remain out of service, however, were sampled for PFAS on a quarterly basis for monitoring purposes only. No water from these sources has been delivered to our customers since the 2014/2015 dates noted above.

NJDEP requires reporting these results in the water quality table within this report. It should be noted however that the detected range for West Deptford sources that supplied water in 2020 was ND-3.59 ppt (PFNA, 13 ppt maximum contaminant level). All 2020 samples above the MCL for PFNA were taken from Well #3. This well has since been inactivated and will no longer be sampled.

The Township is working with Solvay to complete a PFAS treatment system at Well #8, which will be online in 2021.

Q: What are the next steps for West Deptford Township regarding PFAS?

A: West Deptford Township is resolved to continue working with the NJDEP and Solvay to ensure your water meets and exceeds all regulatory standards. Source water sampling will continue on the public water supply system and the results will be published in the annual Water Quality Report. Additional quality control samples will be taken at regular intervals to ensure operational efficiency at the Well #8 treatment plant once that plant is placed back in service. The Township will also monitor the evolution of science around this subject, and any changes that may impact the safety of our customers. The Township will continue to provide property access to Solvay for several West Deptford Township properties in order to assist in ongoing groundwater investigation and remediation projects.

Lead Notice

If present, elevated levels of lead can cause serious health problems, especially for pregnant women and young children. Lead in drinking water is primarily from materials and components associated with service lines and home plumbing. West Deptford is responsible for providing high quality drinking water, but cannot control the variety of materials used in plumbing components. When your water has been sitting for several hours, you can minimize the potential for lead exposure by flushing your tap for 30 seconds to 2 minutes before using water for drinking or cooking. If you are concerned about lead in your water, you may wish to have your water tested. Information on lead in drinking water is available from the Safe Drinking Water Hotline or at

<http://www.epa.gov/safewater/lead>.



People with Special Health Concerns

Some people may be more vulnerable to contaminants in drinking water than the general population. Immuno-compromised persons such as persons with cancer undergoing chemo-therapy, persons who have undergone organ transplants, people with HIV / AIDS or other immune system disorders, some elderly, and infants can be particularly at risk from infections. These people should seek advice about drinking water from their health care providers. EPA / CDC guidelines on appropriate means to lessen the risk of infection by cryptosporidium and other microbiological contaminants are available from the Safe Drinking Water Hotline (800-426-4791).

West Deptford 2020 Water Quality Results					
Radioactive Contaminants	MCLG	MCL	Level Detected	Violation	Likely Source
Combined Radium - 228 & 226 Test Results Year 2020	0 pCi/L	5 pCi/L	Range: ND-3.20 Highest: 3.20	N	Erosion of natural deposits
Gross Alpha Emitters Test Results Year 2020	0 pCi/L	15 pCi/L	Range: ND-5.20 Highest: 5.20	N	Erosion of natural deposits
Inorganic Chemicals	MCLG	MCL	Level Detected	Violation	Likely Source
Arsenic Test Results Year 2020	n/a	5 ppb	Range: ND-0.46 Highest: 0.46	N	Erosion of natural deposits, runoff from orchards, runoff from glass and electronics production waste
Barium Test Results Year 2020	2 ppm	2 ppm	Range: 0.02-0.12 Highest: 0.12	N	Discharge of drilling wastes, metal refineries, and erosion of natural deposits
Fluoride Test Results Year 2020	4 ppm	4 ppm	Range: 0.44-1.40 Highest: 1.40	N	Erosion of natural deposits
Selenium Test Results Year 2020	50 ppb	50 ppb	Range: ND-0.91 Highest: 0.91	N	Discharge from petroleum and metal refineries; Erosion of natural deposits; Discharge from mines
Copper & Lead	MCLG	AL	Level Detected	Violation	Likely Source
Copper Test Results Year 2020	1.3 ppm	1.3 ppm	90th Percentile: 0.281 Samples > AL: 0	N	Corrosion of household plumbing systems and erosion of natural deposits
Lead Test Results Year 2020	0 ppb	15 ppb	90th Percentile: 1.94 Samples > AL: 1	N	Corrosion of household plumbing systems and erosion of natural deposits
Regulated Disinfectants		RUL	Level Detected	Violation	Likely Source
Chlorine Test Results Year 2020		4.0 ppm	Range: 0.05-1.08 RAA: 0.33	N	Treatment process
Volatile Organic Compounds / Disinfection By-products	MCLG	MCL	Level Detected	Violation	Likely Source
HAA5 Haloacetic Acids Test Results Year 2020	n/a	60 ppb	Range: ND-11.1 Highest: 8.53 LRAA	N	Byproduct of drinking water disinfection
TTHM Total Trihalomethanes Test Results Year 2020	n/a	80 ppb	Range: 21.3-58.97 Highest: 52.57 LRAA	N	Byproduct of drinking water disinfection
Dichloromethane ¹ Test Results Year 2020	0 ppb	5 ppb	Range: 0-0.5 Highest: 0.5	N	Discharge from pharmaceutical and chemical factories
¹ Volatile Organic Compounds were sampled during 2020. All 26 contaminants were not detected, last detected dichloromethane result from 2017.					
Secondary Contaminants ²		RUL	Level Found	RUL Exceedance	Likely Source
Iron Test Results Year 2020		0.3 ppm	Range: ND-0.92 Highest: 0.92	Y ³	Erosion of natural deposits
Manganese Test Results Year 2020		50 ppb	Range: ND-44 Highest: 44	N	Erosion of natural deposits
Chloride Test Results Year 2020		250 ppm	Range: 21.4-87.7 Highest: 87.7	N	Erosion of natural deposits
Corrosivity Test Results Year 2020		±1	Range: -0.74 to -1.26 Highest: -1.26	N	Naturally present in the environment
Sodium Test Results Year 2020		50 ppm	Range: 40.8-101 Highest: 101	Y ⁴	Naturally present in the environment
pH Test Results Year 2020		6.5-8.5 Units	Range: 6.62-7.85 Highest: 7.85	N	Naturally present in the environment

West Deptford 2020 Water Quality Results

Secondary Contaminants ²	RUL	Level Found	RUL Exceedance	Likely Source
Sulfate Test Results Year 2020	250 ppm	Range: 11.2-42.5 Highest: 42.5	N	Erosion from natural deposits; Industrial wastes
Hardness, Carbonate Test Results Year 2020	250 ppm	Range: 22.3-78.9 Highest: 78.9	N	Naturally present in the environment
Total Dissolved Solids (TDS) Test Year 2020	500 ppm	Range: 136-276 Highest: 276	N	Erosion from natural deposits
Color Test Results Year 2020	10 CU	Range: ND-10.0 Highest: 10.0	N	Naturally present in the environment
Zinc Test Results Year 2020	5 ppm	Range: 0.004-0.015 Highest: 0.015	N	Naturally present in the environment

² Note on Recommended Upper Limit (RUL) Exceedances: Secondary standards are non-mandatory guidelines to assist public water systems in managing their drinking water for aesthetic considerations, such as taste, color, and odor. These contaminants are not considered to present a risk to human health.

³ Iron: The recommended upper limit for iron is based on unpleasant taste of the water and staining of laundry. Iron is an essential nutrient, but some people who drink water with iron levels well above the recommended upper limit could develop deposits of iron in a number of organs of the body.

⁴ Sodium: For healthy individuals, the sodium intake from water is not important, because a much greater intake of sodium takes place from salt in the diet. However, sodium levels beyond the recommended upper limit may be a concern to individuals on a sodium restricted diet.

Individual Contaminants	MCLG	MCL	Level Found	Violation	Likely Source
Perfluorononanoic Acid (PFNA) Test Results Year 2020	n/a	13 ppt	Range: 0.0-57.0 Highest: 57	Y ⁵	Discharge from industrial chemical factories
Perfluorooctanoic Acid (PFOA) Test Results Year 2020	n/a	14 ppt	Range: ND-10.8 ppt Highest: 10.8 ppt	N	Discharge from industrial chemical factories
Perfluorooctane Sulfonic Acid (PFOS) Test Results Year 2020	n/a	13 ppt	Range: ND-4.55 ppt Highest: 4.55 ppt	N	Discharge from industrial chemical factories
1,4-Dioxane ⁶ Test Results Year 2020	n/a	n/a	Range: ND-0.79 ppb Highest: 0.79 ppb	N	Discharge from industrial chemical factories

⁵ PFNA: The detected levels reported here reflect all available sources during 2020, not the levels delivered to our customers. All water delivered to your tap was below the Maximum Contaminant Level (MCL). Refer to the "Information on Perfluoroalkyl Substances (PFAS)" section for more details about the Township's actions toward PFAS in your drinking water.

⁶ These contaminants were tested in 2020 at the request of NJDEP but did not have regulated contaminant levels in 2020.

Microbiologicals-Revised Total Coliform Rule (RTC)	Number Required	Number Completed	Corrective Actions Required	Corrective Actions Completed
Level 1 Assessment - Total Coliform	0	0	0	0

Total coliform bacteria are generally not harmful themselves. Coliforms are bacteria which are naturally present in the environment and are used as an indicator that other potentially harmful bacteria may be present. 0 of 343 samples tested positive for coliform bacteria.

Unregulated Substances ⁷ for which the EPA requires monitoring	Reference Concentration	Level Detected	Violation
HAA5 Haloacetic Acids Test Results Year 2018	60 ppb	Range: 2.26-9.19 Highest: 9.19	N
HAA6Br Test Results Year 2018	n/a	Range: 1.49-10.47 ppb Highest: 10.47 ppb	N
HAA9 Test Results Year 2018	n/a	Range: 3.21-17.24 ppb Highest: 17.24 ppb	N
Manganese Test Results Year 2015	300 ppb	Range: 1.63-45.47 Average: 0.1	N

⁷ UCMR4 is administered by the United States Environmental Protection Agency. See <https://www.epa.gov/dwucmr/fourth-unregulated-contaminant-monitoring-rule> for additional details.

NJ American Water (NJ0327001) - 2020 Water Quality Results

Regulated Disinfectants	MRDLG	MRDL	Level Detected	Violation	Likely Source
Entry Point Chlorine Residual Test Results Year 2020	4.0 ppm	4.0 ppm	Range: 0.56-1.46 Highest: 1.46	N	Treatment process
Inorganic Chemicals	MCLG	MCL	Level Detected	Violation	Likely Source
Nitrate Test Results Year 2020	10 ppm	10 ppm	Highest: 0.099	N	Runoff from fertilizer use; industrial or domestic wastewater discharges; erosion of natural deposits

NJ American Water (NJ0327001) - 2020 Water Quality Results

Turbidity	MCLG	MCL	Level Found	Violation	Likely Source
Turbidity NTU Test Results Year 2020	n/a	TT= 1 NTU TT=95% < 0.3 NTU	Highest: 0.1 ntu ** Lowest Monthly % of samples ≤ 0.3 ntu: 100%	N	Soil runoff.

** Sample date of highest compliance result: January 3, 2020

100% of the turbidity readings were below the treatment technique requirement of 0.3 NTU. Turbidity is a measure of the cloudiness of water. We monitor turbidity because it is a good indicator of water quality. High turbidity can hinder the effectiveness of disinfectants.

Treatment Byproduct Precursor Removal	MCLG	MCL	Level Found	Violation	Likely Source
TOC Removal Ratio Test Results Year 2020	n/a	TT ≥ 35% Removal	Required Range: 35-45% Achieved Range: 45-66%	N	Naturally present in the environment
Actual/Required TOC Removal Ratio Test Results Year 2020	n/a	TT: RAA ≥ 1.0	Achieved Range: 1.29-1.89	N	Naturally present in the environment

Number of quarters out of compliance for TOC removal: 0

Unregulated Substances for which the EPA requires monitoring (UCMR4)	Reference Concentration	Level Detected	Violation
Manganese *** Test Results Year 2019	300 ppb	Range: ND-1.8 Highest: 1.02 LRAA	N

*** Manganese is regulated as a secondary contaminant with a recommended upper limit (RUL) of 50 ppb

Individual Contaminants	MCLG	MCL	Level Found	Violation	Likely Source
Perfluorooctanoic Acid (PFOA)* Test Results Year 2020	n/a	n/a	Range: 2.4-5.0 ppt Average: 3.46 ppt	N	Used as emulsifier and surfactant in or as fluoropolymers (such as Teflon), fire-fighting foams, cleaners, cosmetics, lubricants, paints, polishes, adhesives and photographic films.
Perfluorooctane Sulfonic Acid (PFOS)* Test Results Year 2020	n/a	n/a	Range: 1.8-4.8 ppt Average: 3.13 ppt	N	Manmade chemical; used in products for stain, grease, heat and water resistance

Definitions

ppm	Parts Per Million: equivalent of one second in 12 days	MCL	Maximum Contaminant Level: The highest level of a disinfectant allowed in drinking water. There is convincing evidence that addition of disinfectant is necessary for control of microbial contaminants.	MRDL	Maximum Residual Disinfection Level The highest level of a disinfectant allowed in drinking water. There is convincing evidence that the addition of a disinfectant is necessary for control of microbial contaminants.
ppb	Parts Per Billion: equivalent of one second in 32 years				
ppt	Parts Per Trillion: equivalent of one second in 32,000 years				
NA	Not Applicable	MCLG	Maximum Contaminant Level Goal: The level of a drinking water disinfectant below which there is no known or expected risk to health. MRDLGs do not reflect the benefits of the use of disinfectant to control microbial contamination.	MRDLG	Maximum Residual Disinfection Level Goal The level of a drinking water disinfectant below which there is no known or expected risk to health. MRDLG's do not reflect the benefit of the use of disinfectants to control microbial contamination.
RUL	Recommended Upper Limit				
ND	Not Detected				
RAA	Running Annual Average	AL	Action Level The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.	Primary Standards: Federal drinking water regulations for substances that are health-related. Water suppliers must meet all primary drinking water standards.	
LRAA	Locational Running Annual Average				
TT	Treatment Technique: A required process intended to reduce the level of a contaminant in drinking water.	CU	Color Unit	Secondary Standards: Federal drinking water measurements for substances that do not have an impact on health. These reflect aesthetic qualities such as taste, odor and appearance. Secondary standards are recommendations, not mandates.	
		pCi/L	Picocuries Per Liter: equivalent of one second in 32 million years		

Notifications

We are required to monitor your drinking water for specific contaminants on a regular basis. Results of regular monitoring are an indicator of whether or not your drinking water meets health standards. WD received a monitoring and reporting violation for CCR content. All information was displayed accurately but we made some formatting changes for this report. During the Jul 2019-Dec 2019 compliance period, we did not complete all monitoring or testing for pH and orthophosphate, and therefore cannot be sure of the quality of your drinking water during that time. During the Jan 2020-June 2020 compliance period, we did not complete all

monitoring or testing for pH, and therefore cannot be sure of the quality of your drinking water during that time. During the Jan 2020-Mar 2020 compliance period, we did not monitor or test for 1,2,3-Trichloropropane, 1,2-Dibromo-3-Chloropropane, and Ethylene Dibromide and therefore cannot be sure of the quality of the drinking water during that time. During the Feb 2020 compliance period, sampling for Chlorine was completed but results were submitted late to NJDEP. The quality of the drinking water is known during that time. Please share this information with all the other people who drink this water, especially those who may not have received this notice directly (for example, people in apartments, nursing homes, schools, and businesses). You can do this by posting this notice in a public place or distributing copies by hand or mail.

Attachment 35



EPA Document No.
822D-21-003

DRAFT

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EXTERNAL PEER REVIEW DRAFT
Draft Framework for Estimating Noncancer Health Risks
Associated with Mixtures of Per- and Polyfluoroalkyl
Substances (PFAS)

**Draft 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

EPA Document Number: EPA 822D-21-003

NOVEMBER 2021

Notices

This document has been reviewed in accordance with U.S. Environmental Protection Agency (EPA) policy and approved for publication for the purpose of external peer review by the EPA Science Advisory Board (SAB).

This document provides a draft framework for estimating the likelihood of noncancer human health risks associated with mixtures of per- and polyfluoroalkyl substances (PFAS), based on longstanding EPA mixtures guidelines and guidance. This document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community, and might not apply to a particular situation based on the circumstances. Based upon peer-review and/or evolving availability of information, EPA may change certain aspects of this document in the future.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Contents

1.0	Introduction and Background	1
1.1	Purpose.....	1
1.2	Background on PFAS	2
1.3	Occurrence of PFAS Mixtures.....	5
1.4	Evidence of PFAS Exposure in Humans	7
1.5	Brief Summary of State, National, and International Approaches to Address PFAS Mixtures in Water.....	8
1.6	Overview of Proposed Framework for Estimating Health Risks for PFAS Mixtures	11
2.0	Background on EPA Mixtures Additivity Guidance	13
2.1	Component-Based Mixtures Assessment Methods	13
2.1.1	Application of Dose Addition as EPA’s Default Assumption.....	15
3.0	Dose Additivity for PFAS	16
3.1	Overview of Assessment Approaches for Chemical Mixtures.....	16
3.2	Examples of Chemical Classes and Toxicological Pathways Utilizing Mixture Assessment Approaches.....	17
3.2.1	Aryl Hydrocarbon Receptor Pathway and Toxic Equivalence Factors of Dioxin-Like Chemicals.....	17
3.2.2	Pyrethroids – Central Nervous System and Behavior.....	18
3.2.3	Organophosphates and Related Pesticides – Lethality, Central Nervous System and Behavior	18
3.2.4	Mixture Effects on the Female Reproductive Tract – Estrogen Agonists	19
3.2.5	Mixture Effects on the Female Reproductive Tract – Phthalates in Utero	19
3.2.6	Male Reproductive Tract Development – Antiandrogens	19
3.3	Systematic Reviews of Mixtures Toxicity: Quantification of Deviations from Dose Additivity.....	22
3.3.1	Deviation from Additivity.....	22
3.4	PFAS Dose Additivity	23
4.0	Introduction to Estimating Noncancer PFAS Mixture Hazard or Risk	26
4.1	Whole Mixtures Approach.....	26
4.2	Component-Based Mixtures Approach for PFAS	26
4.3	Hazard Index.....	31
4.3.1	Illustrative Example Application of Hazard Index to PFAS Mixtures: Screening-Level Hazard Index	35
4.3.1.1	Example 1: PFOA+PFOS	38
4.3.2	Advantages and Challenges of the Approach	40
4.3.3	Target Organ Specific Hazard Index	42

4.4	Relative Potency Factors.....	42
4.4.1	Basic Principle, Data Requirements to Calculate Relative Potency Factors and Corresponding Index Chemical Equivalent Concentrations	42
4.4.2	Illustrative Example Application of Relative Potency Factors and Index Chemical Equivalent Concentrations to PFAS Mixtures.....	44
4.4.2.1	Example 1: PFOA and PFOS Only.....	46
4.4.2.2	Example 2: PFOA, PFOS, PFBS, and GenX Chemicals	48
4.4.3	Advantages and Challenges of the Relative Potency Factor Approach.....	51
4.5	Dose Addition Mixture BMD Approach	53
4.5.1	Basic Principle, Data Requirements for Mixture Benchmark Doses and Corresponding Mixture Health-Based Water Concentrations	53
4.5.2	Illustrative Example Application of Mixture Benchmark Doses and Mixture Health-Based Water Concentrations.....	54
4.5.3	Advantages and Challenges of the Mixture Benchmark Dose Approach.....	56
	References	57
	APPENDIX A: EPA Internal Report, Preliminary Results from a USEPA ORD Study: Cumulative Developmental Toxicity of Combined Exposure to Ammonium Perfluorooctanoic Acid (PFOA, CASRN: 3825-26-1) and Potassium Perfluorooctane Sulfonate (PFOS, CASRN: 2795-39-3) in the Sprague-Dawley Rat	A-1

Figures

Figure 2-1.	Flow chart for evaluating chemical mixtures using component-based additive methods.	14
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.	21
Figure 4-1.	Flow diagram for proposed component-based approaches based on dose additivity to estimate noncancer health risk associated with PFAS mixtures.....	30

Tables

Table 1-1.	Two Primary Categories of PFAS ^a	3
Table 1-2.	Classification and Chemical Structure of Perfluoroalkyl Acids (PFAA) ^a	4
Table 1-3.	Classification of Short-Chain and Long-Chain PFAA ^a	4
Table 1-4.	Summary of 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).....	9

Table 4-1. EPA and ATSDR Peer-Reviewed Human Health Assessments Containing Non-Cancer Toxicity Values (RfDs or MRLs) for PFAS that Are Either Final or Under Development (only final assessment values are provided)	33
Table 4-2. Summary of Information Available for PFAS in the Hypothetical Screening HI Example	36
Table 4-3. Summary of Input Parameters Used to Calculate the 2016 EPA Health Advisories for PFOA and PFOS	39
Table 4-4. Example Screening-Level HQs and HI for PFOA and PFOS at Low Water Concentrations.....	39
Table 4-5. Example Screening-Level HQs and HI for PFOA and PFOS at Higher Water Concentrations.....	40
Table 4-6. Summary of Health Effects/Endpoints Selected for Illustrative RPF Examples	45
Table 4-7. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA and PFOS at Low Water Exposure Concentrations	47
Table 4-8. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA and PFOS at Higher Water Exposure Concentrations	47
Table 4-9. Example Thyroid Effect RPFs for a Mixture of PFOA and PFOS	48
Table 4-10. Example Liver Effect RPFs for a Mixture of PFOA and PFOS.....	48
Table 4-11. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals at Low Water Exposure Concentrations	49
Table 4-12. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals at Higher Water Exposure Concentrations.....	50
Table 4-13. Example Thyroid Effect RPFs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals.....	50
Table 4-14. Example Liver Effect RPFs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals.....	51
Table 4-15. Mixture BMD Approach: Hypothetical Water Sample 1	55
Table 4-16. Mixture BMD Approach: Hypothetical Water Sample 2.....	55

List of Abbreviations and Acronyms

6:2 FTS	6:2 fluorotelomer sulfonic acid	FQPA	Food Quality Protection Act
AFFF	aqueous film forming foam	FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
AhR	aryl hydrocarbon receptor	GenX chemicals	hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt
AOF	adsorbable organofluorine	GD	gestation day
AOP	adverse outcome pathway	HBWC	Health-Based Water Concentration
AR	androgen receptor	HED	human equivalent dose
ATSDR	Agency for Toxic Substances and Disease Registry	HFPO	hexafluoropropylene oxide
AUC	area under the concentration versus time curve	HI	hazard index
BMD	benchmark dose	HQ	hazard quotient
CAR	constitutive androstane receptor	HTTr	high-throughput transcriptomics
C-F	carbon-fluoride bond	IA	integrated addition
CAA	Clean Air Act	IC	index chemical
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act	ICEC	Index Chemical Equivalent Concentration
CPSC CHAP	Consumer Product Safety Commission Chronic Hazard Advisory Panel	ICEC _{MIX}	total mixture ICEC
DA	dose addition	ICED	index chemical equivalent dose
DLC	dioxin-like chemical	IRIS	Integrated Risk Information System
DWI	drinking water intake	IVIVE	in vitro to in vivo extrapolation
E	duration-relevant exposure	KE	key event
EC	effect concentration	LOAEL	lowest observed adverse effect level
ED _x	effective dose in x percent of test animals	MAC	maximum acceptable concentration
EOF	extractable or adsorbable organofluorine	MCL	Maximum Contaminant Level
EPA	U.S. Environmental Protection Agency	MCLG	Maximum Contaminant Level Goal
EU	European Union	mg/kg	milligrams per kilogram

MIE	molecular initiating event	PFDS	perfluorodecanesulfonate
MOA	mode of action	PFECHS	perfluoroethylcyclohexane sulfonate
MRL	minimum risk level		
NAM	New Approach Methodology(ies)	PFHpA	perfluoroheptanoic acid
		PFHpS	perfluoroheptanesulfonic acid
NAS	National Academy of Sciences	PFHxA	perfluorohexanoic acid
NBP2	Nafion byproduct 2	PFHxS	perfluorohexanesulfonic acid
ng/g	nanograms per gram		
ng/L	nanograms per liter	PFNA	perfluorononanoic acid
NHANES	National Health and Nutrition Examination Survey	PFNS	perfluorononanesulfonic acid
NOAEL	no observed adverse effect level	PFOA	perfluorooctanoic acid
		PFOS	perfluorooctanesulfonic acid
NPDWR	National Primary Drinking Water Regulation	PFOSA	perfluorooctane sulfonamide
NTP	National Toxicology Program	PFPA	perfluoropropanoic acid
		PFPeA	perfluoropentanoic acid
		PFPeS	perfluoropentanesulfonic acid
OECD	Organisation for Economic Co-operation and Development		
		PFPIA	perfluoroalkyl phosphinic acid
OP	organophosphate		
ORD	Office of Research and Development	PFPS	perfluoropropane sulfonic acid
osRfV	organ-specific reference value	PFSA	perfluoroalkane sulfonic acid
PCB	polychlorinated biphenyl	PFSIA	perfluoroalkane sulfinic acid
PCDD	polychlorinated dibenzo-p-dioxins		
		PFTA	perfluorotetradecanoic acid
PCDF	polychlorinated dibenzofuran		
		PFTTrDA	perfluorotridecanoic acid
PFAA	perfluoroalkyl acids	PFUnA	perfluoroundecanoic acid
PFAS	per- and polyfluoroalkyl substances	PIL	post-implantation loss
		PND	post-natal day
PFBA	perfluorobutanoic acid	POD	point of departure
PFBS	perfluorobutanesulfonic acid	PPAR α	peroxisome proliferator activated receptor alpha
PFCA	perfluoroalkyl carboxylic acid	PPAR γ	peroxisome proliferator activated receptor gamma
PFDA	perfluorodecanoic acid	ppt	parts per trillion
PFDoDA	perfluorododecanoic acid	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
SARA	Superfund Amendments and Reauthorization Act
SDWA	Safe Drinking Water Act
SEM	standard error of the mean
T4	serum thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TD	toxicodynamic
TEC	toxic equivalent concentrations
TEF	toxic equivalence factor
TEQ	toxic equivalents
TK	toxicokinetic
TOSHI	target organ specific hazard index
TSCA	Toxic Substances Control Act
TTD	target-organ toxicity doses
UCMR	Unregulated Contaminant Monitoring Rule
UF _L	LOAEL-to-NOAEL uncertainty factor
UF _S	extrapolation from subchronic to a chronic exposure duration uncertainty factor

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) is issuing the *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)*. This document is designed to communicate and illustrate the practical application of existent EPA chemical mixtures approaches and methods to two or more PFAS co-occurring in environmental media, such as drinking water. Specifically, this document describes an approach for providing a flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS based on dose additivity. Descriptions of the Hazard Index (HI) approach (Tier 1) and Relative Potency Factor (RPF) and Mixture Benchmark Dose (BMD) (Tier 2) approaches are presented to demonstrate application to PFAS mixtures but they are not intended to provide a comprehensive treatise on the methods themselves; EPA mixtures guidelines and guidance (EPA, 1986, 2000) already exist for such a purpose. The EPA 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 interested in assessing the potential noncancer human health hazards and risks associated with PFAS mixtures.

PFAS are a large and diverse structural family of compounds used in a myriad of 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 that time, hundreds of PFAS have been identified in water, soil, and air. Many PFAS are environmentally persistent, bioaccumulative, and have long half-lives in humans, particularly the longer-chain species such as PFOA and PFOS. PFAS with fewer carbon atoms, such as perfluorobutanesulfonic acid (PFBS) and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (GenX chemicals), were subsequently developed and integrated into various consumer products and industrial applications because they have the desired properties and characteristics associated with this class of compounds but are more quickly eliminated from the human body than PFOA and PFOS; however, shorter-chained compounds are not necessarily less toxic to humans. The landscape of PFAS encountered in environmental media is often a diverse milieu of linear and branched parent species, metabolites, and/or abiotic degradants, leading to significant potential for PFAS mixture exposures in aquatic, terrestrial, and human populations.

As of November 2021, completed EPA human health assessments are available for four PFAS; these include PFOA (EPA, 2016a), PFOS (EPA, 2016b), PFBS (EPA, 2021a), and GenX chemicals (EPA, 2021b). EPA is in the process of updating the assessments for PFOA and PFOS, which are undergoing EPA Science Advisory Board (SAB) review along with this document (EPA, 2021c,d), and developing five additional PFAS toxicity assessments (perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA)), which are expected to be completed by 2024. In May 2021, the Agency for Toxic Substances and Disease Registry (ATSDR) published a “Toxicological Profile for Perfluoroalkyls” that included an

additional nine species that EPA has not yet formally assessed. However, beyond PFOA and PFOS, ATSDR derived quantitative minimal risk levels (MRLs) for only two additional PFAS: PFHxS and PFNA. 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. EPA and the National Institute of Environmental Health Sciences (NIHES) are actively engaged in research and testing to help address data gaps for a broad landscape of PFAS (approximately 150 species at the time of the drafting of this document). Until results from these ongoing research and testing efforts are available, the evaluation of potential toxicity/risk associated with mixtures of PFAS is limited to existing data under the purview of human health assessments by federal, state, and/or international entities. The application of the component-based methods presented in this PFAS mixtures framework document is demonstrated using the four PFAS for which EPA human health assessments are available; however, this framework allows for the integration of information derived from other health assessment data sources (e.g., other federal, state, international), available human epidemiological and experimental animal hazard and dose-response data, and information from New Approach Methodologies (NAMs). Opportunities for integrating additional PFAS into the context of a mixture assessment is 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.

The PFAS “mixture” information selected for the illustrative examples of the HI, RPF, and Mixture BMD approaches in Section 4 of this document were based on final EPA toxicity assessments at the time of drafting this document: PFOA (EPA, 2016a), PFOS (EPA, 2016b), PFBS (EPA, 2021a), and GenX chemicals (EPA, 2021b), and is purely intended for demonstration of methodological application and is not intended to be used directly in risk assessment or remediation, or in a regulatory context. Further, the extent of the framework’s utility for a particular programmatic application will need to be assessed within each specific decision context under different authorities and regulations.

1.0 Introduction and Background

1.1 Purpose

The U.S. Environmental Protection Agency (EPA) has initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) for per- and polyfluoroalkyl substances (PFAS) under the Safe Drinking Water Act (SDWA) (86 FR 12272, March 3, 2021). The agency is seeking comment from the EPA SAB on key scientific issues related to development of the NPDWR. As part of this proposed rulemaking, EPA has prepared this document, *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)*, that illustrates the practical application of EPA chemical mixtures approaches and methods for two or more PFAS co-occurring in environmental media, such as drinking water. This draft framework is being submitted by EPA for scientific review by the SAB along with three other documents:

- EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (EPA, 2021c)
- EPA's *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (EPA, 2021d)
- EPA's *Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water* (EPA, 2021e)

Each of the four documents of which EPA is seeking review will inform development of the MCLGs and NPDWR for PFOA and PFOS. EPA is moving expeditiously to develop the proposed MCLGs and NPDWR, therefore this draft document was developed concurrently with the three other draft documents for SAB review. While qualitative statements on health effects are consistent with the conclusions from the first two documents listed above, this framework does not fully incorporate the updated information described in the proposed approaches for deriving MCLGs for PFOA and PFOS (EPA, 2021c,d). Specifically, all example calculations presented in this document for PFOA and PFOS are based on information from previously issued, final EPA human health assessments and drinking water Health Advisories (EPA, 2016a,b,c,d). EPA will consider the collective input from the SAB on this framework document and the other three draft documents to prepare final documents that will inform the promulgation of MCLGs and NPDWRs.

This document provides a draft approach for a tiered, data-driven framework for estimating the likelihood of noncancer human health risks associated with oral exposures to mixtures of PFAS, based on longstanding EPA guidelines and guidance (EPA, 1986, 2000). Although the framework and illustrative examples provided in this document include examples for PFAS in water, the framework itself is not limited to specific media and may be useful for understanding the potential non-cancer health effects of PFAS mixtures under various authorities or decision contexts. The approach presented here is not intended to be used to assign groups or subclasses or otherwise classify PFAS. Rather, the framework is designed for practical application of EPA chemical mixtures approaches and methods for a particular exposure to gain insight on the potential health risk(s) associated with exposure to mixtures of PFAS. The EPA 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 interested in assessing the potential human health hazards and risks associated with PFAS mixtures.

The framework and example calculations presented here incorporate information for four PFAS for which EPA has finalized toxicological assessments: PFOA, PFOS, PFBS, and GenX chemicals. However, due to the constantly evolving science related to PFAS, the approach has the flexibility to consider information as it becomes available, including forthcoming EPA toxicological assessments, assessments from other sources (e.g., federal, state, international), available hazard and dose-response data in the public domain, and information from high throughput bioassays and other NAMs.

The draft document is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community, and might not apply to a particular situation based on the circumstances. Based upon peer-review and/or evolving availability of information, EPA may change certain aspects of this document in the future.

1.2 Background on PFAS

PFAS are a large group of anthropogenic chemicals that include PFOA, PFOS, and thousands of other chemicals. The universe of environmentally relevant PFAS, including parent chemicals, metabolites, and degradants, is greater than 9,000 compounds.¹ 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, the number of PFAS currently used in commercial products at the time of the drafting of this document is approximately 250 substances (Buck et al., 2021).

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States since the 1940s. PFAS have strong, stable carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Beach et al., 2006; Buck et al., 2011). The chemical structures of PFAS make them repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties; these properties are what make PFAS useful for commercial and industrial applications and purposes, but these are also what make some PFAS extremely persistent in the human body and the environment (Calafat et al., 2007, 2019). Due to their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many PFAS co-occur in exposure media (e.g., air, water, ice, sediment), and in tissues and blood of aquatic and terrestrial organisms, and humans.

There are many families or classes of PFAS, and each contains many individual structural homologues and can exist as either branched-chain or straight-chain isomers (Buck et al., 2011; EPA, 2021f). These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl and polyfluoroalkyl substances. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-1). For the proposed reporting and recordkeeping requirements for PFAS under the Toxic Substances Control Act (TSCA), PFAS are defined as “per- and polyfluorinated

¹ See EPA PFAS Master List of PFAS Substances (Version 2) available at: https://comptox.epa.gov/dashboard/chemical_lists/PFASMASTER

substances that structurally contain the unit R-(CF₂)-C(F)(R')R". Both the CF₂ and CF moieties are saturated carbons and none of the R groups (R, R' or R") can be hydrogen" (86 FR 33926, June 28, 2021). It should be noted however that what defines or constitutes a PFAS may change over time under different purviews (e.g., federal, state, international).

Table 1-1. Two Primary Categories of PFAS^a

PFAS Non-polymers	Structural Elements	Example PFAS Families
Perfluoroalkyl substances	Compounds in which all carbon-hydrogen bonds, except those on the functional group, are replaced with carbon-fluorine bonds	Perfluoroalkyl acids (e.g., PFOA, PFOS), perfluoroalkane sulfonamides, perfluoroalkane sulfonyl fluorides, perfluoroethers
Polyfluoroalkyl substances	Compounds in which all carbon-hydrogen bonds on at least one carbon (but not all) are replaced with carbon-fluorine bonds	Polyfluoroalkane sulfonamido derivatives, semifluorinated <i>n</i> -alkanes and alkenes, fluorotelomers, polyfluoroalkyl ether carboxylic acids
PFAS Polymers	Structural Elements	Example PFAS Families
Fluoropolymers	Carbon-only polymer backbone with fluorines directly attached	Polytetrafluoroethylene, polyvinylidene fluoride
Polymeric perfluoropolyethers	Carbon and oxygen polymer backbone with fluorines directly attached to carbon	F-(C _m F _{2m} O-) _n CF ₃ , where the C _m F _{2m} O represents -CF ₂ O, -CF ₂ CF ₂ O, and/or -CF(CF ₃)CF ₂ O distributed randomly along polymer backbone
Side-chain fluorinated polymers	Non-fluorinated polymer backbone with fluorinated side chains with variable composition	Fluorinated acrylate and methacrylate polymers, fluorinated urethane polymers, and fluorinated oxetane polymers

^a Modified from Buck et al. (2011).

PFOA and PFOS belong to the perfluoroalkyl acids (PFAA) of the non-polymer perfluoroalkyl substances category of PFAS and are among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review see Podder et al., 2021). The PFAA family includes perfluoroalkyl carboxylic, phosphonic, and phosphinic acids and perfluoroalkane sulfonic and sulfinic acids (Table 1-2). PFAA are highly persistent and are frequently found in the environment (Ahrens, 2011; Brendel et al., 2018; Wang et al., 2017). Although 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 FR 45109, July 27, 2020), a more comprehensive delineation of what constitutes short-chain versus 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 are identified 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. Classification and Chemical Structure of Perfluoroalkyl Acids (PFAA)^a

Classification	Functional Group	Examples
Perfluoroalkyl carboxylic acids (PFCAs) Or Perfluoroalkyl carboxylates (PFCAs)	-COOH	Perfluorooctanoic acid (PFOA) ^b
	-COO ⁻	Perfluorooctanoate
Perfluoroalkane sulfonic acids (PFSAs) Or Perfluoroalkane sulfonates (PFSAs)	-SO ₃ H	Perfluorooctane sulfonic acid (PFOS)
	-SO ₃ ⁻	Perfluorooctane sulfonate (PFOS) ^b
Perfluoroalkane sulfinic acids (PFSIAs)	-SO ₂ H	Perfluorooctane sulfinic acid
Perfluoroalkyl phosphonic acids (PFPA)	-P(=O)(OH) ₂	Perfluorooctyl phosphonic acid (C8-PFPA)
Perfluoroalkyl phosphinic acids (PFPIAs)	-P(=O)(OH)(C _m F _{2m+1})	Bis(perfluorooctyl) phosphinic acid (C8/C8-PFPIA)

^a Modified from Buck et al. (2011).

^b The anionic form is most prevalent in the aquatic environment.

Table 1-3. Classification of Short-Chain and Long-Chain PFAA^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		
	PFPA	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				

^a Modification of Table 2-2 (ITRC, 2021)

Notes:

For brevity, Table 1-3 only includes PFAAs of 3–10 carbons; the long-chain class of PFCAs and PFSAs can be expanded considerably.

PFPA = 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.

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. Importantly, the protonated and anionic forms may have different physiochemical and environmental fate and transport properties.

1.3 Occurrence of PFAS Mixtures

As a result of improved monitoring and detection methods, co-occurrence of multiple PFAS has been reported in drinking water, ambient surface waters, aquatic organisms, biosolids (sewage sludge), and other environmental media.² PFOA and PFOS have historically been target analytes, which has partly contributed to their prevalence in environmental monitoring studies. Relatively recent monitoring studies, however, have begun to focus on additional PFAS via advanced analytical instruments/methods and non-targeted analysis (De Silva et al., 2020; McCord et al., 2019, 2020). The proposed framework for estimating the likelihood of human health risks associated with oral exposures to mixtures of 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.

EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are suspected to be present in drinking water and do not have health-based standards set under the Safe Drinking Water Act (SDWA). Between 2013 and 2015, EPA's third UCMR (i.e., UCMR 3) required all large public water systems (PWSs) (serving more than 10,000 people) and a statistically representative national sample of 800 small PWSs (serving 10,000 people or fewer) to monitor for 30 unregulated contaminants in drinking water, including six PFAS: PFOS, PFOA, PFNA, PFHxS, perfluoroheptanoic acid (PFHpA), and PFBS. 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 (EPA, 2019b; Guelfo and Anderson, 2018). EPA found that 4% of PWSs reported results for which one or more of the six UCMR 3 PFAS were measured at or above their respective minimum reporting levels.³ Outside of the UCMR 3 data collection, many states have undertaken individual efforts to monitor for PFAS in both source and finished drinking water. These results show occurrence in multiple geographic locations consistent with what was observed during UCMR 3 monitoring (EPA, 2021g).

PFAS mixtures have also been reported in U.S. ambient surface waters and in aquatic biota (Ahrens, 2011; Benskin et al., 2012; Burkhard, 2021; 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. In addition to PFOA and PFOS, Sinclair and Kannan (2006) also detected PFHxS; however, PFBS and perfluorooctane sulfonamide (PFOSA) were below detection limits in all samples. De Silva et al. (2011) detected PFOS and additional short chain PFAS (i.e., perfluoropentanoic acid (PFPeA) (C5), PFHxA (C6), PFHpA (C7), and PFOA (C8)) co-occurring as mixtures in all surface water samples (n = 32) collected across the five

² For a more detailed discussion of the occurrence of PFOA, PFOS, and other PFAS in potential human exposure sources see the RSC sections in EPA, 2021c and 2021d.

³ 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 (EPA, 2019b,c).

Laurentian Great Lakes. Relatively longer chain PFAS, including PFNA (C9), PFDA (C10), perfluoroundecanoic acid (PFUnA) (C11), PFBS, PFHxS, perfluoroethylcyclohexane sulfonate (PFECHS), and perfluoromethylcyclohexane sulfonate, 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 direct application of manufactured products that contain a specific mixture of PFAS. For example, aqueous film forming foam (AFFF) used in firefighting and training activities can contain hundreds of polyfluoroalkyl precursors (Ruyle et al., 2021). Anderson et al. (2016) quantified PFAS in ambient surface waters across 10 U.S. Air Force bases where there were known historic uses of AFFF. PFOA and PFOS largely co-occurred with one another and were detected in 88% and 96% of samples, respectively. Anderson et al. (2016) also detected PFBA, PFBS, PFPeA, PFHxA, PFHxS, and PFHpA in $\geq 80\%$ 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 several estuaries and in the Laurentian Great Lakes region. 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 = 3.9 nanograms (ng) per gram (g); geometric mean PFOSA = 3.2 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. Out of the six species from Charleston Harbor, PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoDA, PFHxS, and PFOSA were all commonly detected in fish tissues. Charleston Harbor was the more developed of the two sites and had higher overall PFAS concentrations. PFOS and PFDoDA were the only two PFAS that were detected at elevated concentrations in the fish species residing in Sarasota Bay (Houde et al., 2006). 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 lake trout tissues across all of the Great Lakes, with PFOA, PFECHS, and perfluorodecanesulfonic acid (PFDS) also being detected in Lake Ontario (De Silva et al., 2011).

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 = 17.7 ng/liter (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).

1.4 Evidence of PFAS Exposure in Humans

Humans can be exposed to PFAS through a variety of sources, including food that is packaged in PFAS-containing materials, processed with equipment that use 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, AFFF; 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 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. Results, from a nationally representative, cumulative biomonitoring study in which data were gathered from 1999–2000 through 2015–2016, documented measurable serum levels of PFOS, PFOA, PFHxS and PFNA in greater 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, blood levels of PFOA and PFOS dropped 60% to 80% between 1999 and 2014, presumably due to restrictions on their commercial use in the United States. Under EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, by 95% on a global basis by no later than 2010 and eliminate these substances in products by 2015 (EPA, 2021f). However, since the voluntary phase out of these longer-chain PFAS compounds in the United States, manufacturers are shifting to shorter-chain and alternative forms of PFAS compounds such as GenX chemicals. Additionally, other PFAS compounds were found in blood samples from recent (2011–2016) NHANES surveys, for example, PFDA, PFDoDA, PFHpA, PFHxS, PFNA, and 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid. 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 (Yu et al., 2020). There is less publicly available information on the occurrence and health effects of these replacements for PFOA and PFOS and other members of the carboxylic acid and sulfonate PFAS families.

1.5 Brief Summary of State, National, and International Approaches to Address PFAS Mixtures in Water

In 2016, EPA finalized drinking water Health Advisories of 70 parts per trillion (ppt) for PFOA and PFOS, for the individual chemicals and when present as a mixture (EPA, 2016c,d) because the reference doses (RfDs) were based on similar developmental effects and numerically identical. Since then, some states have developed state-specific cleanup levels, drinking water or groundwater guidelines, advisories or standards for PFOS and PFOA. In some cases, the state values are the same as EPA's 2016 drinking water Health Advisory (70 ppt for the individual and/or combined concentration of PFOA and PFOS); in other cases, states have developed different values. As of July 2021, Alaska, Colorado, Connecticut, Delaware, Florida, Montana, New Hampshire, North Carolina, Ohio, and Rhode Island have followed an approach similar to EPA and have adopted or otherwise applied a value of 70 ppt (e.g., as a guideline, advisory, or enforceable standard for water resources) to account for the combined toxicity of PFOA and PFOS (Table 1-4).

Several states have included additional PFAS (beyond PFOA and PFOS) in their combined toxicity approach based on similarity in chemical structure and/or toxicity (Table 1-4). For instance, Connecticut has established a limit of 70 ppt for any combination of the following PFAS: PFOA, PFOS, PFNA, PFHxS, and PFHpA (CT DPH, 2021). In some cases, the combined concentration is set at the higher concentration of either PFOA or PFOS alone. For example, Illinois established a Health Advisory of 21 ppt for PFOA and 14 ppt for PFOS for non-carcinogenic effects, and the combined value is set at 21 ppt (Illinois EPA, 2019). Wisconsin has established a maximum concentration of 20 ppt for combined PFOA and PFOS (WI DHS, 2019), while Massachusetts and Maine derived a maximum concentration of 20 ppt for any combination of the following six PFAS: PFOA, PFOS, PFNA, PFHxS, PFDA and PFHpA based on "close similarities in chemical structure and similar toxicities for this subgroup of PFAS" (Maine DEP, 2021; Mass DEP, 2019). Similarly, Vermont established a limit of 20 ppt for the same PFAS as Massachusetts and Maine with the exclusion of PFDA based on several criteria, including that "PFHxS, PFHpA and PFNA ... are considered sufficiently similar to PFOA and PFOS" (VT DEC, 2021).

International approaches to addressing multiple PFAS in drinking water have resulted in a range of proposed and promulgated standards, guidance values, and a variety of grouping methods (Table 1-4). Canada has adopted a method similar to the HI to estimate cumulative toxicity by adding the ratio of the PFOA concentration to its maximum acceptable concentration (MAC) to the ratio of the PFOS concentration to its MAC. If the sum of the ratios is equal to or lower than one, the drinking water is considered safe to drink. Australia has established a combined level of 70 ppt for PFOS and PFHxS, as a precaution, 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 chemicals. 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 for PFOSA, which Sweden excludes, 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 PFSA and C4-C13 PFCAs and a level of 500 ppt for

all PFAS, as measured by extractable or adsorbable organofluorine (EOF/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 EPA's draft framework (Borg et al., 2013; RIVM, 2018).

Table 1-4. Summary of 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 (EPA, 2016c,d)	2016	70	PFOA and PFOS	Drinking water Health Advisory. Assumes dose additive toxicity of PFOA and PFOS.
Alaska (USA) (Alaska DEC, 2019)	2019	70	PFOA and PFOS	Application of EPA Health Advisory.
Colorado (USA) (CDPHE, 2020)	2020	70	PFOA and PFOS	Application of EPA Health Advisory.
Connecticut (USA) (CT DPH, 2017)	2017	70	Either PFOA and PFOS or the sum of PFOA, PFOS, PFNA, PFHxS and PFHpA	Application of EPA Health Advisory to the sum of five PFAS; assumes toxicity similar to that of PFOS and PFOA.
Delaware (USA) (DE DNREC, 2018)	2018	70	PFOA and PFOS	Application of EPA Health Advisory.
Florida (USA) (Florida Health, 2020)	2019	70	PFOA and PFOS	Application of EPA Health Advisory.
Illinois (USA) (Illinois EPA, 2021)	2019	21	PFOA and PFOS	Same approach as EPA Health Advisory but used different reference doses (MRLs from ATSDR (2021)) and applied additional uncertainty factor for PFOS.
Maine (USA) (Maine DEP, 2021)	2021	20	PFOA, PFOS, PFNA, PFHxS, PFHpA, and PFDA	Based on similarities in chemical structure and toxicities of six PFAS to PFOS and PFOA. Same approach as EPA 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 six PFAS to PFOS and PFOA. Same approach as EPA Health Advisory but includes an additional uncertainty factor.
Montana (USA) (MT DEQ, 2020)	2019	70	PFOA and PFOS	Application of EPA Health Advisory
New Hampshire (USA) (NHDES, 2021)	2016	70 ^c	PFOA and PFOS	Application of EPA Health Advisory

Entity	Date	Conc (ng/L)	Sum of PFAS	Background
North Carolina (USA) (NC DEQ, 2021)	2020	70	PFOA and PFOS	Application of EPA Health Advisory
Ohio (USA) (Ohio EPA, 2019)	2019	70	PFOA and PFOS	Application of EPA Health Advisory
Rhode Island (USA) (RIDEM, 2017)	2019	70	PFOA and PFOS	Application of EPA Health Advisory
Vermont (USA) (VT DEC, 2021)	2019	20	PFOA, PFOS PFNA, PFHxS and PFHpA	PFHxS, PFHpA and PFNA are considered sufficiently similar to PFOA and PFOS. Difference to EPA Health Advisory is due to Vermont's calculation being based on infant consumption rates.
Wisconsin (USA) (WI DHS, 2019a,b)	2019	20	PFOA and PFOS	Based on ATSDR's 2021 intermediate MRL, with additional modifying factor of 10 for immunotoxicity; HI approach.
European Union (EU, 2020)	2020	100 500	100 ng/L for sum of 20 PFAS (C4–C13 PFASs 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 are limited on PFAS other than PFOS and PFOA.
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 are limited on PFAS other than PFOS and PFOA.
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 are limited on PFAS other than PFOS and PFOA.
Canada (Health Canada, 2018)	2018	200 600	PFOA PFOS	When PFOS and PFOA are found together in drinking water, a cumulative toxicity (HI) approach is applied.

^a Modified from Cousins et al. (2020).

^b As of July 2021, several states have passed or proposed compound-specific Maximum Contaminant Levels (MCLs) or Health Advisories, e.g., California, Michigan, Minnesota, New Jersey, New York, Pennsylvania, Texas, and Washington. Some states have applied the EPA 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 New Hampshire established MCLs of 12 and 15 for PFOA and PFOS, respectively; however, there is a court injunction at this time preventing the MCLs from being enforced. In the meantime, New Hampshire is applying EPA's 70 ppt Health Advisory.

1.6 Overview of Proposed Framework for Estimating Health Risks for PFAS Mixtures

This draft document describes a framework for estimating the likelihood of noncancer human health risks associated with mixtures of PFAS, based on longstanding EPA chemical mixtures guidance. To address concerns over health risks from multichemical exposures, EPA issued the *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (EPA, 1986). The 1986 guidelines were followed in 2000 by the *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA, 2000). 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” (EPA, 1986, 2000); this definition is used in this framework document.

Several laws direct 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 (SARA) 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 Chemical Mixtures Guidelines (EPA, 1986) and the 2000 Supplementary Chemical Mixtures Guidance (EPA, 2000) were developed, in part, to be responsive to these laws. When developing risk information for exposures to chemical mixtures, risk assessors and risk managers in EPA’s programs currently implement environmental laws through regulations that rely on the guidance and methods articulated in the 1986 Chemical Mixtures Guidelines and the 2000 Supplementary Chemical Mixtures Guidance. This proposed framework does not supersede previously published EPA guidance on mixtures or longstanding EPA approaches used to assess cumulative effects of chemical mixtures under various environmental statutes (e.g., Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), FQPA).

The objective of this document is to provide a tiered, flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS under an assumption of dose additivity. The approach is broken into two tiers: Tier 1 entails a HI approach that provides an initial indicator screening metric for a PFAS mixture of concern (Section 4.3); and Tier 2 is based on the RPF approach that is designed to provide a mixture toxicity estimate (Section 4.4), and an alternative approach that uses the DA (dose addition) model-based calculation (similar to the Berenbaum equation; Section 4.2.6 in EPA, 2000) of a mixture BMD (e.g., ED₁₀) for a PFAS mixture with a specific mixing-ratio of component chemicals (Section 4.5). The HI is a component-based mixtures methodology that facilitates estimation of potential aggregate toxicity associated with co-occurrence of chemicals in environmental media (e.g., water, soil) (EPA, 2000). 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 mode of action (MOA) or have a conserved toxicological target (e.g., share a common apical endpoint/effect) and (2) the dose-response functions for the effect of concern are similar over the exposure ranges most relevant to the decision context (EPA, 2000). This is illustrated in Section 4.4 using common target organs/pathways including developmental, thyroid, and liver effects. These same assumptions are also inherent when applying the Mixture BMD approach (Section

4.5). Considering that PFAS are an emerging chemical class of note for toxicological evaluations and human health risk assessment, MOA data may be limited or not available at all for many PFAS, including those approved for use under TSCA. As such, this draft framework proposes to focus the biological level of organization for evaluation of potential dose additivity on similarity of toxicity endpoint/effect/adverse outcome rather than similarity in MOA, which is consistent with EPA mixtures guidance (EPA, 2000).

The PFAS used to demonstrate the application of the HI and RPF approaches described in this draft framework are limited to those with final EPA toxicity assessments at the time of drafting this document: PFOA (EPA, 2016a), PFOS (EPA, 2016b), PFBS (EPA, 2021a), and GenX chemicals (EPA, 2021b). Thus, the information provided in the Mixture BMD approach examples is purely hypothetical and provided to illustrate application of the framework. Recognizing the evolving and dynamic nature of PFAS science, the approach described herein is flexible to allow for consideration of new or evolving dose-response data and peer reviewed toxicity assessments as they become available. Additionally, because publicly available traditional (in vivo mammalian) toxicity studies are limited to only a small fraction of the more than 9,000 known PFAS, this framework also provides suggestions for practical integration of validated NAMs such as toxicogenomics (e.g., high-throughput transcriptomics (HTTr), in vitro bioactivity) and in silico platforms (e.g., structure-activity, read-across) into the HI and RPF approaches. The illustrative examples in Section 4 are intended to demonstrate the component-based mixture approaches with available dose-response data from completed EPA human health assessments and hypothetical exposure information.

2.0 Background on EPA Mixtures Additivity Guidance

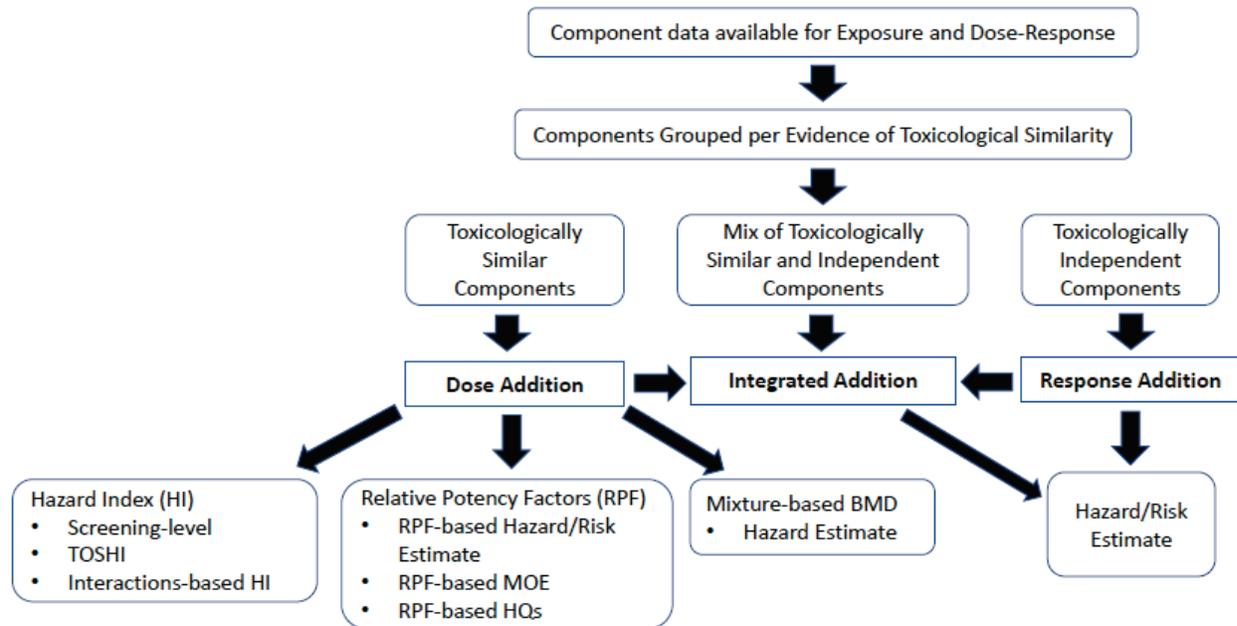
Exposure to mixtures of environmental chemicals occurs in human populations through ingestion, inhalation, and/or dermal contact with contaminated media (e.g., water, air, food). It should be noted that a “mixture” of chemicals may be a function of both co-occurrence in exposure media and/or internal bioaccumulation and persistence in biological matrices. In recognition of the need for methods and approaches that inform evaluation of potential health risks associated with chemical mixtures, EPA developed the 1986 Chemical Mixtures Guidelines and subsequently the 2000 Supplementary Chemical Mixtures Guidance (EPA, 1986, 2000). In those guidance documents, EPA proposed a tiered hierarchy of mixtures approaches where the preferred approach is to evaluate toxicity 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 too 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., limited number of component chemicals at fixed proportions/ratios). As such, EPA also developed multiple component-chemical based mixtures assessment approaches. Component-based methods are used more frequently than whole-mixture methods. These component methods are based on assumptions of how the chemicals behave 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.” Basic tenets of EPA mixtures additivity theory and practice are as follows:

- 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 and guidance documents, development of component-based mixture approaches were 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 mixtures of 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 demonstrate or suggest 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 mixtures of 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 demonstrate or suggest 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 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), specifically the HI, RPF, and Predictive Hazard Estimate (Mixture BMD approach). As noted above, the methods involve different assumptions for component chemical “mixtures” toxicity.



Notes:

Modification of Figure 4-3b (EPA, 2007a)

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.

BMD = benchmark dose; HI = hazard index; HQ = hazard quotient; MOE = margin of exposure; RPF = relative potency factor; TOSHI = target-organ specific hazard index.

Figure 2-1. Flow chart for evaluating chemical mixtures using component-based additive methods.

An important property of DA-based methods is that they can aid in the prediction of effects of a mixture even when all of the individual component chemical exposures are at or below their individual no-observed-adverse-effect levels (NOAELs). In dose additivity models such as the RPF approach, the sum of the scaled index chemical (IC)⁴ equivalent doses/concentrations for each component can exceed the equivalent threshold dose of the mixture and result in a

⁴ An IC is that mixture component that is typically the most toxicologically well-studied member. The qualitative and quantitative hazard and dose-response data for an index chemical 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 index chemical.

detectable response, which has been supported experimentally (Jonker et al., 1996; Silva et al., 2002).

2.1.1 Application of Dose Addition as EPA's Default Assumption

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; EPA, 2007a; 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; 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 (NAS, 2008; Rider et al., 2009). In general, the results of such studies listed here, and many others, support the continued application of DA as EPA's default component-based mixture assessment approach. Further discussion and examples of the basis for use of dose additivity for PFAS is 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 initiating events (MIEs) and/or MOAs interact. As discussed in Section 3.2, evidence demonstrates that mixtures of chemicals that disrupt common pathways typically produce dose additive alterations. In studies that tested model prediction accuracy for mixture components that disrupted common pathways, DA models provided predictions that were better than or equal to Integrated Addition (IA) and RA predictions of the observed mixture effects (Section 3.2).

Consistent with the conclusions of the National Academy of Sciences (NAS) (2008), a review of published studies in the literature (Section 3.2) did not contain a single case where RA was a better predictor of the adverse effects of a mixture than DA, even when the mixtures included chemicals like phthalates and androgen receptor (AR) antagonists with diverse MOAs (but common targets of toxic action). Taken together, this supports the health protective assumption that a mixture of chemicals with similar apical effects should be assumed to also act in a DA manner unless shown otherwise. Further, data demonstrating that PFOS, PFOA, and other PFAS disrupt signaling of multiple biological pathways resulting in common adverse effects on several biological systems including thyroid hormone levels, lipid synthesis and metabolism, developmental toxicity, and immune and liver function, are reviewed in Section 3.4. Finally, in Section 3.4, a summary is provided for two ongoing EPA Office of Research and Development (ORD) PFAS developmental toxicity mixture studies (of which one study uses a mixture of PFOA and PFOS) that provide robust evidence that PFAS behave in a DA manner.

3.1 Overview of Assessment Approaches for Chemical Mixtures

Over 30 years ago, scientists developed quantitative dose metrics and methods to assess the combined toxicity of mixtures of large classes of chemicals that disrupt a common pathway (NATO, 1988). 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 upon their potency relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Many of the lessons learned about assessing the effects of mixtures of dioxin-like chemicals (DLCs) also are applicable to assessing the effects of PFAS mixtures. Since that time, TEF-like approaches have been used to evaluate mixtures of other chemical classes. The emerging picture is that some chemicals, regardless of MIE or MOA, produce cumulative effects on common apical endpoints that generally are well predicted using DA models.

The general applicability of DA models is based on the review of studies specifically designed to evaluate how well different mixture models predict the way chemicals in a mixture interact to cause effects. Studies evaluating mixture effects typically include an evaluation of individual chemical dose response curves and apply this information to different statistical models of mixture interaction. The data from a number of studies (reviewed below) indicate that chemicals that produce common adverse effects will typically interact in a DA 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 of 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

their 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 database to evaluate cumulative mixture models, it should be assumed that any mixture acts in a DA cumulative manner if the individual chemicals produce common effects. This approach was fully endorsed by NAS (2008).

3.2 Examples of Chemical Classes and Toxicological Pathways Utilizing Mixture Assessment Approaches

3.2.1 *Aryl Hydrocarbon Receptor Pathway and Toxic Equivalence Factors of Dioxin-Like Chemicals*

In 2010, EPA published guidance for the use of TEFs for human health risk assessments of DLCs, which produce many of their adverse effects by acting as aryl hydrocarbon receptor (AhR) agonists (EPA, 2010). Hundreds of chemicals are AhR agonists including PCBs, PCDFs, and PCDDs. For DLC mixtures, EPA recommended use of the TEF methodology and the World Health Organization's TEFs to evaluate the risks associated with exposure to mixtures of TCDD and DLCs for human health (EPA, 1987, 1989, 2003) and ecological risk assessments (EPA, 2008). TEFs can be calculated for each DLC based on dietary dose or internal whole body toxic equivalent concentrations (TECs).

The total 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 toxic equivalence factor (TEF) by the corresponding chemical concentration in the mixture. The individual TEQs are then summed to calculate the TEQ of the mixture. The reported 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:

- Chemicals interact in a DA manner
- They all affect a common pathway via the AhR, among other pathways
- Synergistic and antagonistic interactions are uncommon within the group (Safe, 1994)
- TEFs and TEQs for AhR agonism cannot predict toxicities induced by these chemicals that perturb other biological pathways

TEF values have undergone several revisions (Van den Berg et al., 2006); in 2010, EPA published recommended TEFs for human health risk assessment for DLCs (EPA, 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). EPA (2008) stated that the TEQ methodology was appropriate for evaluating risks to fish, birds, and mammals associated with AhR agonists.

Studies of AhR agonists in various species indicate:

- Species and tissues differ in sensitivity to the effects of the mixture
- 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 ED₃₀ 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 in 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 – Central Nervous System and Behavior

Pyrethroids act on the nervous system, and they all alter neuronal excitability and neuronal firing rate; however, there is uncertainty about whether all pyrethroids act via a narrowly defined “common mechanism of toxicity” or if they should all be included in a common mechanism group for assessment of cumulative toxicity. Wolansky et al. (2009) administered a mixture of 11 pyrethroid pesticides to adult male rat acutely by oral gavage 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. These pesticides may disrupt different MIEs, but they all converge on a common key event (KE) within an adverse outcome pathway (AOP) network. Exclusion of chemicals from a common mechanism group for cumulative toxicity assessment based upon purported differences in MIEs is not protective when they all converge in an AOP network on a common KE and induce a common adverse outcome in a DA manner.

3.2.3 Organophosphates and Related Pesticides – 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 given simultaneously. Subsequently, all organophosphate (OP) pesticides in use were evaluated in binary mixture studies to determine if nonadditivity 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 4 pairs showed greater-than-additive effects on lethality (Dubois, 1961). Moser et al. (2005, 2006) reported a range of responses with mixtures of 4 or 5 OPs. The ratios of the predicted-to-observed ED_{20s} and ED_{50s} of the mixtures indicated that several effects displayed small 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 2006, EPA concluded that DA was a reasonable approach for predicting the effect of exposure to OP mixtures (EPA, 2006).

Similarly, EPA (2007b) concluded that DA was a reasonable approach for estimating cumulative risk associated with joint exposure mixtures to another class of pesticides, the N-methyl

carbamate insecticides. 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 Mixture Effects on the Female Reproductive Tract – Estrogen Agonists

Scientists have examined the effects of mixtures of estrogenic chemicals in the female rat using an uterotrophic assay, an EPA Endocrine Disruptor Screening Program Test Guideline that is a sensitive *in vivo* test for estrogenicity (EPA, 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 taken. Exposures can be administered orally or through subcutaneous injections. 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 versus 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, DA is the most appropriate model for mixtures of estrogenic compounds.

3.2.5 Mixture Effects on the Female Reproductive Tract – Phthalates in Utero

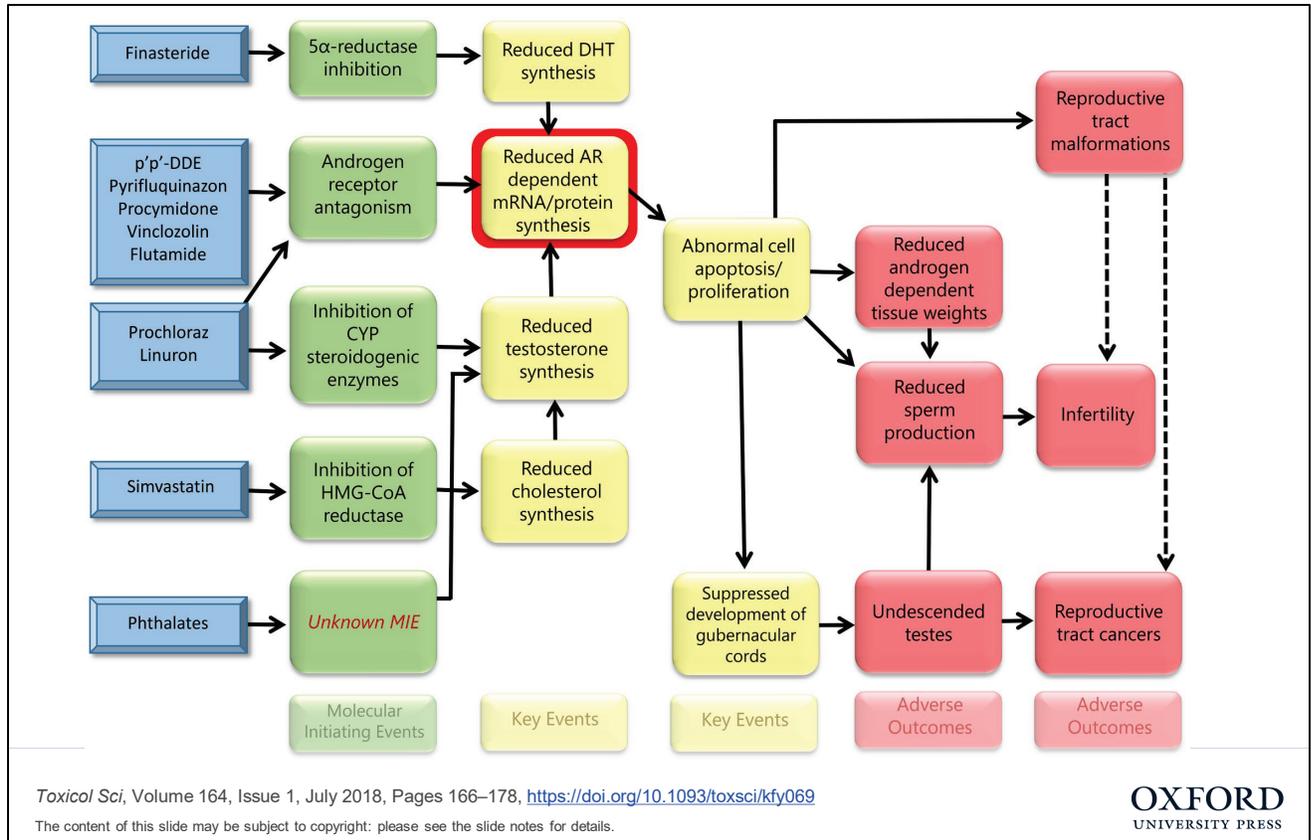
Hannas et al. (2013) reported that administration of a mixture of five phthalates (>520 mg total phthalate) to pregnant rats from gestational days 8 to 13 induced reproductive tract malformations in female rat offspring. These malformations included complete to partial uterine agenesis and agenesis of 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 mg/kg/d) and diethylhexyl (750 mg/kg/d) 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 Male Reproductive Tract Development – Antiandrogens

Historically, it has been hypothesized that mixtures of chemicals with dissimilar MIEs would interact in a 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 NAS (2008). Studies on the effects of mixtures on male reproductive development provide one of the larger databases supporting the use of DA models 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 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 numbers 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 appears 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 their review there were no published studies that addressed whether or not phthalate mixtures exhibited cumulative 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 3 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. For example, 60% of male offspring were found to have penile malformations that resulted in infertility and this effect was accurately predicted by DA, 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.

The validity of using DA models for diverse mixtures of chemicals that disrupt androgen signaling and male rat development is supported by an examination of the effects of these chemicals at the cellular level. All these 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 that are involved in the development of the androgen-dependent tissues. Each of these chemicals reduces the number of AR dimers, AR/AR, activated by an androgen agonist. AR antagonists, like vinclozolin or procymidone, accomplish this by blocking androgens from binding to ARs and

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, di(2-ethylhexyl)phthalate, dipentyl phthalate, butyl benzyl phthalate, diisobutyl phthalate) reduce the levels of androgens available to the cell (Hannas et al., 2011; Howdeshell et al., 2008; Furr et al., 2014), and chemicals like finasteride inhibit the enzyme in the tissues that converts testosterone to dihydrotestosterone (a more active androgen that has higher affinity for the AR) (Clark et al., 1990). 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 the events disrupted in the androgen signaling pathway by all these chemicals at the cellular-molecular level explains why one should expect the mixtures to behave in a DA manner. It is not important to the cell what MIE reduced the androgen signal to the gene; it is only important that the signal is reduced.

In summary, an examination of the literature on the effects of mixtures on male reproductive tract development is as follows:

- Mixtures of chemicals that disrupt common effects can be adequately modeled by DA.
- The chemicals acted in a DA manner regardless of whether or not they shared a common MIE.
- IA and RA models can grossly underestimate the hazard of a mixture of chemicals acting on a common KE or with a common apical effect.

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, 6 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) versus DA or less-than DA (sometimes referred to as antagonistic) interactions was recently reassessed by Martin et al. (2021). The authors conducted a systematic review and quantitative reappraisal of 10 years of a broad range of mixture studies from 2007 to 2017. Martin et al. (2021) identified 1,220 mixture studies, ~65% of which did not incorporate more than 2 components. 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 predicted by 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, it is important to note that greater-than-additive and less-than-additive interactions do occur with co-exposure to chemicals that affect different target organs or different, unrelated AOPs. There are numerous examples of chemical interactions that deviate from DA that are biologically relevant including mixtures in which one chemical alters the metabolism of the other chemical(s), including the ones provided below.

- Twenty years of research has identified at least 85 drugs whose metabolism is inhibited by a chemical in grapefruit, potentially resulting in serious side effects (Bailey et al., 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 versus 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.
- Imidazole and triazole fungicides, used as clinical medicines and pesticides, can influence sterol biosynthesis and retinoic acid metabolism, which have been associated with adverse patient outcomes (Yamazoe et al., 2020).

In addition to metabolic activity leading to synergistic or antagonistic interactions among chemical mixtures, there are other examples of deviations from DA. As with the examples in the list above, these do not include chemicals that disrupt common KEs, AOPs, AOP networks, or target organs. For example, adulteration of pet food with melamine and derivatives, including cyanuric acid, caused kidney failure and death of a large number of cats and dogs in the United States (Jacob et al., 2011). In addition, more than 54,000 infants and young children in China were treated for urinary problems and possible kidney stones related to the melamine contamination of infant formula and related dairy products with several confirmed deaths (WHO, 2008). Although individually these compounds present low toxicity, co-exposure can lead to the formation of melamine cyanurate crystals in the nephrons and eventual kidney failure in mammals.

3.4 PFAS Dose Additivity

PFOA and PFOS, as well as other PFAS with linear or branched alkyl or alkyl ether chains and sulfonic or carboxylic acid functional groups, share common toxicological impacts of exposure on multiple cellular receptors, tissues, 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 disparate molecular mechanisms but common KEs or adverse outcomes support predictions of dose additive effects. Thus, in the absence of detailed molecular mechanisms for most PFAS, it is considered a reasonable health-protective assumption that PFAS which can be demonstrated to share one or more KEs or adverse outcomes will act with toxicological similarity to produce dose-additive effects from co-exposure. 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 straight chain compounds as well as emerging ether-linked compounds. Below is a brief overview of MIEs, KEs, and adverse outcomes that have been shown to be common to several PFAS and evidence which supports dose additivity. This overview highlights results from, among others, the NIEHS 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 endpoints spanning MIEs, KEs, and AOPs. More comprehensive reviews of common PFAS toxicity endpoints in experimental animal studies and observational human studies can be found elsewhere (ATSDR, 2021; EFSA et al., 2018, 2020).

Mechanistically, demonstration of nuclear receptor activation constitutes a principle MIE in the description of PFAS-relevant AOPs. PFAS, such as PFOA and PFOS, have been shown to activate multiple similar nuclear receptors in both in vitro and in vivo studies, thus identifying multiple MIEs relevant to PFAS. In vitro activation of peroxisome proliferator activated receptor alpha (PPAR α) (Behr et al., 2020; Ishibashi et al., 2019; Takacs and Abbott, 2007; Vanden Heuvel et al., 2006) and gamma (PPAR γ) (Houck et al., 2021; Vanden Heuvel et al., 2006) is well described for multiple PFAS. Further, in vivo studies of tissue-specific gene expression patterns have also demonstrated activation of constitutive androstane receptor (CAR) for both PFOA and PFOS due to upregulation of CAR-dependent genes (Rosen et al., 2017) and liver-based profiles of PPAR α -induced gene expression (Bjork et al., 2008; Rosen et al., 2007). Recently, PFOA and PFOS, along with PFHxA, PFNA, PFDA, PFBS, and PFHxS, were shown to upregulate the PPAR α -inducible *Acox1* and *Cyp4a1* and the CAR-inducible *Cyp2b1* and *Cyp2b2* in adult male and female rat livers in 28-day repeat dose guideline studies (NTP, 2019a,b). From a molecular mechanism perspective, PFOA and PFOS both activate similar nuclear receptors and gene transcription pathways, along with several other studied PFAS including those listed above.

KEs downstream of the above potential MIEs are also shared between PFOA, PFOS, and other PFAS. In both rodent and non-human primate studies, serum lipids (cholesterol, triglycerides) are consistently reduced and markers of liver 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 from exposure to PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS (NTP, 2019a,b). Further, all PFAS reduced total and free thyroxine (T4) (NTP, 2019a,b). In combination with the nuclear receptor activity and gene expression profiles, there is a pronounced similarity in the serum clinical chemistry and thyroid hormone-based KEs for PFOA, PFOS, and several other studied PFAS.

Similar adverse outcomes at the organ and whole animal levels have been described for PFOA, PFOS, and several other PFAS. Effects in developmental exposure studies with PFOA, PFOS, PFNA, and GenX chemicals in rats and/or mice have reported consistent effects on pups including reduced F1 survival/viability and reduced F1 body weight (Abbott et al., 2007, 2009; Blake et al., 2020; Butenhoff et al., 2004; Conley et al., 2021b; Das et al., 2015; Lau et al., 2003; Luebker et al., 2005a,b; Thibodeaux et al., 2003). All PFAS studied by NTP (2019a,b) increased rat liver weights and produced 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 is a broad spectrum of adverse effects in laboratory animals that are highly similar and plausibly associated with the common molecular mechanisms and KEs displayed by PFOA, PFOS, and several other PFAS.

Limited work has been conducted on combined exposure to PFAS in experimental systems. An *in vitro* mixture study of PPAR α activation demonstrated cumulative effects of combined exposure to binary combinations of PFOA and PFOS, PFNA, PFHxA, and PFHxS that conformed to models of dose additivity (Wolf et al., 2014). Mammalian studies evaluating exposure to multiple PFAS are limited but two recent studies indicate that exposure to combined PFOA, PFOS, and PFHxS (Marques et al., 2021) and combined PFOA, PFOS, PFNA, PFHxS, and GenX chemicals (Roth et al., 2021) in mice produced numerous significant effects compared to control which were consistent with the spectrum of individual PFAS effects described above. Currently, developmental toxicity studies of PFAS mixtures are ongoing at EPA ORD. Conley et al. (2021a) presented preliminary data on an unpublished mixture study of PFOS, HFPO dimer acid (also known as GenX chemicals), and Nafion byproduct 2 (NBP2) (an emerging polyfluoroethersulfonic acid compound recently detected in human serum (Kotlarz et al. 2020)), which produced neonatal mortality that was accurately predicted by DA modeling, among other cumulative mixture effects. Further, a direct investigation of the cumulative *in vivo* mixture developmental toxicity of combined exposure to PFOA and PFOS was recently conducted by EPA ORD (see Appendix A). A series of experiments were designed to characterize the dose responses across several endpoints for PFOA and PFOS individually, followed by a mixture study of the two chemicals combined. Preliminary results identified numerous effects from each chemical individually as well as the mixture, including reduced maternal gestational weight gain, reduced pup body weight and pup viability, and increased maternal and pup liver weights. As a clear demonstration of cumulative mixture effects, individual exposures to 62.5 milligrams (mg) per kilogram (kg) PFOA and 2 mg/kg PFOS produced $12\pm 7\%$ (mean \pm standard error of the mean (SEM)) and $8\pm 6\%$ post-implantation loss (PIL; a measure of fetal and pup mortality), respectively, while a combination of the two (62.5 mg/kg PFOA+2 mg/kg PFOS) produced $66\pm 15\%$ PIL. Further, when graphed as a function of oral PFOA dose, the dose response curves for the combination of PFOS+PFOA across multiple effects, such as pup body weight and maternal and pup liver weights, were significantly shifted towards effects at lower doses than the curves for PFOA alone, which means that there was an additive effect of PFOS (see Figure 2 in Appendix A). This study is ongoing with multiple analyses still to be conducted on samples collected during the studies. However, these preliminary results provide robust evidence of combined toxicity of PFOA, PFOS, and other PFAS on multiple developmental endpoints. Studies with PFOA alone and PFOS alone demonstrated that both chemicals independently produce numerous similar developmental adverse outcomes including increased PIL, reduced pup body weight, and increased pup and maternal liver weight (see Figure 2 in Appendix A). Co-exposure to PFOA and PFOS in combination produced cumulative mixture effects that are at least dose additive for most endpoints and support the combined toxicity of these compounds.

In summary, the data reported in the literature support an assumption of similarity in toxicity profiles for PFOA and PFOS and other PFAS with linear or branched alkyl or alkyl ether chains and sulfonic or carboxylic acid functional groups and a dose-additive assessment approach. 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 that are shared across the spectrum of PFAS effects.

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 associations and proportions makes whole mixture evaluations extremely 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 abiotic 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 mixtures of different component associations and proportions in the field. In the context of PFAS, increasing environmental evidence (e.g., environmental water, air, and soil sampling results) suggests that the complexities briefly summarized above with regard to the diversity of chemicals co-occurring in different component associations and 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 the real problem of human exposure to multiple PFAS (see Sections 4.2–4.5).

EPA's *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA, 2000) 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 what constitutes a "sufficiently similar mixture," but determinations should be based on a comparison of similarities or differences in the component's chemical fate and transport in the environment, persistence, bioaccumulative potential, kinetics, and toxicity profile. 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 chemical could be used as a surrogate for the mixture of concern.

4.2 Component-Based Mixtures Approach for PFAS

As a result of both the complexities associated with characterization and evaluation of whole mixtures (see Section 4.1 above) and the reality that most toxicological information for chemicals derives from exposure-response studies of individual species, component-based mixtures risk assessment is particularly relevant (see 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 evaluation of PFAS mixtures in other exposure media as well (e.g., soil, air). As outlined in earlier sections of this framework, while EPA component-based methods and approaches are available for evaluation of mixtures of chemicals under different assumptions of additivity (EPA, 2000), the currently available evidence on PFAS supports an assumption of dose additivity (see Section 3). The HI and RPFs are two component-

based mixture approaches based on dose additivity, that are well validated and actively used by EPA. These two approaches are discussed below and include illustrative examples that are based on PFAS with completed EPA human health assessments. An alternative Mixture BMD approach, generally based on the Berenbaum equation (see section 4.2.6 in EPA mixtures guidance (2000)), is also a dose additive approach that is described and illustrated with hypothetical examples. 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; Mumtaz et al., 2021), lending confidence to the direction of this framework document in guiding formal component-based assessment of PFAS mixtures.

A pragmatic tiered approach to application of component-based evaluation of mixtures of PFAS with variable hazard and dose-response databases is presented in Figure 4-1. The entry point into the flow diagram follows the search, collection, and assembly of all available toxicity (including NAM-based data such as cell-based bioactivity) and exposure data available for all mixture component PFAS of potential concern. Off-the-shelf exposure duration-relevant human health hazard data and dose-response assessment values (e.g., POD, RfDs, MRLs) are available from federal (EPA, ATSDR), state, international, or other formal entities for a limited set of PFAS (e.g., EPA final assessments are currently available for PFOA, PFOS, PFBS, and GenX chemicals). Such human health reference or toxicity values may be leveraged to inform PFAS mixture assessment (i.e., using HI, RPF, and Mixture BMD approaches), however with clearly identified nuances and uncertainties that help contextualize such values (see discussion in Section 4.3). For many PFAS, no formal health assessments exist, however human epidemiological and/or experimental animal hazard and dose-response data may be available in the public domain. Should such data be available, de novo derivation of chronic (and/or subchronic) non-cancer toxicity values might be possible; it would be necessary to transparently communicate the targeted fit-for-purpose application and attendant uncertainty(ies) associated with such derived values.

Users of this framework may find that PFAS of interest may be data-poor (i.e., no usable 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 potential hazard and dose-response for PFAS mixture components. For example, read-across is a NAM approach that could potentially be leveraged to identify dose-response metrics (e.g., POD, effect concentration (EC_X), IC_X) for integration into the component-based mixtures assessment approaches presented in the subsections below. Analogue-based read-across, in general, is a process in which chemicals (i.e., analogues) with relatively replete toxicity databases are compared to a data-poor target chemical across similarity domains including structural, physicochemical, toxicokinetic (TK), and/or toxicodynamic (TD) similarity (Wang et al., 2012; Wu et al., 2010). Based on weight-of-evidence for similarity between a target chemical and candidate analogues, hazard and dose-response data (e.g., POD) are then adopted from a selected (single-best) analogue as surrogate for the data-poor target chemical. This read-across approach might facilitate incorporation of data-poor PFAS into the component-based methods presented in this framework, as (surrogate) data that informs similarity of toxic endpoint/health effect and dose-response could potentially: (1) be used in the derivation of a non-cancer RfD (using UFs appropriate for the data-poor target chemical) and subsequent calculation of a HQ, (2) be used in the calculation of RPF(s), or (3) the surrogate health-effect dose-response data could be BMD modeled and included in the calculation of an overall mixture BMD.

PFAS with identified hazard(s) and dose-response inputs (e.g., RfDs, MRLs), as described above, are first screened in Tier 1 of this framework using the HI approach (Section 4.3). In brief, duration-relevant exposure (E) and toxicity values (e.g., reference value (RfV)) for each mixture PFAS are used in a simple ratio (E/RfV) to calculate a hazard quotient (HQ). The component PFAS HQs are then summed to generate a mixture HI (see Equation 4-1). The HI approach is split into three general subtypes: (1) screening-level, (2) target-organ-specific hazard index (TOSHI), and (3) interactions-based HI (see Figure 2-1). In this framework, only the screening level HI and TOSHI are included as dose additive Tier 1 methods; data to inform deviations from dose additivity (e.g., interactions such as synergism or antagonism) are virtually non-existent for PFAS co-occurring in mixture, as such, an interactions-based HI is not feasible at present. The screening level HI involves the use of RfVs for each PFAS mixture component irrespective of health outcome domain. Since each mixture component HQ is calculated using a corresponding RfV, the mixture HI may represent a conservative indicator of potential mixture hazard. Conversely, 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 target-organ toxicity doses (TTDs) (note: some TTDs could also be the overall RfV for a given PFAS). A mixture HI approaching or exceeding 1.0 indicates potential concern for a given environmental media or site and is typically flagged for further evaluation using a more robust evaluation (e.g., Tier 2, RPF). The key to this first HI Tier is that it provides an initial indication of: (1) concern for the overall mixture and (2) potential driver PFAS (i.e., those PFAS with high[er] HQs). Potential “driver” PFAS may be prioritized for further evaluation in a RPF approach. Conversely, those PFAS with low(er) HQs (e.g., $\leq 0.0X$) might be deprioritized for further evaluation as they may not have significant impact(s) on overall mixture risk. It should be noted that a user of this approach should consider the potential exposure (e.g., water concentration), the potency for effect (e.g., low[er] or high[er] PODs), and qualitative and quantitative uncertainty (i.e., totality of uncertainty factor application) for each PFAS mixture component in deciding if a given PFAS should move on to a more robust mixture evaluation (i.e., Tier 2).

In contrast to the HI, the RPF approach in Tier 2 provides a PFAS mixture risk estimate (see Section 4.4). Application of this approach is demonstrated using PFOA, PFOS, PFBS, and GenX chemicals as examples in this document; in practice, it could be expanded to other PFAS with sufficient hazard and dose-response information. In the illustrative examples provided in Section 4.4, potency for an effect across each mixture PFAS is normalized to a selected IC for three critical health effect domains – developmental, thyroid, and liver – resulting in three sets of RPFs. These three health effect domains were selected primarily because: (1) the effects were the basis for RfD derivation for each respective PFAS (i.e., PFOA/PFOS = developmental effect (EPA, 2016a,b); PFBS = thyroid effect (EPA, 2021a); and GenX chemicals = liver effect (EPA, 2021b)) and (2) each example PFAS has sufficient hazard and dose-response data across all three health effect domains to facilitate demonstration of the RPF methodology. In addition, these three health effect domains have been identified for other PFAS for which data are available. 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 4-3), 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., total dose of IC) is compared directly to a Health-Based Water Concentration (HBWC) (e.g., Health Advisory, MCLG) based on the relevant health effect domain (e.g., developmental, thyroid, liver) for the IC. If the mixture ICEC for one or more of the three 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, if the mixture ICEC for all three effect domains is below the IC HBWC, risk is not anticipated. Additionally, individual mixture 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.

An additional option, represented as an alternative Tier 2 approach (Figure 4-1) in the decision flow diagram, entails calculation of a mixture BMD (see Section 4.5). In contrast to the Tier 2 RPF approach, there is no need for identification of mixture ICs, calculation of RPFs or ICECs, or existence of HBWCs. The approach results in a mixture BMD (i.e., POD) that could be converted into a mixture RfD using appropriate uncertainty factor application, and subsequently 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).

As previously noted, the HI and RPF examples presented for PFOA and PFOS rely on information from EPA’s 2016 health effects support documents (EPA, 2016a,b). To support the NPDWR for these chemicals, EPA has reevaluated the current toxicological literature knowledgebase and developed draft updated RfDs for PFOA and PFOS, which are currently undergoing review by EPA’s SAB. This document was developed concurrently with the draft MCLG documents for PFOA and PFOS and does not reflect the updated health information (i.e., RfDs) presented in those specific documents.

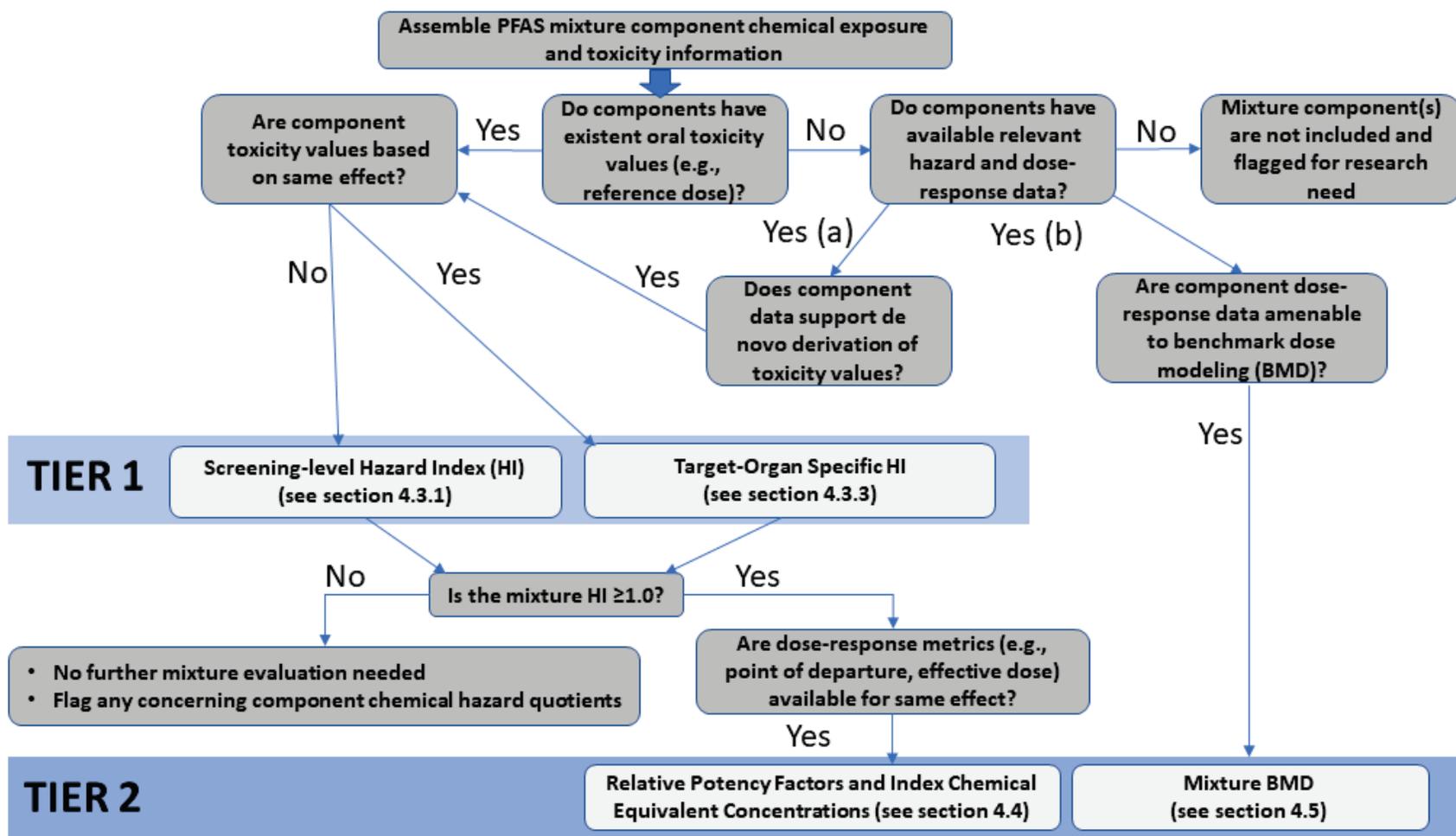


Figure 4-1. Flow diagram for proposed component-based approaches based on dose additivity to estimate noncancer health risk associated with PFAS mixtures.

4.3 Hazard Index

The HI is the most commonly used component-based mixture risk assessment method in EPA. Because the HI employs a population level exposure and human health assessment value, such as an oral RfD, this ratio provides an indication of potential health hazard(s). That is, the HI is a decision aid; it is not a mixture risk estimate in that it is not expressed as a probability, nor is it an estimate of specific toxicity (e.g., embryo toxicity). The HI is based on an assumption of DA among the mixture components (EPA, 2000; Svendsgaard and Hertzberg, 1994). In the HI approach, an HQ is calculated as the ratio of human exposure to a health hazard RfV for each mixture component chemical (i) (EPA, 1986). The HI is dimensionless, so in the HI formula, E and the RfV must be in the same units (Equation 4-1). For example, if E is the oral intake rate (mg/kg/d), then the RfV could be the RfD, which has the same units. Alternatively, the exposure metric can be a water concentration and the toxicity value is best represented as an HBWC, for example, an EPA drinking water Health Advisory (e.g., EPA 2016c,d) or MCLG, or a similar value (e.g., developed by a state). The component chemical HQs are then summed across the mixture to yield the HI, as illustrated in Equation 4-1.

$$HI = \sum_{i=1}^n HQ_i = \sum_{i=1}^n \frac{E_i}{RfV_i} \quad (4-1)$$

Where:

HI = Hazard Index

HQ_i = Hazard Quotient for chemical i

E_i = Exposure, i.e., dose (mg/kg/d) or occurrence concentration, such as in drinking water (mg/L), for chemical i

RfV_i = Reference value (e.g., oral RfD or MRL (mg/kg/d), or corresponding health-based, media-specific value; e.g., such as an HBWC, that is, a drinking water Health Advisory or MCLG) for chemical i (mg/L)

Because the numerator of each component chemical HQ is the estimated population-level human exposure, the non-cancer health RfVs used in the denominator must be based on human toxicity. These RfVs are derived either directly from human epidemiological/occupational study PODs (or measured or modeled effective dose (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 a human equivalent dose (POD_{HED}) using cross-species TK-based modeling or allometric body-weight scaling).

The HI approach in practical application may be subdivided into a “screening-level” HI and a “target-organ specific” HI (TOSHI). In the screening level HI, the RfV for each mixture component chemical is used in the calculation of a 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 screening-level HI is generally a conservative indicator because often the most sensitive health effects are used as the basis for each respective chemical HQ. Conversely, the TOSHI entails derivation of HQs for each mixture component chemical based on a “similar” effect. For example, in the case of a

liver-specific HI, for some mixture components the liver effect(s) may indeed be the basis for the RfD (e.g., GenX chemicals) whereas for other components, the liver might be among the least sensitive of effects (e.g., PFBS). To use this approach, organ-specific reference values (osRfVs) or TTDs are needed (note: these are the same type of non-cancer values, just with different naming conventions) for each mixture component of potential concern. For chemicals lacking hazard and dose-response data from traditional or NAM-based data streams for the selected effect, it may not be possible to determine their potential contribution to the mixture, which may result in an underestimation of the overall mixture risk.

In a screening-level context, an HI less than or equal to one is regarded as being of minor or no concern (recall that an RfV, like an oral RfD, represents an estimate at which no appreciable risk of deleterious effects exists), typically requiring no further analysis (EPA, 1986, 1991, 2000). An HI greater than one is generally regarded as suggestive of possible toxicity. Further analysis could provide a refined assessment of the potential for health effects associated with the individual chemicals and their contributions to the potential joint toxicity associated with the mixture (see Section 4.4).

In the case of PFAS, final peer-reviewed toxicity assessments are only available for a small proportion of the more than 9,000 environmentally relevant PFAS (e.g., see summary of EPA and ATSDR PFAS assessments in Table 4-1). EPA's primary source of peer-reviewed toxicity assessments is its Integrated Risk Information System (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 toxicological assessments, such as EPA's IRIS⁵, Provisional Peer-Reviewed Toxicity Values (PPRTV)⁶, EPA Office of Water toxicity assessments⁷, TSCA risk evaluations⁸, and ATSDR's ToxProfiles⁹, undergo rigorous peer and public review processes; as a result, they are considered to be of high scientific quality. The chronic RfDs for PFOA (EPA, 2016a), PFOS (EPA, 2016b), PFBS (EPA, 2021a), and GenX chemicals (EPA, 2021b) represent the only currently available/final EPA toxicity values, although several more PFAS assessments are under development in EPA ORD (e.g., PFBA, PFHxA, PFHxS, PFNA, and PFDA; see Table 4-1 below) that can be considered in the future. Additionally, use of this approach could consider other PFAS toxicity values (e.g., ATSDR MRLs) for which EPA has not yet developed values.

⁵ <https://www.epa.gov/iris>

⁶ <https://www.epa.gov/pprtv>

⁷ e.g., <https://www.epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>

⁸ <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluations-existing-chemicals-under-tsca>

⁹ <https://www.atsdr.cdc.gov/toxprofiledocs/index.html>

Table 4-1. EPA and ATSDR Peer-Reviewed Human Health Assessments Containing Non-Cancer Toxicity Values (RfDs or MRLs) for PFAS that Are Either Final or Under Development (only final assessment values are provided)

Chemical	EPA Chronic Oral RfD	ATSDR Intermediate MRL ^a
PFOA	2016 RfD = 2×10^{-5} mg/kg/d; Draft updated toxicity assessment undergoing SAB review (EPA, 2021c)	2021 MRL = 3×10^{-6} mg/kg/d
PFOS	2016 RfD = 2×10^{-5} mg/kg/d; Draft toxicity assessment undergoing SAB review (EPA, 2021d)	2021 MRL = 2×10^{-6} mg/kg/d
PFNA	Under development in the EPA IRIS program	2021 MRL = 3×10^{-6} mg/kg/d
PFDA	Under development in the EPA IRIS program	N/A
PFBA	Public Comment and External Review Draft released August 2021	N/A
PFBS	2021 RfD = 3×10^{-4} mg/kg/d	N/A
PFHxA	Under development in the EPA IRIS program	N/A
PFHxS	Under development in the EPA IRIS program	2021 MRL = 2×10^{-5} mg/kg/d
GenX chemicals	2021 RfD = 3×10^{-6} mg/kg/d	N/A

^aNote that MRLs and RfDs are not necessarily equivalent (e.g., intermediate duration MRL vs. chronic duration RfD; EPA and ATSDR may apply different uncertainty/modifying factors) and are developed for different purposes.
N/A = Not available

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 EPA or ATSDR assessments.

There may be scenarios where a final peer-reviewed toxicity assessment for one or more component chemicals is not available for a mixture. In these cases, an evaluation of available hazard and dose-response information for PFAS in the mixture may be necessary under a 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 have a need to develop a targeted, fit-for-purpose assessment, if possible (i.e., based on 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 underestimation of the potential risk 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, the associated uncertainties (e.g., data gaps) transparently characterized, and that the assessment undergoes independent external peer review. EPA has published several peer-reviewed guidance 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 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, 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 animal bioassay and human epidemiological information, validated NAMs could potentially play a pivotal and transformational role in human health (and ecological) risk assessment, particularly in evaluating hazard and dose-response of PFAS that co-occur in mixtures.

Individually or in concert, NAMs such as toxicogenomics (e.g., HTTr, in vitro cell bioactivity) and in silico platforms (e.g., read-across) might inform identification or prediction of metrics 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 estimated in vivo exposure-response using in vitro to in vivo extrapolation (IVIVE) (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), with expert-driven application of appropriate uncertainty factors and corresponding non-cancer 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.

A critical consideration in using NAM-based hazard and concentration/dose-response data is recognizing that for some high(er) throughput platforms or bioassays, perturbations of underlying biological pathways may not be readily identifiable as being directly related to specific apical toxic effects per se. 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, transcriptomics) may be considered in an in vivo 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 reasonable quantitative approximation for dose-response (e.g., POD) associated with traditional apical

effects (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 (EC₅₀), inhibitory concentration 50 (IC₅₀), or other biologically supported response levels of interest), could potentially be leveraged and applied in the mixture component approaches proposed in this chapter (e.g., HI, RPF, mixture BMD), irrespective of direct linkage(s) to a phenotypic apical effect.

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, leveraging NAMs could potentially serve an important role for PFAS screening and assessment, including in a mixture context. It is recognized that 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 appropriately contextualize the applicability of results, and that they transparently communicate uncertainties associated with a given platform or assay output(s) in human health assessment.

4.3.1 Illustrative Example Application of Hazard Index to PFAS Mixtures: Screening-Level Hazard Index

As mentioned previously, in addition to PFOA and PFOS, final EPA human health assessments exist for PFBS and GenX chemicals. EPA has derived chronic oral RfDs for all four of these PFAS. Further, ATSDR has published a ToxProfile for several PFAS (ATSDR, 2021); beyond PFOA and PFOS, ATSDR has derived MRLs for PFNA and PFHxS (see Table 4-1). Many states and others (e.g., international entities) are addressing rapidly evolving PFAS issues under their respective purviews, including the development of toxicological assessment documents. Although there is overlap in the landscape of PFAS evaluated (or currently being evaluated) across federal, state, and international agencies, at the state/international level, there may be assessment values available for a broader array of PFAS; in the context of this framework, these will be collectively referred to as “PFAS 1” (Table 4-2). Several other PFAS have varying levels of human epidemiological and/or experimental animal toxicity information available, but do not currently (at the time of the drafting of this framework) have completed human health assessments or associated RfVs (e.g., EPA ORD is currently assessing PFBA, PFHxA, PFHxS, PFNA, and PFDA); any PFAS with available hazard and dose response data but no final RfV will be collectively referred to as “PFAS 2” (Table 4-2). Lastly, information coming from the tiered testing collaboration between EPA and NIEHS¹⁰, or other sources of bioactivity data, and/or read-across may provide opportunities to calculate a value similar to an oral RfD when traditional human epidemiological and experimental animal study data are lacking; in the context of this framework, these PFAS will be collectively referred to as “PFAS 3” (Table 4-2). However, because there are no EPA-published HBWCs (e.g., Health Advisories, MCLGs) at this time for other PFAS with federal or state assessments/RfVs (e.g., PFBS (EPA), GenX chemicals (EPA), PFHxS (ATSDR), and PFNA (ATSDR)) or chemicals categorized under PFAS 1 or

¹⁰ See: <https://www.epa.gov/chemical-research/pfas-chemical-lists-and-tiered-testing-methods-descriptions#2>

PFAS 2, these values would need to be calculated in order to develop component HQs and an overall PFAS mixture HI (Equation 4-1).

Table 4-2. Summary of Information Available for PFAS in the Hypothetical Screening HI Example

Chemical	Final U.S. Federal Assessment(s) with RfV Available	No Final U.S. Federal Assessment(s) with RfV; Final State or Other (e.g., International) Assessment(s) with RfV Available	No Final Assessment with RfV; Hazard and Dose-Response Information ^a Available	No Hazard and Dose-Response Information ^a ; NAM Information Available	EPA Published HBWC (Health Advisory, MCLG)
PFOA, PFOS	X				X
EPA: PFBS, GenX chemicals; ATSDR: PFNA, PFHxS	X				
PFAS 1		X			
PFAS 2			X		
PFAS 3				X	

^a Specifically refers to traditional human epidemiological and experimental animal study data.

The basic steps for calculating a *screening-level HI* for mixtures of PFAS with varying levels of available hazard and dose-response data are as follows:

Step 1. Identify Chronic Oral RfDs.

- PFOA, PFOS, PFBS, GenX chemicals, PFHxS, and PFNA:** Review federal assessments containing oral RfDs or MRLs and select the appropriate values. Again, for screening-level HI, this will be the overall RfD (or MRL), regardless of underlying critical health effect. If an MRL is only available for an intermediate duration (akin to subchronic for EPA purposes), additional uncertainty may be considered for extrapolation to a corresponding chronic duration value.
- PFAS 1:** No final U.S. federal assessments with RfV are available. Review state or other PFAS assessment documents for qualitative confidence in hazard identification and uncertainty(ies) in quantitative derivation of health/toxicity values for PFAS. Considerations should also include the level of rigor of peer-review associated with the published assessments/values.
- PFAS 2:** No federal, state, or other assessments with RfV are available, but traditional hazard and dose-response (i.e., human epidemiological and/or experimental animal study) data are judged to support derivation. Thus, the user may choose to calculate a screening-level RfV using appropriate dose-response metrics (i.e., POD) and application of

uncertainty factors. It is recommended that study hazard effect and dose-response data be systematically evaluated for suitability in supporting the derivation of screening-level RfVs using accepted EPA approaches and practice. Appropriate characterization and denoting of confidence and uncertainty(ies) in screening-level RfVs for PFAS in this category is imperative. Consultation with experts in the field and independent external peer review is recommended.

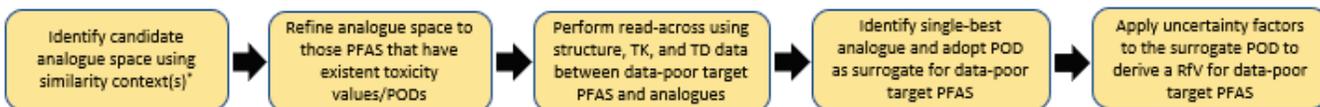
- PFAS 3:** Because no final federal, state, or other RfD or MRL or traditional hazard and dose-response data are available, NAM data streams could be surveyed and leveraged for PFAS information that might facilitate development of a POD, and potentially, derivation of a NAM-based RfV using application of uncertainty factors consistent with the data scenario (Judson et al., 2011; Parish et al., 2020). It is recommended that data be systematically evaluated for suitability in supporting the derivation of screening-level RfVs using accepted EPA approaches and practice. Appropriate characterization and denoting of confidence and uncertainty(ies) in screening-level RfVs for PFAS in this category is imperative. Consultation with experts in the field of NAM data interpretation and risk assessment application and independent external peer review is recommended.

Currently, the general process proposed for translating/integrating NAM into a human health assessment context is as follows for: (a) bioactivity (where “bioactivity” includes any/all cell-based assays/platforms, as well as HTTr); or (b) analogue-based read-across:

(a) Bioactivity-based (e.g., ToxCast, Tox21; HTTr)



(b) Read-across



*similarity contexts include structure/physicochemical, TK, and/or TD

Step 2. Identify or Calculate HBWCs.

- PFOA/PFOS:** Review and select an HBWC for PFOA and PFOS from available sources (e.g., EPA, 2016c,d).
- PFBS, GenX chemicals, PFHxS, PFNA, or species under PFAS 1, PFAS 2, or PFAS 3:** Calculate HBWCs using approach similar to that described in the 2016 Health Advisories for PFOA and PFOS (EPA, 2016c,d). Once all possible RfVs are assembled across PFAS mixture components, HBWCs are then derived where and when feasible.

Step 3. Select Exposure Estimates.

- Select appropriate exposure estimates from monitoring data.

Step 4. Calculate Screening-Level HI.

- Calculate individual component HQs for PFOA, PFOS, PFBS, GenX chemicals, PFHxS, PFNA, and/or species categorized under PFAS 1, 2, and 3, depending on the PFAS mixture of interest. In the drinking water context, HQs are first calculated as a ratio of the exposure concentration to the corresponding PFAS HBWC. The component PFAS HQs are then added together to determine the screening-level HI. HI values in exceedance of 1.0 indicates potential mixture hazard.

Application of the TOSHI is essentially identical to the steps for the screening-level HI. The critical nuance is that identification and assembly of human health/toxicity values are effect/endpoint specific. For some PFAS, this might entail the overall RfD or MRL; for other PFAS, this will involve TTDs (i.e., an RfD for a specific health effect). In the TOSHI, 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. In those instances, TTDs could potentially be derived 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.

4.3.1.1 Example 1: PFOA+PFOS

PFOA and PFOS are included in this illustrative example to demonstrate a screening-level HI approach for these chemicals specifically in the context of drinking water. For this example, EPA is relying on hypothetical exposure estimates and the 2016 EPA drinking water Health Advisories (Table 4-3) for PFOA and PFOS (EPA, 2016c,d). As stated previously, EPA finalized drinking water Health Advisories of 70 ng/L (ppt) for PFOA and PFOS, for the individual chemicals and when present as a mixture based on an assumption of dose additivity, because the RfDs were based on similar developmental effects and numerically identical (EPA, 2016c,d). [Note: EPA's draft updated toxicity assessments and MCLG documents for PFOA and PFOS are currently undergoing SAB review, and thus, these values are subject to change. These HI calculations would need to be updated following finalization of updated RfDs].

Table 4-3. Summary of Input Parameters Used to Calculate the 2016 EPA Health Advisories for PFOA and PFOS

Chemical	RfD; Critical Effect (Developmental)	Drinking Water Intake (DWI) Rate/Body Weight (L/kg-d)	Relative Source Contribution (RSC) ^a	2016 EPA Health Advisory (ng/L) ^b
PFOA	2 x 10 ⁻⁵ mg/kg/d; Reduced ossification of the proximal phalanges and accelerated puberty in mice (EPA, 2016c)	0.054 ^b	0.2	70
PFOS	2 x 10 ⁻⁵ mg/kg/d; Decreased pup body weight in rats (EPA, 2016d)	0.054 ^b	0.2	70

^a RSC is the portion of an exposure for the target (sub)population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources (see EPA, 2016c,d for details regarding PFOA/PFOS RSC = 0.2).

^b Health Advisory = (RfD/(DWI/bw)) x RSC.

^c 0.054 L/kg/d is the 90th percentile consumers only estimate of combined direct and indirect community water ingestion for lactating women (see EPA, 2016c,d). This value has been updated with the publication of the 2019 edition of Chapter 3 of EPA's Exposure Factors Handbook (EPA, 2019a)

For illustration purposes, example screening-level HQs are calculated using hypothetical low (Table 4-4) and higher (Table 4-5) exposure estimates for drinking water and the 2016 EPA Health Advisories for PFOA and PFOS.

Table 4-4. Example Screening-Level HQs and HI for PFOA and PFOS at Low Water Concentrations

Chemical	Hypothetical Exposure Estimate (ng/L) ^a	2016 EPA Health Advisory (ng/L)	Example HQ ^b
PFOA	20	70	0.29
PFOS	20	70	0.29
SCREENING LEVEL HAZARD INDEX			0.6

^a The hypothetical water exposure estimates provided represent a 5-fold increase over the minimum reporting level listed in UCMR 5 (<https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule>).

^b HQ = Exposure Estimate/Example HBWC (in this case the 2016 EPA Health Advisory). HI = the sum of individual HQs.

Table 4-5. Example Screening-Level HQs and HI for PFOA and PFOS at Higher Water Concentrations

Chemical	Hypothetical Exposure Estimate (ng/L)	2016 EPA Health Advisory (ng/L)	Example HQ ^b
PFOA	400	70	5.7
PFOS	400	70	5.7
SCREENING LEVEL HAZARD INDEX			11

^a The hypothetical water exposure estimates provided represent a 100-fold increase over the minimum reporting level listed in UCMR 5 (<https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule>).

^b HQ = Exposure Estimate/Example HBWC (in this case the 2016 EPA Health Advisory). HI = the sum of individual HQs.

In the first hypothetical application of the screening-level HI approach, the HQs for PFOA and PFOS individually are below the threshold of 1.0, indicating hazard is below a level of concern for either PFAS in isolation. Further, when the PFOA HQ is summed with the PFOS HQ, the screening-level HI for the mixture is also less than 1.0 (Table 4-4). This indicates that the potential for health outcomes in exposed populations is not expected, at the low water concentrations indicated, for the mixture of [PFOA+PFOS].

In the second example, the HQs for PFOA and PFOS individually are above the threshold of 1.0, indicating a potential human health hazard associated with either PFAS in isolation. Further, when the PFOA HQ is summed with the PFOS HQ, the screening-level HI for the mixture is 11 (Table 4-5). This indicates that there is potential for health outcomes in exposed populations, at the higher water concentrations indicated, for the mixture of [PFOA+PFOS].

Please note that the magnitude of an HI, 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 10.0 is not necessarily of 2-fold greater concern than a mixture HI of 5.0. The practical interpretation is that both mixtures would be of concern and should be prioritized for further evaluation. In the illustrative example of PFOA+PFOS only, further analysis would be needed to better understand the potential for health effects associated with PFOA and PFOS individually and their relative contributions to the combined toxicity associated with the mixture.

4.3.2 Advantages and Challenges of the Approach

The HI approach provides an indication of the combined 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 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 where the HQs have greater contribution to a HI ≥ 1.0 , relative to other PFAS members of the mixture).

Another advantage is that the “hazard” does not necessarily have to be the same for screening-level HI (e.g., all liver or all kidney effects). Specifically, the screening-level HI approach can be used where the individual HQ calculated for each mixture PFAS is based on the most well-characterized, and oftentimes most sensitive, toxic effect and corresponding non-cancer RfV

(e.g., oral RfD). As such, a screening-level HI will typically represent the most conservative indicator of mixture risk, as each component HQ is based on its health-protective RfV.

A disadvantage of this approach is that the HI is an indication of potential hazard, 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 scenarios can be misleading. 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 endpoints, and similar receptor (exposed population) age), the two exposure scenarios could be judged to have the same potential for causing toxic effects. This interpretation has the strongest scientific foundation when there are only minor differences in the component exposures (thus, same exposure route, similar exposure duration for specific receptors, and roughly similar estimates of the individual HQs) between the two scenarios. Interpretation is more difficult when the underlying information is poor. For example, if the dominant chemical (highest HQ) has a highly uncertain exposure estimate, or its RfV was derived using a large UF, then the associated HI is also highly uncertain.

Another disadvantage of the application of this HI approach 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). Development of these values 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 per se, there is a recognized gradation of confidence across the range of possible PFAS values. Specifically, it would clearly be preferable to use RfVs obtained from assessment sources that use transparent systematic approaches and standardized protocols and have been vetted in rigorous peer-review processes. The level of confidence or certainty in such values would be greater than RfVs deriving from questionable toxicity data sources, entails non-transparent decision-making, and/or is associated with higher levels of qualitative and quantitative uncertainty.

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 NAM, dose-response metrics obtained from read-across and/or bioactivity-based assays/platforms may inappropriately be assigned some level of a priori uncertainty simply because of lack of confidence by end-users in interpretation and risk assessment application of such data and outputs. As mentioned previously in this framework, NAM may represent the only opportunity to integrate a data-poor PFAS into mixtures assessment. The end-user of this framework, in consultation with experts/practitioners in NAM development and application, would be advised to leverage NAM when and where possible, but 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 screening-level RfV and subsequent HQ and HI calculations. The disadvantage to 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.

4.3.3 *Target Organ Specific Hazard Index*

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). 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 for that particular PFAS. Another limitation is that so many PFAS species lack human epidemiological or experimental animal hazard and dose-response information across a broad(er) effect range thus limiting derivation of TTD values. As with the screening-level HI, a TOSHI approach might benefit from consideration of NAM data and approaches that can inform organ/tissue-specific dose-response.

4.4 Relative Potency Factors

4.4.1 *Basic Principle, Data Requirements to Calculate Relative Potency Factors and Corresponding Index Chemical Equivalent Concentrations*

RPF approaches comprise the second basic dose-addition method used most commonly by 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, and a few other chemical groups 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 or most robust toxicological database and is considered to be most representative of the type of toxicity caused by the mixture components (EPA, 1986, 2000). Further, the IC must have dose-response data for the dose range of interest; chemicals with steep slopes that cause effect and/or induce significant toxicity at all doses tested are not ideal for IC selection. 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 (EPA, 2000). 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 NOAELs, 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, non-human 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 the calculation of RPFs across

mixture PFAS, BMDs (i.e., the central tendency estimate) would be optimal. BMDs incorporate the totality of a given dose-response and facilitate identification of a dose at a pre-defined benchmark response level (e.g., 0.5SD or 1SD over control; 10% change in some effect/endpoint). BMD modeling would optimize comparison of “same” as a function of dose across mixture PFAS for a given health effect or endpoint. It is recognized that dose-response data for chemicals is sometimes not amenable to BMD modeling. Human equivalent NOAELs or ED_X values are perfectly 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 4-2:

$$RPF_j = \frac{ED10_{IC}}{ED10_j} \quad (4-2)$$

where IC refers to the index chemical.

For example, if mixture component chemical 2 is twice as potent as the IC, its NOAEL, 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 NOAEL, BMD_X, or ED_X will be twice as large and the RPF would be 0.5. In practice, 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 versus chronic exposure, oral versus 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 (e.g., human equivalent NOAEL, BMD_X, ED_X) in Equation 4-2, IC equivalent concentrations (ICEC) are then calculated by multiplying each respective RPF_j by the corresponding component chemical’s concentration (d_j), as shown in Equation 4-3:

$$ICEC_{MIX} = \sum_{j=1}^n d_j * RPF_j \quad (4-3)$$

The total mixture ICEC (ICEC_{MIX}) is then obtained by taking the sum of the component chemical ICECs (including that of the IC) (Equation 4-4). A numerical estimate of risk for non-cancer 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:

$$y_{MIX} = f(ICEC_{MIX}) \quad (4-4)$$

where the ICEC is derived from Equation 4-3. In the context of this PFAS mixture framework, there are important modifications or adaptations of this approach to note that include: (1) use of Index Chemical Effect Concentrations (ICECs), which are water-specific, correlates to index chemical equivalent doses (ICEDs) (EPA, 2000) and (2) using effect-specific HBWCs for the IC (e.g., 70 ppt for PFOS-induced developmental effects (decreased body weight in offspring)) 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} ≥ IC HBWC), or “no,” there is no anticipated effect-specific risk (e.g., ICEC_{MIX} ≤ IC HBWC), as well

as magnitude of health effect concern and identification of potential component chemical drivers of an ICEC.

EPA's supplementary guidance (EPA, 2000) states: "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 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, EPA has developed RPFs for only a few chemical groups, largely pesticides (organophosphorous pesticides, triazines, N-methyl carbamates, chloroacetanilides, and pyrethrins/pyrethroids), which in each case were based on MOA-level information (EPA, 2018). However, considering that PFAS are an emerging chemical class of concern, 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. This is the approach taken in the illustrative RPF examples below and is consistent with previous NAS recommendations pertaining to the evaluation of chemicals that cause common adverse health outcomes presumably through diverse biological pathways (NAS, 2008).

4.4.2 Illustrative Example Application of Relative Potency Factors and Index Chemical Equivalent Concentrations to PFAS Mixtures

The examples below demonstrate the application of RPF and ICEC calculations for PFOA, PFOS, PFBS, and GenX chemicals as these have existing final EPA hazard assessments and contain databases suitable for estimation of PODs for multiple health effect domains. Specifically, among the spectrum of adverse effects reported in these final EPA assessments, these PFAS have been shown to have dose-dependent effects on several common target organs/pathways including, but not limited to, developmental, thyroid, and liver effects (Table 4-6). These same three health outcome domains have also been reported as potential targets of other compounds within the broader class of PFAS (ATSDR, 2021; EFSA et al., 2020; ITRC, 2021). The approach here is to use a grouping construct that allows for combination of PFAS with shared, common apical effects (e.g., decreased pup body weight), as opposed to a stringent requirement of same MOA, to calculate RPFs across one or more health effect domains. Inclusion of 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 PFOA relative to PFOS 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 seek those that are shared among the PFAS in the mixture being assessed. However, for purposes of evaluating mixture risk using the RPF approach, it is critical to have an IC effect-specific HBWC so that the mixture ICEC can be compared to a benchmark point or dose. 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.

Critically, this is an illustrative example only and does not represent final RPFs. In the present example, for each PFAS and each effect domain the lowest POD_{HED} was selected from studies included in final EPA human health assessments, with the exception of the thyroid effect endpoint for PFOA, which is based on preliminary data from the 2021 EPA ORD studies (Appendix A). It is possible that different endpoints may be appropriate for different combinations of PFAS and/or updated effect and dose-response data (i.e., BMDs) may become available. For the examples in this section, BMDs were not available across all PFAS and/or health effects; as such, human equivalent NOAELs (or LOAELs) were harvested from the respective completed human health assessments as the dose response metric for potency comparisons of health effect/endpoint (Table 4-6).

Table 4-6. Summary of Health Effects/Endpoints Selected for Illustrative RPF Examples

Chemical	Reference	Species/life stage-sex	POD_{HED}^a (mg/kg/d)
DEVELOPMENTAL EFFECT: Decreased Pup Body Weight			
PFOA ^b	EPA, 2016a (Table 4-9); Wolf et al., 2007	Mouse; gestation days (GDs) 1–17	0.0109 (LOAEL)
PFOS ^b	EPA, 2016b (Table 4-9); Luebker et al., 2005c	Rat; 2 generation (84 days)	0.00051 (NOAEL)
PFBS	EPA, 2021a (Table 6); Feng et al., 2017	Mouse; neonatal female	0.21 (NOAEL)
GenX Chemicals	EPA, 2021b (Table 12); DuPont-18405-1037, 2010	Mouse; neonatal	0.07 (NOAEL)
THYROID EFFECT: Decreased Total T4 (TT4)			
PFOA ^b	ORD studies 2021 (see Appendix A)	Rat; PND2 neonatal male and female	0.24 (LOAEL)
PFOS ^b	EPA, 2016b (Section 3.4.1.5); Lau et al., 2003	Rat; neonatal male and female	0.24 (LOAEL)
PFBS	EPA, 2021a (Table 9); Feng et al., 2017	Mouse; PND 1 neonatal female	0.21 (NOAEL)
GenX Chemicals	EPA, 2021b (Table 12); Conley et al., 2021b	Rat; female maternal on post-pregnancy Day 2	7.0 (NOAEL)
LIVER EFFECT: Increase in Liver Weight^c			
PFOA ^b	EPA, 2016a (Table 4-9); Perkins et al., 2004	Rat; male	0.0044 (NOAEL)
PFOS ^b	EPA, 2016b (Table 4-8); Seacat et al., 2003	Rat; male	0.0013 (NOAEL)
PFBS	EPA, 2021a (Table 6); 3M Company, 2001	Rat; male	72.0 (NOAEL)
GenX Chemicals	EPA, 2021b (Table 12); DuPont-18405-1037, 2010	Mouse; male and female	0.01 (NOAEL)

^a Following EPA (2011) and EPA (2014) guidance, animal doses from selected studies were converted to HEDs through the application of a dosimetric adjustment factor (DAF), where $HED = \text{animal exposure dose} \times DAF$.

^b PFOA and PFOS health effects presented here, with the exception of the PFOA thyroid effect example, are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and do not reflect the updated information currently under review by the

EPA SAB. The PFOA thyroid effect example relies on preliminary data from the 2021 EPA ORD studies (Appendix A) because this information was not available in the 2016 assessments.
 ° Includes absolute and/or relative liver weight(s).

4.4.2.1 Example 1: PFOA and PFOS Only

An example illustration of the application of the RPF approach to a mixture of PFOA and PFOS is provided below using information for PFOA and PFOS (Table 4-6) from the 2016 EPA health effects support documents (EPA, 2016a,b), preliminary thyroid effect data for PFOA (Appendix A), and hypothetical exposure estimates.

Step 1. Identify Mixture IC And Effect-Specific HBWCs.

As both PFOA and PFOS are well-characterized PFAS and share an identical Health Advisory, selection of either chemical as the IC would be reasonable regardless of toxicity effect/endpoint. For this example, we selected PFOS as the IC for developmental and thyroid effects; PFOA was selected as the IC for liver effects as it has a more well-informed database for this effect domain, compared to PFOS. The only currently available HBWC for PFOA and PFOS derives from the EPA 2016 Health Advisories, which are based on developmental effects (70 ppt). Effect-specific HBWCs are not available for thyroid or liver effects and would need to be calculated using an approach similar to that described in the EPA 2016 Health Advisory.

Step 2. Calculate RPFs And ICECs For Each Effect Domain.

The RPF for the IC is always 1. The developmental effect RPF in this example is calculated for an effect of decreased body weight in offspring. Both PFOA and PFOS were shown to decrease body weight in offspring (EPA, 2016a,b). However, while this effect was the basis for the 2016 RfD for PFOS, the PFOA RfD was based on a different developmental effect, i.e., reduced ossification of the proximal phalanges and accelerated puberty in mice, because that was the more sensitive effect. Offspring body weight decreases were selected as a common developmental effect for the purposes of this RPF illustration, and PFOS was selected as the IC. The developmental effect RPF for PFOA is calculated by dividing the PFOS POD_{HED} by the PFOA POD_{HED} , resulting in a RPF of 0.5. Each component RPF is multiplied by the corresponding chemical-specific estimated water exposure concentration to derive a PFOS ICEC (e.g., Table 4-7). The example developmental RPF Mixture Total PFOS ICECs (Table 4-7 and 4-8) are compared to the EPA 2016 Health Advisory for PFOS (70 ppt), which is based on the developmental effect of decreased body weight in offspring (EPA, 2016d). Effect-specific HBWCs are not available for PFOA or PFOS for the thyroid or liver, and so the illustrative examples for thyroid and liver stop at the point of RPF calculation (Tables 4-9 and 4-10).

Table 4-7. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA and PFOS at Low Water Exposure Concentrations

Mixture Component	POD _{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L) ^a	PFOS ICEC (ng/L)
PFOA	0.001 (NOAEL _{HED}) ^{b, c} (EPA, 2016a)	0.5	20	10
PFOS (IC)	0.00051 (NOAEL _{HED}) ^b (EPA, 2016b)	1	20	20
Mixture Total PFOS ICEC (ppt)				30

^a The hypothetical water exposure estimates provided represent a 5-fold increase over the minimum reporting level listed in UCMR 5 (EPA, 2021g).

^b PFOA and PFOS health effects presented here are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and does not reflect the updated information currently under review by the EPA SAB.

^c The POD for PFOA-induced effects on pup body weight is a LOAEL; as such, a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 was applied to convert the POD to a NOAEL.

The PFOA+PFOS mixture equivalent water concentration is then compared to the IC HBWC for the specified effect or hazard domain (e.g., for this example, decreased body weight in offspring). In this case, the mixture total PFOS ICEC of 30 ppt does not exceed the PFOS HBWC of 70 ppt, indicating no potential risk of body weight effects in birthed offspring associated with exposure to a mixture of PFOA+PFOS at the hypothetical water exposure estimates provided.

At higher hypothetical water exposure estimates (Table 4-8), the mixture total PFOS ICEC of 600 ppt exceeds the PFOS HBWC of 70 ppt by nearly an order of magnitude, indicating potential risk of body weight effects in offspring associated with exposure to a mixture of PFOA+PFOS.

Table 4-8. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA and PFOS at Higher Water Exposure Concentrations

Mixture Component	POD _{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L) ^a	PFOS ICEC (ng/L)
PFOA	0.001 (NOAEL _{HED}) ^{b, c} (EPA, 2016a)	0.5	400	200
PFOS (IC)	0.00051 (NOAEL _{HED}) ^b (EPA, 2016b)	1	400	400
Mixture Total PFOS ICEC (ppt)				600

^a The hypothetical water exposure estimates provided represent a 100-fold increase over the minimum reporting level listed in UCMR 5 (EPA, 2021g).

^b PFOA and PFOS health effects presented here are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and does not reflect the updated information currently under review by the EPA SAB.

^c The POD for PFOA-induced effects on pup body weight is a LOAEL; as such, a UF_L of 10 was applied to convert the POD to a NOAEL.

For thyroid effects (decreased total T4), PFOS is identified as the IC. The POD_{HEDs} are identical indicating equipotency between PFOA and PFOS for effect on total T4; thus, both RPFs are 1 (Table 4-9). There is currently no thyroid effect HBWC available for PFOS so this would need to be calculated following the steps described in Section 4.3.1 and compared to the PFOS ICEC to determine the potential risk of the mixture.

Table 4-9. Example Thyroid Effect RPFs for a Mixture of PFOA and PFOS

Mixture Component	POD_{HED} (mg/kg/d); Decrease in Total T4	Example RPF
PFOA	0.024 ($NOAEL_{HED}$) ^{a, b} (EPA, 2016a)	1
PFOS (IC)	0.024 ($NOAEL_{HED}$) ^{a, b} (EPA, 2016b)	1

^aThe $PODs$ for PFOA- and PFOS-induced decrease in total T4 are $LOAELs$; as such, a UFL of 10 was applied to convert the $PODs$ to corresponding $NOAELs$.

^bPFOA and PFOS health effects presented here are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and does not reflect the updated information currently under review by the EPA SAB.

For liver effects (increased liver weight(s)), PFOA is identified as the IC. Based on the identified POD_{HEDs} , PFOS is approximately 3-fold more potent than PFOA for effects on liver weight (Table 4-10). There is currently no liver effect HBWC available for PFOA so this would need to be calculated following the steps described in Section 4.3.1 and compared to the PFOA ICEC to determine the potential risk of the mixture.

Table 4-10. Example Liver Effect RPFs for a Mixture of PFOA and PFOS

Mixture Component	POD_{HED} (mg/kg/d); Increase in Liver Weight	Example RPF
PFOA (IC)	0.0044 ($NOAEL_{HED}$) ^a (EPA, 2016a)	1
PFOS	0.0013 ($NOAEL_{HED}$) ^a (EPA, 2016b)	3

^aPFOA and PFOS health effects presented here are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and does not reflect the updated information currently under review by the EPA SAB.

4.4.2.2 Example 2: PFOA, PFOS, PFBS, and GenX Chemicals

Example application of the RPF approach to a mixture of PFOA, PFOS, PFBS, and GenX chemicals is provided below using information from EPA's 2016 Health Effects Support Documents for PFOA and PFOS (EPA, 2016a,b), preliminary thyroid effect data for PFOA from the 2021 EPA ORD study (Appendix A), final PFBS toxicological assessment (EPA, 2021a), final GenX chemicals toxicological assessment (EPA, 2021b), and hypothetical low or high(er) exposure estimates. Illustrative RPFs and corresponding ICECs are shown for a developmental health effect only, as the only HBWC available for comparison to the mixture total ICEC is for this effect domain (Tables 4-11 and 4-12). RPFs are presented for thyroid (Table 4-13) and liver

(Table 4-14) effects for the same four-component mixture, however mixture ICECs are not derived as there are no effect-specific HBWCs available for comparison purposes.

Table 4-11. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals at Low Water Exposure Concentrations

Mixture Component	POD _{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L) ^a	PFOS ICEC (ng/L)
PFOA	0.001 (NOAEL _{HED}) ^{b, c} (EPA, 2016a)	0.5	20	10
PFOS (IC)	0.00051 (NOAEL _{HED}) ^b (EPA, 2016b)	1	20	20
PFBS	0.21 (NOAEL _{HED}) (EPA, 2021a)	0.002	15	0.04
GenX chemicals	0.07 (NOAEL _{HED}) (EPA, 2021b)	0.007	25	0.2
Mixture Total PFOS ICEC (ppt)				30

^a The hypothetical water exposure estimates provided represent a 5-fold increase over the minimum reporting level listed in UCMR 5 (EPA, 2021g).

^b PFOA and PFOS health effects presented here are drawn from EPA's 2016 health effects support documents (EPA, 2016a,b) and does not reflect the updated information currently under review by the EPA SAB.

^c The POD for PFOA-induced effects on pup body weight is a LOAEL; as such, a UFL of 10 was applied to convert the POD to a NOAEL.

In this illustrative example, the addition of PFBS and GenX chemicals to the example mixture of PFOA+PFOS, at the hypothetical low exposure concentrations, did not meaningfully impact the overall PFOA ICEC for developmental effects. This is primarily a function of significantly lower potency of PFBS and GenX chemicals for effects on body weight in offspring, compared to PFOA and PFOS. In this case, the total mixture PFOS ICEC of 30 ppt does not exceed the PFOS HBWC of 70 ppt, indicating minor concern for risk of body weight effects in offspring associated with exposure to the four PFAS mixture at the hypothetical low water exposure estimates provided.

Similarly, the addition of PFBS and GenX chemicals to the example mixture of PFOA+PFOS, at the hypothetical higher water exposure concentrations (Table 4-12), did not meaningfully impact the overall PFOS ICEC for developmental effects. Again, this is primarily a function of significantly lower potency of PFBS and GenX chemicals for effects on body weight in offspring, compared to PFOA and PFOS. In this case, the total mixture PFOS ICEC of 603 ppt exceeds the PFOS HBWC of 70 ppt by nearly an order of magnitude, indicating the potential risk of body weight effects in offspring. Further, based on the PFOS ICECs, both PFOA and PFOS are significant drivers for the mixture risk to offspring (200 and 400 ng/L, respectively).

Table 4-12. Example Developmental Effect RPFs and ICECs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals at Higher Water Exposure Concentrations

Mixture Component	POD _{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L) ^a	PFOS ICEC (ng/L)
PFOA	0.001 (NOAEL _{HED}) ^b (EPA, 2016a)	0.5	400	200
PFOS (IC)	0.00051 (NOAEL _{HED}); (EPA, 2016b)	1	400	400
PFBS	0.21 (NOAEL _{HED}); (EPA, 2021a)	0.002	300	0.7
GenX chemicals	0.07 (NOAEL _{HED}) (EPA, 2021b)	0.007	500	2.1
Mixture Total PFOS ICEC (ppt)				603

^a The hypothetical water exposure estimates provided represent a 100-fold increase over the minimum reporting level listed in UCMR 5 (EPA, 2021e).

^b The POD for PFOA-induced effects on pup body weight is a LOAEL; as such, a UFL of 10 was applied to convert the POD to a NOAEL.

Based on the POD_{HEDs} for thyroid effect (i.e., decreased total T4) across the four-component mixture, PFOA and PFOS are approximately 1-2 orders of magnitude more potent than PFBS or GenX chemicals, and are reflected in the corresponding RPFs (Table 4-13). As such, at approximately equivalent water concentrations, in mixture, PFOA and PFOS would contribute equipotently to an effect on total T4, whereas PFBS would contribute 1/10th of the mixture potency, and the contribution of GenX chemicals to thyroid effect would be virtually negligible.

Table 4-13. Example Thyroid Effect RPFs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals

Mixture Component	POD _{HED} (mg/kg/d); Decrease in Total T4	Example RPF
PFOA	0.024 (NOAEL _{HED}) ^a (ORD studies 2021; see Appendix A)	1
PFOS (IC)	0.024 (NOAEL _{HED}) ^a (EPA, 2016b)	1
PFBS	0.21 (NOAEL _{HED}) (EPA, 2021a)	0.1
GenX chemicals	7.0 (NOAEL _{HED}) (EPA, 2021b)	0.003

^a The PODs for PFOA- and PFOS-induced decrease in total T4 are LOAELs; as such, a UFL of 10 was applied to convert the PODs to corresponding NOAELs.

Based on the POD_{HEDs} for liver effect (i.e., increased liver weight) across the four-component mixture, GenX chemicals are approximately 4-fold more potent and PFOS is approximately 3-fold more potent than PFOA for this effect (Table 4-14). The contribution of PFBS is effectively

negligible in the mixture. At approximately equivalent water concentrations, GenX chemicals and PFOS might be significant drivers for mixture risk of liver weight effects.

Table 4-14. Example Liver Effect RPFs for a Mixture of PFOA, PFOS, PFBS, and GenX Chemicals

Mixture Component	POD _{HED} (mg/kg/d); Increase in Liver Weight	Example RPF
PFOA (IC)	0.0044 (NOAEL _{HED}) (EPA, 2016a)	1
PFOS	0.0013 (NOAEL _{HED}) (EPA, 2016b)	3
PFBS	7.2 (NOAEL _{HED}) ^a (EPA, 2021a)	0.0006
GenX chemicals	0.001 (NOAEL _{HED}) ^a (EPA, 2021b)	4

^a The PODs for PFBS and GenX chemicals-induced effects on increase in liver weight are derived from subchronic studies; as such, a UF_s of 10 was applied to convert the PODs to corresponding “chronic duration” NOAELs.

As illustrated in the RPF examples above, PFAS can have different potencies across health effect domains. Due to differences in both TKs and TDs, PFAS may exhibit complex gradations of potency for different effects, and this 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. 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 dependent 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). 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, a limitation for PFAS is the availability of human health assessment grade toxicity data; Section 4.5 offers an alternative to the RPF approach in such a scenario.

4.4.3 Advantages and Challenges of the Relative Potency Factor Approach

A significant advantage of the RPF approach is that formal toxicity or RfV derivation is not necessary 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 same effect/endpoint using same dose metrics from same study design/durations, calculation of RPFs across PFAS may in practical application entail (i.e., necessitate) use of effect data deriving from diverse study designs and exposure durations. As such, in some cases, there may be a need to selectively apply uncertainty factors in the RPF method, in particular, the LOAEL-to-NOAEL (UF_L) and/or subchronic-to-chronic duration (UF_s) factors. For example, in many of the

illustrative RPF calculations in this chapter, the effect data for PFBS, GenX chemicals, PFOA, and PFOS came from a mixture of reproductive/developmental study design 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 (e.g., PFOA, PFOS). For the expressed purpose of deriving RPFs, applying a UF_S of 10 to convert a subchronic $NOAEL_{HED}$ to a corresponding chronic $NOAEL_{HED}$, or, converting a $LOAEL_{HED}$ to a $NOAEL_{HED}$, provides the opportunity for a more 1:1 comparison of potency for a given effect (e.g., developmental body weight, increase in liver weight) between PFOA, PFOS, PFBS, and GenX chemicals. In practice, users of this framework document may find the need to apply $UF(s)$ consistent with the examples summarized here. A critical facet to this is to be transparent about such 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 presents a problem as it pertains to practical application of RPF methodology in that a vast majority of environmental chemicals have limited-to-no MOA data available. This is particularly true of PFAS as an emerging chemical class of concern. EPA mixtures guidance does provide flexibility in use of 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 greater probability of identifying effect/endpoint and associated dose-response data (e.g., effect-specific PODs) for mixture components than there is for MOA type data. However, as the data for PFAS evolve over time, the toxicity profiles including 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 calculation of an actual mixture toxicity dose or concentration estimate, as opposed to the HI which is considered an indicator of potential hazard/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 a HBWC).

A clear challenge, not uniquely associated with the RPF approach per se, is the use of potentially disparate hazard and dose-response data across mixture components. The implicit assumption for dose-response data selection in the calculation of RPFs in this tiered framework, is that the same dose-response data that underpinned the derivation of corresponding RfVs (overall RfDs or effect-specific TTDs) for use as input(s) for HQs and HIs (i.e., Tier 1) would also be leveraged in Tier 2 (be it for RPF and/or Mixture BMD approaches (see Section 4.5)). 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 NOAELs are acceptable), and qualitative and quantitative uncertainties or confidence in what could potentially be a diverse assembly of data/metrics to support RPF application(s).

An additional potential challenge, that actually may present an opportunity for advancing the science of mixtures risk assessment is the use of NAM data. The constantly evolving information coming from alternative toxicity testing assays and platforms may be of paramount importance

to human health assessment of environmental chemicals in general (not just for mixtures applications), however there are inherent challenges associated with 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 selection of “effect” data, a key requirement is that the “effect” on which RPFs are based be the same. For example, one mixture 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 the 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 administered equivalent dose) is suitable for traditional RPF calculation simply based on the optics of “same” effect. Further investigation is needed to investigate 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.

4.5 Dose Addition Mixture BMD Approach

4.5.1 *Basic Principle, Data Requirements for Mixture Benchmark Doses and Corresponding Mixture Health-Based Water Concentrations*

Both the HI/Quotient and RPF steps described above require published or user-derived human health assessment values, such as oral RfDs or individual chemical HBWCs. For example, in the HI approach, 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 corresponding human health assessment values (e.g., RfDs) for use in mixture-based risk calculation. Further, for the RPF approach, for a given effect domain being considered, there may not be a corresponding dose-response assessment value (e.g., POD_{HED}) for the common effect being used to derive relative potencies. In these cases, a third approach, described in EPA supplementary guidance (2000) (Section 4.2.6) and NAS (2008) (Appendix C), employs a DA model-based calculation of a mixture BMD based on a defined benchmark response (e.g., ED_{10}) for a PFAS mixture with a specific mixing-ratio of component chemicals. As previously discussed, DA has broadly been viewed as the most appropriate model for estimating combined effects of “toxicologically similar” compounds. The rationale for the use of DA as a default model for estimating combined effects of exposure to multiple PFAS is reviewed in Section 3.

DA modeling of a PFAS mixture requires empirical data-driven or reasonable estimation (e.g., read-across between structures) of effect-equivalent endpoints for all PFAS in the mixture. Importantly, the endpoint selected must be the same for all PFAS included in the calculation, for example BMD_{10S} for the same liver effect, or NOAELs for the same developmental effect. The model output will produce an equivalent metric (i.e., BMD_{10} or NOAEL) for the specific total mixture of PFAS being evaluated. Effect equivalent BMDs are more statistically robust and the

equation explanation and example below will reference BMD_X as the model components using Equation 4-5, where t_{add} is the total mixture dose in mg/kg/d, 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). Similar to the RPF approach above, 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.

$$t_{add} = \left(\sum_{i=1}^n \frac{a_i}{BMD_i} \right)^{-1} \quad (4-5)$$

4.5.2 Illustrative Example Application of Mixture Benchmark Doses and Mixture Health-Based Water Concentrations

An example is described here and in Tables 4-15 and 4-16 for two hypothetical mixtures of four different PFAS. The two samples have the same four PFAS, which have existing dose response data on liver weight (i.e., liver endpoint), pup body weight (i.e., developmental endpoint), and reduced serum thyroid hormone concentrations (i.e., thyroid endpoint) in rodent models for each compound. The difference in the two samples are the specific concentrations of each of the four PFAS and therefore the mixing ratios and the overall total mixture PFAS concentration. Dose responses for each chemical and each endpoint are modeled and effect-appropriate BMDs calculated for each compound (e.g., ED_5 , ED_{10} , ED_{20}). These values serve as the denominator values in Equation 4-5. The numerator values are the proportions of each component PFAS in the precise mixture. The total mixture BMD (t_{add}) is the inverse of the sum of the relative ratio divided by the BMD_X for each PFAS in the mixture. The total mixture BMD_X represents an equivalent BMD_X as each of the individual chemical effects that were used in the calculations (i.e., if the individual chemical data were ED_{10} values, the DA calculation derives an ED_{10} for the mixture of PFAS with that specific mixing ratio).

The total mixture BMD, which is in the same units as the component chemical BMDs (e.g., oral dose in a rodent study such as mg/kg/d), can then be adjusted based on user-defined extrapolation factors (e.g., dosimetric adjustment, RSC correction, 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). The derived “mixture-HBWC” can then be compared to the actual (measured) mixture concentration and if the actual mixture concentration exceeds the mixture-HBWC there is risk of the specific effect from exposure to that mixture at the measured concentrations.

In practice, the lowest mixture-specific endpoint indicates the most sensitive domain for the mixture and this endpoint can then be used for the derivation of an equivalent mixture-HBWC and estimation of risk. In the present example, for Water Sample 1 (Table 4-15) the liver specific domain produced the lowest mixture BMD (i.e., 0.094 mg/kg/d), representing the most sensitive effect domain. This mixture dose could then be used to derive a mixture-HBWC for comparison to the actual mixture concentration of the sample (i.e., 470 ng/L). If the mixture-HBWC is greater than 470 ng/L, in this instance, then there is potential risk of liver effects in the exposed population. For Water Sample 2 (Table 4-16), the different mixing ratio resulted in the

developmental domain having the lowest mixture BMD (0.0068 mg/kg/d) and this would be the mixture BMD used to calculate the mixture-HBWC and for comparison to the measured water concentration (70 ng/L).

Table 4-15. Mixture BMD Approach: Hypothetical Water Sample 1

	Measured Water Concentration (ng/L)	Mixing Ratio (Proportion)	Thyroid BMD (mg/kg/d)	Liver BMD (mg/kg/d)	Developmental BMD (mg/kg/d)
PFAS 1	10	0.02	0.24	0.044	0.01
PFAS 2	10	0.02	0.24	0.013	0.0051
PFAS 3	50	0.11	2.1	720	2.1
PFAS 4	400	0.85	70	0.1	0.7
Mixture Total	470	1.0			
DA Mixture BMD Calculation			4.16	0.094*	0.132

*The lowest mixture BMD is converted to a mixture-HBWC for comparison to the measured concentration (i.e., 470 ng/L).

Application of Equation 4-5 to the example water sample in Table 4-15 to derive the DA Mixture BMD. This example is for the liver domain as it was the lowest mixture BMD in this example.

$$t_{add} = \left(\sum_{i=1}^4 \frac{a_i}{BMD_i} \right)^{-1} = \left(\frac{0.02}{0.044} + \frac{0.02}{0.013} + \frac{0.11}{720} + \frac{0.85}{0.1} \right)^{-1} = 0.094 \text{ mg/kg/d}$$

Table 4-16. Mixture BMD Approach: Hypothetical Water Sample 2

	Measured Water Concentration (ng/L)	Mixing Ratio (Proportion)	Thyroid BMD (mg/kg/d)	Liver BMD (mg/kg/d)	Developmental BMD (mg/kg/d)
PFAS 1	5	0.07	0.24	0.044	0.01
PFAS 2	50	0.71	0.24	0.013	0.0051
PFAS 3	10	0.14	2.1	720	2.1
PFAS 4	5	0.07	70	0.1	0.7
Mixture Total	70	1.0			
DA Mixture BMD Calculation			0.299	0.017	0.0068*

*The lowest mixture BMD is converted to a mixture-HBWC for comparison to the measured concentration (i.e., 70 ng/L).

4.5.3 *Advantages and Challenges of the Mixture Benchmark Dose Approach*

There are several advantages to the Mixture 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 effect endpoints (i.e., BMDs or NOAELs) for each of the 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 mixture 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 combined mixture effect (mixture BMD) is different for each PFAS mixture depending on: (1) the specific PFAS in the mixture, (2) the mixing ratio, and (3) the effect endpoint being modeled. For example, one could expect that a mixture of PFAS that has a greater amount of a more potent compound and a lower amount of a less potent compound would have a lower (i.e., more potent) mixture BMD than a similar assortment of compounds that has less of the more potent PFAS and more of the less potent PFAS. It is also advantageous that the Mixture BMD approach does not actually require or assume that the component PFAS in a given mixture have congruent dose response curves for each effect being evaluated (reviewed in NAS (2008)). Finally, it is ideal to have well resolved dose response curves for each component PFAS in a mixture to estimate equivalent BMDs (e.g., ED₁₀); however, DA modeling is also amenable to simple point estimates such as NOAELs, as long as they are toxicologically similar across component chemicals (i.e., for same endpoint, such as increased liver weight).

There are also several challenges with the Mixture BMD approach. Like 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. For certain mixtures with less well-studied PFAS there may be limited or no available dose response data comparable to other PFAS in the mixture for calculating the mixture BMD. In that case, similar to the RPF approach, read-across or NAM data may be the only source of data for estimating an effect endpoint and with associated uncertainty. Another challenge is that the mixture BMD and subsequent mixture-HBWC is unique for each specific mixture based on PFAS assortment and ratios, which is conceptually different from the classical approach of a single value specific to a single chemical (e.g., an RfD for a specific compound). Further, it is assumed that the PFAS mixture composition is fairly constant, when in actuality, PFAS mixtures may change over time in environmental media. However, the calculation can readily be repeated for different mixing ratios and mixture concentrations once the effect endpoint values have been determined. Finally, for both the RPF and Mixture BMD approach, 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 liver effects are the most sensitive and would produce the lowest mixture BMD, but data are only available for the component PFAS to calculate mixture BMDs for developmental and thyroid effects).

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APPENDIX A: EPA Internal Report, Preliminary Results from a USEPA ORD Study: Cumulative Developmental Toxicity of Combined Exposure to Ammonium Perfluorooctanoic Acid (PFOA, CASRN: 3825-26-1) and Potassium Perfluorooctane Sulfonate (PFOS, CASRN: 2795-39-3) in the Sprague-Dawley Rat

INTERNAL REPORT

August 26, 2021

Cumulative Developmental Toxicity of Combined Exposure to Ammonium Perfluorooctanoic Acid (PFOA, CASRN: 3825-26-1) and Potassium Perfluorooctane Sulfonate (PFOS, CASRN: 2795-39-3) in the Sprague-Dawley Rat:

Preliminary Results from a USEPA ORD Study

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A.1 Executive Summary

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the two most commonly detected and studied PFAS found in environmental media, however experimental evidence of their combined toxicity is lacking in the literature. As part of our on-going research program and at the request of the USEPA Office of Water we performed a series of oral exposure experiments in pregnant Sprague-Dawley rats beginning with single chemical dose response evaluations of PFOA and PFOS, followed by a binary mixture study in which we repeated a dose response of PFOA but combined with a fixed dose of PFOS added to each PFOA dose. Preliminary results identified numerous significant adverse effects from each chemical individually and the mixture including reduced maternal gestational weight gain, reduced pup body weight, reduced pup viability, and increased maternal and pup liver weights. As a clear demonstration of cumulative mixture effects, individual exposures to 62.5 mg/kg PFOA and 2 mg/kg PFOS produced 12% and 8% post-implantation loss (PIL; a measure of fetal and pup mortality), respectively, while a combination of the two (62.5 mg/kg PFOA+2 mg/kg PFOS) produced 65% PIL. Further, by combining PFOS with PFOA, the dose response curves for PFOA across multiple effects such as pup bodyweight and maternal and pup liver weights were significantly shifted towards effects at lower doses meaning that because of the additive effect of PFOS, PFOA produced a similar effect but at a lower dose than PFOA alone. Study is on-going with multiple analyses still to be conducted on samples collected during the studies, but preliminary results clearly support additive effects of combined exposure to PFOA and PFOS during pregnancy in the Sprague-Dawley rat.

A.2 Background

The U.S. Environmental Protection Agency announced on March 3, 2021 the final decision to regulate perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) under the Safe Drinking Water Act. EPA is now working to establish a National Primary Drinking Water Regulation (NPDWR) and has up to two years from the final decision to propose health-based Maximum Contaminant Level Goals (MCLGs) and NPDWR. The current PFOA and PFOS Drinking Water Health Advisories are 70 ng/L each or 70 ng/L combined concentration of PFOA and PFOS because the reference doses were determined to both be based on critical effects that were developmental and thus should be considered cumulative. The Health Advisories were set at levels that EPA concluded would “not result in adverse developmental effects to fetuses during pregnancy or breastfed infants, who are the groups most sensitive to the potential harmful effects of PFOA and PFOS” (81 FR 33250).

The USEPA Office of Water Office of Science and Technology is currently updating and revising the existing Health Effects Support Documents (HESDs) for PFOA and PFOS. As part of this process, OST scientists identified a literature database deficiency pertaining to cumulative mixture effects associated with combined exposures to two or more PFAS, including a lack of *in vivo* studies directly investigating co-exposure to PFOA and PFOS. On April 14, 2021 OST formally requested additional research and assistance from ORD scientists to support the SDWA regulatory effort for PFOA and PFOS. Specifically, OST requested that ORD prioritize *in vivo* studies regarding the combined toxicity of PFOA and PFOS (and potentially other PFAS) by characterizing the maternal and fetal/postnatal effects of combined exposure to PFOA and PFOS during pregnancy in a rodent model. The study design was expected to assess a hypothesis of dose additivity to inform a combined toxicity approach for the PFOA and PFOS NPDWR.

Our research group in ORD/CPHEA/PHITD/RDTB has nearly two decades of laboratory research experience investigating the mixture-based developmental and reproductive effects of co-exposure to multiple compounds that perturb the androgen signaling pathway, notably multiple phthalates and pesticides. Further, our group has been studying the developmental toxicity of several emerging PFAS (e.g., hexafluoropropylene oxide dimer acid (HFPO-DA or GenX), Nafion byproduct 2 (NBP2), and perfluoromethoxyacetic acid (PFMOAA)) for the past three years and has already conducted a mixture-based experiment on the combined toxicity of HFPO-DA, NBP2, and PFOS (manuscript in preparation).

Here, at the request of OW/OST, we designed and conducted a series of three *in vivo* experiments designed to robustly assess the combined toxicity of PFOA and PFOS on multiple endpoints associated with the spectrum of PFAS adverse effects, specifically poor birth outcomes, liver effects, and thyroid effects from exposure during pregnancy. The in-life phases of the studies were conducted May-July 2021 and have been completed. This report describes the methods, study design, and preliminary findings on apical effects and evidence of combined toxicity. The studies are on-going with multiple sample analyses in-process (e.g., histopathology, clinical chemistry, tissue gene expression, thyroid hormone concentrations, analytical chemical determinations of test chemical in serum and tissues). Once complete, the studies will be published in one or more peer-reviewed journal articles (expected by FY23).

A.3 Methods

Dosing solutions were prepared in high performance liquid chromatography-grade water (Honeywell - Burdick&Jackson, CASRN: 7732-18-5, Cat. No. 356-4, Lot: BC128) containing 0.5% (by volume) Tween-80 (Sigma-Aldrich, CASRN: 9005-65-6, Cat. No.: P1754, Lot: BCCB5237). Pentadecafluorooctanoic acid ammonium salt (PFOA, CASRN: 3825-26-1, Cat. No. 77262, Lot: BCBW7054, Purity: 100%) and Heptadecafluorooctanesulfonic acid potassium salt (PFOS, CASRN: 2795-39-3, Cat. No. 77282, Lot: BCBX5798, Purity: >98%) were purchased from Sigma-Aldrich. Dosing was administered once daily via oral gavage at 2.5 mL/kg body weight from gestation day (GD) 8 to postnatal day (PND) 2 across the range of doses specified in Table 1. Dosing solutions are reported as nominal and not corrected for the conjugate cation.

Table 1. Study dosing design for in vivo studies of PFOA, PFOS, and combined PFOA+PFOS

Study 1 – PFOA dose response							
PFOA (mg/kg/d)	0	10	30	62.5	125	250	--
Study 2 – PFOS dose response							
PFOS (mg/kg/d)	0	0.1	0.3	1	2	5	--
Study 3 – Mixture study – PFOA dose response with PFOS fixed							
PFOA (mg/kg/d)	0	3	10	30	40	62.5	80
PFOS (mg/kg/d)	0	2	2	2	2	2	2

Time-mated Sprague-Dawley rats (CrI:CD(SD), 78 days old) were purchased from Charles River Laboratories (Raleigh, NC, USA) and shipped to USEPA (Research Triangle Park, NC, USA) on

GD 2 (GD0 = bred date; GD1 = plug positive date). Dams and their offspring were housed individually in clear polycarbonate cages (20 x 25 x 47 cm) with heat-treated, laboratory-grade pine shavings (Northeast Products, Warrensburg, NY) and fed NIH07 Rat Chow and filtered (5 μ m) municipal tap water (Durham, NC) ad libitum. Dams were weight-ranked and stratified then randomly assigned to treatment groups to produce similar mean weights and variances given the range of dam body weights. This study was conducted in accordance with a protocol approved by the USEPA Center for Public Health and Environmental Assessment Institutional Animal Care and Use Committee (ACUP 22-03-001). Animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and maintained at 20-22°C, 45-55% humidity, and a 12:12 h photoperiod (lights off 18:00 EDST).

Three blocks of 30 dams each were used to assess the neonatal effects of *in utero* and short-term postnatal exposure to PFOA, PFOS and combined PFOA+PFOS (as described in Table 1). Dams gave birth naturally and were checked for parturition hourly beginning at 6AM on the morning of GD22 (i.e., PND0) until all dams had delivered. Hourly checks on maternal and neonatal pup health continued until 5PM on PND1 and dead pups were removed and those that were moribund were removed and euthanized via decapitation. As dams delivered, we recorded the time the first pup was observed in a cage and then the assumed time of completion of delivery based on normal maternal behaviors (e.g., retrieving pups to the nest, licking/grooming of pups, hovering over nest to stimulate nursing). Upon assumed completion of delivery, all pups were removed, counted and whole litter weight was recorded. All pups were returned to their nest except for two randomly selected pups, which were euthanized via decapitation and pooled trunk blood was collected. One pup had the thoracic and abdominal cavities exposed and then the whole carcass was fixed in 10% formalin. Subsequently, livers from formalin fixed pup carcasses were removed and shipped to Experimental Pathology Laboratories, Inc. (Durham, NC) where they will be embedded, sectioned, stained with hematoxylin and eosin (H&E), and evaluated by a Diplomate of the American College of Veterinary Pathology. For the second euthanized pup we removed liver tissue for RNA extraction and gene expression by homogenizing in TRI reagent and a second liver sample was weighed and snap frozen in liquid nitrogen for glycogen assay.

Dams were weighed and dosed on the morning of PND2 and time of dosing was recorded. Dams were euthanized via decapitation and necropsied 2 – 6 hr after dosing and time of euthanasia was recorded. Euthanasia order was stratified such that the timing of necropsy was equally distributed across dose groups. Maternal trunk blood was collected for serum isolation, liver weight was recorded and subsamples of maternal liver were collected for RNA extraction, chemical determination, and fixation in 10% formalin for histopathology. Kidneys were weighed, section longitudinally and fixed in 10% formalin for histopathology. The uterus was removed and implantation sites were scored. All pups were sexed and weighed then all pups were euthanized via decapitation and trunk blood was collected for serum isolation, liver weight was recorded for 1 male and 1 female pup per litter, both livers were fixed in 10% formalin for histopathology (for Study 3 only). Maternal serum (PND2) will be analyzed for clinical chemistry parameters, thyroid hormone concentrations (free and total T3 and T4), test chemical determination via LC-MS, and multiplex metabolomics assays (Biocrates). Neonatal serum will be analyzed for clinical chemistry parameters and thyroid hormone concentrations (total T3 and T4).

All values are reported as mean \pm standard error of the mean (SEM) and all statistical comparisons were conducted at $\alpha=0.05$ significance level analysis of variance (ANOVA),

followed by pairwise comparison at $\alpha=0.05$ to determine differences of treatment compared to control for significant genes. Data were log₁₀-transformed and treatment effects were identified by ANOVA using the PROC GLM statement in SAS (v.9.4, SAS Institute, Cary, NC, USA) and pairwise comparison versus control was performed using the least squares means (LSMEANS) statement using the PDIF option. For all fetal and neonatal data, litter means were used as the statistical unit to account for the nested effects of individuals within litters. GraphPad Prism was used to generate all figures and to conduct sigmoidal dose-response curve analyses.

A.4 Preliminary Results

The individual dose response studies for PFOA and PFOS both produced statistically significant reduced maternal gestational weight gain, increased maternal and pup relative liver weight, increased post-implantation loss (i.e., reduced pup survival), reduced pup body weight, reduced pup liver glycogen content, and reduced maternal and pup serum total T3 and T4 concentrations. In the PFOA only experiment overt maternal toxicity occurred in some or all of the top two dose groups (125 and 250 mg/kg/d) and those animals were removed from the study.

The mixture experiment (Study 3) was based on dose response data from the individual chemical experiments with PFOA and PFOS (Studies 1 and 2, respectively). The design of PFOS fixed at 2 mg/kg/d combined with increasing doses of PFOA 3-80 mg/kg/d provided the greatest difference in dose addition and response addition model predictions for some endpoints. This design allows for direct comparison of the magnitude of effect at several single doses of PFOA (10, 30, and 62.5 mg/kg/d) and a single dose of PFOS (2 mg/kg/d) with the mixed doses (e.g., 62.5 mg/kg/d PFOA+2 mg/kg/d PFOS) across multiple endpoints. We also predicted that a fixed dose of PFOS mixed with increasing doses of PFOA would significantly shift the PFOA dose response curves towards effects at lower doses as compared to PFOA alone. Overall, this study design allows for multiple complementary approaches for statistically evaluating whether the two chemicals produced cumulative mixture adverse developmental effects.

Mixture toxicity was clearly demonstrated for the effect of post-implantation loss (PIL) (Figure 1). PFOA at 62.5 mg/kg/d alone produced $12.2\pm 7.2\%$ PIL, while PFOS at 2 mg/kg/d alone produced $8.2\pm 5.9\%$ PIL. In contrast, the mixture of 62.5 mg/kg PFOA plus 2 mg/kg PFOS produced $65.6\pm 15.0\%$ PIL, which was significantly greater than PFOA or PFOS alone at the same oral doses (Figure 1A). The full PIL dose response curves for PFOA plus 2 mg/kg/d PFOS and PFOA alone resulted in an ED₅₀ parameter that was significantly lower, by a factor of 2.2-fold (Figure 1B), for PFOA combined with PFOS (58.4 mg/kg) compared to PFOA alone (129.7 mg/kg/d).

As described above, the mixture study was designed in part based on the hypothesis that combined exposure to PFOA with a fixed amount of PFOS would shift the PFOA dose response curves towards effects at lower doses compared to PFOA alone. This was clearly demonstrated by the effects on pup bodyweight (Figure 2A), pup liver weight (Figure 2B), and maternal liver weight (Figure 2C). Measurements were normalized to vehicle controls and plotted as percent (%) of control then fit with linear or nonlinear models providing the best fit of the observed data. For all three endpoints the co-exposure of 2 mg/kg/d PFOS with varying doses of PFOA significantly shifted the data and best fit lines towards lower x-axis doses resulting in similar dose response trend lines but at significantly lower oral doses of PFOA due to the combined effects with PFOS.

A.5 Conclusion

These preliminary results provide robust evidence of combined toxicity of PFOA and PFOS on multiple developmental endpoints. Studies 1 and 2 (PFOA alone and PFOS alone) demonstrated that both PFOA and PFOS independently produce numerous similar developmental adverse outcomes including increased post-implantation loss, reduced pup bodyweight, and increased pup and maternal liver weight. Study 3 demonstrated that co-exposure to PFOA and PFOS produced cumulative mixture effects that are at least dose additive for most endpoints and support the combined toxicity of these compounds.

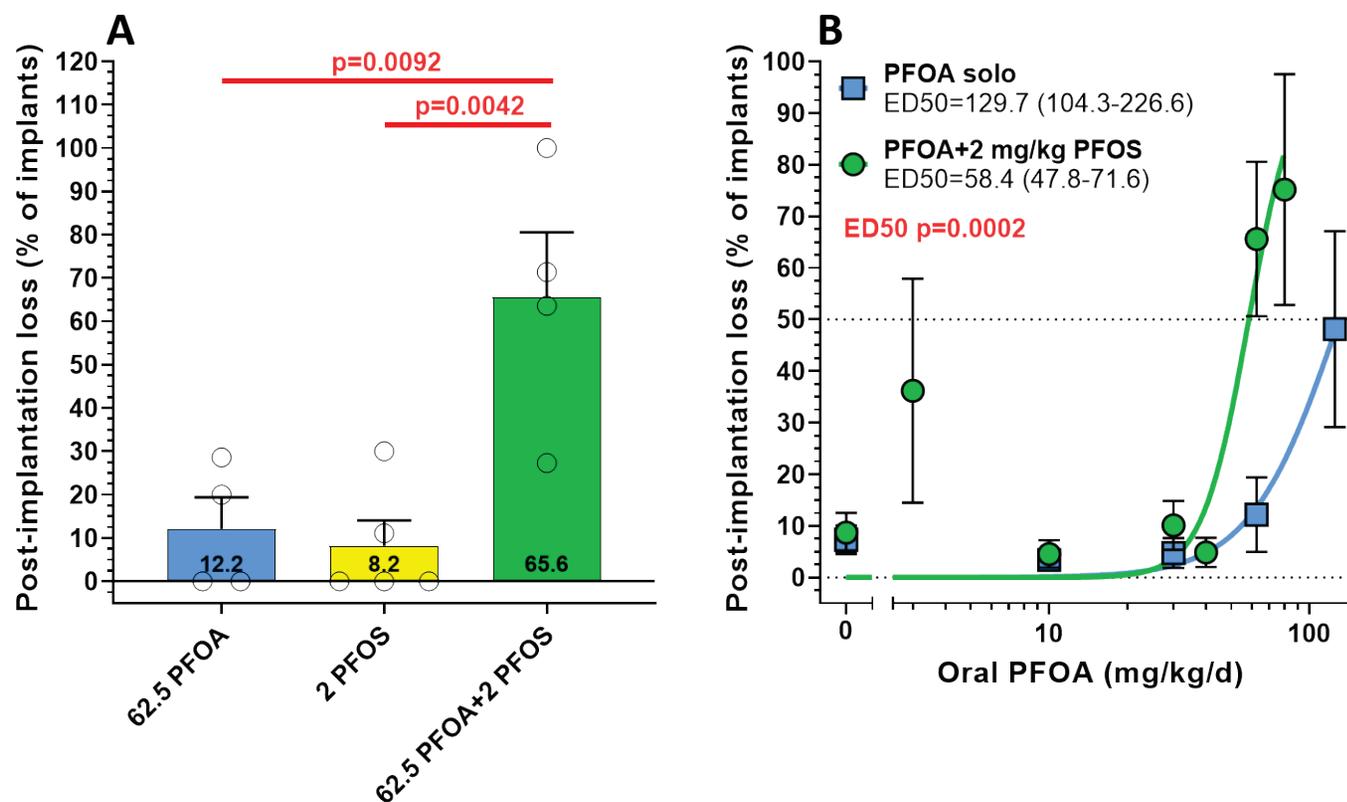


Figure 1. (A) Post-implantation loss from GD8-PND2 oral maternal exposure to PFOA alone (62.5 mg/kg/d) or PFOS alone (2 mg/kg/d) compared to the mixture of 62.5 mg/kg/d PFOA plus 2 mg/kg/d PFOS. The mixture exposure produced a response that was significantly greater than PFOA alone ($p=0.0092$) or PFOS alone ($p=0.0042$). (B) Post-implantation loss dose response curves for PFOA alone (blue points and line) compared to PFOA combined with 2 mg/kg PFOS (green points and line). The dose response effective dose 50% (ED50) value for PFOA alone (129.7 mg/kg/d, 95% CI 104.3-226.6 mg/kg/d) was significantly greater ($p=0.0002$) than PFOA combined with PFOS (58.4 mg/kg/d, 95% CI 47.8-71.6 mg/kg/d), representing greater potency of PFOA when combined with PFOS.

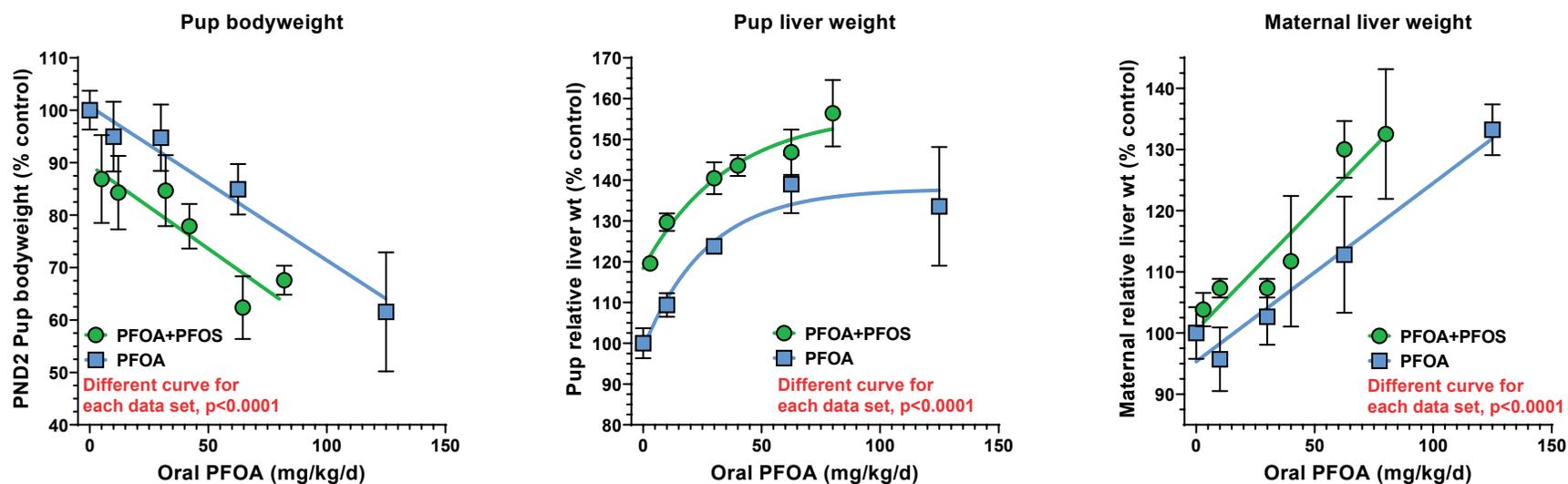


Figure 2. Comparisons of best-fit regressions between PFOA only exposure (blue points and lines) and PFOA combined with 2 mg/kg PFOS (green points and lines) for PND2 pup bodyweight (A), PND2 pup relative liver weight (B), and PND2 maternal relative liver weight (C) normalized to respective controls. For all endpoints the best fit regressions were highly significantly different ($p < 0.0001$) with the combined exposure shifted towards effects at lower doses than when PFOA exposure occurred alone, demonstrating combined toxicity of PFOA and PFOS for these endpoints.

Attachment 36

Per- and Polyfluoroalkyl Substances

Informational Links

[Chemicals Dashboard](#)

[Analytical Method](#)

Contaminant Navigation

[Overview](#)

[Treatment
Processes](#)

[Properties](#)

[Fate and Transport](#)

[References](#)

Treatment Processes

The following processes were found to be effective for the removal of PFASs: granular activated carbon (GAC) (up to > 99 percent), membrane separation (up to > 99 percent), and ion exchange (up to > 99 percent). Various types of novel adsorptive media have also been found to effectively remove PFASs (up to > 99 percent removal), but results for these media published to date have been limited to batch tests at bench scale. These results cover the removal of specific PFASs including PFTriA, PFDoA, PFUnA, PFDA, PFNA, PFHpA, PFHxA, PFPeA, PFBA, PFDS, PFNS, PFHpS, PFHxS, PFPeS, PFBS, PFPrS, PFOSA, PFHxSA, PFBSA, PFMOBA, PFMOPrA, PFMOAA, PFO4DA, PFO3OA, PFO2HxA, FtS 8:2, FtS 6:2, FtS 4:2, N-EtFOSAA, N-MeFOSAA, ADONA, PFECHS, F35-B, Nafion BP2, and GenX. For results on the removal of PFOS and PFOA, see the separate treatability database entries for those specific contaminants.

Studies were identified evaluating the following treatment technologies for the removal of PFASs:

Adsorptive Media

Bench-scale studies tested adsorption of PFASs using novel media including: magnetic nanoparticles with different polymer coatings [2535], functionalized and hybrid hydrogel sorbents [2617, 2606], swellable modified silica with an entrapped cation...

[See more](#)

Aeration and Air Stripping

At a full-scale site, packed tower aeration was not effective for removing PFASs [2441].

[See more](#)

Biological Filtration

A full-scale study [2175] of a plant treating reclaimed domestic wastewater found biological filtration to be ineffective for removing per- and polyfluoroalkyl substances.

[See more](#)

Biological Treatment

One bench-scale study [2161] examined removal of various PFAS using supernatant from a domestic wastewater activated sludge process as a microbial source in both aerobic and anaerobic test conditions. Although decreases in some of the PF...

[See more](#)

Chlorine

Chlorination was ineffective for removing PFASs at full-scale sites [2441, 2619].

[See more](#)

Chlorine Dioxide

Chlorine dioxide was ineffective for removing per- and polyfluoroalkyl substances at a full-scale site [2441].

[See more](#)

Conventional Treatment

Sampling at full scale [2174, 2175, 2441, 2508, 2518, 2619] and pilot [2518] drinking water treatment facilities observed either no removal or inconsistent removal of per- and polyfluoroalkyl substances by conventional treatment.

[See more](#)

GAC Isotherm

Isotherm data are available for adsorption onto various types of granular activated carbon and onto other media, powdered activated carbon [2637], including anion exchange resin [2426, 2611, 2621, 2637] and novel adsorbents [2522].

[See more](#)

Granular Activated Carbon

Removal of per- and polyfluoroalkyl substances (PFASs) by granular activated carbon (GAC) has been examined in a number of bench and pilot studies and in full scale application. For some of these studies, the primary focus was optimizing the remov...

[See more](#)

Hydrogen Peroxide

[See more](#)

Ion Exchange

Removal of per- and polyfluoroalkyl substances (PFASs) using anion exchange resins can be effective. Bench- and pilot-scale studies found removals up to greater than 99 percent [2427, 2503, 2504, 2515, 2523, 2534, 2535, 2538, 2559, 2560, 2563...]

[See more](#)

Membrane Filtration

A single bench scale study [2524] observed moderate to high (69 to 84 percent) removal of perfluorohexanoic acid (PFHxA) from spiked lab water by ultrafiltration membranes. Sampling at full scale [2175, 2441] drinking water treatment facilities, h...

[See more](#)

Membrane Separation

Removal of per- and polyfluoroalkyl substances (PFASs) from water using membrane separation was found to be quite effective. Bench [2423, 2514, 2524, 2530, 2547], pilot [2569, 2571, 2573], and full-scale [2175, 2424, 2428, 2441] studies ...

[See more](#)

Other Treatment

Other processes that have been evaluated for the treatment of PFASs in groundwater or at environmentally relevant concentrations (e.g., 1 milligram per liter or less) include electrochemical treatment [2630], electrocoagulation [2608], and plasm...

[See more](#)

Ozone

Several full-scale studies [2174, 2175, 2441, 2508, 2509, 2518] found conventional ozonation ineffective for removal of per- and polyfluoroalkyl substances (PFAS). A demonstration study of a patented ozofractionation process, which used ozone gas ...

[See more](#)

Ozone and Hydrogen Peroxide

A bench-scale study [2635] using ozone, followed by increasing pH to 11, followed by hydrogen peroxide addition consistently achieved reductions (14 percent to greater than 92 percent) of PFASs including perfluorobutanoic acid (PFBA), perfluoronon...

[See more](#)

Powdered Activated Carbon

Bench [2158, 2521, 2542, 2544] and pilot scale [2518] tests have shown that PAC can be effective for removal of some per- and polyfluoroalkyl substances. Removal depends on factors including PAC dosage, PAC particle size, contact time, and in...

[See more](#)

Precipitative Softening

[See more](#)

Ultraviolet Irradiation

A full-scale study showed ultraviolet irradiation to be ineffective for removing most of the PFASs sampled. Partial removals (32 to 56 percent) were observed in a few samples, however, for perfluorohexylsulfonate (PFHxS) and fluorotelome...

[See more](#)

Ultraviolet Irradiation and Hydrogen Peroxide

In a full-scale study, advanced oxidation with ultraviolet irradiation and hydrogen peroxide was ineffective for removing any of the PFASs sampled [2441].

[See more](#)

LAST UPDATED ON {MONTH DAY, YYYY}

Attachment 37



Spokane: (509) 838-3999

Moscow: (208) 883-2839

UCMR5 & PFAS Testing – PFCs/PFOS/PFOA:

Anatek Labs is approved for PFAS analysis by EPA Methods 533 and 537.1 for Fifth Unregulated Contaminant Monitoring Rule (UCMR5).

Anatek Labs is accredited by the [Florida Department of Health \(NELAP\)](#), [Washington Department of Ecology](#), [Oregon Environmental Lab Accreditation Program \(ORELAP\)](#), and [New Mexico Environmental Department](#) to perform testing for PFOA, PFOS, and other perfluorinated compounds.

Anatek Labs was also [approved by the EPA for PFAS/PFC analysis](#) during the Third Unregulated Contaminant Monitoring Rule (UCMR3) survey of public water systems.

Perfluorinated compounds have been found in ground water and wells near airbases and other sites where fire-retardant foam (also called aqueous film-forming foam) has been used in the past. If you would like to test your drinking water for PFOA, PFOS, or other perfluorinated compounds, please contact either our [Spokane](#) or [Moscow](#) offices for more information on sample bottles, sample collection procedures, and analysis. Specific sampling instructions are available – select [PFAS Sampling Instructions](#).

Pricing:

- EPA 533 – 25 perfluorinated compounds, including PFOS and PFOA: \$375/sample

PFAS/PFC analyte lists for each method are available here: [PFAS Analytes](#)

Call the laboratory for quotes on large or long-term monitoring projects, or if your regulatory requirements call for EPA 537.1 (18 PFAS compounds).



Everest Project

Anatek Labs has performed PFAS analysis for a project that collected snow and meltwater samples from Mt. Everest. This study found significant levels of PFAS contamination on the world's tallest peak.

Read more in articles from [GQ](#) and [Science Direct](#).

Other Links:

- These [EPA links](#) provide more information about PFOA, PFOS, and other perfluorinated compounds (also known as perfluoroalkyl substances (PFASs)).
- [Basic Information](#) (Health Effects listed at the bottom of the linked page) C

- [Click here to watch a CBS News report about PFCs at Peterson Air Force Base in Colorado.](#)
- [Click here to watch an NBC News report about PFAS contamination near military bases in Pennsylvania.](#)



ANATEK LABS

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Hours:

Monday - Friday: 8AM - 5PM

Quick Dial:

Moscow: (208) 883-2839

Spokane: (509) 838-3999

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[Quality Assurance](#)
[State Certifications](#)
[About Us](#)
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Attachment 38

CLINICAL
TESTING

ABOUT
WSLH

WSLH
SERVICES

LAB NETWORKS
& SURVEILLANCE

RESEARCH
SUPPORT CENTER

NEWS
& PUBLICATIONS

PAY
YOUR BILL

WATER & OTHER
ENVIRONMENTAL TESTING

OCCUPATIONAL HEALTH
& SAFETY SERVICES

FORENSIC
TOXICOLOGY

PROFICIENCY
TESTING

Environmental Testing

Water Testing Info
Environmental Toxicology
Flow Cytometry
Inorganic Chemistry
Mold for Homeowners
Organic Chemistry
Radiochemistry
Trace Elements Lab
Water Microbiology
Educational Resources
WI Emergency Response
Drinking Water Collection Kits
COVID-19 wastewater

PFAS

PFAS in Drinking Water Testing

The detection of PFAS and associated emerging contaminants in drinking water is a new and rapidly evolving field. Information on this page is subject to change without notice.

For questions about PFAS testing, please call us 608-262-3750 or email us at WSLHPFAS@slh.wisc.edu

Test Options and Pricing

- **EPA 537.1** – 18 compounds – Total Cost: \$420
 - Sample Cost – \$300
 - Field Reagent Blank (if required) – \$120
- **PFAS in Water** – 33 compounds (includes all 18 compounds in EPA 537.1) – Total Cost: \$585
 - Sample Cost – \$330
 - Field Reagent Blank (if required) – \$255

Sampling Instruction Video

PFAS Sampling video - Wisconsin State Laboratory of Hygi...



FAQs

What's the difference between the two tests?

- **EPA 537.1** includes 18 compounds. It is a drinking water-specific test method developed and published by the US Environmental Protection Agency (EPA).

- **PFAS in Water** includes 33 compounds. It is a test method developed and validated by WSLH scientists. The Wisconsin Department of Natural Resources (DNR) Laboratory Certification Program has granted the WSLH certification in the aqueous matrix for 33 compounds extracted and analyzed by this test method.

Which test is recommended?

You can choose either test. DNR and the Wisconsin Department of Health Services (DHS) recommend the PFAS in Water test because it includes more compounds.

What's the difference between PFAS, PFOS and PFOA?

PFAS stands for per- and polyfluoroalkyl substances. PFAS are a group of synthetic chemicals used in a wide array of consumer and industrial products since the 1950s. Perfluorooctane sulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) are two types of PFAS. You can learn more about PFAS [here](#).

Are there health-based standards for these compounds?

There are currently no federal drinking water standards for PFAS. The DNR is currently undergoing rule-making to adopt groundwater and drinking water standards for some PFAS. The recommended standards can be found [here](#).

How do I request a test kit?

Please call us at 608-262-3750 (Monday – Friday, 7:45 AM – 4:30 PM or leave a voicemail after hours) or email us at WSLHPFAS@slh.wisc.edu.

What is the current turnaround time for test results?

It is approximately 1 month. You can call 608-262-3750 or email WSLHPFAS@slh.wisc.edu to get an estimate for your situation.

Why is the turnaround time so long?

This is a very labor intensive test. When the lab is not running at capacity, the turnaround time is approximately 1 month.

Will I be contacted before my kit is shipped?

Yes, we will reach out to you the week before we ship your kit to make sure you are ready to receive it, answer any questions you may have, and confirm you still want to receive the kit.

I have my kit, when should I collect my sample?

Each kit is shipped with a Test Request form to fill out. There are certain sections of the form highlighted in yellow, including the bottom right portion where a sample collection date range is listed. You **MUST** collect your sample and return it to the lab during that sample collection date range. If you fall outside that date range, you may be asked to resample at a later date.

How will I get my results?

Results will be sent to the email address you provide when setting up your account. If you do not provide an email address, your results will be sent via US Postal Service.

How/when do I pay for my test?

An invoice will be sent through US Postal Service to the address you provide when setting up your account. This occurs around the 5th of the month after you have received your results.

I have my results, what do they mean?

The lab does not interpret results. If you have questions about what your test results mean for your health, please contact DHS at DHSEnvHealth@dhs.wisconsin.gov and one of their experts will get in touch with you.

I have other questions about PFAS, who can I contact for more information?

DNR PFAS contacts by subject area can be found [here](#).