Group A Public Water Supplies • Chapter 246-290 WAC

Recommended State Action Levels for Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water: Approach, Methods, and Supporting Information

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Glossary

ADD	Acceptable Daily Dose. An estimated maximum daily dose of a chemical (in mg/kg-day) that can be consumed by humans for an entire lifetime without adverse effects. Health protective value derived by CA OEHHA.
ATSDR	Agency for Toxic Substances and Disease Registry atsdr.cdc.gov/pfas/index
BMD	Benchmark Dose modeled on the dose-response data from one or more studies. A BMD_5 is a modelled estimate of a 5 percent change in the effect.
BMDL	Benchmark Dose Lower Bound is the lower bound of the 95 percent confidence interval in benchmark dose modelling.
CA OEHHA	The California Environmental Protection Agency's Office of Environmental Health Hazard Assessment.
Critical Effect	The most sensitive adverse effect from human clinical or epidemiological studies or the most sensitive outcome in animal studies deemed relevant to adverse outcomes in humans.
Critical Study	The study that best identifies the lowest dose at which these effects first occur or the no observable effect level.
EPA	U.S. Environmental Protection Agency
LOD	Limit of detection for laboratory analysis.
LOAEL	Lowest Observed Adverse Effect Level is the lowest administered dose in an experiment with an observed adverse effect.
LOEL	Lowest Observed Effect Level is the lowest administered dose in an experiment with an observed effect, including effects that are not clearly adverse.
MDH	Minnesota Department of Health
MDHHS	Michigan Department of Health and Human Services
MCL	Maximum Contaminant Level is the highest concentration of a regulated contaminant allowed in drinking water by the Safe Drinking Water Act. An MCL is a legally enforceable standard that applies to public water systems. It is set as close to the MCLG as feasible.
MCLG	Maximum Contaminant Level Goal is a concentration in drinking water generally considered safe under the Safe Drinking Water Act. The MCLG of carcinogens is generally set at zero. The MCLG of non-cancer effects are

	calculated by dividing the RfD by an upper-bound drinking water intake rate and multiplying by a relative source contribution.
mg/kg-day	Milligrams of chemical per kilogram body weight per day.
m/L	Milligrams of chemical per liter. Equivalent to parts per million (or ppm).
MRL	Minimal Risk Level is an estimate of the amount of a chemical a person can eat, drink, or breathe each day without a detectable risk to health. MRLs are developed for non-cancer endpoints.
MSWG	Michigan Science Advisory Workgroup. An appointed group of toxicology, epidemiology and risk assessment experts that recommended health-based drinking water values for PFAS in Michigan.
NTP	National Toxicology Program
NHDES	New Hampshire Department of Environmental Services
ng/kg-day	Nanograms of chemical per kilogram body weight per day.
ng/L	Nanograms of chemical per liter. Equivalent to parts per trillion (or ppt).
NJ DWQI	New Jersey Drinking Water Quality Institute, the scientific body that conducted the PFAS risk assessment and developed recommendations for drinking water standards for the State of New Jersey Department of Environmental Protection.
NOAEL	No Observed Adverse Effect Level is the highest administered dose in an experiment with no observed adverse effects.
PFBS	Perfluorobutane sulfonic acid (anion: perfluorobutane sulfonate)
PFHxS	Perfluorohexane sulfonic acid (anion: perfluorohexane sulfonate)
PFOA	Perfluorooctanoic acid also known as C8 (anion: perfluorooctanoate)
PFOS	Perfluorooctane sulfonic acid (anion: perfluorooctane sulfonate)
PFNA	Perfluorononanoic acid (anion: perfluorononanoate)
POD	Point of Departure. The NOAEL, LOAEL or benchmark dose that defines the minimal or no effect level in animals in the critical study. For PFBS, this was a dose in mg/kg-day. For the other PFAS, it was a serum level in mg/L.
PPAR	Peroxisome Proliferator-Activated Receptors are nuclear receptors that regulate many genes. There are several subtypes including alpha (α) and gamma (γ).
RfD	An Oral Reference Dose is an estimate of a daily oral intake not anticipated to cause adverse health effects over a lifetime (including in sensitive subgroups). RfDs are developed for non-cancer endpoints.

RSC	The Relative Source Contribution is the proportion of the RfD allocated to come from drinking water sources under the Safe Drinking Water Act.
Steady state	PFAS risk assessors use predictive models to estimate average serum levels in a population with PFAS in their daily drinking water. These models predict that in adults exposed daily over many years, serum levels will rise until the serum level reaches a plateau. This plateau is called steady state. See figure 3.
Target serum	The serum level (internal dose) at the RfD in µg/L. For PFOA, PFOS, PFNA and PFHxS this equals the serum concentration in rodents from the critical study at the point of departure divided by the uncertainty factors. This is not a clinical or diagnostic value and should not be interpreted as such.
T4	Thyroxine is a hormone the thyroid gland produces and releases into the blood. It converts to T3 in cells. Most circulating T4 is bound to transport proteins. The small fraction of unbound and biologically active T4 is called free T4 (fT4). The sum of bound and unbound T4 is called total T4 or tT4.
T3	Triiodothyronine is a thyroid hormone three to five times more active than T4. It stimulates metabolism and is critical to growth and differentiation of cells and tissues. T3 measurements may target the fraction of free fT3 or the total of bound and free T3 (tT3).
TSH	Thyroid-stimulating hormone is a hormone produced in the pituitary gland that stimulates the thyroid gland to produce T4.
TWA	Time-Weighted Average is the average concentration of a substance over a specified amount of time.
µ/L	Micrograms of chemical per liter. Equivalent to parts per billion (or ppb).

Summary

In October 2017, the State Board of Health (board) accepted a petition from ten organizations to establish drinking water standards for per- and polyfluoroalkyl substances (PFAS). Board authority to adopt such standards comes under RCW 43.20.050(2), RCW 70.119.080(1), and RCW 70.142.010.

To support the board, the Washington Department of Health (department) released draft state action levels (SALs) for five PFAS in November 2019. These PFAS occur in Washington drinking water and had sufficient scientific information to recommend a value. We presented the draft rule language at stakeholder workshops and at numerous meetings with stakeholders. After evaluating the feedback from these events and from two public comment periods, we updated our technical document and lowered our recommendation for the PFBS SAL from 1,300 to 345 ng/L to better protect infants. We also revised the PFNA SAL from 14 to 9 ng/L based on new evidence of serum half-life in humans. The PFHxS SAL was revised slightly to correct our calculation of average maternal body weight used in the infant exposure model. The revised SAL values are part of the proposed rule being considered for adoption by the State Board of Health in 2021.

The recommended SALs for PFOA, PFOS, PFNA, PFHxS, and PFBS are shown in Table 1. The department developed these recommended values after evaluating primary scientific literature on PFAS and reviewing health protective values in recent toxicological assessments by U.S. federal and state agencies. The health protective values we selected are based on immune, developmental and thyroid hormone effects observed in toxicity testing in laboratory animals. While epidemiological data were not used quantitatively to derive these values, they were considered as part of the evidence base.

The SALs were calculated like a maximum contaminant level goal (MCLG) under the Safe Drinking Water Act. They assume that 20-50 percent of the daily acceptable exposure can come from a drinking water source. Because four of these PFAS are highly bioaccumulative, we used a model to estimate accumulated exposure over many years of drinking water consumption. Specifically, we used a model developed by the Minnesota Department of Health, to estimate age-specific exposure to PFOS, PFOA, PFNA, and PFHxS when they occur in community drinking water. This model accounts for maternal transfer of PFAS to the growing child at the time of birth and during breastfeeding.

The SALs represent the maximum level in tap water that we consider to be without health concern for long-term consumption in daily drinking water. The SALs were developed to specifically protect early life stages of development (fetal, infancy) because these periods of rapid development are potentially more susceptible to adverse effects of these PFAS, and infants have relatively high exposure to contaminants in drinking water. Acting at these levels is consistent with the mission of providing safe and reliable drinking water and consistent with the SDWA.

Table 1 Recommended State Action Levels (SALs) for per- and polyfluoroalkyl substances (PFAS) in Drinking Water

Individual PFAS	State Action Level	CAS# for Test Analyte
PFOA	10 ng/L	335-67-1
PFOS	15 ng/L	1763-23-1, as acid
PFNA	9 ng/L	375-95-1
PFHxS	65 ng/L	355-46-4, as acid
PFBS	345 ng/L	375-73-5, as acid

PFAS are frequently detected as mixtures in drinking water. We did not identify sufficient toxicological information to provide science-based health protective values for mixtures of PFAS. PFAS mixtures detected in Washington drinking water to date have nearly always included one of the five PFAS with a SAL. As such they should serve as indicators of drinking water impacted by a PFAS source. When water systems employ mitigation to remove PFAS from a water source, we encourage them to employ broad approaches that effectively remove many PFAS. Combining a science-based level for state action with a broad management approach to PFAS mitigation provides a reasonable interim approach to protect the public from PFAS mixtures in drinking water.

Ultimately, a more comprehensive, grouped approach to regulation is preferred to a chemical-by-chemical approach given the large size of the PFAS class of chemicals and the frequent detections of PFAS mixtures in environmental media, food, and drinking water. As science advances, PFAS could be grouped in subclasses based on key characteristics, such as chemical structure, bioavailability, bioaccumulation potential, toxicity, or mechanism of action. We use this type of grouped approach to regulate other complex mixtures, such as PCBs, dioxins, PAHs and total petroleum hydrocarbons. We will continue to monitor progress and consider adopting a broader grouped approach to regulating PFAS mixtures as the science and methodology evolve.

Background

PFAS impact drinking water supplies in several areas of our state.

Limited testing in our state shows PFAS contamination in drinking water supplies in the areas shown in Figure 1. In several of these areas, the levels of PFOA+PFOS exceeded the current EPA health advisory level of 70 ng/L in drinking water. In response, public water systems and the military have taken voluntary action to provide alternate drinking water or bring concentrations in tap water below the federal advisory level.[1] Two sites in central Washington have detected PFAS in groundwater monitoring wells not drinking water wells. The State Board of Health rule

under consideration would expand testing of public water systems and may uncover additional impacted areas.

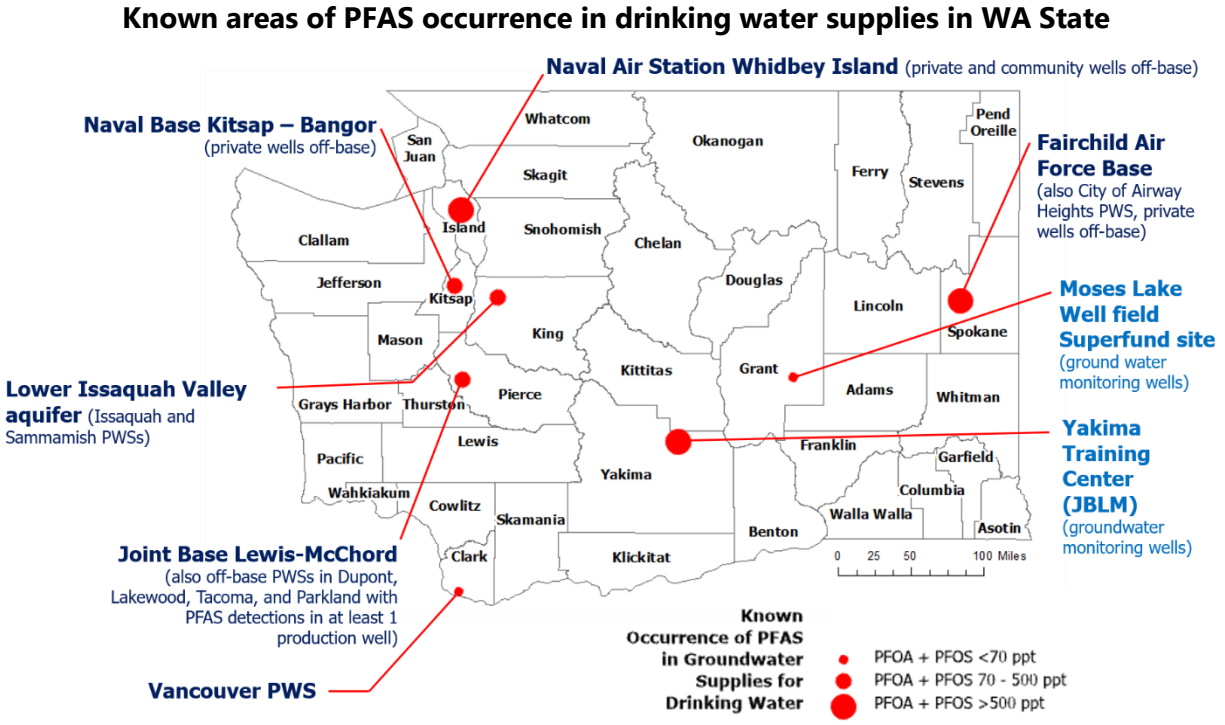

***Figure 1**: Known areas of PFAS occurrence in drinking water supplies in WA State, as of July 2021. The size of the dot indicates the publicly reported concentration of PFOS + PFOA. Other PFAS are typically also present. The Moses Lake and Yakima sites involve groundwater but not necessarily drinking water. PWS = Public Water System. Sources of data include the third unregulated contaminant monitoring rule (UCMR3) that sampled mostly larger public water systems in 2013-2015 across the nation, and publicly available results from voluntary testing by the military and public water systems collected from 2016-2021.[1]*

The primary source of PFAS suspected at the above Washington sites is a type of firefighting foam called aqueous film-forming foam, or AFFF.[1] Nationwide, other major sources of PFAS contamination in drinking water include: discharge from fluoropolymer manufacturing plants, industrial paper and textile manufacturing sites, chrome plating operations, land-applied biosolids derived from industrial PFAS waste streams; and landfills that accepted industrial PFAS waste.[2, 3]

PFAS are highly persistent in the environment.

Perfluoroalkyl acids (PFAAs), such as PFOS, PFOA, PFNA, PFHxS, and PFBS, have no known natural degradation pathways and thus persist in the environment and in groundwater.[3] Other PFAS compounds can break down partly in the environment to form persistent PFAS.[3]

Some PFAS are highly bioaccumulative in people.

The human body absorbs some PFAS much faster than it excretes them.[4] As a result, they accumulate in human blood serum, liver, lung, bone, and other locations in the body.[5, 6] These PFAS can pass from mothers to their babies in the womb.[7] They can also pass into breastmilk.[8] PFOS, PFOA, PFNA, and PFHxS are highly bioaccumulative in humans. PFBS is more rapidly excreted, but several studies indicate that when PFBS is in daily drinking water it increases serum levels of PFBS in consumers.[9-11]

Most people tested have detectable levels of four PFAS in their blood serum.

In national surveys, the U.S. Centers of Disease Control and Prevention (CDC) found that nearly all people tested had detectable levels of PFOS, PFOA, PFNA, and PFHxS in their blood serum. In contrast, PFBS was detected in serum of less than 1 percent of older children and adults and approximately 9 percent of children six to eleven years old.[12] Figure 1 shows average U.S. serum levels of four PFAS over time. More information is available in CDC's PFAS biomonitoring factsheet.

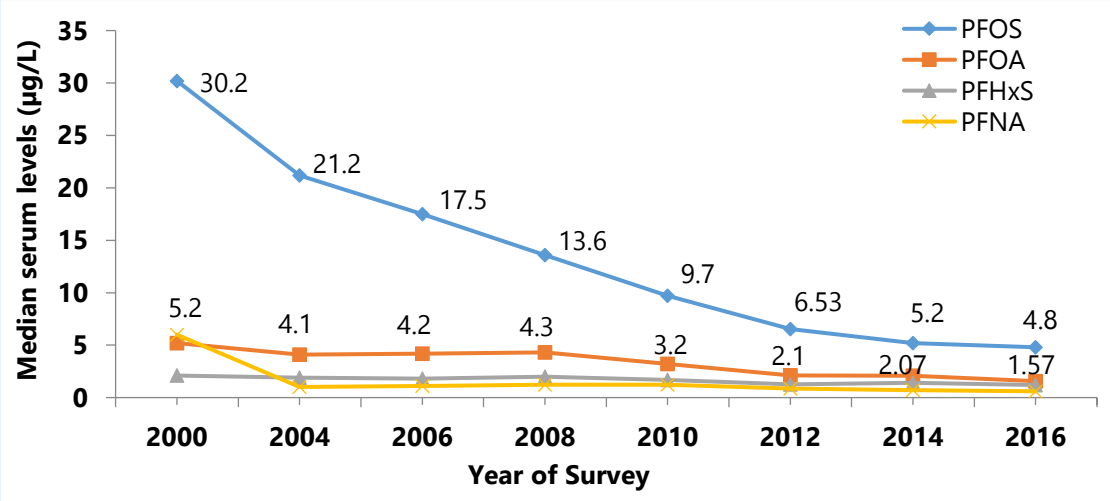

Figure 2: *Time trend of median serum levels of four PFAS in representative samples of U.S. residents >12 years old. Source: CDC National Health and Nutrition Examination Survey (NHANES).*[13, 14]

PFOS, PFOA, PFHxS, and PFNA have been largely phased out in the USA.

Over the last twenty years, major U.S. industries phased out a number of highly bioaccumulative PFAS from production and most uses.[15] These PFAS continue to be produced in other countries and may occur in imported materials and products.[15] Serum levels of these PFAS, especially PFOS, declined over time in the U.S. population following phase-out (Figure 2). Other PFAS have taken their place. For example, PFBS replaced some uses of PFOS.

PFAS in drinking water can contribute significantly to consumer exposure.

A number of studies show that PFAS in drinking water can contribute significantly to human exposure.[9, 16-18] For example, after high levels of PFAS were discovered and removed from a

community drinking water system in Airway Heights, WA, an ATSDR exposure study showed that residents had average blood levels of some PFAS that were much higher than national norms.[19]

People without PFAS in their drinking water are most likely exposed through their diet, certain consumer products, certain occupations, indoor dust, and air.[20-23]

PFAS can stay in the body long after exposure stops.

Estimates of average PFAS half-lives in human serum from different study populations are listed below. A half-life is the time it takes for the serum concentration of a PFAS to drop by half after exposure stops (e.g., after occupational exposure stops or drinking water contamination is mitigated).

- PFOA: 2.3 to 3.9 years[17]
- PFOS: 3.3 to 4.6 years[17, 24]
- PFNA: 2.5 to 4.3 years[25, 26]
- PFHxS: 5.3 to 7.1 years[17]
- PFBS: 27 to 44 days[27, 28]

It can take a while for low levels of PFAS in drinking water to accumulate in serum.

PFAS risk assessors use predictive models to estimate average serum levels in a population with PFAS in their daily drinking water. These models predict that in adults, serum levels increase gradually over a period of years until the serum level reaches a plateau (a condition models call steady state). Figure 3 shows an example of predicted serum level of PFOA in adults consuming drinking water with PFOA at 10 ng/L.

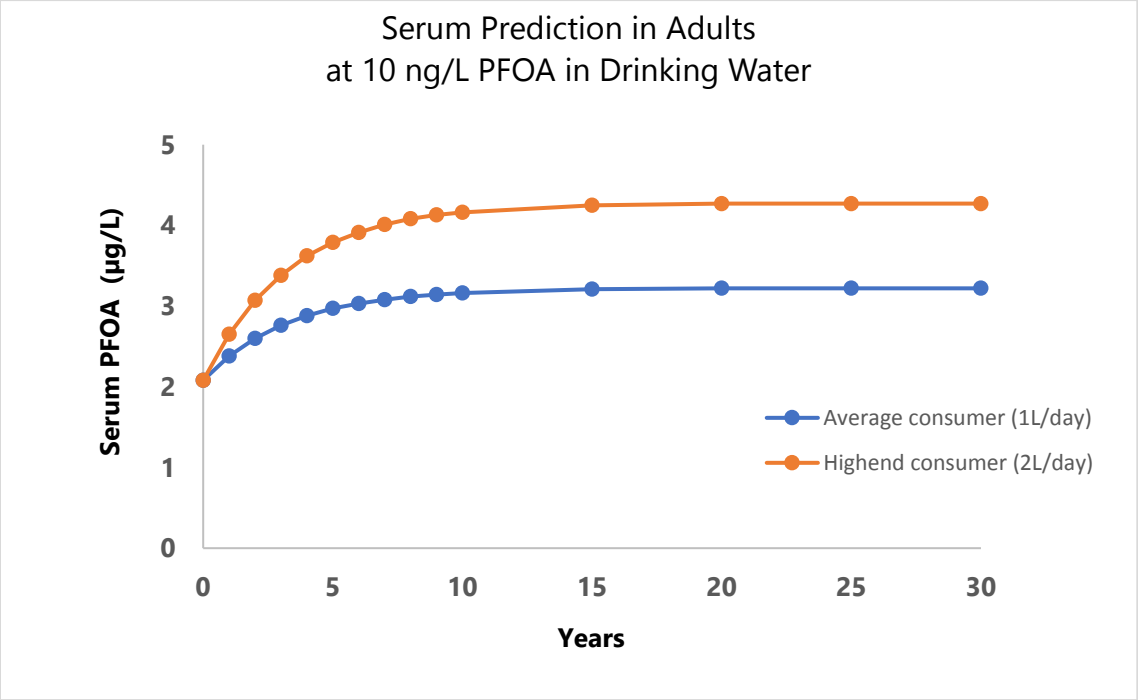

Figure 3 *Predicted average PFOA serum levels in adults with PFOA in residential drinking water at 10 ng/L using the Bartell model.[29] The graph, based on an average starting PFOA serum level of 2.0 µg/L, represents average background levels of PFOA in serum in the U.S. population. It plots two scenarios of adult drinking water intake: an average drinking water ingestion rate of one liter per day and a high-end consumer drinking two liters per day.*

Infants have higher exposure than adults to PFAS in drinking water.
Infants consume more drinking water and other fluids per pound of body weight than adults.[30] This means that infants who drink contaminated tap water or formula mixed with that tap water ingest more contaminant per pound of body weight than adults sharing the same drinking water.

In addition, mothers with PFAS in their daily drinking water pass some of the PFAS they absorb on to their babies during pregnancy and through breastfeeding.[31] PFOS, PFOA, PFHxS, and PFNA occur in breast milk at concentrations that are 1–12 percent of the mother's serum concentration.[4] At birth, these PFAS levels in infant serum are generally lower than or similar to their mothers, but can increase sharply during periods of exclusive breastfeeding.[32-34]

The SALs support public health recommendations for breastfeeding.
We strongly recommend that nursing mothers breastfeed because of the many known health benefits of breastfeeding. Our SALs protect the healthy practice of breastfeeding by modelling this specific pathway of exposure and keeping estimated infant exposure at or below health protective limits. It is important to protect infants from the types of toxicity observed with PFAS. Thyroid hormones are critical to normal brain and body development and the immune system is also developing during this time. Adverse effects incurred during critical stages of development can result in health conditions that persist into adulthood.[35-37]

Health researchers are still learning about how exposure to PFAS may affect people's health. The strongest evidence from studies of exposed human populations indicates that some PFAS may increase serum cholesterol levels and alter liver enzyme levels, slightly lower birth weights, and reduce immune response to childhood vaccines.[4] More limited support is available for increased risk of having thyroid disease, hypertension disorders during pregnancy, reproductive problems, altered hormone levels, and metabolic issues.[4] There is limited but growing evidence from occupational and non-occupational studies that PFOA may increase risk of kidney and testicular cancer.[38, 39]

In laboratory animals (such as rats, mice, monkeys), some PFAS produce toxicity in the liver, kidney, thyroid, and reproductive organs, impair the immune system, alter the growth and development of offspring, reduce thyroid hormone levels, and alter reproductive function. Higher rates of certain tumors have been observed in rodents administered PFOA, PFOS, or GenX but not PFHxA over their lifetime. Other PFAS are less studied.[4]

The strength of the evidence and types of effects observed vary by PFAS. For details, see the supporting information for each PFAS SAL.

We still have limited ability to identify all PFAS in water or assess their health impacts.
The PFAS class contains thousands of chemicals and it is unknown how many of these could occur in drinking water. While some research laboratories have identified hundreds of PFAS in groundwater contaminated by PFAS containing firefighting foam,[40] commercial laboratories testing drinking water typically test for 14-18 PFAS associated with the most commonly produced PFAS (using EPA Method 537 1.1). A new validated method (EPA Method 533) can detect 25 PFAS in drinking water.[41]

We still know relatively little about the potential toxicity of many PFAS. EPA has completed assessments for PFOA, PFOS and PFBS; a draft assessment of GenX; and is currently assessing PFDA, PFNA, PFHxS, PFHxA, and PFBA.[41, 42] ATSDR developed health-based values for PFOA, PFOS, PFNA, and PFHxS.[4] Several states have developed independent assessments.[43]

Our recommendations for five SALs focus on the most commonly detected PFAS in Washington state drinking water with sufficient toxicity information. While our capacity develops to more fully characterize and assess PFAS in water, we recommend using these five SALs to decide when to act on PFAS in drinking water. When a water system decides to take action to reduce PFAS, we recommend broad mitigation options effective for many PFAS briefly discussed below.

Current water filtration technologies can remove many PFAS from drinking water.
EPA maintains a database of water treatment options for PFOA, PFOS, and other PFAS. The database contains information about treatment efficacy of various technologies.[44] Current PFAS removal technologies for water systems include granular activated carbon, reverse osmosis membranes and anion exchange resins.[45] These can remove 90 to 99 percent of most PFAS listed in the EPA database. Drinking water filtration requires ongoing water monitoring for efficacy, maintenance, and periodic replacement of filter media. Active research into additional PFAS removal and destruction technologies is underway.

Federal toxicology research underway may allow a grouped approach to regulating PFAS mixtures.
Investigators from EPA and the National Toxicology Program (NTP) are studying 150 PFAS using rapid high throughput testing to inform toxicity assessments.[41, 46] The list includes PFAS from 75 different subclasses of PFAS. Results from this additional research could inform a regulatory approach based on subclasses. We will continue to monitor progress and consider adopting a broader grouped approach to regulating PFAS mixtures as the science and methodology evolve.

Introduction to Approach and Methods

The state action levels (SALs) were derived using the same basic approach as a Maximum Contaminant Level Goal (MCLG) under the Safe Drinking Water Act. The MCLG equation for non-cancer health concerns (shown below) divides a health-based value of an acceptable daily intake called the Reference Dose (RfD) by an upper-bound drinking water ingestion rate usually associated with the most sensitive group in the population. This term is then adjusted with the Relative Source Contribution (RSC) to account for other sources of daily exposure from non-drinking water sources such as food and consumer products. This yields an MCLG that is a concentration of a contaminant in drinking water protective of human health, including sensitive groups, assuming long-term, year-round exposure (365 days/year).

$$MCLG\ (\frac{mg}{L}) = \frac{RfD\ \left(\frac{mg}{kg-day}\right)}{Drinking\ water\ ingestion\ rate\ \left(\frac{L}{kg-day}\right)}\ x\ RSC(\%)$$

We describe the approach that we used to identify a health-based value (such as a RfD), the drinking water ingestion rate and the RSC in more detail below. Briefly, we calculated SALs for PFOS, PFOA, PFNA and PFHxS, substituting internal dose for external dose in the equation above. This is because these PFAS readily bioaccumulate in our bodies over time. Parts per trillion concentrations of these PFAS in drinking water (external dose) result over time in parts per billion levels in serum (internal dose). We used an exposure model developed by the Minnesota Department of Health that accounts for age-specific intake of drinking water as well as important indirect pathways of infant exposure to PFAS in drinking water via maternal transfer (placental and lactational). We used the serum PFAS level to define internal dose associated with our health protective value. For PFBS, we used EPA's RfD and a water ingestion rate for infants. Finally, we used an EPA decision tree to derive the RSC for each PFAS.

Selecting health-based values of acceptable daily intake for five PFAS in drinking water. We reviewed scientific literature and the available health protective values for acceptable daily human intake of five PFAS detected in Washington drinking water. We focused on government risk evaluations that were high quality, peer-reviewed, comprehensive and based on current scientific research. The health protective values we identified included U.S. EPA and U.S. state reference doses (RfDs), ATSDR minimal risk levels (MRLs) and California Acceptable Daily Doses (ADD). We describe the values specific to each of the five PFAS chemicals in the section "Supporting information—How we derived each SAL" (see page 26).

Information to assess carcinogenicity of these five PFAS was available for only PFOA and PFOS. EPA and the New Jersey Drinking Water Quality Institute (NJ DWQI) based their PFOA cancer

assessment on testicular cancer, which has supporting evidence in rodents and in humans. EPA and NJ evaluators found that their reference doses based on non-cancer endpoints were also protective against cancer risk.[47-49] California EPA Office of Environmental Health Hazard Assessment derived lower cancer risk levels for PFOA and PFOS based primarily on liver cancer in rodents. The strongest evidence for cancer risk in humans is between PFOA exposure and kidney and testicular cancer.[38, 50] In contrast, liver cancer has not been associated with PFOA in exposed populations.[38] For these reasons, our review focused on non-cancer outcomes. This approach aligns with conclusions by EPA, ATSDR, and several other states that noncancer endpoints had the best evidence base for human health protective values.

The health-based values we selected are based on adverse effects observed in laboratory animals on immune function, altered development of offspring, and reduced serum levels of thyroid hormones. Points of departure for other clearly adverse effects in the liver, kidney, and for reproductive toxicity, were sometimes only slightly less sensitive.

All the U.S. risk assessors concluded that the limitations of epidemiological studies meant they couldn't be used quantitatively as the basis for an RfD. A major concern is teasing out associations between individual PFAS (e.g. PFOA) and health outcomes in populations with simultaneous exposure to multiple PFAS. When multiple PFAS occur in public drinking water, the individual PFAS are often highly correlated with each other in serum samples. In addition, epidemiological studies typically measure only about a dozen PFAS in both water and serum. Unmeasured PFAS in drinking water may also contribute to community exposure and may confound associations between health outcomes and measured PFAS. For example, four new (previously unmeasured) PFAS were recently identified in the drinking water and human serum of residents in Wilmington, NC.[51]

Another concern is that the cross-sectional study design of many PFAS epidemiological studies limits their use in determining causality. In fact, some health outcomes associated with serum levels of PFAS could be due to reverse causation. For example, earlier menopause and shorter breast-feeding duration may result in increased serum PFAS since menstruation and lactation are PFAS excretion pathways in women.[52] Conditions like kidney disease that can reduce glomular filtration rate may lead to higher serum PFAS because it impairs a major PFAS excretion pathway.[52, 53] Another concern is using a single serum sample to quantify PFAS exposure. Serum levels reflect exposure across recent months to years, but will not necessarily reflect the level in serum that preceded the onset of a disease or condition. Some studies, like the large C8 Health Project did exhaustive exposure reconstruction to overcome this limitation.[54] A final concern was that a number of the outcomes with the most robust evidence in people—increased cholesterol, reduced birth weight, immunosuppression—have many possible causes, which are difficult to control for in community-wide observational studies. Still,

U.S. risk assessors considered epidemiological data qualitatively when evaluating the relevance of animal testing on human health and the weight-of-evidence for specific health outcomes.

It is important to acknowledge the uncertainty of relying on studies in laboratory animals as well. Laboratory animals differ from humans in how rapidly they excrete a number of PFAS (serum half-lives in hours and days in rodents vs. years in humans)[55] and in how a chemical effects specific tissues (PPARα activation[1] in rodent vs. human liver tissue). If we rely solely on experimental animals, we can miss characterizing toxicity that is uniquely a human response.

For all five PFAS, there are large differences between humans and laboratory animals in how external dose (the amount of intake) translates to internal dose (the amount in blood and organs). Humans retain PFOA, PFOS, PFHxS, and PFNA much longer than laboratory rats, mice, or monkeys, which leads to a higher internal dose in humans given the same external dose.[57] For this reason, internal dose (serum level) rather than administered dose was generally used to determine the point of departure in animal studies (such as a NOAEL, LOAEL, BMDL). When the critical study was based on effects in offspring exposed during gestation and lactation, maternal serum level was typically used as the most relevant measurement of internal dose. The critical study selection was often limited to studies that measured internal dose or had sufficient data to model serum level across the dose-range.

Relative source contribution (RSC).

When setting drinking water standards, EPA considers daily exposure expected from non-drinking water sources and apportions a relative source contribution (RSC) for drinking water. When significant exposures occur from other sources, such as food and consumer products, water quality criteria must be more stringent to allow for these other exposures. The sum of all exposure sources should not exceed the RfD or other health protective value in the most sensitive populations.

EPA provides a decision tree for deriving the RSC for water quality standards.[58] EPA recommends a default RSC of 0.20 (20 percent) contribution from drinking water when little information exists about other exposure sources and pathways. EPA recommends a maximum RSC of 0.80 to account for unknown or unexpected exposures. We used the EPA Decision Tree to derive RSCs (Figure 4).

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[1]PPARα is perioxisome proliferation activated receptor subtype alpha. This is a nuclear receptor that is more prevalent in rodent liver than human liver and mediates certain biological responses in rodent liver that are not thought to be relevant for human liver. 56. Corton, J.C., J.M. Peters, and J.E. Klaunig, *The PPARalpha-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions.* Arch Toxicol, 2018. **92**(1): p. 83-119.

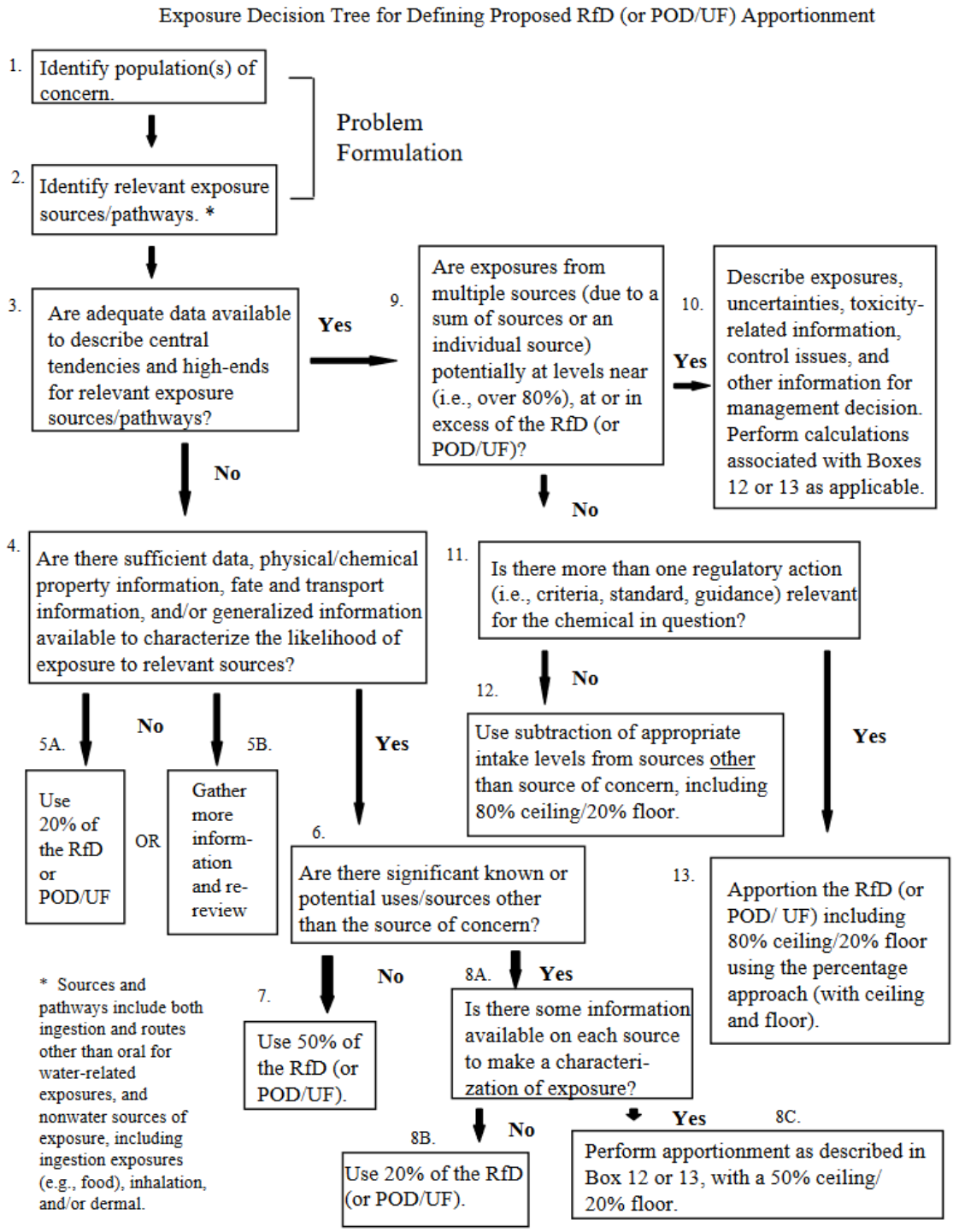


Figure 4. *EPA Exposure Decision Tree for defining apportionment of the RfD between different regulated sources of exposure.*[58]

Box 1 populations of concern. We identified the developing fetus and infant as sensitive life stages of concern. The most sensitive endpoint for PFOA, PFNA, PFHxS, and PFBS were developmental and thyroid hormone concerns. The critical role that thyroid hormones play

during rapid growth and brain development makes altered thyroid hormone levels a concern for developmental effects. For PFOS, the rodent serum level (internal dose) at the NOAEL for developmental toxicity was only slightly higher than the point of departure for immune toxicity (see PFOS summary for more details).

Since maternal serum drives fetal exposure and lactational exposure in infants, women of reproductive age were considered a sensitive population.

We considered adults a sensitive population for PFOS as the RfD was based on immune suppression in adult mice.

RSCs for infants

In **Box 2**, pathways of early life exposure included direct ingestion of drinking water and indirect exposures due to placental and lactational transfer from maternal serum.

Exposure pathways other than drinking water included diet, indoor dust and air, and direct contact with PFAS in products (cosmetics, waterproofing sprays, stain proof treatments for carpets and textiles). We did not identify sufficient data to describe the central tendencies and high-end exposures for individual PFAS exposure pathways ("no" to **Box 3**). There was, however, infant exposure information to inform estimates of the most relevant sources of exposure ("yes" to **Box 4**).

Box 6 asks about significant sources of exposure other than drinking water. Infants (birth to six months) rely heavily on breast milk or formula for nutrition. A 2011 Norwegian Institute of Public Health study assessed PFAS levels in indoor air, dust, and breast milk for a number of six-month-old infants.[59] The study estimated that breast milk contributed on average 83 percent of an infant's total daily intake of PFOA and 94 percent of PFOS. Since we were able to model breastmilk exposure associated with four PFAS in drinking water, we assumed few other exposure sources for this age group (birth to six months) and answered "no" to Box 6. For infant exposure to PFOA, PFOS, PFHxS, and PFNA the decision tree recommends an RSC of 50 percent (**Box 7**).

For infant exposure to PFBS, we are unable to model breastmilk exposure when PFBS occurs in a community water supply. In addition, PFBS is part of the PFAS chemistry replacing phased-out PFAS in the current U.S. marketplace and there are significant potential PFAS sources other than drinking water.[11, 20, 60, 61] We answered "yes" to Box 6. In **Box 8A**, we lacked enough information on specific applications of PFBS-based chemistry to estimate exposure from indoor environments, air and food pathways. We answered "no" to Box 8A and selected the 20 percent default (Box 8B) for the PFBS RSC for all life stages.

RSCs for older children, women of childbearing age and adults

In **Box 2**, pathways of exposure included ingestion of drinking water, and intakes associated with foods, indoor dust and air, and direct contact with PFAS in products (cosmetics, waterproofing sprays, stain proof treatments for carpets and textiles). We did not identify sufficient data to describe the central tendencies and high-end exposures for individual PFAS

exposure pathways ("no" to **Box 3**). There was, however, biomonitoring information to inform estimates of exposure to sources other than drinking water ("yes" to **Box 4**). Biomonitoring data are discussed below (see Box 8). We answered yes to **Box 6** as non-drinking water sources are likely significant.

We used several lines of evidence to answer "yes" to **Box 8A** and estimate the total amount of exposure from non-drinking water sources (**Box 8C**). CDC biomonitoring surveys provide distributions for PFOA, PFOS, PFNA and PFHxS in the serum of the U.S. population three years old and older.[62] Biomonitoring data provides an indication of total exposure from all sources. We assumed that the 95th percentile of PFAS in serum of the general U.S. population adequately represents exposure to sources other than drinking water. This assumption has limited support from a study by Hu et al. that estimated 16.5 million people (or about 5 percent of the U.S. population in 2014) had detectable levels of at least one of six PFAS measured in their drinking water in a survey of mostly large public water systems in the U.S.[2] A comprehensive Michigan state survey also provides limited support for this assumption. Michigan tested for 18 PFAS and with lower detection limits at 1,741 drinking water sources (including public water systems, at schools, at childcare providers, and in Tribal communities). Six percent of the total population served by the systems tested had total PFAS levels in at least one water supply source between 10-70 ng/L. Only two water systems (<0.1 percent of population served in this survey) had more than 70 ng/L of PFOA and PFOS combined.[63, 64] Our assumption that the 95th percentile serum level in the general U.S. populations adequately represents non-drinking water sources is conservative. If the true contribution from drinking water is higher, we will have overestimated the non-drinking water sources and thus underestimated the RSC for drinking water (a lower RSC is more protective).

Box 8C recommends the subtraction method to calculate an RSC with a ceiling of 50 percent and a floor of 20 percent. In the equation for the subtraction method below, the target serum level is the human serum concentration associated with PFAS intake at the RfD or MRL, and the serum level from sources other than drinking water is the 95th percentile serum level for the age group from NHANES.

$$RSC = \frac{Target\ serum\ level - serum\ level\ from\ non\ drinking\ water\ sources}{Target\ serum\ level}$$

For the subtraction method, we used the target serum level identified for each chemical (see chemical summaries) and the 95th percentile serum level that the 2015-16 CDC NHANES reported for the U.S. general population > twelve years of age. An NHANES survey of children aged three to eleven years in 2013-14 provided estimates for ages three to eleven years. We used these national estimates because we expect that serum levels of PFAS in Washington residents will be similar. A 2004 study by Olsen et al., measured seven PFAS compounds in stored blood serum of 238 men and women in an elderly Seattle population.[65] Levels measured

in this population were comparable to the distribution in NHANES for the same time period.[66]Another study of American Red Cross blood donors in six U.S. cities showed that PFOA levels in donors living in Portland, OR were equal to or lower than donors in the other cities tested.[67]

We then applied the ceilings and defaults recommended in the EPA Exposure Decision Tree to derive RSCs for each age group (see Table 2, next page).

Table 2. Relative Source Contribution (RSC) for each PFAS by Age Group.

Reference Population	95th Percentile Serum Level from NHANES[a] (ng/mL)	Target Serum Level[b] (ng/mL)	Subtraction Method RSC	RSC Using Ceilings and Defaults from Exposure Decision Tree[c]
PFOA				
Ages ≥ 12 yrs.	4.17	27.6	85%	50%
Females ≥ 12 yrs.[d]	4.17	27.6	85%	50%
6-11 year olds	3.84	27.6	86%	50%
3-5 year olds	5.58	27.6	80%	50%
Infants	-		Box 7	50%
PFOS				
Ages ≥ 12 yrs.	18.3	23.8	22%	20%
Females ≥ 12 yrs.[d]	15.1	23.8	36%	35%
6-11 year olds	12.4	23.8	47%	45%
3-5 year olds	8.82	23.8	63%	50%
Infants	-		Box 7	50%
PFHxS				
Ages ≥ 12 years	4.9	108	95%	50%
Females >12 yrs.[d]	3.8	108	97%	50%
6-11 year olds	4.4	108	96%	50%
3-5 year olds	1.62	108	99%	50%
Infants	-		Box 7	50%
PFNA				
Ages ≥ 12 yrs.	1.90	22.7	92%	50%
Females ≥ 12 yrs.[d]	1.80	22.7	92%	50%
6-11 year olds	3.19	22.7	86%	50%
3-5 year olds	3.49	22.7	85%	50%
Infants	-		Box 7	50%
PFBS				
Ages ≥ 12 yrs.	< 0.1		Box 8B	20%
Females ≥ 12 yrs.[d]	< 0.1		Box 8B	20%
6-11 year olds	0.13		Box 8B	20%
3-5 year olds	< 0.1		Box 8B	20%
Infants	-		Box 8B	20%

[a]NHANES data on PFAS serum levels in 3-11 year olds are from a 2013-14 nationally representative sample. For ages 12 and up, serum levels of PFOA, PFOS, PFHxS and PFNA are from the 2015-16 NHANES survey and serum PFBS is from the 2013-14 NHANES survey [68]. < 0.1 means less than the limit of detection of 0.1 ug/L.

[b]Target serum levels are the concentration of the PFAS in serum associated with an oral intake rate at the RfD or MRL. More information about the target serums are in the supporting information for each SAL.

[c]The RSCs in the right hand column were derived using the subtraction method and the EPA Exposure Decision Tree [58].

[d]Serum levels of female >12 years old were used to represent women of childbearing age.

Drinking water ingestion rate.

EPA calculates a health protective level in drinking water by dividing the RfD with a drinking water ingestion rate that is protective of the population, including sensitive groups. For chronic criteria meant to cover a lifetime of exposure, EPA typically uses the 90th percentile of adult drinking water ingestion rates from the EPA Exposure Factors Handbook. If a sensitive subpopulation is identified, drinking water ingestion rates are selected specific to their expected consumption.

Infants are considered a sensitive population for PFAS, so we applied their drinking water ingestion rates. In addition, breastfeeding infants will have a secondary pathway of exposure if their mothers are consuming PFAS in their daily tap water. PFAS ingested by the mother will contribute to PFAS in her breastmilk. Several studies have observed rapid accumulation of PFOA and other bioaccumulative PFAS in the serum of breastfed infants during the first year of life.[32, 69-71] Additionally, higher serum levels in older children (eight years old) correlate with breast feeding history.[33, 72]

In order to account for higher drinking water intake rates of children and the breastfeeding pathway, the Minnesota Department of Health (MDH) developed a toxicokinetic model for age-specific intake of PFOA in drinking water that includes infant exposures via breastfeeding. The method was peer reviewed by academic, government, and private industry experts and published in a peer-reviewed journal.[33]

MDH employed the model instead of a standard drinking water ingestion rate in their Health-Based Guidance Values for PFOA, PFOS, and PFHxS in drinking water.[73-75] Michigan Department of Health and Human Services (MDHHS)[76] and the New Hampshire Department of Environmental Services (NHDES)[77] recently adapted the model for PFNA and employed the model to derive their state recommendations on PFOA, PFOS, PFHxS, and PFNA.

Brief summary of the MDH transgenerational toxicokinetic model.[33]

- Assumes that exposure to PFAS contamination in a community water supply is chronic, that maternal exposure begins at birth, and that her serum level at the time of pregnancy reflects her accumulated lifetime consumption.
- Calculates serum concentrations from the dose and clearance rate for each PFAS using the equation below. Where dose = water or breastmilk intake (L/kg-day) x water or breastmilk PFAS concentration (mg/L) and clearance = volume of distribution (L/kg) x (Ln 2/human half-life of PFAS, in days).

$$Serum\ Concentration\ \left(\frac{mg}{L}\right) = \frac{Dose\left(\frac{mg}{kg-day}\right)}{Clearance\ rate\ \left(\frac{L}{kg-day}\right)}$$

- Predicts infant serum at birth as a proportion of maternal serum concentration. The ratio applied is a mean or median placental transfer ratio from empirical studies of PFAS in paired maternal and cord serum.

- Includes two scenarios of infant nutrition. The first scenario assumes exclusive breastfeeding through twelve months and the second scenario assumes formula feeding with infant formula prepared with tap water. In both scenarios, MDH applied age-specific drinking water ingestion rates throughout childhood to predict serum levels into adulthood for a given concentration of a PFAS in drinking water.
- Models lactational transfer to infants with mean or median breastmilk transfer ratios from empirical observations of PFAS in paired breast milk vs. maternal serum levels.
- Assumes upper bound ingestion rate (mean plus two standard deviations) for breast milk intake by infants and 95th percentile drinking water ingestion by all age groups.
- Models a gradual decline in breast milk concentration of PFAS over the course of lactation.
- Applies age-adjusted factors to the chemical-specific volume of distribution, to account for differences in the extracellular water content in children as a percentage of their body weight. The age adjustment factors range from 2.4 for newborns to 1.0 for children over one year old.
- Addresses non-drinking water sources of exposure to PFAS within the relative source contribution parameter. Specifically, serum levels of infants and children must remain below the proportion of the RfD allotted to drinking water sources.

We modified several parameters in the MDH model based on the following evidence.

- Duration of exclusive breastfeeding: The American Academy of Pediatrics (AAP) recommends that infants be exclusively breastfed for the first six months with continued breastfeeding alongside introduction of appropriate complementary foods for one year or longer. This includes complementary foods and beverages mixed with tap water. The department actively supports these recommendations and conducts outreach and support activities every year to help families follow them. According to the CDC Breast-feeding Report Card for Washington State Infants Born In 2017, 58 percent of Washington mothers reported exclusive breast-feeding through three months and 29 percent reported exclusive breast-feeding through six months. Seventy-one percent of Washington infants are not exclusively breastfed through six months.

  These data and the AAP recommendation support a model assumption of gradually phasing out breast milk after six months while phasing in other dietary sources of nutrition and drinking water. We assumed exclusive breastfeeding for the first six months followed by a six-month period when breastmilk intake declines as other sources of nutrition increase. During the breastmilk phase-out, tap water intake increases so the combined liquid intake from both sources remains at the 95th percentile intake for this age group (133 mL/kg-day).

- Estimate of high-end drinking water ingestion rate. In the 2019 EPA exposure factors handbook, drinking water ingestion rates come from surveys of a representative population asked about their water consumption in the last two days.[30] Survey results

are a measure of high-end consumption by individuals on any given day in a population but do not represent consumption over long periods by an individual who represents the 95th percentile for chronic intake. EPA prefers the 90th percentile to represent upper-end consumption over long periods of time.

We applied age-specific 90th percentile water-ingestion rates for chronic intake of water after one year of age. This included women of childbearing age in the years prior to pregnancy. We retained the MDH model assumption of 95th percentile water ingestion by mothers over twelve-months of lactation, 95th percentile ingestion of water for formula-fed infants, and upper bound intake estimates for breastmilk intake (mean plus two standard deviations) by breastfed infants.

Table 3, below, shows our model inputs for the MDH model. We retained MDH's other assumptions on half-life, volume of distribution, and lactational transfer ratios. MDH did not derive drinking water advice for PFNA. For PFNA we used the model inputs developed by the Michigan Department of Health and Human Services,[76] with the exception of a more recent half-life estimate from Yu et al., 2021.[26]

We did not use the MDH model for PFBS because we had insufficient information to model infant lactational exposures. For PFBS, we used the 95th percentile estimate for drinking water intake for infants from birth to one year old (from the 2019 EPA Exposure Factors Handbook Table 3-3) as this life stage is the most exposed sensitive population.

Table 3. Washington Department of Health model parameters for the MDH transgenerational exposure model of PFAS in infancy and childhood.

Model Parameter	Central or Upper Tendency of Parameter	PFOA[a]	PFOS[a]	PFHxS[a]	PFNA[b]
Half-life (years)	Central	2.3	3.4	5.3	3.5[c]
Placental Transfer Ratio	Central	0.87	0.40	0.70	0.69
Breastmilk Transfer Ratio	Central	0.052	0.017	0.014	0.032
Volume of Distribution (L/kg)	Central	0.17	0.230	0.25	0.20
Relative Source Contribution (%)	Upper	50	50	50	50
All PFAS scenarios					
Duration of exclusive breast feeding (months)	Mid-upper	6			
Duration of breastmilk phase out with addition of solid foods and liquids based on drinking water (months)	Mid-upper	6			
Age-specific water ingestion rates (mL/kg-day)[d]					
Birth to <1 month	Upper (95th)	224			
1 to <3 months	Upper (95th)	267			
3 to <6 months	Upper (95th)	158			
6 to <12 months	Upper (95th)	133			
Birth to <1 year	Upper (95th)	174			
1 to <2 years	Upper (90th)	49			
2 to <3 years	Upper (90th)	51			
3 to <6 years	Upper (90th)	39			
6 to <11 years	Upper (90th)	31			
11 to <16 years	Upper (90th)	25			
16 to <21 years	Upper (90th)	25			
Adults 21 <50 years	Upper (90th)	35			
Lactating women[e]	Upper (95th)	47			
Women of childbearing age[e]	Upper (90th)	35			
Breastmilk ingestion rates[f] (mL/kg-day)					
Birth to <1 month	Upper	220			
1 to <3 months	Upper	190			
3 to <6 months	Upper	150			
6 to <12 months	Phase-out	150->0			

[a] *Model inputs that MDH developed based on review of empirical epidemiological studies.*

[b] *Model inputs developed by the Michigan Department of Health and Human Services.*

[c] *Mean serum half-life observed over three annual serum measurements in 68 highly exposed participants after PFNA was removed from community drinking water system (Yu et al. 2021).*

[d] *2019 update to Chapter 3 EPA Exposure Factors Handbook, Table 3-1 Recommended values for drinking water ingestion rates (2 day average community intake) and Table 3-21 Two-day average, consumer-only estimates of combined direct and indirect water ingestion based on National Health and Nutrition Examination Survey (NHANES) 2005–2010: community water (mL/kg-day).*

[e] *2019 update to Chapter 3 EPA Exposure factors Handbook. Table 3-3 recommended values for water ingestion rates of community water of pregnant and lactating women and women of childbearing age (13 to <50 years) and Table 3-63.*

Two-day average consumer-only drinking water intake: pregnant and lactating women, and women of child-bearing age (13 to <50 years).

[f] 2011 EPA Exposure factors Handbook, Table 15-1. Upper percentile is reported as the mean plus 2 standard deviations.

We used the process described above for determining health protective values, drinking water intake, and the relative source contribution, to derive SALs for five PFAS shown in Table 4. We used the MDH model to ensure that serum levels in infants and children remained below the serum equivalent of the dose allotted to drinking water sources. We provide details on how we derived each SAL in the Supporting Information for each PFAS.

Table 4 Recommended health protective values and state action levels (SALs) for five PFAS in Washington drinking water

PFAS	RfD/MRL (ng/kg-day)	Source (year)	Basis	Relative Source Contribution	Ingestion rate	SAL in drinking water
PFOA	3	ATSDR MRL (2021)	Developmental effects in mice.	50%	MDH model[a]	10 ng/L
PFOS	3	MDH, NHDES[b] RfD (2019)	Immune effects in mice. Also protective of developmental effects in rats.	20% Adults 50% Children	MDH model[a]	15 ng/L
PFNA	2.5[c]	Modified ATSDR MRL[c] (2021)	Developmental effects in mice.	50%	MDH model w/MDHHS inputs[d]	9 ng/L
PFHxS	9.7	MDH RfD (2019)	Reduced thyroid hormone (T4) in rats (developmental concern).[e]	50%	MDH model[a]	65 ng/L
PFBS	300	EPA RfD (2021)	Reduced thyroid hormone (T4) in mice (developmental concern).[e]	20%	0.174 L/kg-d	345 ng/L

[a]The MDH model is the Minnesota Department of Health toxicokinetic model for infant intake of bioaccumulative PFAS in drinking water. It includes age-specific drinking water ingestion rates as well as placental and lactational transfer pathways from mother to child.

[b]NHDES is the New Hampshire Department of Environmental Services.

[c]We modified the ATSRD MRL by substituting a serum half-life estimate of 3.5 years from Yu et al, 2021.

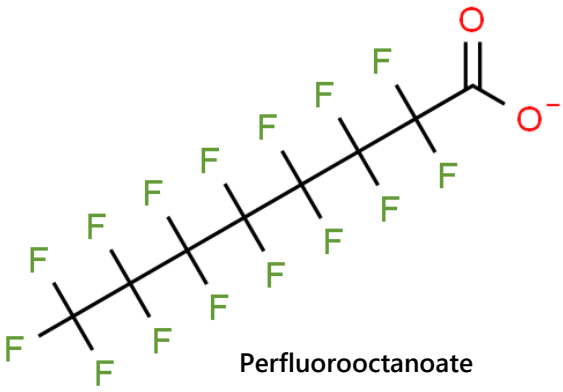
[d]MDHHS is the Michigan Department of Health and Human Services. We used MDHHS inputs but substituted serum half-life estimate of 3.5 years from Yu et al 2021.

[e]T4 is thyroxine, a thyroid hormone.

Supporting Information—How We Derived Each SAL

Deriving the State Action Level for PFOA

Perfluorooctanoic acid (PFOA) has seven fully fluorinated carbons and a carboxylic acid group at one end. In drinking water, PFOA occurs in the form of its anion shown here. PFOA was used as a processing aid to make products that repel water and oil, resist heat, and have extreme durability. These include a wide array of household and industrial products such as non-stick cookware, stain-resistant carpets, waterproof fabrics, and clothing. Chemicals that can breakdown to PFOA (called PFOA precursors) were used in coated paper and cardboard, food packaging such as fast food wrappers and parchment papers, and in certain types of firefighting foam.[4, 15, 78] Under a stewardship agreement with the U.S. EPA, major domestic manufacturers of PFOA voluntarily phased-out their production between 2006 and 2015. In 2020, EPA restricted significant new uses of PFOA and import of certain products containing PFOA unless EPA reviews and approves them.[79] PFOA and precursor chemicals may still be produced and released globally. PFOA has no known natural degradation pathway. Its persistence and water solubility enable it to leach into groundwater from surface soils.



Perfluorooctanoate

In national surveys, nearly every person tested had detectable levels of PFOA in their blood serum. The average serum level in the U.S. has declined by 60 percent since the phase-out of PFOA and precursors in the U.S. began in 2006.[68] The CDC NHANES survey from 2015-16 reported 1.56 µg/L as the mean serum level of PFOA in the U.S. general population (aged twelve and older) and 4.17 µg/L as the 95th percentile of the population distribution.[62] PFOA accumulates in our bodies because it is readily absorbed orally and only slowly excreted. Estimates of median or average serum half-life of PFOA in human studies ranged from 2.3 to 3.9 years with very little difference reported between sexes.[17] The long half-life of PFOA in humans is attributed to resorption of PFOA following filtration by the kidney.[80] In humans, PFOA appears to accumulate most in liver, kidney, blood serum, lung, and bone.[5, 81]

Food and drinking water contamination are thought to be the major pathways of nonoccupational exposure to PFOA.[4] People may also be directly exposed to PFOA or precursors chemicals when handling certain products and indirectly exposed when indoor dust and air becomes contaminated by products that release PFOA.[4] Exposure to PFOA may also be higher in young children because of age-specific behaviors (e.g., mouthing of treated textiles, closer contact with treated carpets, higher incidental ingestion of house dust, higher consumption of food and water per pound body weight).

The primary effects observed in laboratory animals following PFOA exposure are liver toxicity,[82-85] immunotoxicity,[86-88] reproductive and developmental toxicity,[84, 89-93] and altered thyroid hormones.[94] Numerous health effects are associated with PFOA exposure in humans. Epidemiological studies have assessed health outcomes in PFOA-exposed workers from manufacturing plants, large communities with high levels of PFOA in drinking water, and the general population with background exposures from diet and consumer products.[4] The adverse health effects in humans with the strongest and most consistent associations with higher PFOA exposure are elevated serum cholesterol,[95, 96] reduced birth weight,[97, 98] reduced antibody response to vaccines,[99, 100] and increased serum liver enzymes.[101-105] Studies also report associations between PFOA exposure and altered development of reproductive tissue and delayed puberty,[106, 107] higher serum uric acid,[108-110] altered thyroid hormone levels and thyroid disorders,[111-114] pregnancy-induced hypertension and preeclampsia,[115-117] and ulcerative colitis.[118, 119]

PFOA is not considered genotoxic or mutagenic but studies in laboratory animals show increased incidence of tumors in liver, testicular, and pancreatic tissues as well as ovarian tubular hyperplasia.[120-123] PFOA exposure was positively associated with increased incidence of kidney and testicular cancers in a large study of people with high levels of a PFOA exposure from their drinking water (the C8 Health Project).[124-126] A study in the U.S. general population also reported a higher risk of renal cell carcinoma associated with higher serum PFOA levels.[39] The prospective design of this second study allowed the authors to control for reverse causation due to diminished kidney function. Most other studies in the general population have looked for but not found associations between serum PFOA levels and a range of human cancers.[127-130] In 2016, EPA classified PFOA as having "suggestive evidence" of carcinogenic potential in humans.[81] The International Agency for Research on Cancer (IARC) has classified PFOA as possibly carcinogenic to humans (Group 2B)[38]

Review of Health Protective Values

DOH reviewed the available health protective values for daily chronic human intake of PFOA. We focused on high-quality and comprehensive risk evaluations that considered current scientific research and were conducted by U.S. federal and state agencies. We focused on noncancer endpoints as discussed on page 13. Health protective values identified included an EPA reference dose (RfD), an ATSDR minimal risk level (MRL), an acceptable daily dose (ADD) developed by CA OEHHA, and target serum levels developed by NJ DWQI and NHDES. A target serum is equivalent to an RfD except on a serum basis.

These health protective values are shown in Table 5 below. EPA and ATSDR selected developmental endpoints as the basis for their health-based values for PFOA while NJ, NH, and CA based their health-based values on liver effects.

Table 5. Health Protective Values for PFOA Reviewed by WA

Source	Critical Study	Critical Effect	Human Equivalent Dose	Uncertainty Factors (UF)[a]	Oral RfD, MRL, ADD[b], or Target Serum Level	Exposure Duration
EPA 2016[120]	Lau et al. 2006	LOAEL (1 mg/kg-day) for developmental effects of gestational exposure in mice (reduced bone ossification, earlier puberty in males). Estimated maternal serum level at LOAEL: 38 mg/L.	0.0053 mg/kg-day (38 mg/L x 0.000139 L/kg-day)	UF_{total} 300 UF_H =10 UF_A = 3 UF_L =10	20 ng/kg-day (RfD)	Chronic
NJ DWQI 2017 [78, 96]	Loveless et al. 2006	$BMDL_{10}$ for 10% increase in relative liver weight in male adult mice following a 14-day exposure. LOAEL: 0.3 mg/kg-day Estimated serum at $BMDL_{10}$: 4.35 mg/L		UF_{total} 300 UF_H =10 UF_A = 3 UF_D =10	14.5 µg/L (target serum level)[c] 2 ng/kg-day[d] (RfD)	Chronic
ATSDR 2021 [4]	Koskela et al. 2016; Onishchenko et al. 2011	LOAEL (0.3 mg/kg-day) for neurodevelopmental and skeletal effects in mouse offspring following gestational exposure Predicted time-weighted average maternal serum level: 8.29 mg/L.	0.000821 mg/kg-day (8.29 mg/L x 0.000099 L/kg-day)	UF_{total} 300 UF_H =10 UF_A = 3 UF_L =10	3 ng/kg-day (MRL)	Inter-mediate (2-52 wks.)
NH DES 2019 [77, 131]	Loveless et al. 2006	$BMDL_{10}$ for 10% increase in relative liver weight in male adult mice following a 14-day exposure. LOAEL=0.3 mg/kg-day. Estimated serum at the $BMDL_{10}$: 4.35 mg/L		UF_{total} 100 UF_H =10 UF_A = 3 UF_D = 3	43.5 µg/L (target serum level)[c] Or 6.1 ng/kg-day[e] (RfD)	Chronic
CA OEHHA 2019[132]	Li et al. 2017	LOAEL (0.05 mg/kg-day) for physiological changes in liver that could lead to adverse effects observed in female adult mice in a 28-day gavage study. Measured mean serum level at LOAEL: 0.97 mg/L.		UF_{total} 300 UF_H =10 UF_A = 3 UF_L = 3[f] UF_D = 3[f]	3.2 µg/L (target serum level)[g] Or 0.45 ng/kg-day (ADD)	Chronic

[a]Uncertainty factors: UF_H=intra-species uncertainty factor (human variability); UF_A= inter-species uncertainty factor; UF_S= subchronic to chronic uncertainty factor; UF_L= LOAEL to NOAEL uncertainty factor; UF_D= incomplete database uncertainty factor; UF_{total}= total (multiplied) uncertainty factor. Uncertainty factors are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing a 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10.

[b]RfD= Reference dose. MRL = minimal risk level. Target serum level is the concentration of the PFAS in serum associated with an oral intake rate at the RfD or MRL. ADD = acceptable daily dose.

[c]NJ DWQI and NHDES expressed their health-based value as a target serum level rather than a daily dose.

[d]For purposes of comparison, NJ calculated an RfD for their target serum level by multiplying their target serum by the EPA-derived clearance factor for PFOA. 0.0145 mg/L x 0.000139 L/kg-day = 0.000002 mg/kg-day.

[e]NHDES used a dosimetric adjustment factor of 0.000149 L/kg-day to calculate an RfD from the target serum level. 0.0435 mg/L x 0.000149 = 0.000006 mg/kg-day.

[f]CA OEHHA applied a UF_l of 3 instead of 10 for use of a LOAEL because the critical effects are upstream physiological changes that can lead to adverse effects. The UF_D reflects their concern about development toxicity (reduced female pup weights) reported by van Esterik et al. 2016 at dietary doses of 0.01 mg/kg-day in pregnant mice (serum levels were not measured).

[g]CA OEHHA calculated a target human serum of 3.2 µg/L (=0.97 mg/L ÷300 UF). The target serum was then multiplied by EPA's daily clearance factor for PFOA (0.00014 L/kg-day) to express the ADD as a daily dose.

EPA RfD

EPA based its RfD on **Lau et al. 2006**,[89] which was a developmental study in mice that administered oral doses of 1, 3, 5, 10, 20, and 40 mg/kg-day PFOA on gestation days (GD) 1-17. Severe reproductive toxicity (increased incidence of full litter absorptions) was observed ≥5 mg/kg-day dose (external dose). Most neonates in the dose groups ≥10 mg/kg-day PFOA died shortly after birth. Dams showed less weight gain at the end of pregnancy and higher maternal liver weight at all PFOA dose groups. Teratological examination of "at term" fetuses showed reduced ossification of bones at several sites at 1 mg/kg-day with progression to limb and tail defects ≥ 5 mg/kg-day. In live pups, the study observed retarded growth ≥3 mg/kg-day and delayed development of eye opening ≥5 mg/kg-day. Female pups showed slightly altered timing of pubertal maturation compared to controls. Surviving male pups reached puberty early at all doses including almost four days early at 1 mg/kg-day despite a body weight deficit of 25–30 percent compared to controls. The LOAEL for reduced ossification and early puberty in males was 1 mg/kg-day. There was no NOAEL for developmental effects or for liver weight increase in dams.[89]

EPA's point of departure was the LOAEL of 1.0 mg/kg-day. EPA evaluators concluded that alterations in bone development and in timing of puberty observed were unlikely to be secondary to reduced growth. EPA used a toxicokinetic model developed by Wambaugh et al. 2013, to estimate an average maternal rodent serum level of 38 mg/L (internal dose) associated with the LOAEL (external dose). EPA calculated a daily intake in humans that would produce this same average serum level in a human population at steady state. Assumptions included a human serum half-life for PFOA of 2.3 years and a volume of distribution for adults of 0.17 L/kg. The human equivalent dose was 0.0053 mg/kg-day. EPA applied an uncertainty factor of 300 to derive a chronic oral RfD of 20 ng/kg-day.[120]

EPA also calculated candidate RfDs for several other critical effects observed in animal studies including signs of liver necrosis in rats from Perkins et al. 2004, kidney weight changes in adult rats in a two-generation reproductive study by Butenhoff et al. 2004, and reduced immune

response to an antigen challenge in mice by De Witt et al. 2008.[82, 86, 133] The candidate RfD based on developmental effects from Lau et al. 2006 was as low or lower than the other RfDs.

ATSDR MRL

ATSDR selected a different developmental study as the basis of their minimal risk level (MRL): **Koskela et al. 2016 and Onishchenko et al. 2011**. This single study published in two papers,[134, 135] dosed pregnant mice daily from gestation days 1–21 with 0.3 mg/kg-day via their food. Offspring were not administered PFOA doses, but they were allowed to nurse until postnatal day 21. ATSDR estimated a time-weighted average maternal serum level to be 8.29 mg/L (internal dose). Offspring were tested for neurobehavioral and skeletal effects into their adulthood. Subtle measures of physical activity level were increased in PFOA-exposed pups at five to eight weeks of age. Measures of strength, coordination, and response to novelty or response to an adverse task did not differ between controls and treated offspring. Two groups of five offspring were sacrificed at 13 months and 17 months of age and their bones analyzed for skeletal effects. Concentration of PFOA in femurs and tibias of treated animals was four to five times higher than in controls. Subtle changes in bone morphology and mineral density were observed. Skeletal changes in this study extend the observations of Lau et al. 2006 and add additional weight to skeletal effects as a sensitive developmental effect for PFOA in rodents. A 2019 study by NTP provides further support. In this study, adult male rats dosed with 10 mg/kg-day PFOA (plasma concentration was 148.6 mg/L) had signs of bone marrow hypocellularity of mild severity after 28 days of oral exposure.[94]

ATSDR selected the LOAEL (0.3 mg/kg-day; serum level of 8.29 mg/L) as the point of departure and used the same model employed by EPA (with modifications) to calculate a daily intake in humans predicted to produce the same serum level in humans assuming years of exposure. Modified inputs to the model included a volume of distribution for PFOA of 0.2 L/kg and a half-life of 3.8 years in human serum based on observations in an occupational cohort by Olsen et al. 2007.[101] The Olsen study had a longer follow-up time than the Bartell et al. 2010 study[136] used by EPA. ATSDR reasoned that a study with longer follow-up is more likely to represent the initial and terminal rates of serum elimination of PFOA in humans. On the other hand, Olsen et al. was a small (n=24) and mostly male population of retired fluorochemical workers whereas Bartell et al. studied a larger population (n=200) of men and women whose main exposure to PFOA was via drinking water.[136] The resulting human equivalent dose was 0.00082 mg/kg-day, which was divided by an uncertainty factor of 300 to derive an MRL of 2.7 rounded to 3 ng/kg-day.

NJ DWQI and NHDES Target Serums

Both NJ and NH based their health-based value on increased relative liver weight observed in **Loveless et al. 2006**. This was a fourteen-day oral dosing study in adult male mice and rats that tested for toxicity of different mixtures of linear and branched isomers of the ammonium salt of PFOA, ammonium perfluorooctanoate (APFO). There was a 17–20 percent increase in liver weight relative to body weight in male mice at the lowest dose tested (0.3 mg/kg-day). Mean

serum levels of PFOA at this dose were 10–14 mg/L depending on the composition of branched vs. linear PFOA in the test mixture. This was accompanied by a significant increase in peroxisomal β-oxidation activity indicating that PPARα activation played a role in the liver effects observed at the LOEL.[137] Male rats were not as sensitive as mice (LOEL for increased relative liver weight in male rats was 1 mg/kg-day; serum level 48–65 mg/L). Declines in serum lipids were a more sensitive outcome than liver weight in the rat.[137]

These results were supported by similar liver observations in a four-week immune toxicity study in adult male rats and mice by **Loveless et al. 2008** conducted with linear chain AFPO. Daily doses of 0, 0.3, 1, 10, or 30 mg/kg-day PFOA were administered by oral gavage. Serum cholesterol and triglycerides were reduced at 0.3 mg/kg-day in rats and 10 mg/kg-day in mice, liver weight was increased at 1 mg/kg-day in both rats and mice. Signs of liver injury (focal necrosis) were observed at higher doses in rats and mice. Serum PFOA was not measured in this experiment. The LOAEL for immunotoxicity outcomes (suppressed antibody response to sheep red blood cell antigen and atrophy in thymus and spleen) was 10 mg/kg-day in mice. The rat was not sensitive to PFOA immunotoxicity consistent with several other longer duration studies in rats.[83]

New Jersey DWQI used benchmark dose methodology to estimate the serum level associated with a BMDL for 10 percent increase in relative liver weight in mice.[96] A mouse serum level of 4.35 mg/L was their point of departure. They divided this by an uncertainty factor of 300 to derive a target serum level of 14.5 µg/L for humans. Although NJ developed a target serum level rather than a daily dose as the basis for their drinking water MCL, they calculated an RfD of 2 ng/kg-day for comparison purposes using the same model as EPA to estimate an average daily human intake that would result in the target serum level.

NH DES used the same critical study and BMDL analysis as NJ DWQI but applied a smaller uncertainty factor for other toxicities in calculating a human equivalent serum. NH's resulting target serum level was 43.5 µg/L and its RfD was 6.1 ng/ kg-day.

CA OEHHA ADD

CA OEHHA based their acceptable daily dose on adverse effects in liver cells in **Li et al., 2017**. In this study, male and female mice were dosed daily with PFOA by oral gavage for 28 days.[138] Dose groups were 0, 0.05, 0.5, or 2.5 mg/kg-day. At the end of experiment, PFOA was measured in serum and the livers were evaluated for histopathology and levels of protein expression. Significant increases in liver weight were observed in all treatment groups except in low dose males. Histopathology in both sexes showed hepatocellular hypertrophy at doses of 0.5 and 2.5 mg/kg-day and apoptosis (programmed cell death) in hepatocytes at 2.5 mg/kg-day. The study investigated the underlying mechanisms and concluded that apoptosis was triggered by hyperaccumulation of reactive oxygen species in liver cells.[138] While PPARα activation appeared to explain much of the reactive oxygen species generation, PPARα activation was not evident in the low dose females. The mechanism in the low dose females appeared to be leakage of reactive oxygen species from Complex 1 of the electron transport chain in the mitochondria.

CA OEHHA's critical liver effect was loss in mitochondrial membrane potential and increased biomarkers of apoptosis and of oxidative DNA damage in hepatocytes in the low dose female mice. An increase in relative liver weight was also observed in this group. CA evaluators acknowledged that the critical effects selected are upstream physiological changes in liver cells that can lead to adverse effects. CA OEHHA's point of departure was a LOAEL of 0.97 mg/L PFOA serum level in low dose female mice (0.05 mg/kg-day dose group).[132] Their uncertainty factor of 300 included a three-fold factor for database uncertainty based on concern about developmental toxicity observed at a lower oral dose (LOAEL 0.01 mg/kg-day, NOAEL 0.003 mg/kg-day) in a developmental mouse study by van Esterik et al. 2016.[139] That study administered PFOA in maternal feed and did not measure serum levels of PFOA so the internal dose of dams and offspring could not be confirmed. CA OEHHA calculated a target human serum of 3.2 µg/L (0.97 mg/L ÷ 300 UF) and multiplied this by EPA's daily clearance factor for PFOA (0.00014 L/kg-day) to express the ADD as a daily dose (0.45 ng/kg-day). See Table 5.

Discussion of uncertainties

In rodents, especially mice, liver is a sensitive target of PFOA toxicity.[81] PPARα activation has been shown to play a major role in mediating these effects in mouse liver, although PPARα activation is not the exclusive mechanism responsible.[140-142] The human liver is less responsive to PPARα agonists and this introduces uncertainty in extrapolating to humans from dose-response relationships in mice.[56, 143] EPA and ATSDR followed the Hall criteria established by an expert group of scientists to determine adversity of liver effects.[144] Both EPA and ATSDR evaluators concluded that the liver weight changes observed at low doses in mice did not meet criteria for being an adverse effect. Liver weight increase was considered adverse only when accompanied by histological findings of cellular necrosis, inflammation, fibrosis, or steatosis in liver tissue. Specifically, EPA considered clearly adverse liver effects (low-level necrotic cell damage) observed in rats and mice in three studies.[82, 83, 133] The liver effects considered adverse by EPA and ATSDR had higher LOAELs than developmental effects used as the basis of the EPA RfD or the ATSDR MRL.[4, 81] As more information emerges about the mechanisms of action and their applicability to humans, we may reevaluate this endpoint as a point of departure.

Another area of uncertainty for PFOA is the functional significance and relevance to humans of delayed and reduced mammary gland development observed in certain strains of mice.[91-93, 145-148] NJ DWQI added a ten-fold database uncertainty factor for this finding. ATSDR and EPA concluded that this endpoint needed further investigation before it could be interpreted for risk assessment. EPA noted variability in the dose-response between strains of mice and in the scoring of mammary gland development across studies. They also noted that the developmental delay observed at low doses did not have an adverse effect on lactational support of offspring in a two-generation mouse study by White et al. 2011.[93] In their assessment of this endpoint, EPA derived a human equivalent dose of 0.0017 mg/kg-day based on mammary gland effects in Macon et al. 2011.[92] This HED is not as low as the ATSDR HED (0.000821 mg/kg-day) based on

other developmental effects (see Table 5). This endpoint may need to be reconsidered as more data emerges.

Human Relevance

In human observational studies, modest increases in serum liver enzyme levels and other markers suggestive of liver damage have been associated with higher serum levels of PFOA in adults. [103, 104, 149-151] In studies of children, higher prenatal and mid-childhood PFOA concentrations were associated with slight decreases in the liver enzyme ALT indicating potentially better liver function in a Boston birth cohort of children aged eight years old (serum PFOA ranged from 0.1-14.3 ng/L).[152] A cross-sectional study in Atlanta children (aged seven to nineteen years old) with nonalcoholic fatty liver disease found positive associations between the level of serum PFOS and PFHxS, but not PFOA, and increased severity observed in liver histology (liver steatosis, inflammation, fibrosis).[153] PFOA exposure was not associated with an increase of clinically diagnosed liver disease in adults in the large C8 study.[149]

Development effects with some supporting epidemiological evidence are described below. A number of studies have reported small but consistently inverse relationships between maternal PFOA level and birth weight.[97, 98, 154, 155] A downward shift in birthweights across an exposed population might result in more children classified as low birth weight or small for gestational age. These are clinically important risk factors for metabolic and cardiovascular diseases later in life.[156] Confounding by maternal glomerular filtration rate appears to explain some of the association with birth weight observed in studies that measured prenatal PFOA exposure in maternal serum later in pregnancy or in cord blood.[97, 157-159] Two recent high quality studies measured maternal PFOA level early in pregnancy, however, and reported significant inverse associations with birth weight.[98, 155] In addition, Zhu and Bartell (2020) reported that lower average birth weights in county level data correlated with higher concentrations of PFAS (including PFOA) reported in public water systems.[160] Wikstrom et al., 2020, reported a positive association between higher maternal serum PFOA and having a baby that was small for gestational age in girls only.[155] Savitz et al. 2012 did not see an association between prior PFOA exposure and term births that met the definition of low birthweight in the large C8 Health Project.[115, 116] Recent analysis of the Flemish Environmental Health Survey suggested that PFOA might amplify effects of other environmental pollutants on low birth weight.[161]

A small number of epidemiological studies have evaluated associations between PFOA exposure and altered development of reproductive tissue or time of puberty onset. A 2017 review of six studies found that the most consistent evidence associated with higher PFOA exposure was later age at menarche.[162] Early onset or delayed onset puberty are outcomes of concern because of their association with risk of adult diseases such as diabetes, heart disease, and bone disease.[162] Associations between higher serum PFOA and reduced testosterone levels in boys (six to nine years old) and delayed puberty in girls (eight to eighteen years old) were observed in two cross-

sectional studies of children in the C8 Health Project.[106, 163] Another study population with high levels of PFOA in drinking water near a fluoropolymer plant in Veneto, Italy reported that young adult males exposed to high levels of PFOA prenatally and throughout childhood had reduced testicular volume and penile length and shorter anogenital distance compared to a reference population).[107] In studies in the general population with lower serum PFOA levels, age of menarche in girls was associated with prenatal exposure in one study[164] but not another.[165] A study of young adult Danish males (aged 19-21 years) born in 1988-89 reported associations between prenatal PFOA exposure and lower sperm concentration, total sperm counts, and higher serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH).[166] Another Danish study by Ernst et al., 2019, evaluated a range of markers of pubertal development in boys and girls in the longitudinal Danish National Birth Cohort. They reported that prenatal PFOA level was not associated with any of the markers evaluated. However, both inverse and positive associations were observed between various puberty markers and other PFAS.[167] No association between maternal serum PFOA and anogenital distance in three-month-old male Danish infants was observed in the longitudinal Odense Child Cohort.[168]

Several longitudinal birth cohort studies have investigated breastfeeding duration as an outcome potentially associated with impaired mammary gland differentiation and development. Fei et al. 2010, reported that higher serum PFOA levels in mothers during pregnancy was associated with shorter durations of breastfeeding among only multiparous women in the Danish national birth cohort. Previous breastfeeding duration was not controlled for.[169] Since pregnancy and lactation are demonstrated PFOA excretion pathways for women, studies examining breastfeeding duration must carefully control for parity and prior breastfeeding duration. Timmerman et al. 2017 reported that PFOA serum level in primiparous women in a Faroe Islands cohort was associated with reduced duration of breastfeeding.[170] Ramono et al. 2016 also reported an association with reduced duration of breastfeeding in a Cincinnati cohort that controlled for previous breastfeeding duration.[171] A larger cohort study by Rosen et al. 2018 in the Norwegian MoBa Cohort found no association between maternal serum PFOA and breastfeeding duration and in fact observed longer breastfeeding durations associated with some other PFAS. This study controlled for confounding by parity and prior breastfeeding duration.[172]

There are limited skeletal observations in human studies. PFOA has been measured in bone in adult human cadavers[5, 6] and associations have been reported between serum PFOA level and lower bone density in women,[173] smaller bone mass and size in British girls,[174] and lower bone mineral density in children in the Project Viva cohort assessed during mid-childhood. Impaired bone accrual during childhood increases the risk of osteoporosis later in life.[175] In a case control study of a Saudi women with osteoporosis, those with higher serum PFAS, including PFOA, had higher odds of an osteoporosis diagnosis.[176]

A number of epidemiological studies have investigated neurodevelopmental outcomes associated with elevated PFOA exposure. While a few studies reported positive associations with hyperactivity[177, 178] most reported null or even inverse associations.[179-182]

Washington State Recommendation: 3 ng/kg-day

We selected the ATSDR's MRL of 3 ng/kg–day based on developmental effects in mice as the best basis for drinking water state action levels. In both the EPA and ATSDR evaluations, developmental endpoints yielded health protective values that were as low as or lower than liver injury and immunotoxicity endpoints.

There are sufficient supporting toxicity data demonstrating PFOA's developmental toxicity in fish, rats, mice, and monkeys.[4, 90] Epidemiological studies (discussed above) support an association between gestational exposure to PFOA and small reductions in fetal growth in humans. Epidemiological evidence is still limited regarding PFOA's potential to alter time of puberty, impair development of reproductive tissues, alter skeletal development, or produce neurodevelopmental outcomes.

Sensitive subpopulations. While most studies of developmental toxicity in animals administered PFOA during gestation, some studies have demonstrated that postnatal exposure alone resulted in decreased postnatal growth and altered behavior in adulthood mature mice.[91, 183] Overall, toxicity studies available for PFOA demonstrate that early life stages are sensitive to PFOA-induced toxicity.[81] Based on the rodent data, we expect fetal and infant periods to have the highest sensitivity to developmental effects. Later childhood developmental periods such as puberty could also be sensitive as these are periods of rapid growth and development.

Relative Source Contribution (RSC): 50 percent

RSCs were developed for children and adults (see Table 2) with the subtraction method and the EPA Exposure Decision Tree described in EPA's methodology.[58] The RSCs for PFOA were 50 percent for infants, children, and adults. The target or reference serum at the PFOA MRL is 27.6 µg/L. The serum contribution from drinking water sources should not exceed 50 percent of that target serum level: 13.8 µg/L (27.6 µg/L x 0.50).

Water Intake Rate: MDH model

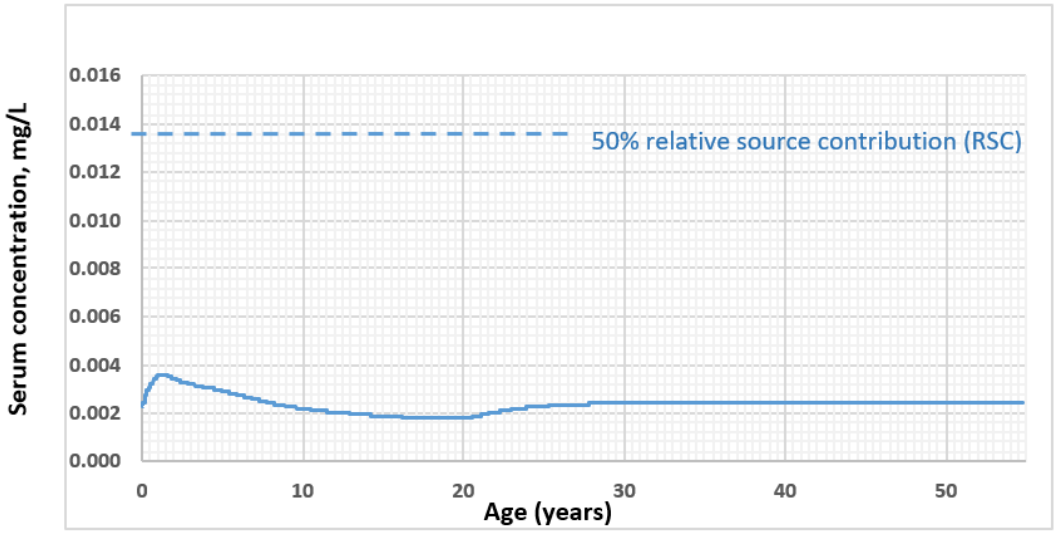
As discussed in the Introduction to Approach and Methods, we used a Minnesota Department of Health model with age-specific drinking water rates that includes transplacental and lactational exposure routes in estimating exposure from PFOA in drinking water across the life course.

We assumed age-specific water intake rates at the 90th percentile for chronic periods of exposure (children >one year old and adulthood). We assumed 95th percentile drinking water intake rates for lactating women and for formula-fed infants (assuming powdered formula is mixed with tap water). Breastfed infants were assumed to be exclusively breastfed at the 95th percentile intake rate for six months and then gradually tapered off breastmilk over the

following six months while other foods and drinks are introduced. Foods introduced include juices or infant formula mixed with tap water.

The model outputs are provided below (Figure 5). A chronic drinking water level of 10 ng/L PFOA was the maximum concentration that allowed serum levels of infants and children to remain within the 50 percent RSC for drinking water sources. The peak serum level predicted for breastfed infants as a result of 10 ng/L PFOA in drinking water was 13.4 µg/L. Formula-fed infants peaked at 3.6 µg/L PFOA in serum. Maternal serum level attributed to drinking water at the time of pregnancy was 2.6 µg/L and the expected starting serum for infants at birth was 2.3 µg/L.

A) Formula-fed Scenario for 10 ng/L PFOA in Drinking Water



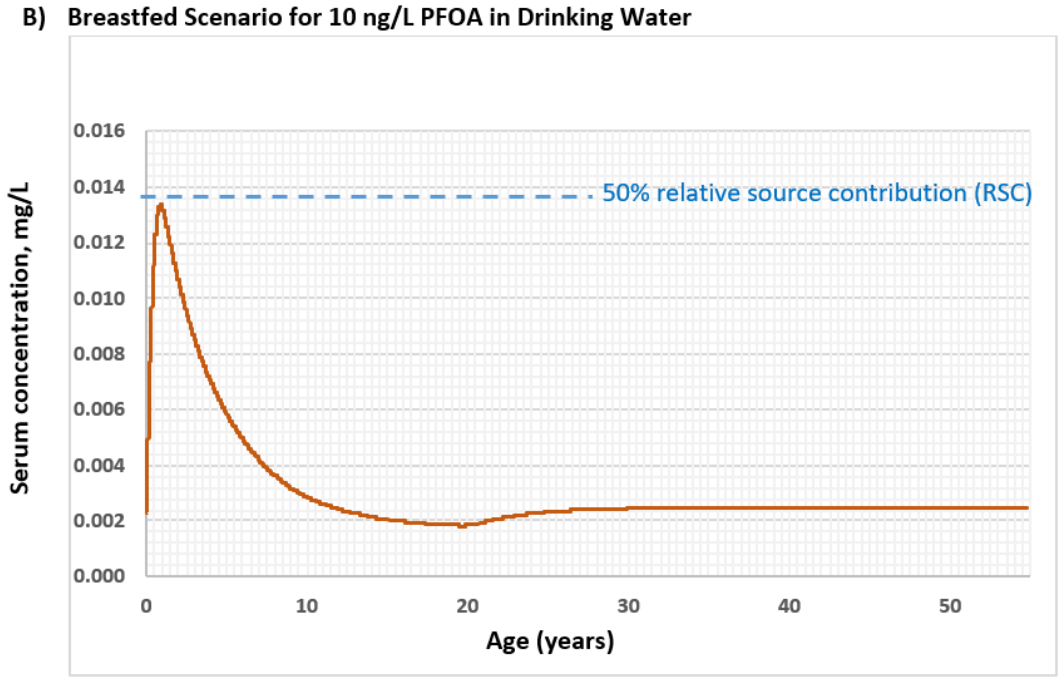
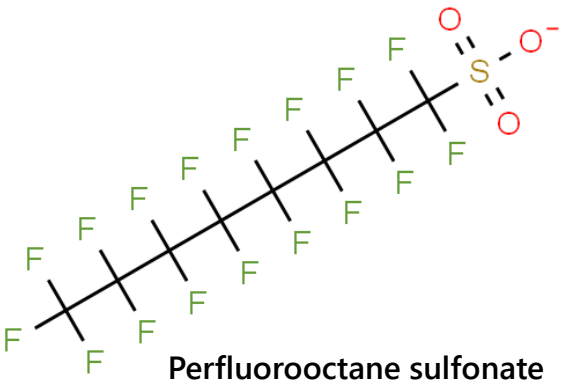


Figure 5. *Model predicted PFOA serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFOA in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the rest of childhood and adulthood. For breastfed infants, exclusive breastfeeding was assumed for the first six months with gradual tapering until one year of age. After one year, breastfed infants are assumed to drink water at the 90th percentile intake rate for their age group. The dotted lines represent the maximum allowable PFOA serum level from drinking water only, as determined by the RSC for the age group. It represents the percentage allotted to drinking water sources of the acceptable daily PFOA intake from all sources.*

Deriving the State Action Level for PFOS



Perfluorooctane sulfonate

Perfluorooctane sulfonic acid (PFOS) has eight fully fluorinated carbons with a sulfonic acid group at one end. In drinking water, PFOS dissociates into its anion form: perfluorooctane sulfonate (shown here). PFAS substances that can breakdown to PFOS in the environment are referred to as precursors. PFOS and precursors were used to make consumer products such as stain and water repellent textiles (clothing, carpets, upholstery, tents, etc.), aftermarket stain and waterproofing sprays, and food contact papers and containers.[4] PFOS and precursors have also been used in aqueous film forming foams (AFFF) for firefighting and for a wide range of industrial and consumer uses as surfactants and emulsifiers. In the U.S., manufacturing of PFOS and precursors began in the 1940s and was mostly discontinued by the end of 2002.[15, 184] Some U.S. commercial uses were allowed to continue (e.g., AFFF, metal plating, aviation fluids, photograph development).[185] PFOS production also continued in other countries.[186] PFOS has no known natural degradation pathway. It persists in the environment and can leach into groundwater from surface soils.[48]

In national surveys, nearly every person tested has detectable levels of PFOS in their blood serum.[14] The phase-out in U.S. production resulted in a decade of steady declines in serum levels in the U.S. (see Figure 2). Between 1999-2000 and 2011-2012, there was a 78 percent decline in the median serum PFOS level in the U.S. population. Since 2012, declines in mean PFOS serum levels have flattened suggesting ongoing exposure.[13, 14] The latest CDC NHANES survey of the U.S. general population (aged 12 and older) from 2017-18 reported 4.25 µg/L as the mean PFOS serum level and 14.6 µg/L as the 95th percentile of the population distribution.[14] Current U.S. exposures are thought to stem primarily from environmental and industrial contamination of food and drinking water and from release of PFOS and precursors from older products such as treated carpets and textiles in our homes.[4] PFOS bioaccumulates in humans because it is so slowly excreted from the body. Estimates of average PFOS half-life in human serum were 3.3—3.4 years in two studies of populations exposed to PFOS via contaminated water.[17, 187] Men appear to have slower elimination rates than women.[17, 24]

The primary types of toxicity observed in experimental animals exposed to PFOS are developmental toxicity,[188-190] immune suppression,[191-195] liver and kidney toxicity,[196-198] and disruption of thyroid and other hormones.[199-203] PFOS does not appear to be mutagenic or genotoxic but chronic rodent studies observed liver, thyroid and mammary gland tumors,[204]

The most consistent findings from human epidemiological studies are positive associations between serum PFOS and higher serum cholesterol,[111, 205-207] reduced antibody response to

vaccines,[35, 99] and reduced birth weight.[208] Other endpoints of concern with less evidence include elevated uric acid,[108, 109] altered energy metabolism and glucose intolerance,[209-211] altered hormone levels,[212-214] thyroid disease,[113, 215, 216] and chronic kidney disease.[108, 217]

Data relevant to cancer risk of PFOS are limited. The EPA concluded there is "suggestive evidence for carcinogenic potential" in humans based on liver and thyroid adenomas observed in the chronic rat study by Butenhoff et al. 2012.[204] This study reported a dose-dependent increase in hepatocellular adenomas in both male and female rats at the highest dose.[204] Thyroid follicular cell adenomas and carcinomas were observed in both the male and female rats; however, NJ DWQI and EPA evaluators concluded they were of unclear biological significance and lacking a clear dose-response relationship.[184, 218] Mammary gland tumors in female rats were observed but also lacked a dose-response pattern.[184] Epidemiological evidence reviewed by ATSDR mostly did not find statistically significant increases in cancers associated with PFOS exposure in occupational cohorts, the C8 health project, or the general population.[4] The exception is mixed evidence of associations between breast cancer and PFOS in four case-control studies. PFOS was positively associated with breast cancer in Greenland Inuit and Taiwanese women.[219, 220] Two larger case-control studies of breast cancer in California and Danish women found no such association.[127, 221]

Reviewing Health Protective Values

DOH reviewed the available health protective values (RfD, MRL, target serum level) for daily ongoing human intake of PFOS. We focused on high-quality and comprehensive risk evaluations that considered current scientific research and were conducted by U.S. federal and state agencies. We focused on noncancer effects (see page 13). Health protective values included reference doses (RfDs) derived by EPA and the Minnesota Department of Health, a minimal risk level (MRL) derived by ATSDR, an acceptable daily dose (ADD) derived by CA OEHHA and target serum levels derived by NJ DWQI and NHDES. Target serum levels are analogous to an RfD except on a serum basis. These health protective values are shown in Table 6 below.

Table 6: Health Protective Values for PFOS Reviewed by Washington

Source	Critical study	Critical effect	Human Equivalent dose	Uncertainty Factors (UF)[a]	Oral RfD, MRL, ADD, Target Serum Level[b]	Exposure duration
EPA 2016[184]	Luebker et al. 2005a	NOAEL (0.1 mg/kg-day) for reduced pup weight and developmental delays in rats in a 2-generation rat study Average maternal serum level at NOAEL: 6.26 mg/L	0.00051 mg/kg-day	UF_{total} 30 UF^H =10 UF^A =3	20 ng/kg-day (RfD)	Chronic
NJ 2018[218, 222] Also CA OEHHA 2019[132]	Dong et al. 2009	NOAEL (0.0083 mg/kg-day) for reduced immune response in adult mice (decreased plaque-forming cell response) dosed for 60-days. Serum level measured 24 hrs. after last dose at the NOAEL: 0.675 mg/L LOAEL: 7.1 mg/L		UF_{total} 30 UF^H=10 UF^A=3	22.5 µg/L (target serum level)[c] 1.8 ng/kg-day (RfD/ADD)[d]	Chronic
ATSDR 2021[4]	Luebker et al. 2005a	NOAEL (0.1 mg/kg-day) for reduced pup weight and developmental delays in rats in a 2-generation rat study TWA maternal serum level at the NOAEL: 7.4 mg/L LOAEL: 29.7 mg/L	0.000515 mg/kg-day	UF_{total} 300 UF^H=10 UF^A=3 MF=10	2 ng/kg-day (MRL)	Intermediate (2-52 wks.)
MN 2019[73]	Dong et al. 2011	NOAEL (0.0167 mg/kg-day) for immune endpoints (increased IL-4 cytokine production, reduced IgM antibody response to immunization) in adult male mice dosed for 60 days. Serum level measured 24 hrs. after last dose at the NOAEL: 2.36 mg/L LOAEL: 10.75 mg/L	0.000307 mg/kg-day (2.36 mg/L x 0.000128 L/kg-day DAF)[e]	UF_{total} 100 UF^H=10 UF^A=3 UF^D=3	3.1 ng/kg-day (RfD)	Short-term and chronic
NH 2019[77, 131]	Dong et al. 2011	NOAEL (0.0167 mg/kg-day) for immune endpoints (increased IL-4 cytokine production, reduced IgM antibody response to immunization) in adult male mice dosed for 60 days. Serum level measured 24 hrs. after last dose at the NOAEL: 2.36 mg/L LOAEL: 10.75 mg/L		UF_{total} 100 UF^H=10 UF^A=3 UF^D=3	23.6 µg/L (target serum level) 3.0 ng/kg-day (RfD)[e]	Chronic

[a] Uncertainty factors: UF_H= intra-speciesl uncertainty factor; UF_A= inter-species uncertainty factor; UF_S= subchronic to chronic uncertainty factor; UF_L= LOAEL to NOAEL uncertainty factor; UF_D= incomplete database uncertainty factor; UF_{total}= total (multiplied) uncertainty factor.

Uncertainty factors are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing a 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10. MF = Modifying factor.

[b] RfD= Reference dose, MRL = minimal risk level, ADD = acceptable daily dose, target serum level is the concentration of the PFAS in serum associated with an oral intake rate at the RfD or MRL.

[c] The target serum was calculated by dividing 0.675 mg/L (NOAEL) by 30 (UF_{total}).

[d] The RfD of 1.8 ng/kg-day was calculated by multiplying the target serum by the clearance factor developed by EPA (2016): (8.1 x 10^{-5} L/kg-day x 22.5 µg/L = 0.00182 µg/kg-day or 1.8 ng/kg-day). CA OEHHA concurred with this approach.

e Minnesota derived a PFOS clearance factor for PFOS of 0.000128 L/Kg-day derived from a serum half-life of 1241 days (Li et al 2018) and volume of distribution 0.023 L/kg (EPA, 2016). New Hampshire concurred with this approach.

EPA RfD and ATSDR MRL

EPA and ATSDR both conducted detailed evaluations of the scientific literature relevant to PFOS. They derived their health protective values for PFOS from a NOAEL of 0.1 mg/kg-day for developmental effects (decreased pup body weight) in a two-generation rat study by Luebker et al. 2005a[189] with support from Luebker et al. 2005b.[190]

The **Luebker et al. 2005a** study exposed rats to PFOS over two generations and studied reproductive parameters, pup growth, developmental milestones, and neurobehavioral function. At the 0.4 mg/kg-day dose, the first generation of offspring had slight delays in eye opening and the second generation had slightly lower birthweights. At the two higher doses (1.6 and 3.2 mg/kg-day), impaired growth, development and mortality in newborn pups was observed. All the pups at the higher dose died. Only the pups from the 0.1 and 0.4 mg/kg-day doses were in acceptable condition to continue in the study and complete the second cycle of breeding. After weaning, a subset of males and females from the first generation of offspring were tested on learning and memory tasks. No differences were observed on tasks related to learning or memory at PFOS doses of 0.1 and 0.4 mg/kg-day. The LOAEL for slight developmental effects was 0.4 mg/kg-day and the NOAEL was 0.1 mg/kg-day.[189]

A second reproductive and developmental study (one-generation study design) by **Luebker et al. 2005b** used additional doses in the low dose range to better define the dose-response and to support benchmark dose modeling of a minimal response in the observed outcome.[190] Administered doses were 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg-day. Dosing of female rats occurred for six weeks prior to mating with untreated males, through mating, gestation, and four days of lactation. Reduced birth weight and weight gain was observed in pups at all PFOS doses in the absence of any differences in maternal weight gain during pregnancy. The BMDL for a 5 percent reduction in the mean birth weight per litter was a maternal dose of 0.39 mg/kg-day. Again, reduced pup survival at the higher doses was observed. Over 70 percent of the dams at the 2.0 mg/kg-day dose had all pups die within five days of birth. The BMDL for a 5 percent decrease in survival of pups between postnatal days one and five was a maternal dose of 0.89 mg/kg-day. Serum total thyroxine (tT4), measured at lactation day five, was sharply reduced in dams and pups at all doses tested without a statistically significant change in TSH. However, thyroid hormone results were not consistent across two measurement methods employed.

Gestational length was also shorter in a dose-dependent manner in both Luebker et al. experiments.

The NOAEL in Luebker et al. 2005a was 0.1 mg/kg-day. EPA applied toxicokinetic models to estimate an average maternal serum level at the NOAEL (6.26 mg/L) and to estimate daily intake in people that would result in an average equivalent serum level. Their clearance factor was 8.1 x 10^{-5} L/Kg-day (assumed volume of distribution was 0.23 L/kg and serum half-life was 5.4 years). This estimated daily intake, called the human equivalent dose, was 0.00051 mg/kg-day. It is much lower than the daily dose in rats required to reach this same average serum level because PFOS is much more bioaccumulative in humans than in rats. EPA applied a 30-fold uncertainty factor consisting of a ten-fold factor (UF_H) to account for variability in individual human responses and a three-fold factor (UF_A) to account for differences between rats and humans.[184]

EPA also evaluated other endpoints and derived candidate RfDs based on elevated biomarkers of liver damage in rats and monkeys from Seacat et al. 2002 and 2003,[198, 223] developmental neurotoxicity in rats from Butenhoff et al. 2009,[224] and reduced pup weight and neonatal mortality in another rat study by Lau et al. 2003.[188] The RfD from the Luebker et al. study was lower than or equal to the other RfDs and was carried forward in the risk assessment. For immunotoxicity, EPA concluded that "Taken together, the lower antibody titers associated with PFOS levels in humans and the consistent suppression of sheep red blood cell response in animals indicates a concern for adverse effects on the immune system. However, lack of human dosing information and lack of low-dose confirmation of effects in animals for the short duration study precludes the use of these immunotoxicity data in setting the RfD."[184]

Risk assessors at ATSDR also selected developmental effects as the most sensitive effect that they were confident modeling. ATSDR modeled a time-weighted average for maternal serum level at the LOAEL and NOAEL in Luebker et al. 2005a and reported these as 29.7 and 7.4 mg/L respectively.[4] They concurred with EPA that immunotoxicity was observed in mice at doses 1-10 times lower than the developmental endpoint selected. ATSDR did not have the necessary pharmacological data to estimate time-weighted average serum concentration over the 60-day dosing period in the strain of mice used by Dong et al. Instead they applied a ten-fold modifying factor to account for insufficient pharmacological data in critical studies for immunotoxicity. In support of this 10-fold factor ATSDR calculated a "candidate MRL" of 3 ng/kg-day based on the NOAEL for immune toxicity in Dong et al 2011.[4]

New Jersey Target Serum and CA OEHHA ADD

Mouse studies have shown that PFOS exposure reduces antibody responses to sheep red blood cell antigen, reduces survival after exposure to influenza A virus, alters immune cell populations, and suppresses immune function in adult mice.[191-195] When mice received PFOS exposure during pregnancy, similar immune effects were observed in their offspring at eight weeks of age.[225] State risk assessments (MN, NJ, NH, CA) based their health protective values on immunotoxicity endpoints in mice in Dong et al. 2009 or 2011, which are descried briefly below. A key assay used in these studies, the sheep erythrocyte T-dependent antibody response (or

TDAR), evaluates the ability of animals sensitized *in vivo* to produce primary IgM antibodies to sheep red blood cells (SRBC). This assay is highly regarded as a sensitive indicator of functional immunosuppression in animals and is relevant to adaptive humoral immunity in humans. Assay response requires antigen recognition and presentation, T cell and B cell signaling, and class switching, and thus can detect immunosuppression across a range of cell types and signals.[35]

NJ DWQI selected **Dong et al. 2009** as the critical study to derive an RfD based on evidence of immune suppression in adult male mice. This study dosed male C57BL/6N mice for 60 days by oral gavage. The NOAEL (0.008 mg/kg-day) and LOAEL (0.083 mg/kg-day) resulted in serum PFOS levels at the completion of the dosing of 0.67 and 7.1 mg/L, respectively. At and above the LOAEL, SRBC-specific IgM plaque forming cell response was reduced in a dose-dependent manner. Natural killer cell activity was increased by 38 percent at the LOAEL but was decreased compared to controls at the higher doses. Higher doses also reduced body weights, organ weights (kidney, thymus and spleen), and reduced thymic and splenic cellularity. The LOAELs for immune suppression were also LOAELs for increased liver weight in this study. NJ applied a ten-fold uncertainty factor for human variability (UF_H) and a three-fold factor to account for uncertainty in applying mouse data to humans (UF_A).[191] CA OEHHA concurred with this approach and adopted 1.8 ng/kg-day and their ADD for PFOS (see Table 6).

Minnesota RfD and New Hampshire Target Serum

Minnesota Department of Health (MDH) selected a different critical study by **Dong et al. 2011.**[192] This 60-day companion study was similar to Dong et al. 2009 but it evaluated the balance of cytokines associated with T-helper cell subsets (T_H1 and T_H2) that may underlie the reduced IgM response to SRBC antigen. The study observed a dose-related suppression of SRBC-specific IgM synthesis in adult male mice immunized with SRBC. In this experiment, IgM antibodies were assessed in the serum by ELIZA rather than by plaque forming cell response. Serum levels of IgG and IgE were elevated at the highest dose.[192] Cytokine evaluation showed increased IL-4 cytokine levels in the spleen and a pronounced shift to a more T_H2-type dominant immune state with excess type 2 immune responses and deficient type 1 immune responses. Suppressed IgM response in this TDAR study had the same LOAEL as Dong et al. 2009 (0.0083 mg/kg-day). The NOAEL (0.0167 mg/kg-day) in Dong et al 2011 was slightly higher as the second experiment added an extra dose group in the low dose range.

MDH derived an RfD as follows. The serum concentration at the NOAEL (2.36 mg/L) was multiplied by a dosimetric adjustment factor (DAF) to calculate a human equivalent dose. The DAF (0.00013 L/kg-day) assumed a half-life of 1241 days (3.4 years) for PFOS in human serum and a volume of distribution of 0.23 L/kg. The human equivalent dose was 0.000307 mg/kg-day. MDH applied a total uncertainty factor of 100 that included a three-fold factor for database uncertainty (UF_D) based on the need for a more complete assessment of developmental exposures and immune effects and T4 thyroid hormone reductions. MDH noted that two studies in developing rats reported decreased serum thyroxine (T4) in dams and pups at serum levels equivalent to the NOAEL of Dong et al 2011.[203, 226] MDH's resulting RfD was 3.1 ng/kg-day.[73]

NHDES concurred with Minnesota's inputs and approach. The NHDES target serum was 24 ug/L and their associated RfD was 3.0 ng/kg-day. The NHDES RfD differs slightly because MDH rounded their DAF and NHDES did not.[77]

Discussion of Uncertainties

Risk evaluators relied on different estimates of half-life for PFOS in humans when deriving human equivalent doses. ATSDR and EPA relied on an estimate of serum half-life reported from retired fluorochemical workers in Olsen et al 2007.[101] This study collected periodic blood samples from 24 male and two female workers over a five-year period. Initial mean PFOS level in serum of participants was 799 µg/L. MDH and NHDES used a serum half-life from Li et al. 2018, a study of 106 residents of Ronneby, Sweden (age 4–84 years, 53 percent female) who were exposed to PFOS in their municipal drinking water.[17] Samples were collected periodically over a two year period. Median initial serum PFOS level in the group was 345 µg/L (range 24–1500 µg/L). The drinking water exposure scenario and the wider representation of all ages and women in this cohort make it better suited to our transgenerational model for exposure assessment which includes maternal placental transfer and breastfeeding exposures from drinking water.

Risk evaluators differed in their selection of the critical study for suppressed immune response. Both Dong et al. 2009 and Dong et al. 2011 observed a LOAEL at 0.083 mg/kg-day for impaired immune response to SRBC in male adult mice exposed to PFOS for 60 days. Both metrics measured, IgM suppression and plaque forming cell response, describe different aspects of the same immune process and taken together support a POD at the NOAEL for this endpoint. ATSDR, MN and NH concurred that the intermediate dose included in the Dong et al. 2011 study provides more granular information about the NOAEL and was suitable as a point of departure.

MDH and NHDES concurred on a 3-fold UF for database uncertainty in part due to the emerging evidence on thyroid hormone disruption and its potential impact on neurodevelopment which has not been well studied. We agree with this UF and note that a number of *in vitro* and *in vivo* animal models, recently reviewed by Coperchini et al. 2021, indicate that PFOS has thyroid disrupting effects on circulating thyroid hormone levels, thyroid signaling, the structure of thyroid follicular cells, and early brain development in Xenopus embyros.[227]

Human Relevance

Both immune and developmental endpoints have supporting epidemiological data to indicate their relevance for humans.

In adults and children, PFOS exposure has been associated with suppressed antibody response to vaccines in a number of studies in different populations.[228-232] For example, an investigation of childhood response to vaccines from birth cohorts in the Faroe Islands showed that PFOS exposure was associated with lower antibody responses to childhood diphtheria and tetanus

immunizations.[228] These authors reported a 39 percent decrease in diphtheria antibody concentrations at age five for each doubling of the PFOS exposure in maternal serum. Additionally, higher serum PFOS at age five correlated with greater risk of falling below clinically protective serum levels for both tetanus and diphtheria antibodies at age seven.[228]

There are limited studies of PFOS exposure and the risk of infectious disease. In several longitudinal birth cohort studies, higher prenatal PFOS exposure (measured in maternal or cord blood) correlated with indicators of increased infectious disease during childhood including: higher risk of hospitalization for infectious disease for girls but not boys,[233] higher number of days with fever,[234] and more lower respiratory tract infections.[235, 236] No association with prevalence of allergies and infectious diseases was observed in children followed to age seven in the Hokkaido longitudinal study.[237] A cross-sectional study in the C8 study population did not find associations between PFOS and the frequency of cold or flu infections in adults.[238] Overall, the evidence for altered risk of infectious disease is mixed and inconclusive. More studies that stratify by sex may be important in clarifying whether PFOS exposure affects the risk of infections.

The National Toxicology Program conducted a systematic review in 2016 of evidence for immune toxicity from epidemiological studies and studies in experimental animals and concluded that PFOS met their criteria of a "presumed immune hazard" in humans.[99] This was based on high confidence that PFOS is immunotoxic in rodents and moderate evidence of immunotoxicity in humans. Specifically that "the results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOS were associated with suppression in at least one measure of the anti-vaccine antibody response to common vaccines across multiple studies."[99]

A large number of epidemiological studies have evaluated associations between PFOS and developmental/reproductive outcomes. See reviews by EPA in 2016 and ATSDR in 2021.[4, 184]

Two meta-analyses of epidemiological studies support an association between PFOS and lower birth weights. A systematic review by Koustas et al. 2014 found higher PFOS exposure was consistently associated with lower birth weights.[208] A meta-analysis of seven studies by Verner et al. 2015 reported that overall, for every increase of 1 µg/L in prenatal serum PFOS, there was a five gram reduction in birthweight of babies.[157] Verner et al. investigated possible confounding of this association by the mother's glomerular filtration rate (i.e., women with lower GFR during pregnancy would tend to have smaller babies and higher blood PFOS levels). Their results indicate that GFR may explain some but not all of the association.[157] Meng et al. 2018 reported that for every doubling of PFOS in maternal serum, birthweight declined 45 grams in a study in the Danish National Birth Cohort.[98] Wikstrom et al. 2020, reported that maternal serum PFOS was associated with reduced birth weight and small for gestational age in a prospective birth cohort. In age-stratified results, the association was stronger in girls.[155] Both Meng et al. and

Wikstrom et al. measured maternal PFOS early in pregnancy when GFR is less likely to confound results on birthweight.

In the Danish National Birth Cohort, the odds ratios of preterm birth were about two-fold higher in the top three quartiles for maternal PFOS exposure compared to the lowest quartile.[98] In the large C8 Health Project cohort, PFOS serum level was associated with self-reported preeclampsia and low birth weight (defined as birth weight < 2,500 g), but not with preterm-birth or miscarriage in the previous five-year period within the cohort.[117] Two follow-up studies by Darrow et al. evaluated reproductive outcomes following serum PFAS measurement in women (99 percent of the births occurred within three years of serum collection).[239, 240] These studies found no association between preconception PFOS maternal serum level and low birth weight babies (defined as < 2,500 g) or pre-term births. However, higher PFOS exposure was associated with slightly lower birth weights and with higher risk of pregnancy-induced hypertension.[239] There was no association with miscarriage among pregnancies overall but a slight association with PFOS and miscarriage in nulliparous women.[240] There was no evidence of birth defects or increased risk of stillbirths evident in over 10,000 births evaluated as part of the C8 Health Study cohort.[117, 165]

Only limited and mixed evidence is available on timing of pubertal developmental in children. PFOS serum level in girls was associated with delayed menarche in a cross-sectional study in the C8 study cohort.[106] Doubling of serum PFOS level was inversely associated with serum testosterone in boys and estradiol in girls indicating delayed sexual maturation.[106] A prepubertal cohort (ages six to nine years) from the same C8 study population had similar inverse associations between serum PFOS and estradiol, testosterone, and insulin-like growth factor-1 in boys. Girls had similar results for testosterone and insulin-like growth factor-1.[163] Prenatal PFOS exposure was associated with decreased odds of earlier age at menarche in a British birth cohort[165] and no association with markers of puberty in girls or boys in two other studies.[164, 166] Ernst et al. 2019 reported a non-monotonic pattern for prenatal PFOS exposure and markers of puberty in girls in the Danish National Birth Cohort (n= 1167 children).[167] Compared to the lowest tertile of exposure, girls in the middle tertile had lower age of onset for most pubertal milestones measured. Some of the markers however showed higher age at onset when comparing the third tertile with the lowest tertile. In boys, the estimated average age of onset for most pubertal markers was slightly reduced in the second and third tertiles of PFOS prenatal exposure compared to the lowest exposure tertile.[167]

Washington State Recommendation: 3.0 ng/kg-day

We concurred with MDH and NHDES on their derivation of the RfD for PFOS based on immune effects in Dong et al. 2011. The RfD without rounding of the DAF is 3.0 ng/kg-day. While rodents are sensitive to both immune and developmental effects of PFOS, reduced antibody response to an antigen appears to be a more sensitive endpoint. Serum levels in mice at the LOAEL in Dong et al. 2011 were similar to the serum levels in rats at the NOAEL for developmental effects in

Luebker et al 2005a. While there are uncertainties in the toxicokinetics for the mouse strains used in various immune studies, the critical study, Dong et al. 2011, measured PFOS levels in mouse serum at the end of the experiment. The experiment was 60 days long and was supported by two other 60-day studies in the same strain of mouse with similar serum measurements indicating reproducibility (Dong et al. 2009 and 2012).[191, 241]

TDAR assays, like the one used in Dong et al. 2011, are validated, well regarded evidence of immune suppression and are relevant to humans.[35] In adults and children, PFOS exposure has been associated with suppressed antibody response to vaccines in a number of studies in different populations (see discussion under Human Relevance). The 2016 systematic review by the National Toxicology Program supports the relevance of reduced antigen response in laboratory animals to reduced antibody response to vaccines in children and adults.[99]

Sensitive populations. Infants and children are sensitive life stages for immune effects associated with PFOS exposure. Infants and children receive a number of vaccinations to protect them from serious infectious diseases before the age of five. Suppressed antibody production erodes the protection of vaccines and represents a functional decrease in interception and clearance of infectious agents. Failure to reach a clinically protective antibody response puts children at risk for serious infectious diseases. The studies in mice indicate that adult male mice are sensitive to antibody suppression associated with PFOS exposure so we considered human adults a target population for protection. Sensitive subgroups of adults may include people with autoimmune and other immune deficits. Immune function naturally declines with age so older adults could also be at increased risk.

Relative Source contribution (RSC): 50 percent infants, 20 percent adults

RSCs were developed for children and adults (see Table 2) with the subtraction method and the EPA Exposure Decision Tree as described in our Introduction to Approach and Methods section. The RSCs for PFOS were 50 percent for infants and children, 35 percent for women of childbearing age, and 20 percent for all adults (both sexes). The lower RSC for adults is a result of the higher background PFOS serum levels in men in the general population. Because the immune effects in rodents were observed in adult male rodents, we used a 20 percent RSC for all adult populations (the lower of the two RSCs for adults). The target or reference serum at the RfD is 23.6 µg/L. At 20 percent RSC for adults, the contribution from drinking water should not exceed 4.7 µg/L in the serum (23.6 µg/L x 0.20). For infants and children, we used a 50 percent RSC. The serum contribution from drinking water should not exceed 11.8 µg/L for PFOS.

Minnesota and New Hampshire also followed the Exposure Decision Tree approach described in EPA's methodology (USEPA 2000) and the subtraction or the percentage method to derive RSCs. Minnesota derived an RSC of 50 percent for infant and young children and an RSC of 20 percent for chronic exposure in adult populations. New Hampshire derived an RSC of 50 percent. EPA. ATSDR, and CA OEHHA all set the PFOS RSC at 20 percent.

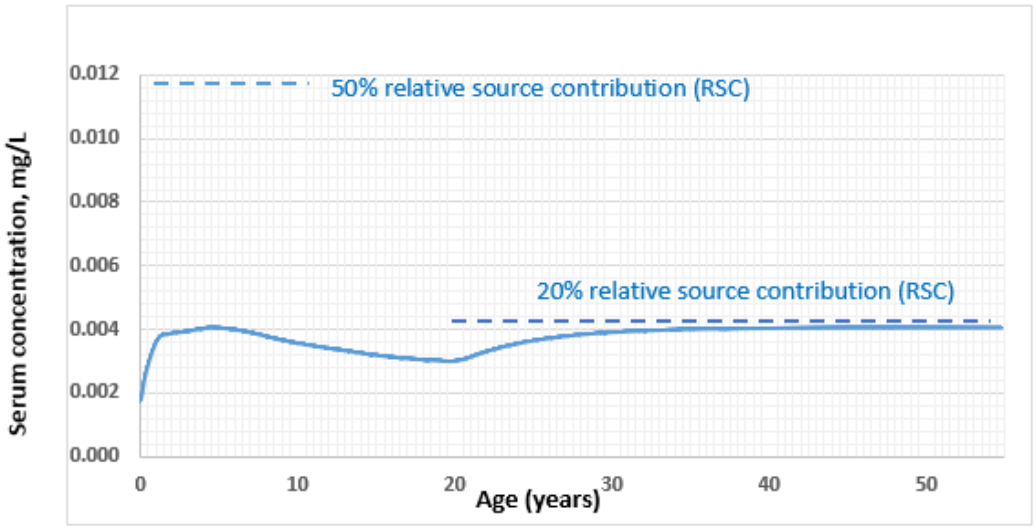
Water Intake Rate: MDH model

As discussed in the Introduction to Approach and Methods section, we used a Minnesota Department of Health model and age-specific drinking water intake rates to estimate serum levels in children, resulting from PFOS in community drinking water. The model includes placental and lactational transfer to offspring of mothers that are chronically exposed to the drinking the water. The model also estimates exposure to infants fed formula mixed with drinking water that contains PFOS.[73]

We assumed age-specific drinking water intake rates at the 90th percentile for chronic periods of exposure (children >one year old through adulthood). Following birth, we assumed 95th percentile drinking water intake for lactating women, and the 95th percentile drinking water ingestion rates for formula-fed infants (assuming powdered formula is mixed with tap water). Breastfed infants were assumed to be exclusively breastfed for six months and then gradually tapered off breastmilk over the following six months with other foods and drinks introduced including juices or infant formula mixed with tap water.

The model outputs are shown in Figure 6. A chronic drinking water level of 15 ng/L PFOS was the maximum concentration that allowed serum levels of adults to remain within the 20 percent RSC for drinking water sources. At this concentration in drinking water, infants and children remained below the 50 percent RSC. Maternal PFOS level at time of pregnancy (4.3 µg/L) was used to calculate the starting serum at birth for infants (maternal serum x placental transfer ratio), which was 1.7 µg/L. The peak serum level predicted for breastfed infants resulting from 15 ng/L PFOS in drinking water was 8.6 µg/L. Formula-fed infants peaked at reached 4.1 µg/L PFOS in serum.

A) Formula-fed Scenario for 15 ng/L PFOS in Drinking Water



B) Breastfed Scenario for 15 ng/L PFOS in Drinking Water

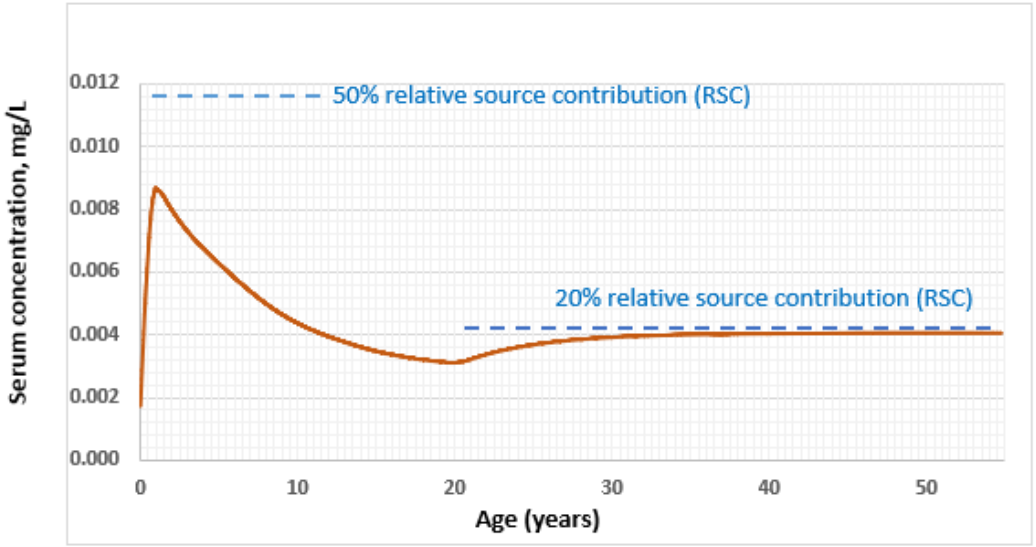
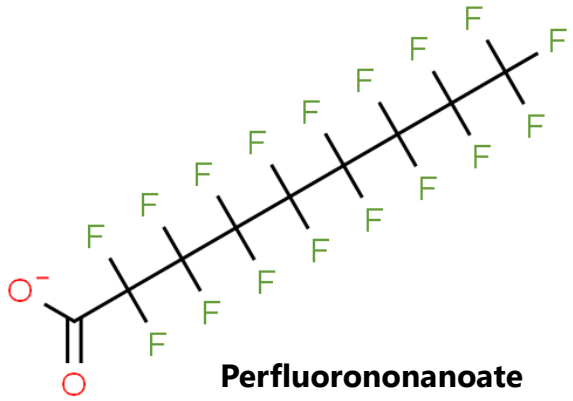


Figure 6. *Model predicted PFOS serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFOS in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the rest of childhood and adulthood. For breastfed infants, exclusive breastfeeding was assumed for the first six months with gradual tapering until one year of age. After one year, breast-fed infants are assumed to drink water at the 90th percentile intake rate. The dotted lines represent the maximum allowable PFOS serum level from drinking water only, as determined by the RSC for the age group. It represents the percentage allotted to drinking water sources of the acceptable daily PFOS intake from all sources.*

Deriving the State Action Level for PFNA

Perfluorononanoic acid (PFNA) has eight fully fluorinated carbons and a carboxylic acid group at one end. In drinking water, PFNA typically occurs as its anion perfluorononanoate (shown here). PFNA was primarily used as a processing aid to make a fluoropolymer called polyvinylidene fluoride (PVDF).[242] PFNA may be present in PVDF at low concentrations (100-200 ppm). PVDF lines industrial chemical tanks and pipes, coats internal electronic components, and was also used for biomedical membranes, monofilament fishing line, and architectural coatings.[242] PFNA was phased out of U.S. production in 2015 under an EPA stewardship agreement.[15] Possible PFNA precursor chemicals include 8:2 FTOH (fluorotelomer alcohol) and 8:2 diPAP (polyfluoroalkyl phosphoric acid diester) that are used in textile coatings and grease proof food contact papers.[242]



Perfluorononanoate

PFNA and precursors have been released to the environment from manufacturing plants and from industrial, commercial and consumer products.[3, 242] Once released into the environment, PFNA can persist for decades, bind to and leach from soil, and be transported in ground, surface and ocean waters.[4, 242] Additionally, volatile precursors, such as 8:2 FTOH, can be transported great distances in air and degrade to PFNA under specific conditions.[243] PFNA has been detected in drinking water near a PVDF manufacturing plant in NJ.[242] PFNA has been occasionally detected in public water systems impacted by AFFF firefighting foam, including in Washington State.[244, 245]

PFNA is widely detected at low levels in blood serum of the general U.S. population. In the 2015-16 NHANES survey by the CDC, mean and 95th percentile serum concentrations were 0.58 µg/L and 1.90 µg/L respectively.[68] Diet is considered the major source of exposure in humans.[246] Drinking water may also be a significant contributor to human exposure. For example, residents in Paulsboro, New Jersey who had PFNA in their drinking water had a mean serum level of PFNA nearly four times higher than the national norm.[18] PFNA bioaccumulates in people. Estimates of elimination half-life in human serum range from 2.5-4.3 years.[25, 26] PFNA concentration in maternal blood serum correlates with PFNA in umbilical cord serum and in breastmilk.[11]

The toxicity of PFNA is less studied than PFOA or PFOS, but the general types of rodent toxicity observed are similar.[4, 94, 247] Mice and rats are sensitive to liver toxicity from PFNA. Liver effects include increased liver weight, hepatocellular hypertrophy, increased serum liver enzymes, and liver cell damage/necrosis.[94, 248-251] PFNA also affects reproductive tissues and function. Oral PFNA administration reduced testosterone levels, altered sperm concentration and motility, reduced male fertility, and produced degenerative changes in the testes and seminiferous tubules of male rodents.[94, 252-254] In female rodents, it reduced the fertility index, pregnancy rate,

and the number of live pups at birth.[251, 255] Developmental toxicity observed with PFNA exposure included reduced growth, delayed development, and reduced survival of pups.[249, 255-257] Immunotoxicity in male rodents includes findings of reduced spleen and thymus weights, apoptosis in thymocytes and splenocytes, and altered cytokines involved with immune system function in the spleen.[94, 258-260] PFNA reduced serum thyroid hormones total T4 and free T4 in male and female rats orally exposed to PFNA for 28 days without a concomitant rise in TSH.[2] [94]

Epidemiological studies relevant to PFNA were reviewed by NJ DWQI in 2015 and ATSDR in 2021.[4, 261] There is limited evidence that higher PFNA exposure in humans is associated with increased serum cholesterol, increased serum liver enzymes, and decreased bilirubin levels;[150, 262, 263] immune suppression including reduced antibody response to vaccinations in children and adults;[229, 231, 236, 264] reproductive effects including preterm birth;[98, 265-267] and developmental effects including lower birth weight, altered bone density and timing of puberty.[163, 167, 174, 265] These findings have not been sufficiently studied or consistently observed. Most of the epidemiological studies compared serum levels of multiple PFAS to the endpoint of concern. Associations between PFNA and a health outcome were often reported for other PFAS as well.

No lifetime rodent assay for cancer was identified. A single case-control study in humans found no association between serum levels of PFNA and prostate cancer.[129]

Review of Health Protective Values

DOH reviewed the available health protective values for daily chronic human intake of PFNA. We focused on high-quality and comprehensive risk evaluations that considered current scientific research and were conducted by U.S. federal and state agencies. These included a target serum level derived by NJ DWQI, a minimal risk level (MRL) derived by ATSDR, and RfDs developed NHDES and the Michigan Science Advisory Workgroup (MSWG).

These values are presented in Table 7 below.

[2]Thyroxine (T4) is the primary thyroid hormone produced by the thyroid gland. Most of serum T4 is bound to proteins, but free T4 is unbound and can travel into tissues where it is converted to triiodothyronine (T3), which is the active form of the hormone. Thyroid stimulating hormone (TSH) is produced by the pituitary gland and stimulates hormone production by the thyroid gland.

Table 7. Health Protective Values for PFNA Reviewed by Washington

Source	Critical study	Critical effect	Human Equivalent Dose	Uncertainty Factors (UF)[a]	Oral RfD, MRL, Target Serum Level[b]	Exposure duration
NJ 2015[242]	Das et al. 2015	$BMDL_{10}$ for increased liver weight in mouse dams at GD-17 following gestational exposure. LOEL: 1 mg/kg-day Maternal serum level at $BMDL_{10}$: 4.9 mg/L		UF_{total} 1000 UF_H=10 UF_A=3 UF_S=10 UF_D=3	4.9 µg/L (target serum level)	Chronic
ATSDR 2021[4]	Das et al. 2015	NOAEL of 1 mg/kg-day for reduced pup weight and developmental delays in mice. Modeled TWA maternal serum at NOAEL: 6.8 mg/L LOAEL: 10.9 mg/L	0.001 mg/kg-day (6.8 mg/L x DAF[c])	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10	3 ng/kg-day (MRL)	Intermediate (2–52 wks.)
NH 2019 [77, 131]	Das et al. 2015	$BMDL_{10}$ for increased liver weight in mouse dams at GD-17 following gestational exposure. LOEL: 1 mg/kg-day. Maternal serum level at $BMDL_{10}$: 4..9 mg/L		UF_{total} 100 UF_H=10 UF_A=3 UF_D=3	49 µg/L (target serum level) 4.3 ng/kg-day (RfD)[d]	Chronic
MSWG 2019 [268]	Das et al. 2015	NOAEL of 1 mg/kg-day for reduced pup weight and developmental delays in mice. Modeled TWA maternal serum at NOAEL: 6.8 mg/L LOAEL: 10.9 mg/L	0.000665 mg/kg-day (6.8 mg/L x DAF[e])	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10	2.2 ng/kg-day (RfD)	Chronic

[a]Uncertainty factors: UF_H= intra-species uncertainty factor; UF_A= inter-species uncertainty factor; UF_S= subchronic to chronic uncertainty factor; UF_L= LOAEL to NOAEL uncertainty factor; UF_D= incomplete database uncertainty factor; UF_{total}= total (multiplied) uncertainty factor. Uncertainty factors are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing a 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10.

[b]RfD is a reference dose, MRL is a minimal risk level, target serum level is analogous to an RfD except on a serum basis.

[c]ATSDR dosimetric adjustment factor (DAF) = V_d x (Ln(2)/$T_{1/2}$) = 0.2 L/kg x (Ln(2)/900 days) =15.40 x 10^{-4} L/kg – day. ATSDR paired their developmental POD with the shorter serum half-life for women of reproductive age from Zhang et al 2013.

[d]NHDES dosimetric adjustment factor = V_d x (Ln(2)/$T_{1/2}$) = 0.2L/kg x (Ln(2)/1570 days) = 0.883 x 10^{-4} L/kg-day. NH used a half-life estimate for older women and men from Zhang et al 2013.

[e]MSWG dosimetric adjustment factor (DAF) = DAF = V_d x (Ln(2)/$T_{1/2}$) = 0.2L/kg x (Ln(2)/1417 days) = 0.978 x 10^{-4} L/kg-day. Serum half-life of 1417 days the arithmetic mean from Zhang et al 2013 of serum half-life in women of reproductive years and serum half-life in older women and men.

ATSDR, NJ DWQI, NHDES, and MSWG selected the same critical study, but the three assessments differed in the endpoint selected, the human serum half-life estimate for PFNA and/or the uncertainty factors applied. The critical study is described below.

Das et al. 2015,[249] is a development study in which bred female CD-1 mice received daily oral PFNA dosing (at 1, 3, 5 or 10 mg/kg-day) from gestation days (GD) 1-17. Dams were evaluated for overt signs of toxicity, growth, and reproductive impairment. Some fetuses were evaluated for skeletal and visceral birth anomalies on GD 16. Live-born pups were evaluated for abnormal development through puberty and their growth and survival was monitored through postnatal day (PND) 287. Serum and liver levels of PFNA were measured at multiple time points throughout the experiment in dams, fetuses, and pups.

Pregnant mice at the highest dose (10 mg/kg-day) lost weight and all had full litter resorptions. They were sacrificed at GD 13 and removed from the rest of the experiment.

Eighty percent of the pups in the 5 mg/kg-day dose group died between PND 2 -10. At and above 3 mg/kg-day, surviving pups showed statistically significant dose-dependent delays in reaching developmental milestones (eye opening, preputial separation, and vaginal opening). Pups alive at PND 24 (time of weaning) showed dose-dependent reductions in body weight. Reduced growth in male mice persisted to PND 287 (statistically significant ≥ 3 mg/kg-day). Female body weights were less affected and recovered to control levels by seven weeks of age.[249]

Maternal and fetal liver weights were higher than controls in all treatment groups. At delivery, there were no reductions in birthweight, skeletal abnormalities, or visceral abnormalities (except liver). The study did not report whether elevated liver enzymes or other signs of liver damage accompanied an increase in liver weight. Gene expression in liver tissue was evaluated at five time points in fetal and pup livers. PFNA induced a clear PPARα-dependent gene expression profile, but activation of other nuclear receptors (CAR and PXR[3]) were also evident in the liver of the mouse offspring. Upregulation of genes waned after PND 24 as body burden declined in pups.[249] This observation was similar to a fuller investigation of gene expression by Rosen et al. 2017, which showed that PPARα was the main target for PFNA in mouse liver with minor activation of genes associated with CAR, ERα, and PPARγ.[4] [269]

[3]Constitutive androstane receptor (CAR) and Pregane X receptor (PXR) are nuclear receptors and function as sensors of endogenous and xenobiotic substances. When activated, they upregulate genes involved with metabolism and excretion and are important receptors for detoxification and clearance of drugs and other foreign substances.

[4]Estrogen receptor alpha (ERα) is a nuclear receptor activated by estrogen. Peroxiosme proliferator-activated receptor gamma (PPARγ) is a nuclear receptor that controls expression of a number of genes related to metabolism and development.

ATSDR MRL

ATSDR selected the NOAEL for adverse effects on development (reduced growth and delayed development in pups exposed prenatally) in the Das et al. study as the point of departure. They estimated time-weighted average (TWA) maternal serum levels of PFNA across pregnancy to define the serum level associated with each dose group. The TWA serum level was 6.8 mg/L at the NOAEL. ATSDR also considered two other developmental toxicity studies for developmental points of departure.[255, 257] Wolf et al. 2010, observed decreased litter size and pup survival in mice exposed during gestation (GD 1-18). The TWA maternal serum level was estimated to be 11.6 mg/L at the LOAEL (1.1 mg/kg-day) and 4.47 mg/L at the NOAEL (0.83 mg/kg-day).[255] Rogers et al. 2014 reported decreased birthweight and increased blood pressure and kidney effects at ten weeks of age in rat offspring exposed to PFNA during gestation (GD 1-20) at a LOAEL of 5 mg/kg-day. A TWA maternal serum could not be calculated.[257]

ATSDR derived a human equivalent dose (0.001 mg/kg day) which is daily intake rate expected to result in a serum level of 6.8 mg/L at steady state. ATSDR selected a half-life estimate of 2.5 years for women of reproductive age in a study by Zhang et al 2013.[25] ATSDR acknowledged that the MRL is not specific to this subpopulation, but deemed it appropriate to use a half-life associated with women of childbearing age when calculating the human equivalent dose expected to produce the same PFNA concentration in women's serum at the time of pregnancy. The human equivalent dose was divided by a 300-fold uncertainty factor to derive an MRL of 0.000003 mg/kg-day or 3 ng/kg-day. Uncertainty factors included a ten-fold factor for human variability, a three-fold factor for differences between mice and humans, and a ten-fold factor for database uncertainty. Database uncertainty included the limited scope and number of studies that evaluated intermediate-chronic duration exposures and the lack of longer duration immunotoxicity testing for PFNA. Average steady state human serum level at the MRL was estimated to be 22.7 µg/L (6.8 mg/L ÷ 300 = 0.0227 mg/L).

ATSDR did not consider increased liver weight at the lowest dose in Das et al. as adverse or relevant for human health risk assessment. ATSDR applied the Hall et al., 2012 criteria[144] to liver effects observed and concluded that "doses associated with increases in liver weight and hepatocellular hypertrophy were not considered adverse effect levels for the purpose of human risk assessment unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present."[4]

New Jersey Target Serum

NJ DWQI based their health protective value on increased maternal liver weight at GD 17 in Das et al 2015.[242] Liver weight increased in a dose-dependent manner in maternal, fetal, and postnatal mouse pups and was statistically significant at 1 mg/kg/day. NJ evaluators cited a larger literature of evidence showing that increased liver weight can progress to more severe hepatic toxicity and neoplastic lesions over longer duration exposures. NJ chose to model maternal liver because both serum level and liver effects were measured at the same time point

(dose-response modelling between PFNA in maternal serum and liver weight in pups measured later would be less certain). New Jersey evaluators derived a $BMDL_{10\%}$ (lower 95th percentile confidence limit on the benchmark dose) for a 10 percent increase in liver weight. This was 4.9 mg/L in maternal serum. An uncertainty factor of 1,000 was applied to the $BMDL_{10\%}$ to derive a target serum level in humans of 4.9 µg/L. This included an uncertainty factor of three for gaps in the toxicological database (UF_D) including lack of chronic or cancer studies and variability in animal model response. In support of this factor, they cited two studies in rats, a 13-week sub-chronic study and a two-generation study (18-21 weeks), that administered a commercial mixture of PFNA. The commercial mixture was not pure PFNA; it contained 74 percent PFNA and 26 percent other PFAS with carbon lengths of C8-C13. The PFNA mixture produced liver and kidney effects at lower administered daily doses compared to Das et al. 2015.[250, 251] Further uncertainty factors were ten for human variability (UF_H), three for differences between mice and humans (UF_A), and ten to account for extrapolation of a chronic standard from a short-term (17-day) study (UF_S).

New Hampshire RfD

New Hampshire used the $BMDL_{10}$ derived by NJ as their point of departure but applied a smaller total uncertainty factor of 100 to derive a target serum of 49 µg/L.[77] Their uncertainty factors included: a ten-fold factor for human variability, a three-fold factor for differences between mice and humans, and a three-fold factor for database limitations. New Hampshire did not apply an uncertainty factor for use of a short-term study to derive a chronic health protective value. They considered hepatic hypertrophy to be the onset of adverse critical effects in the liver and were comfortable that the mouse is a highly sensitive animal model for this response to PFAS. NHDES acknowledged database uncertainties cited by NJ DWQI and added lack of immunotoxicity testing results suitable for establishing a dose-response relationship. NHDES used a dosimetric adjustment factor (DAF) to calculate an RfD (4.3 ng/kg-day) that would produce the target serum at steady state. Their calculation used a more conservative serum half-life estimate of 4.3 years also from the study by Zhang et al 2013. This half-life was associated with men and women >50 years old. This was considered reasonable for a liver endpoint that is assumed to apply to the entire population.

Michigan Science Advisory Workgroup RfD

Michigan evaluators concurred with the point of departure and approach developed by ATSDR.[268] The one exception was their use of a different serum half-life estimate for PFNA of 3.9 years. This value is the arithmetic mean of PFNA half-life estimates for two groups 1) women of reproductive years and 2) men and women > 50 years old in a study by Zhang et al. 2013. Michigan selected a developmental endpoint that is associated with maternal serum level of PFNA during pregnancy. However, they reasoned that this approach would better represent the entire population.[268]

Discussion of uncertainties

The serum elimination half-life estimates used by the risk assessors above were derived from a study of Chinese adults by Zhang et al. 2013 that estimated daily clearance of PFAS in paired blood and urine samples.[25] This was a novel method and conducted in a general population with low serum levels of PFNA (median 0.37 ug/L). In the Zhang et al. study population, younger females (age ≤50 years), had significantly lower levels of serum PFNA than women>50 years old or men. The estimated arithmetic mean elimination half-life for the young female group was 2.5 years (913 days) and for the combined male and older female group was 4.3 years (1,570 days). Recently, Yu et al. 2021 published a three-year biomonitoring study in a New Jersey community exposed to elevated PFNA in their drinking water.[26] The geometric mean of the study group was five times higher than the mean PFNA levels in U.S. adults as measured in 2015-2016 by the CDC. The study collected three blood samples one year apart in 99 participants from 2017 to 2020. Residents ranged in age between 20 – 74 years old and were 68% female. Half-life estimates of PFNA in serum were 3.52 years for the 68 most highly exposed participants. Focus on the more highly exposed members minimized bias from ongoing background exposure to PFNA including at least one transient detection of 7-11 ppt PFNA in tap water at all 41 homes tested.[26]

Emerging evidence in mice and rats shows that PFNA reduces serum and testicular testosterone and injures male reproductive tissues and function. In Feng et al. 2009, serum testosterone levels were increased at 1 mg/kg-day and sharply decreased at 5 mg/kg-day in Sprague Dawley adult male rats dosed for 14 days. At ≥3 mg/kg-day, estradiol levels were increased and testicular cells contained apoptotic features including crescent chromatin condensation and chromatin margination.[270] A study by NTP reported an 81 percent drop in serum testosterone in adult male rats dosed 2.5 mg/kg-day for 28 days (serum level measured at day 29 was 380 mg/L). In the same experiment, testosterone levels were increased in females rats ≥ 1.56 mg/kg-day (measured serum level at day 29 was 26.4 mg/L).[94] Recently published studies in Parkes mice by Singh and Singh reported that PFNA reduced serum testosterone levels, altered sperm viability and sperm production, and produced degenerative changes in the seminiferous tubules.[253, 254, 271] The LOAELs for these outcomes in mice were: 5 mg/kg-day in the gestation exposure study (NOAEL: 2 mg/kg-day)[271]; 2 mg/kg-day in the 14-day prepubertal exposure study (no NOAEL),[253] and 0.5 mg/kg-day in the 90 day study (NOAEL: 0.2 mg/kg-day).[254] In addition, the male mice were tested for fertility at the end of the 90-day PFNA exposure by mating them to unexposed female mice. No effect was seen on their ability to mate but reduced numbers of pups per litter was observed in the litters sired by the 0.5 mg/kg-day dose group. This was likely due to reduced sperm motility, viability, and sperm count observed in this group.[254] Singh and Singh did not measure serum PFNA at any time points in their studies. Without an indication of internal dose or more information about toxicokinetics of PFNA in this strain of mice, the study

results are not suitable for dose-response modelling. It does support use of an uncertainty factor for database deficiencies.

Immune toxicity of PFNA has been demonstrated at low doses (LOAELs were 1-3 mg/kg-day) in several 14-day rodent studies. For example, Fang et al. 2008 reported decreased thymus and spleen weights, impairment of cell cycle progression, decreases in specific types of innate immune cells, and decreased interleukin-4 secretion in the spleen in male mice.[258] Studies by the same authors in male rats showed decreased thymus and spleen weights, dose-dependent levels of apoptosis in spleen, increased production of pro-inflammatory and decreased production of anti-inflammatory cytokines in spleen.[259, 260]

Human Relevance

PFNA is structurally very similar to PFOA, a much better studied PFAS with both animal and human data supporting the human relevance of development endpoints. See our discussion of human relevance in Deriving the State Action Level for PFOA.

Reviews by ATSDR and New Jersey showed limited evidence, with both positive and null associations, between PFNA exposure in human populations and 1) increased serum cholesterol or other lipids (HDL, LDL, triglycerides) and 2) higher levels of serum liver enzymes (ALT, ALP, and GGT[5]).[4, 242] Positive associations between serum PFNA level and higher serum ALT, ALP or GGT were also reported in several recent studies of populations with background PFAS exposures in Sweden, China, and the U.S.[150, 262, 263]

In rodents, the liver and developmental effects produced by PFNA at low doses appear to be largely (but not entirely) mediated by activation of PPARα. Wolf et al. 2010 dosed bred wild-type (WT) and PPARα knockout (KO) female mice with five oral doses of PFNA ranging from 0.83 to 2 mg/kg/day on GD 1-18. In WT litters, PFNA increased pup liver weight at PND 21 at a dose of 0.83 mg/kg-day, reduced the number of live pups at birth and decreased survival at weaning at the 1.1 mg/kg-day dose group, and reduced pup weight gain and delayed eye opening at the 2 mg/kg-day dose group. In KO litters, no developmental effects were observed and pup liver weight was increased only at the highest dose.[255] This study underscores that the dose-response curve could look very different in tissues that are less responsive to PPARα. Human liver has lower expression of PPARα compared to mouse liver and is not as prone to proliferative changes mediated by PPARα.[56, 144, 184] This does not rule out the relevance of the effect but it introduces uncertainty in interpreting the dose-response relationship for liver outcomes in mice.

The evidence underlying this argument is specific to liver responses and does not extend to the many other tissues in the human body that express PPARα and other PPARs that may be minor

[5] Alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT) are liver enzymes measured in serum that indicate liver injury.

targets of PFAS. PPARα and PPARγ are centrally involved in lipid and glucose regulation in a number of other tissues and are widely expressed in immune cells, endocrine organs, and reproductive tissue including the placenta.[272, 273] As such, a PPARα-mediated pathway of developmental effects in rodents should be considered potentially relevant to human reproduction and fetal and child development.

Some associations between PFNA exposure measures and reproductive and developmental outcomes have been reported in epidemiological studies. Maternal serum PFNA early in pregnancy was associated with higher risk of preterm birth in two prospective cohorts in Denmark and Massachusetts.[98, 265] Maternal serum levels of PFNA were associated with gestational diabetes in healthy, non-obese women with a family history of type 2 diabetes in one study.[266] Other reports include associations in prospective studies between higher serum PFNA and increased risk of miscarriage,[267] lower birth weights,[265] altered timing of puberty onset for boys and girls,[167] and altered bone mineral density in girls at 17 years old.[174] In addition, a cross-sectional study in the C8 health Project cohort found that PFNA in childhood serum was associated with lower levels of sex hormones and insulin-like growth factor (IGF-1) in boys and girls six to nine years old.[163]

Although there exists a growing body of evidence for male reproductive toxicity in rodents, it is not known whether PFNA lowers testosterone levels or impairs male reproductive function in humans. A few epidemiological studies have looked but not found associations between serum level of PFNA and serum testosterone or impaired sperm parameters.[163, 274-277] These studies were conducted in populations with no obvious source of elevated PFNA exposure with average serum levels of PFNA reported to be 1.0-1.7 µg/L. Studies of more highly exposed populations are needed.

Investigations of PFNA and immune endpoints in humans are also limited. Associations have been reported between higher PFNA exposure and decreased antibody response to a vaccine,[229, 231] higher number of reported respiratory infections or common cold in children,[229, 236] and asthma in children.[264] Asthma and allergic diseases were not associated with PFNA exposure in a number of other studies.[229, 235, 236, 278]

Washington State Recommendation: 2.5 ng/kg-day

We selected the ATSDR MRL as the basis for our public health advice for PFNA in public drinking water. We modified it slightly with the new half-life estimate of 3.52 years (1,285 days) from Yu et al. 2021, as follows:

MRL (mg/kg-day) = POD (mg/L) x DAF (L/Kg-day) ÷ UF

- *Where the POD = 6.8 mg/L PFNA in serum*

- *Where the DAF = V_d x (Ln(2)/$T_{1/2}$) = 0.2 L/kg x (Ln(2)/1,285 days) =1.08 x 10^{-4} L/kg – day.*
- *Where the UF = 300*

WA health protective value = 6.8 mg/L x 1.08 x 10^{-4} L/kg – day ÷ 300 = 2.45 x10^{-6} mg/kg-day (or 2.5 ng/kg-day)

The ATSDR MRL is based on sensitive developmental effects in mice considered relevant to humans. The MRL derived from developmental effects was lower than candidate MRLs derived from clearly adverse liver effects. Although liver weight is a sensitive target of PFNA activity in rodents, human liver has lower expression of PPARα compared to mouse liver and is not as prone to proliferative changes mediated by PPARα.[56, 144, 184] ATSDR did not consider this endpoint suitable for dose-response extrapolation from mice to humans.

Compared to the earlier estimate from Zhang et al. 2013, we had higher confidence in the serum half-life calculated from Yu et al. 2021. The latter study directly measured declining PFNA in serum over three years in a community exposed through drinking water and whose serum levels of PFNA were high enough to minimize bias from low background exposures. We used the estimate in the highly exposed male and female participants from this study (3.52 years or 1,285 days) to further minimize this potential bias. Forty-two percent of these participants were women between 20–55 years old, which supports our use of the estimate to model maternal serum levels. The half-life estimate for younger women was not statistically different than the estimate for older women and men.

We concurred with ATSDR's ten-fold factor for database uncertainty (UF_D). Data gaps for PFNA include lack of chronic testing for immune, liver and cancer endpoints. There is emerging evidence of male reproductive toxicity in rodents. PFNA is less studied but similar to PFOA in both chemical structure and observed rodent toxicity. PFOA has a similar equivalent RfD based on a more robust toxicological dataset including epidemiological studies that support the relevance of adverse effects on growth and development for human populations.

Sensitive subpopulations: We expect the fetal period to have the highest sensitivity to developmental effects. Infancy and childhood may also be sensitive windows for any PFNA-mediated alterations in hormones and effects on pubertal development. Rodent data show that pubertal development may be a sensitive window for PFNA.

Relative Source Contribution: 50 percent

RSCs were developed for children and adults (see Table 2) using the subtraction method and the EPA Exposure Decision Tree described in EPA's methodology.[58] The RSCs for PFNA were 50 percent for infants, children, and adults. The target serum level at the PFNA MRL is 22.7 µg/L.

The serum contribution from drinking water sources should not exceed 50 percent of that target serum level or 11.4 µg/L.

An RSC of 50 percent is concordant with RSC determinations in three other states: NJ, NH and Michigan. [76]

Water Ingestion Rate: MDH model

As discussed in the Introduction to Approach and Methods section, we used a Minnesota Department of Health model with age-specific drinking water ingestion rates that includes placental and lactational transfer to children from mothers with chronic exposure to PFAS in community drinking water.[75] This approach was adopted by MI and NH in deriving their MCLs for PFNA.

We assumed age-specific drinking water ingestion rates at the 90th percentile for chronic periods of exposure (children >one years old and adulthood). We assumed 95th percentile drinking water intake for lactating women and for formula-fed infants (assuming powdered formula is mixed with tap water). Breastfed infants were assumed to be exclusively breastfed at the upper-end intake rate for six months and then gradually tapered off breastmilk over the following six months while other foods and drinks are introduced, including juices or infant formula mixed with tap water.

The model outputs are provided below in Figure 7. A chronic drinking water level of 9 ng/L PFNA was the maximum concentration that allowed serum levels of infants and children to remain within the 50 percent RSC for drinking water sources. The peak serum level predicted for breastfed infants as a result of 9 ng/L PFNA in community drinking water was 11.1 µg/L. Formula-fed infants peaked at 3.6 µg/L PFNA in serum. Maternal serum level of PFNA attributed to the drinking water source at the time of pregnancy was 3.1 µg/L. and the expected starting serum for infants at birth 2.1 µg/L.

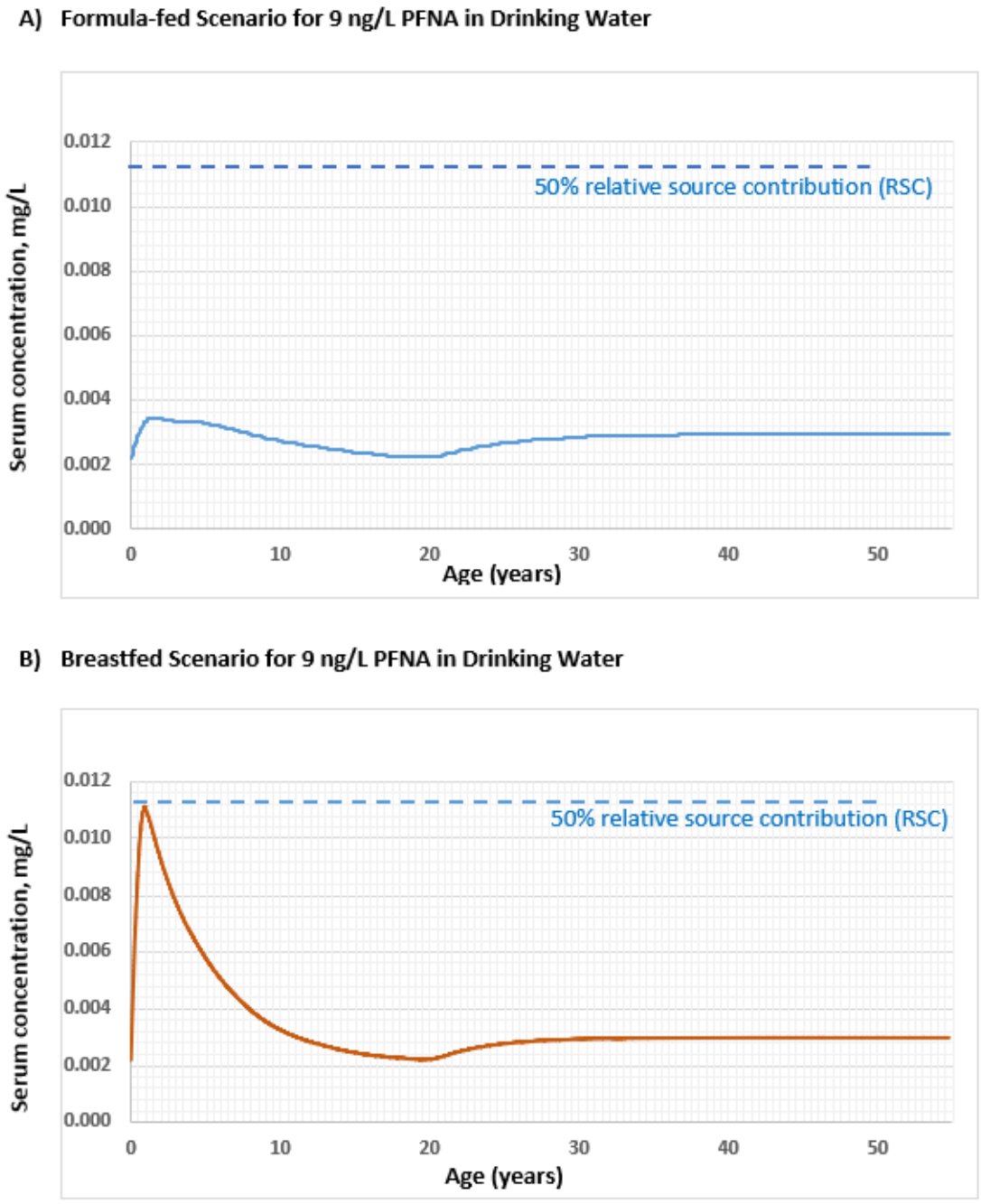
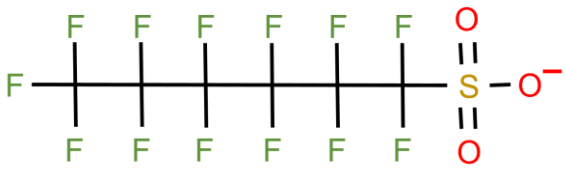


Figure 7. *Model predicted PFNA serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFNA in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the rest of childhood and adulthood. For breastfed infants, exclusive breastfeeding was assumed for the first six months with gradual tapering until one year of age. After one year, breast-fed infants are assumed to drink water at the 90th percentile intake rate. The dotted lines represent the maximum allowable PFNA serum level from drinking water only, as determined by the RSC for the age group. It represents the percentage allotted to drinking water sources of the acceptable daily PFNA intake from all sources.*

Deriving the State Action Level for PFHxS

PFHxS is structurally similar to PFOS, but has six rather than eight fully fluorinated carbons. In water it occurs as its anion perfluorohexane sulfonate (shown here). PFHxS along with its salts and commercial precursor compounds has been used in certain class B firefighting foams; in waterproof and stainproof coatings for carpet, apparel, leather, upholstery and other textiles; in cleaning and polishing agents; as a mist suppressant in metal plating; and in electronics and semiconductors manufacturing.[279] It was phased out of domestic production by its major U.S. producer (3M) in 2002, but may still be produced globally.[279]



Perfluorohexane sulfonate

PFHxS and/or precursors have been released to the environment from manufacturing plants[280] from commercial products such as aqueous film-forming foam used at military bases and airports,[40] and may be released from products like carpet protection treatments.[281] PFHxS is very persistent in the environment and can be detected in soils and groundwater years after release.[282] Volatile precursors can be transported long distances by air.[283] PFHxS frequently occurs with PFOS when detected in U.S. public drinking water samples.[244] In Washington state drinking water, PFHxS has been found along with PFOS in several areas where firefighting foam is the suspected source of PFAS contamination.[1]

PFHxS is one of four PFAS routinely measured in people. The general population is exposed to PFHxS through diet, drinking water, use of certain consumer products, and inhalation of indoor air and dust.[4] Drinking water can be a significant contributor to exposure[4, 19] In the 2015–2016 CDC NHANES survey, the mean serum level of PFHxS in a representative sample of the U.S. population was 1.18 µg/L. Ninety-five percent of the population had serum levels below 4.9 µg/L.[68] PFHxS is poorly excreted from the human body. Mean serum half-life was 7.3 years in a group of retired fluorochemical workers and 5.3 years in over 100 men and women followed after PFHxS was removed from their drinking water.[17, 24]

The liver is the primary target of PFHxS toxicity in rodent studies. Effects observed include increased liver weight, hepatocellular hypertrophy, altered lipid metabolism, steatosis, and necrosis.[284-286] Several studies have reported thyroid cell damage and reduced T4 and T3 thyroid hormone levels in rodent studies.[284, 287, 288] Reproductive and developmental effects have been reported in some studies, such as reduced litter size [286] and reduced birth weight,[287] but have not been consistently observed. Altered spontaneous behavior and habituation was observed in adult mice following PFHxS dosing on postnatal day ten[289] but similar results were not observed in mice exposed as adults,[286] in rats exposed as adults[284] or in adult rats exposed during gestational and pre-weaning periods.[290] A key data gap is the lack of immune toxicity testing in animal studies.

According to ATSDR's 2021 assessment, the weight-of-evidence from epidemiological studies suggests associations between higher PFHxS exposure in humans and decreased antibody response to vaccines, increased serum liver enzymes (particularly alanine aminotransferase or ALT), and decreased serum bilirubin levels.[4]

In addition, studies have reported associations between higher PFHxS exposure and increased risk of hyperactivity in children,[177, 179] reduced T4 levels in pregnant women and male infants,[213, 291, 292] increased serum lipids,[293] and lower birth weights.[160] These associations are inconsistently observed and have not been studied enough to reach conclusions.

The carcinogenicity of PFHxS has not been investigated.

Review of Health Protective Values

DOH reviewed the available health protective values for daily chronic human intake of PFHxS. We focused on high-quality and comprehensive risk evaluations that considered recent scientific research and were conducted by U.S. federal and state agencies. These included a minimal risk level (MRL) derived by ATSDR, a reference dose (RfD) derived by the NHDES, an RfD derived by the MDH and a RfD developed by the MSWG. These are presented in Table 8 and discussed below.

Table 8. Health Protective Values for PFHxS Reviewed by Washington

Source	Critical study	Critical effect	Human Equivalent dose	Uncertainty factors (UFs)[a]	Oral RfD, MRL, Target serum[b]	Exposure duration
ATSDR 2021[4]	Butenhoff et al. 2009; Hoberman and York 2003[284]	NOAEL of 1 mg/kg-day for thyroid follicular cell hypertrophy and hyperplasia in adult male rats treated for 42 days. LOAEL: 3 mg/kg-day TWA serum level for adult males at the NOAEL: 73.22 mg/L.	0.0047 mg/kg-day[c]	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10	20 ng/kg-day (MRL)	Intermediate (2–52 wks.)
NH 2019 [77]	Chang et al. 2018[286]	BMDL for decreased litter size in mice exposed from preconception through gestation. NOAEL: 0.3 mg/kg-day LOAEL: 1.0 mg/kg-day Maternal serum level at BMDL: 13.9 mg/L		UF_{total} 300 UF_H=10 UF_A=3 UF_s=3 UF_D=3	46.3 µg/L (target serum level) 4.0 ng/kg-day (RfD)[d]	Chronic
MDH 2019[74]	NTP 2019[247]	$BMDL_{20\%}$ for reduced serum thyroxine (free T4) in adult male rats in a 28-day oral toxicity study. LOAEL: 0.625 mg/kg-day. No NOAEL. Serum level at $BMDL_{20\%}$ was 32.4 mg/L	0.00292[e] mg/kg-day	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10	9.7 ng/kg-day (RfD)	Short-term and chronic

MSWG 2019[268]	NTP 2019	$BMDL_{20\%}$ for reduced serum thyroxine (free T4) in adult male rats in a 28-day oral toxicity study. LOAEL: 0.625 mg/kg-day. No NOAEL. Serum level at $BMDL_{20\%}$ was 32.4 mg/L	0.00292[e] mg/kg-day	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10	9.7 ng/kg-day (RfD)	Chronic

[a] Uncertainty factors: UF_H= intra-species uncertainty factor; UF_A= inter-species uncertainty factor; UF_S= subchronic to chronic uncertainty factor; UF_L= LOAEL to NOAEL uncertainty factor; UF_D= incomplete database uncertainty factor; UF_{total}= total (multiplied) uncertainty factor. Uncertainty factors are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing a 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10.

[b] RfD is Reference dose, MRL is minimal risk level, target serum level is analogous to an RfD but on a serum basis.

[c] The derivation of the human equivalent dose from the serum level at the NOAEL assumed a human serum half-life of 3,102 days (8.5 years) and a volume of distribution of 0.287 L/Kg for PFHxS.

[d] RfD = target serum x dosimetric adjustment factor = 46.3 ng/mL x 8.61 $x10^{-2}$ mL/kg-day) = 4.0 ng/kg-day. The DAF assumed a human serum half-life of 1716 days for women in Li et al. 2018 and a volume of distribution of 0.213 L/kg.

[e] The human equivalent dose was 32.4 mg/L x 0.000090 L/kg-day = 0.00292 mg/kg-day. The dosimetric adjustment factor assumed a human serum half-life of 1,935 days (5.3 years) from Li et al. 2018 and a volume of distribution of 0.25 L/kg.

ATSDR MRL

ATSDR conducted an extensive review of the available epidemiological and toxicological data and based their minimal risk level on a reproductive and developmental rat study by **Butenhoff et al., 2009**. This study administered PFHxS by gavage at 0, 0.3, 1, 3, and 10 mg/kg-day to adult female rats for 14 days prior to pregnancy and through gestation to postnatal day (PND) 22. Adult males were treated 14 days prior to mating and for a minimum of 42 days. Offspring were not dosed directly but were exposed by placental transfer *in utero* and via nursing. The study reported no significant changes to the fertility index, the mating index, or estrous cycling. There were no signs of neurotoxicity or altered motor activity in the parental rats (F0) when assessed by the functional observational battery. In the adult F0 males, total serum cholesterol was reduced in all treatment groups. At 3 and 10 mg/kg-day, males had increased liver weight, centrilobular hepatocellular hypertrophy, and hypertrophy/hyperplasia of thyroid follicular cells.[284] Increased serum levels of alkaline phosphatase was also seen in males at 10 mg/kg-day. These liver and thyroid effects were not observed in the F0 females.[284] Thyroid hormones were not measured. Pups (F1) did not have lower birthweights or reduced growth at PND 22. Pups were not evaluated for developmental delays, thyroid gland weight, serum thyroid hormones, or for neurobehavioral outcomes.

ATSDR considered the thyroid effects in F0 male rats adverse and relevant to humans. The absence of similar effects in F0 female rats was possibly related to their lower serum levels of PFHxS resulting from more rapid excretion of PFHxS compared to males. Thyroid follicular cell adenomas have been observed in rats with long-term oral exposures to the structurally similar compound PFOS.[121]

The ATSDR MRL is based on the time-weighted average serum concentration (73.2 mg/L) in male rats at the NOAEL of 1 mg/kg-day. ATSDR derived an equivalent human dose by using a

first-order single-compartment model. They divided the $NOAEL_{HED}$ by a total uncertainty factor of 300 (10x for human variability, 3x for extrapolation from animals to humans, plus a modifying factor of 10x for database limitations). The primary database limitations noted were lack of immunotoxicity testing and lack of longer duration studies for PFHxS.[4]

Since the ATSDR assessment was derived in 2018, new high-quality studies on PFHxS have become available and have served as the basis for later assessments by U.S. states.

NHDES RfD

New Hampshire selected reproductive toxicity in mice as the critical effect and Chang et al. 2018 as the critical study for their target serum and RfD. The **Chang et al. 2018 study**[286] administered PFHxS to female mice for 14 days prior to pregnancy, through pregnancy, and through lactation. Males were dosed for 42 days starting 14 days prior to mating. Pups were observed until PND 36 for pubertal development benchmarks. The administered doses were 0, 0.3, 1.0 and 3.0 mg/kg-day. In the parent generation (F0), dose-dependent hepatocellular hypertrophy was observed starting at the lowest dose tested (0.3 mg/kg-day), increased absolute and relative liver weights in both male and female mice were statistically significant ≥ 1.0 mg/kg-day, and liver necrosis, decreased serum cholesterol, decreased bilirubin and increased alkaline phosphatase were observed in F0 males at 3 mg/kg-day.[286]

There was a slight but statistically significant decrease in the mean number of pups per litter at 1.0 and 3.0 mg/kg-day, which appeared to be related to a slight decrease in number of implant sites rather than loss of implanted embryos. The fertility index for F0 males and females was not significantly altered at any dose. There were no significant alterations in sperm motility, count, density, or morphology in F0 males. In offspring (F1), there were no treatment-related effects on postnatal survival or developmental delays noted. Anogenital distance in F1 males at PND 1 was increased in all treated groups but did not show a dose-response relationship. Males and females had increased relative liver weight at 3.0 mg/kg-day, and females had increased relative thyroid weight at 3.0 mg/kg-day dose.

Serum TSH levels were measured at multiple time points and were not altered in F0 or F1 mice. The study did not measure for other serum thyroid hormones T4 and T3. Neurobehavioral testing conducted in the F0 generation was negative for dose-related effects. Mechanistically, PFHxS was biologically active in mice on the same receptors activated by other PFAS as evidenced by mRNA transcripts associated with PPARα activation, CAR activation, PXR activation, and fatty acid metabolism.[286]

The study authors considered all differences observed between treated groups and controls, except liver effects, to be equivocal or of unclear significance. The internal doses in females at the time of mating (study day 14) were 27, 89, and 179 mg/L at 0.3, 1.0, and 3.0 mg/kg-day, respectively.[286]

New Hampshire risk assessors selected the slight decrease in mean litter size at ≥ 1.0 mg/kg-day in Chang et al. 2018 as the critical effect. At the LOAEL, maternal serum at the end of the experiment was 89 mg/L. New Hampshire used a benchmark dose method to derive a BMDL of 13.9 mg/L in serum in female mice from the Chang et al. data. They applied a total uncertainty factor of 300 (10 for human variability, 3 for uncertainties between rodents and humans, 3 for extrapolation from a subacute study to a chronic standard, and 3 for database uncertainties). Database limitations included a lack of multigenerational rodent studies and a lack of immune toxicity testing. The resulting target serum in humans was 46.3 µg/L and the RfD was 4.0 ng/kg-day. They noted that a lack of similar findings in rats in Butenhoff et al, 2009, may be due to faster excretion and lower serum levels of PFHxS in the female rats.

MDH RfD and MSWG RfD

MDH selected reductions in thyroid hormones as their critical effect and a 2019 study by the National Toxicology Program (described below) as their critical study. Similar results from Ramhøj et al. 2018 (described below) were considered supporting evidence. Supporting evidence also included thyroid cell damage observed in male rats (Butenhoff et al. 2009) and increased relative thyroid weight in developmentally exposed female mice (Chang et al. 2018).

The **National Toxicology Program (NTP) 2019** conducted a 28-day oral gavage study in adult male and female Harlan Sprague Dawley rats. The study measured growth and gross behavior, serum hormone levels, and evaluated all organs for gross and histopathological findings at the end of 28 days. Serum measurements of PFHxS were collected for assessment of internal dose at the end of the experiment.[247]

There was a dose-dependent decrease in serum thyroid hormone levels in both sexes with more marked reductions in T3, fT4 and tT4 in males. Reductions were statistically significant in males at the lowest dose tested (LOAEL: 0.625 mg/kg-day; mean serum level of PFHxS was 66.8 mg/L). In males, thyroid hormone effects appeared to plateau above the 2.5 mg/kg-day dose (serum level of 129 mg/L). Males at this dose level had 36 percent reductions in mean serum T3, 65 percent reductions in serum tT4, and 79 percent reductions in fT4. In females, the declines were more gradual. TSH was only slightly increased and did not reach statistical significance in either males or females. In males, increased liver weights and reduced cholesterol was evident at the 1.25 mg/kg-day dose group (mean serum level 92.1 mg/L) and hepatocyte hypertrophy was significant in the 2.5 mg/kg-day dose group (129 mg/L in serum). Internal doses in male rats were much higher than in females reflecting the faster excretion of PFHxS by female rats.[247]

Ramhøj et al. 2018 conducted complimentary reproductive toxicity assays with oral administration of PFHxS in pregnant Wistar rats and collected endocrine measurements in dams and pups. No effect on litter size or post-implantation loss was observed at doses up to 45 mg/kg-day PFHxS. Serum total T4 was markedly reduced in a dose-dependent manner in pregnant and lactating dams and in pups at doses ≥ 5 mg/kg-day. At the LOAEL of 5 mg/kg-

day, maternal serum total T4 was reduced 18 percent compared to controls after only seven days of exposure (at GD 15) and reduced 26 percent after the lactation period (PND 22). Pups at the LOAEL had 31 percent reductions in serum T4 at PND 16. Thyroid hormone changes at the LOAEL were noted in the absence of altered maternal body weight or increased maternal liver weight and only equivocal changes in these two measures in pups. Histological examination of liver tissue was not performed. The NOAEL was 0.05 mg/kg day. Maternal serum level of PFHxS was not measured. This study suggests that reduction of T4 is a sensitive effect in both pregnant rats and their offspring.[287]

The MDH conducted benchmark dose modeling of the total and free T4 data in males and females in the NTP study. $BMDL_{20\%}$ for 20 percent reduction in T4 in males was similar for total T4 (33.6 mg/L) and free T4 (32.4 mg/L). MDH applied a dosimetric adjustment to the $BMDL_{20}$ for fT4 to calculate a human equivalent dose. MDH applied an uncertainty factor of 300 (10 for uncertainty factor for human variability, 3 for interspecies differences, and 10 for database uncertainty). Noted database deficiencies were lack of immunotoxicity testing and lack of a two-generation developmental study. Their final RfD is 9.7 ng/kg-day (corresponding target serum level = 108 µg/L).[74]

Michigan evaluators concurred with Minnesota's derivation of RfD and adopted it as the basis for their MCL in drinking water for PFHxS.

Discussion of Uncertainties

The reduction in litter size observed in mice by Chang et al. was not observed in two studies in rats. The absence of reproductive toxicity in Butenhoff et al. 2009 and Ramhøj et al. 2018 could possibly be explained by lower serum levels in the rat studies. Female rats have been shown to have a much shorter serum elimination half-life for PFHxS (~2 hours) compared to serum half-lives of one month in male rats and male and female mice.[294] A second study that replicates this finding in mice would increase confidence in this effect.

It is not clear whether PFHxS-mediated reductions in circulating thyroid hormone levels adversely affect brain development, cognitive function or behavior in rodents. In a follow-up study by Ramhøj et al. 2020, the thyroid hormone disruptions observed in Ramhøj et al. 2018 were not correlated with significant changes to motor activity or deficits in learning and memory in offspring as adults.[290] Similarly, they were not associated with changes in cortical gene expression in the brains of offspring.[290] We agree with Ramhøj et al. 2020 however that significant reduction in T4 alone should warrant concern and that the metrics currently applied in rodent models my not be sufficiently sensitive to detect adverse neurodevelopmental effect of PFHxS-induced maternal and perinatal hypothyroxinemia.

Human Relevance

In rats, PFHxS lowered serum thyroid hormones T3 and T4 and fT4 without a compensatory rise in TSH. In humans, low serum fT4 with normal levels of TSH is known clinically as hypothyroxinemia and is of special concern when it occurs in pregnant women. Thyroid hormones are critical to normal fetal growth and brain development and even subclinical maternal hypothyroxinemia early in pregnancy may adversely affect neurodevelopment and cognitive function in children (e.g., delayed psychomotor development, delayed language development, and lower IQ).[295-301] In the first trimester of pregnancy, thyroid hormone is supplied from the mother to the fetus via the placenta. In the second trimester, although the fetus begins to synthesize thyroid hormone, it still obtains thyroid hormone mainly from the mother.[295]

Ballesteros 2017[213] conducted a systematic review of ten epidemiological studies in populations of pregnant women, infants, and older children that investigated levels of thyroid hormones in relation to PFAS exposure. Five of these studies measured PFHxS. Associations between maternal serum PFHxS and maternal free T4 and total T4 were generally inverse but not statistically significant.[213] Boesen et al., 2020,[302] reviewed fifteen more recent studies of maternal and infant thyroid hormone levels in relation to maternal PFAS exposure and reported that "overall, most studies supported a positive association of maternal TSH and a possible negative association of maternal T4 and T3 upon PFAS exposure." For PFHxS specifically, the associations between maternal exposure and maternal TSH were positive in seven of eight studies; but only two were statistically significant. No clear trend was evident between PFHxS and T3 or T4.[302]

In the general adult population, two large studies in NHANES populations found no association between PFHxS serum levels and TSH, free or total T4 or T3.[114, 303] Another study in the NHANES population found that higher serum PFHxS was associated with increased rates of subclinical hypothyroidism or hyperthyroidism in women but not in men.[113] A meta-analysis of six studies in the general population by Kim et al. 2018 showed that serum PFHxS correlated with slightly lower serum total T4 but not with fT4, T3, or TSH.[212] A recent cohort study by Andersson et al. 2019, observed no difference in incidence of hypothyroidism or hyperthyroidism between a highly exposed community of Ronneby, Sweden and a reference community. This study evaluated hospital diagnosis records and prescriptions for thyroid medications in the two areas.[304]

Overall, there is limited evidence for PFHxS-associated thyroid hormone level perturbations in human populations. Inconsistency may be due to the age and gender of the population studied or the co-occurrence of other PFAS in serum that appear to be biologically active on these measures.[212] Two small studies by Webster et al. also showed that a marker of thyroid autoimmune disease (TPaO) and iodine insufficiency influenced the strength of the associations

for other PFAS-mediated reductions in T4. These conditions are known stressors for thyroid hormones and may impair the ability of the body to compensate for reductions in T4.[214, 216]

Evidence for neurobehavioral effects is mixed in human observational studies. Positive associations between serum level of PFHxS in children (12–15 years old) and attention deficit hyperactivity disorder (ADHD) were reported in a large cohort (*n*=10,546) of children in the C8 Health Project and in a smaller group (*n*=571) in the NHANES population.[177, 179] In the C8 study, having a doctor diagnosis of ADHD was positively associated with PFHxS serum levels in all quartiles compared to the lowest quartile. The association remained, but was slightly weaker when restricted to those who currently used medication to treat the condition.[179] Several studies have looked for and not found associations between prenatal PFHxS exposure (maternal serum) and ADHD or autism in school-aged children.[305-307]

Washington State Recommendation: 9.7 ng/kg-day

We concurred with the MDH RfD of 9.7 ng/kg-day based on thyroxinemia in adult male rats in the NTP study. This is supported by observations of hypertrophy/hyperplasia of thyroid follicular cells in Butenhoff et al. 2009 and reduced T4 in pregnant rats and their offspring in Ramhøi et al. 2018. The slight reduction in litter size observed in mice by Chang et al. was not observed in two studies in rats and has not been replicated in mice.

We support MDH's application of a ten-fold uncertainty factor for database limitations. In addition to the lack of immunotoxicity testing, there is a lack of testing for developmental neurobehavioral effects in mice exposed during gestation and nursing. A study by Viberg et al. 2013, reported altered spontaneous behavior and habituation behavior in mature laboratory mice after a single dose of PFHxS at postnatal day ten. The LOAEL and NOAEL for this study were 9.2 mg/kg and 6.1 mg/kg.[289]

Sensitive populations. Maternal thyroid insufficiency during pregnancy can affect the neurodevelopment of children. Women of childbearing age and developing fetuses are sensitive subgroups for this outcome. It is not clear whether lower T4 in infants confers a risk to development, but we made a protective assumption to include the infant as a sensitive subgroup. PFHxS is found in breast milk, indicating a potential for lactational exposure. Papadopoulou et al. 2016, found that serum levels of PFHxS (and other PFAS) increased in breastfeeding infants by 3–5 percent per month.[308]

Relative Source Contribution: 50 percent

RSCs were developed for children and adults (see Table 2) with the subtraction method and the EPA Exposure Decision Tree. The RSCs for PFHxS were 50 percent for infants, children, and women of childbearing age. The reference serum at the RfD is 108 µg/L. At 50 percent RSC, the contribution from drinking water should not exceed 54 µg/L in the serum (108 µg/L x 0.50).

Minnesota, New Hampshire, and Michigan also derived an RSC of 50 percent for PFHxS.

Water Intake Rate: MDH model

As discussed in the Introduction to Approach and Methods, we used a Minnesota Department of Health model with age-specific drinking water rates, which includes placental and lactational exposure routes when estimating exposure from PFHxS in community drinking water.[73]

We assumed age-specific water intake rates at the 90th percentile for chronic periods of exposure (children>1 year old through adulthood). We assumed 95th percentile drinking water intake for lactating women and formula-fed infants (assuming powdered formula is mixed with tap water). We assumed breastfed infants were exclusively breastfed for six months and then gradually tapered off breastmilk over the following six months with other foods and drinks introduced, including juices or infant formula mixed with tap water.

We provide the model outputs below (Figure 8). A chronic drinking water level of 65 ng/L PFHxS was the maximum concentration that allowed serum levels of infants and children to remain within the 50 percent RSC for drinking water sources. The peak serum level predicted as a result of 65 ng/L in drinking water was 52.6 µg/L in breastfed children and 26.5 µg/L in formula fed children. The maternal serum level of PFHxS attributed to drinking water at the time of pregnancy was 26.9 µg/L, and the expected starting serum for infants at birth was 18.8 µg/L.

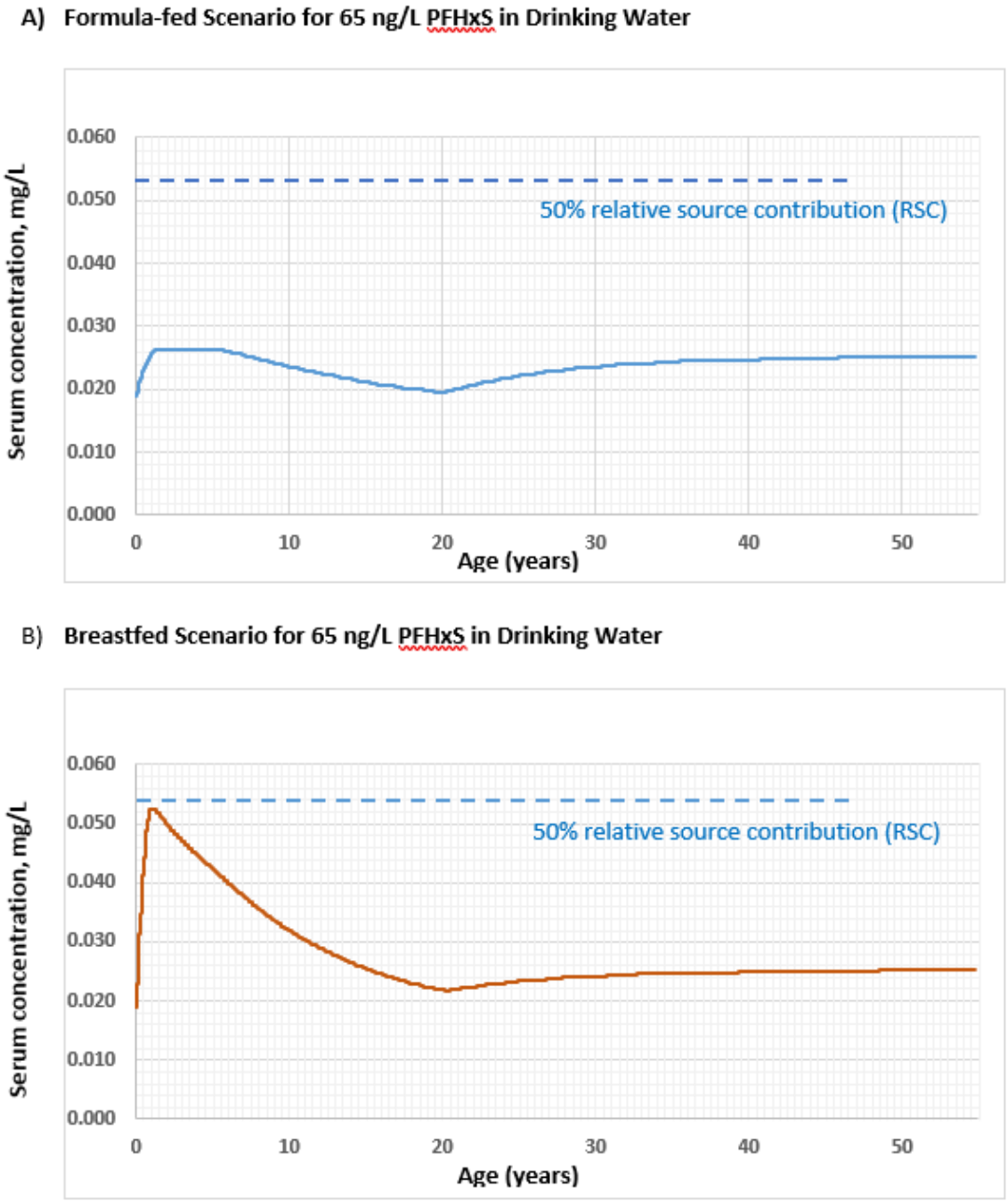
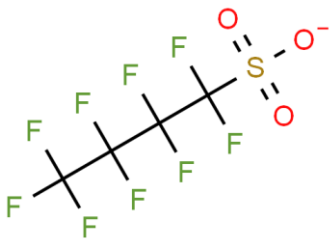


Figure 8. *Model predicted PFHxS serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFHxS in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year, followed by 90th percentile water intake during the rest of childhood and adulthood. For breastfed infants, exclusive breastfeeding was assumed for the first six months, with gradual tapering until one year of age. After one-year, breastfed infants are assumed to drink water at the 90th percentile intake rate. The dotted lines represent the maximum allowable PFHxS serum level from drinking water only, as determined by the RSC for the age group. It represents the percentage allotted to drinking water sources of the acceptable daily PFHxS intake from all sources*

Deriving the State Action Level for PFBS

Perfluorobutane Sulfonate



Perfluorobutane sulfonic acid (PFBS) has four fully fluorinated carbons and a sulfonic acid group on one end. In drinking water PFBS occurs as its anion perfluorobutane sulfonate (shown here). PFBS is a surfactant and a potential degradation product of PFBS-based chemicals. PFBS-based compounds are used primarily as water and stain repellents for leather, textiles and carpets.[309] They are also used to seal porous hard surfaces like concrete, granite and tile grout.[309] They are used in the manufacture of paints, waxes, and electronics and in some fume suppressant products used by chrome plating operations.[42, 310] PFBS is associated with older firefighting foams made by 3M but not with newer fluorotelomer formulations.[311] PFBS has been detected in foods, food contact papers, indoor dust, and drinking water.[42]

PFBS has been detected in limited sampling of drinking water in Washington state.[1] It is the fourth most frequently detected PFAS in testing of drinking water sources near California fire-training areas and municipal landfills.[312] PFBS is cleared from human serum much more rapidly than PFOA, PFOS, PFHxS, and PFNA. The average half-life of PFBS in human serum was estimated to be 27 days and 44 days in two small occupational studies.[28, 313] PFBS is infrequently detected in human serum or urine in the general U.S. population (aged 12 years and older).[12, 68] Communities with PFBS in their drinking water have higher reported frequencies of detection,[9, 10] including over 80% of adults in one study.[28] PFBS serum concentrations measured in people without occupational exposure are generally less than 2 µg/L when detected. Much higher serum levels (92—921 µg/L) have been measured in fluoropolymer manufacturing workers.[313]

PFBS and its potassium salt have been studied for toxicity in mice, rats, and monkeys. The evidence includes a two-generation study of reproduction and development in rats,[314] three gestational exposure studies in mice and rats,[315-317] 90-day oral studies in rats[318] and mice,[285] and 28-day oral studies in rats.[247, 319] Adverse effects observed include reduced thyroid hormones, kidney toxicity such as hyperplasia, developmental toxicity including delayed growth and maturation, hypertrophy in liver tissue, increased serum liver enzymes, and altered lipid and hematological profiles. Data gaps include lack of immune toxicity studies, chronic toxicity studies and cancer testing in laboratory animals.[42] EPA conducted a structured review of studies that investigated adverse effects of PFBS. This included 19 epidemiological studies that met EPA criteria for data quality. PFBS levels in serum were positively associated in at least one study with the following outcomes: adiposity in girls but not boys, asthma, serum cholesterol, cardiovascular disease and hypertensive disorders of pregnancy. Evidence from these human studies was considered *equivocal* by EPA evaluators.[42]

Review of Health Protective Values

DOH reviewed the available health protective values for daily ongoing human oral intake of PFBS. We focused on high-quality and comprehensive risk evaluations that considered recent scientific research and were conducted by U.S. federal and state agencies. Specifically, we evaluated reference doses by EPA, MDH, and MSWG and an acceptable daily dose by CA OEHHA. These are presented in Table 9 below. None of the risk assessments found sufficient information to evaluate PFBS for cancer outcomes.

Table 9. Health Protective Values for PFBS Reviewed by WA

Source	Critical study	Point of Departure and Critical effect	Human Equivalent dose (mg/kg-day)	Uncertainty factors (UF)[a]	Oral RfD or ADD[b] (mg/kg-day)	Exposure duration
EPA 2021 [42]	Feng et al 2017[315]	$BMDL_{0.5\ SD}$ (22.1 mg/kg-day) for reduction of thyroid hormone (total T4) in newborn female offspring of mice dosed during pregnancy (GD 1-20)	0.095[c] (=22.1 x 0.0043 DAF)	UF_{total} 100 UF_H=10 UF_A=3 UF_D =3[d]	0.001 RfD	Subchronic
EPA 2021	Feng et al 2017	$BMDL_{0.5\ SD}$ (22.1 mg/kg-day) for reduction of thyroid hormone (total T4) in newborn female offspring of mice dosed during pregnancy (GD 1-20).	0.095[c] (=22.1 x 0.0043 DAF)	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10[e]	0.0003 RfD	Chronic
CA OEHHA 2021 [312]	Feng et al 2017	$BMDL_{1SD}$ (22.1 mg/kg-day) for reduction of thyroid hormone (total T4) in pregnant female mice on gestation day 20. Mice were dosed during pregnancy (GD 1-20).	0.06[f] (=22.1/345 DAF)	UF_{total} 100 UF_H=10 UF_A=3 UF_D=3[g]	0.0006 ADD	Chronic
MSWG 2019[268]	Feng et al 2017	$BMDL_{20}$ (28.19 mg/kg-day) for 20% reduction of thyroid hormones (total T4) in newborn female offspring of mice dosed during pregnancy (GD 1-20)	0.0892 (=28.19/316 DAF)[h]	UF_{total} 300 UF_H=10 UF_A=3 UF_D=10[i]	0.0003 RfD	Chronic
MDH 2017[320]	Feng et al. 2017	NOAEL (50 mg/kg-day) for altered maternal thyroid hormones, reduced pup growth and developmental delays in female mice dosed (GD 1-20). LOAEL: 200 mg/kg-day	0.158 (=50/317 DAF)[h]	UF_{total} 100 UF_H=10 UF_A= 3 UF_D= 3[k]	0.0016 RfD	Short-term (1-30 days)
MDH 2017[320]	Leider et al 2009a; York et al. 2003[314, 317]	$BMDL_{10}$ (45 mg/kg-day) for 10% increase in mild hyperplasia in kidney in female rats in a 10 week, 2-generation rat study.	0.129 (=45/350 DAF)[j]	UF_{total} 100 UF_H=10 UF_A= 3 UF_D= 3[k]	0.0013 RfD	Subchronic (>30 days – 10% lifetime)
MDH 2017	Leider et al 2009a; York et al. 2003	$BMDL_{10}$ (45 mg/kg-day) for 10% increase in mild hyperplasia in	0.129	UF_{total} 300 UF_H=10 UF_A=3	0.00043 RfD	Chronic (>10% lifetime)

	kidney in female rats in a 10-week, 2-generation rat study.	(=45/350 DAF)[j]	$UF_D=3$[k] $UF_s=3$

[a] Uncertainty factors: UF_H= intra-species uncertainty factor; UF_A= inter-species uncertainty factor; UF_S= subchronic to chronic uncertainty factor; UF_L= LOAEL to NOAEL uncertainty factor; UF_D= incomplete database uncertainty factor; UF_{total}= total (multiplied) uncertainty factor. Uncertainty factors are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing a 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10. UFs that are not listed in this table were equal to 1.

[b] RfD is a reference dose. ADD is acceptable daily dose.

[c] Prior to BMD modelling, animal doses from Feng et al 2017 were converted to HEDs by applying a dosimetric adjustment factor (DAF), where HED = dose × DAF. EPA DAF for this POD= average serum half-life in female mice/average half-life in humans = 4.5 hours (from Lau et al 2020)/ 1050 hours (from Xu et al 2020) = 0.0043. To compare EPA to the other DAFs in the table, we must express the EPA as 1/DAF = 233.

[d] Database deficiencies included lack of developmental neurotoxicity studies, lack of testing for immunotoxicity and mammary gland development.

[e] Database deficiencies for chronic exposure also included lack of long-term toxicity studies and uncertainty about hazard identification and dose-response assessment for PFBS following chronic exposures.

[f] DAF is derived from average serum clearance in mouse from Lau et al. (2020) divided by mean human clearance from Olsen et al. (2009). 1344 mL/kg-d (mouse)÷3.90 mL/kg-day (human)= 345.

[g] Database deficiencies identified by OEHHA included lack of studies for specific endpoints developmental neurotoxicity, immunotoxicity and carcinogenicity and uncertainty about whether longer exposure duration would have exacerbated the critical effect on thyroid or resulted in other effects at lower doses.

[h] The DAF derived by MDH is the ratio of human serum half -life/female mouse serum half-life = 665 hours /2.1 hours = 316.6. The half- life in humans comes from Olsen et al 2009. The half-life in mice comes from Rumpler et al. 2016 and personal communication with Lau, C 2017. Michigan used this same DAF but rounded down to 316.

[i] Database uncertainty based on lack of developmental neurotoxicity study.

[j] MDH applied a DAF based on serum half-life of PFBS in humans (665 hours) and female Sprague-Dawley rats (1.9 hours) to derive an HED. DAF = 665 hours/1.9 hours = 350.

[k] Database uncertainty factor of 3 was applied for lack of neurodevelopmental, immunological and chronic studies.

EPA, Michigan, California and Minnesota recommended chronic health protective values in the range of 0.3 – 0.6 µg/kg-day for PFBS. Evaluators generally concurred that reduction in thyroid hormones was the most sensitive adverse effect of PFBS exposure in animal studies. The three most recent assessments based their chronic health protective value on reductions in thyroid hormones observed in mice in the same critical study (Feng et al. 2017). They also cited a 28-day rat toxicity study by the National Toxicology Program (2019) as providing support for selection of the thyroid hormone reductions as a critical effect. MDH's short-term RfD was based on Feng et al. 2017, but their subchronic and chronic oral RfDs were based on kidney toxicity observed in a developmental rat study (Leider et al 2009; York 2003) (see Table 9).

We describe the critical studies of these assessments below.

Feng et al. 2017 administered oral doses (50, 200, and 500 mg/kg-day) of potassium perfluorobutane sulfonate (K-PFBS) daily to pregnant mice on gestation days 1-20 and allowed offspring to nurse. Female offspring were monitored at birth (postnatal day 1), puberty (postnatal day 30), and adulthood (postnatal day 60) for growth, developmental benchmarks, and hormone levels. Male offspring were used for another study and were not evaluated. At the

LOAEL (200 mg/kg-day), K-PFBS altered thyroid hormones in the dams and the offspring into adulthood. Specifically dams at gestation day 20 had 21 percent lower total thyroxine (T4), 17 percent lower triiodothyronine (T3) and 21 percent higher thyroid stimulating hormone (TSH) at the LOAEL. Female offspring exposed to PFBS *in utero* had reductions up to ~30 percent in T3, and reductions of up to 42 percent in T4 across the three time points evaluated. TSH was elevated in offspring at puberty and adulthood, but was only statistically significant at puberty. The NOAEL for altered thyroid hormones was 50 mg/kg-day.

A number of other adverse effects had the same LOAEL (200 mg/kg-day) in the Feng et al. study. Pups were underweight compared to controls into adulthood and had delayed eye opening, delayed vaginal opening, and delayed first estrous. At adulthood, ovaries were smaller with reduced follicle development and there was reduced thickness of uterine lining indicating reduced development of both organs. Abnormal estrous cycling (prolongation of diestrus) was observed between puberty and adulthood. Serum estradiol was lower and luteinizing hormone was higher at puberty compared to controls. Serum progesterone was decreased in adulthood. The NOAEL was 50 mg/kg-d for all adverse effects noted. Maternal serum PFBS was measured twelve hours after the last dose on gestation day 20 but given the very short half-life of PFBS in female mice, EPA used the administered dose rather than the serum measurement to derive their point of departure for risk assessment.[42, 315]

The **National Toxicology Program, 2019** conducted a 28-day oral gavage study with PFBS in adult male and female rats. Thyroid hormone levels (free T4, total T4, and T3) were reduced in both male and female rats while TSH levels were highly variable and not statistically different from controls at any dose. The magnitude of declines observed in T4 and T3 was more dramatic than declines observed in mice in the Feng et al study. At the lowest dose tested in the NTP study (62.6 mg/kg-day), free T4, and total T4 were at least 50 percent lower than controls in females, and 70 percent lower than controls in males. Reductions in T3 were approximately 30 percent lower than controls in both male and female rats. No changes in thyroid histopathology or weight were reported. A dose-dependent prolongation of diestrus at and above doses of 250 mg/kg-day was observed in female rats with marginal significance at the lowest dose tested (125 mg/kg-day). The NTP study also reported increased kidney weights in males and increased liver weights in females. The lowest dose administered (62.6 mg/kg-day) was the LOAEL for significant reductions in T4 and T3 hormone levels.[247]

Lieder et al. 2009 and York et al. 2003 conducted a two-generation reproductive study in rats with 0, 30, 100, 300, and 1000 mg/kg-day of K-PFBS administered by gavage to males and females for 10 weeks prior to and through mating. Females continued to be dosed daily through gestation and lactation. The first generation of offspring (F1) nursed until PND 22 and then dosed from weaning through mating, gestation and a lactation period. The second generation of offspring (F2) was not dosed directly, but were allowed to nurse and sacrificed at three weeks.

The most sensitive effect observed was increased kidney hyperplasia and focal papillary edema in males and females of the parent generation and F1 adults. MDH derived a $BMDL_{10}$ for this outcome of 45 mg/kg-day. Thyroid hormones T3 and T4 were not measured.[314, 317]

EPA RfD

EPA evaluators noted that similar patterns of reduced T3, total T4, and free T4 levels were observed in the Feng et al. and NTP studies in pregnant mice, their offspring, and in adult male and female rats exposed to PFBS. EPA also recognized that thyroid hormones are essential for proper growth and development across species and that reductions in T3 and T4 observed following gestational exposure in the Feng et al. study plausibly explained altered development observed in female offspring.[42] EPA derived their PFBS RfD by first applying a dosimetric adjustment factor (DAF) to the administered doses in Feng et al. to estimate human equivalent doses. The EPA DAF was a ratio of average serum half-life in female mice (from Lau et al. 2020)[321] over the average serum half-life in humans (from Xu et al. 2020).[28]

$$EPA's\ DAF = \frac{4.5\ hrs}{1050\ hrs} = 0.0043$$

Both studies supporting EPA's DAF are recently published. Lau et al. 2020 evaluated the pharmacokinetics of oral PFBS dosing in CD-1 male and female mice at eight weeks of age. Xu et al. 2020 monitored PFAS in the serum of a group of airport workers for five months. This monitoring began two weeks after PFAS contamination was removed from their workplace drinking water supply. The mean PFBS serum half-life for the group was 44 days and ranged from 21 to 87 days in individual participants.[28]

EPA conducted benchmark dose (BMD) modelling on the human equivalent doses from the Feng et al study. The point of departure was a T4 reduction of one half the standard deviation ($BMDL_{0.5\ SD}$) compared to controls in newborn pups. EPA applied a 100-fold uncertainty factor for subchronic exposures and a 300-fold uncertainty factor for chronic duration exposure. Uncertainty factors (UF) included ten for human variability and three for interspecies uncertainty. For subchronic exposures EPA applied a UF of three for database deficiencies, noting the lack of developmental neurotoxicity studies and the lack of immune toxicity studies. For chronic duration exposures, EPA applied a UF of ten for database deficiencies citing an additional concern that long-term exposure studies in animals are lacking.

CA OEHHA ADD, MSWG and MDH RfDs

Michigan and California assessments differed from EPA in three areas: dose-response modelling, dosimetric adjustment factors, and uncertainty factors (see Table 7). California modelled a $BMDL_{1.0\ SD}$ on the thyroid hormone (T4) reduction in pregnant female mice rather than in their day-old pups. Michigan Science Advisory workgroup relied on earlier EPA modelling of a $BMDL_{20}$ for 20% reduction of thyroid hormones (total T4) in newborn female offspring of mice. For dosimetric adjustment factors, Michigan relied on Rumpler et al. 2016

which reported a shorter serum half-life (2.1 hours) in mice.[322] California and Michigan evaluators also relied on an earlier study for human serum half-life, by Olsen et al. 2009. This earlier study monitored a small group of manufacturing workers (five men, one woman) during a six-month break in PFBS production. The mean serum elimination half-life for PFBS in these workers was 27.7 days and ranged 13–46 days for the six individuals.[27] Minnesota evaluators selected a different critical effect from a longer duration study for the basis of its chronic RfD. This kidney endpoint appears to be less sensitive than the BMDLs for altered thyroid hormone level used as points of departure in the other assessments. EPA, Michigan and Minnesota evaluators applied a 300-fold UF for their chronic health protective value. California evaluators applied a 100-fold UF that differed only in selecting a 3-fold rather than a 10-fold UF for database deficiency. California cited the same elements of concern regarding database uncertainties.

We concurred with the 2021 EPA assessment. The EPA DAF relied on Lau et al. 2020 appears to be the final peer-reviewed published study reported in the conference abstract by Rumpler et al. 2016. The half-life study in humans by Xu et al. 2020 had several advantages over the study in six workers by Olsen et al. 2009. The newer study followed more workers (n=17), included more females (n=6), and was more representative of an environmental drinking water exposure. We also agreed that a database uncertainty factor of 10 was justified given the lack of chronic toxicity testing and key data gaps. Lack of developmental neurobehavioral testing is a concern given the thyroid effects observed. Preliminary studies provide some indication that PFBS may act similarly to PFOS on immunoregulation.[323, 324]

Relevance to Humans

EPA conducted a structured review of the evidence for PFBS and thyroid effects. EPA concluded that the evidence in animals for thyroid effects *supports a hazard* and that the thyroid is a potential target for PFBS toxicity in humans. EPA also concluded that the animal evidence for developmental toxicity *supports a hazard*.[42]

Several lines of supporting evidence were cited by EPA. Consistent reduced T4 and T3 were observed across two species of rodents (mice and rats), adult and early life stages, and different exposure durations (20–90 days). Maternal and neonatal thyroid hormone insufficiency were considered coherent with pronounced and persistent developmental effects in female mouse pups as they grew into adulthood. Altered estrous cycling in mature female pups was deemed biologically consistent with these effects and was also observed in rats exposed as adults.

Although direct human evidence was lacking or deemed *equivocal*, EPA reviewed the considerable evidence that supports the importance of T4 and thyroid hormone sufficiency for proper human development. “Thyroid hormones play a critical role in coordinating complex developmental processes for various organs/systems (e.g., reproductive and nervous system),

and disruption of thyroid hormone production/levels in a pregnant woman or neonate can have persistent adverse health effects for the developing offspring."[42]

Other PFAS with similar chemical structures to PFBS have been investigated for their potential impact on thyroid hormone levels in pregnant women and their offspring. Some but not all of these studies report associations between PFAS serum levels and thyroid hormone perturbations.[213, 302]

EPA also evaluated the available evidence for other adverse effects of PFBS. Their synthesis of rodent and human data on other outcomes concluded that PFBS animal evidence *supports a hazard* for kidney toxicity and was "equivocal" for reproductive toxicity, hepatic effects, and effects on lipid or lipoprotein homeostasis. Two other endpoints, immune effects and cardiovascular effects, had *equivocal* evidence in human studies and no studies in laboratory animals.[42]

Washington State recommendation: 300 ng/kg- day (EPA RfD)

We recommend using the chronic toxicity value in the April 2021 EPA assessment of PFBS. The EPA toxicological assessment is comprehensive, high quality and incorporates the most recent data available for PFBS. We concurred with EPA and others on thyroid hormone reduction as the most sensitive critical effect and with selection of Feng et al, 2017 as the critical study. Results from this study are supported by the 2019 NTP study that showed male and female rats exposed to PFBS for 28 days as adults also had significantly reduced thyroid hormones at the lowest dose tested.

Sensitive populations. Pregnant women, fetuses and infants are potentially susceptible life stages for the types of effects observed in animal testing with PFBS. PFBS caused developmental toxicity and persistent changes in thyroid hormone levels in gestationally exposed mice. In humans, maternal thyroid hormones are critical for normal fetal growth and neurodevelopment especially before the fetal thyroid gland has developed. Following birth, neonatal thyroid function also supports infant growth and neurodevelopment.[36, 227, 325] Thyroid tissue stores of T4 are low in newborn children making them less able than adults to compensate for reductions in T4.[326] Thyroid hormones are also important to maternal health and maintaining pregnancy.[327]

Relative source contribution (RSC): 20 percent

PFBS-based chemistries are still used in manufacturing and in commercial and consumer products.[309] PFBS-based surfactants and polymers may degrade to PFBS as the products age or undergo environmental degradation.[309] PFBS has been detected widely in surface waters and aquatic and terrestrial biota including in remote areas.[309] Field studies show that PFBS has potential for uptake into plants and livestock,[328-332] but PFBS detections in dietary samples were infrequent in European and U.S. food surveys.[333, 334] A Swedish study, which measured PFBS in serum samples collected between 1996 and 2010 from lactating women, showed that market

shifts in PFAS use aligned with trends in serum concentration. While long-chain PFAS showed declining trends, the trend for PFBS showed a doubling of serum level every six years over this time period.[335]

Although infant exposure to PFBS through breastmilk is possible, we did not find sufficient information to model this pathway of exposure for infants. PFBS was detected in less than half the studies identified by the MDH that evaluated breastmilk for its presence.[320] Cord blood PFBS did not correlate well with paired maternal serum in a study by Wang et al. 2019.[336] For other PFAS SALs, we were able to justify a 50 percent RSC for infants by accounting for their breastmilk exposure when PFAS occurred in maternal tap water.

Overall, ongoing exposure to PFBS from non-drinking water sources is likely, but we found insufficient data to quantify exposure from all sources other than tap water. Using the EPA Exposure Decision Tree (Figure 4), we derived a default RSC of 0.2 for all life stages for PFBS (see Table 2).

Drinking water intake rate: 0.174 L/kg-day (Infants)

We used assumptions consistent with our other PFAS SALs for upper-bound drinking water intake rates for sensitive populations and life stages. Specifically, we considered 90th percentile water intake rates for women of reproductive age and 95th percentile water intake rates for pregnant and lactating persons and infants (birth to <1 year old) to protect the developing child. These are shown in Table 10. The SAL calculated for infants adequately protects the other sensitive groups and is selected to protect the population as a whole. Michigan MSWG and California OEHHA also used infant drinking water intake rates to derive their state regulations for PFBS in drinking water based on this same endpoint. The EPA assessment did not calculate drinking water advice.

Table 10: PFBS SAL calculations for four potentially sensitive populations/life stages

Sensitive population	RfD[a] (mg/kg-day)	Drinking water Intake rate (L/kg-day)[b]	Relative Source contribution or RSC (%)	Candidate SALs (mg/L)
Infants (<1 year)	0.0003	0.174 (95th)	20	0.000345
Pregnant women	0.0003	0.038 (95th)	20	0.001579
Lactating women	0.0003	0.047 (95th)	20	0.001277
Women of reproductive age (15-44 y/o)	0.0003	0.035 (90th)	20	0.001714

[a]*RfD = chronic oral Reference Dose for PFBS (EPA 2021).*

[b]*Intake rates from 2019 EPA Exposure Factors Handbook Chapter 3 (based on consumers only population and two-day average consumption).*

Derivation of the PFBS SAL

$$\text{SAL} = \frac{RfD}{Drinking\ Water\ Intake\ Rate}\ x\ RSC$$

$$\text{SAL} = \frac{0.0003\,\frac{mg}{kg-day}}{0.174\,\frac{L}{kg-day}}\ x\ 0.20 = 0.000345\ mg/L$$

SAL = 345 ng/L (parts per trillion)

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