Group A Public Water Supplies • Chapter 246-290 WAC

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

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Glossary

ATSDR	Agency for Toxic Substances and Disease Registry atsdr.cdc.gov/pfas/index.html
BMD	Benchmark Dose modeled on the dose-response data from one or more study. 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 for the benchmark dose.
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.
EPA	U.S. Environmental Protection Agency, epa.gov/pfas
GAC	Granular activated carbon
IRIS	Integrated Risk Information System at EPA
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 concentration of a regulated contaminant the Safe Drinking Water Act allows in drinking water. 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	Units used for doses of PFAS in animal experiments meaning milligrams of chemical per kilogram body weight per day.

m/L	Units of PFAS concentration in blood serum used for reporting results in animal studies meaning milligrams of chemical per liter of serum. Equivalent to parts per million (or ppm).
MPART	Michigan PFAS Action Response Team, a temporary body established in 2017 by an executive directive to investigate sources and locations of PFAS and protect drinking water and public health.
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.
NH DES	New Hampshire Department of Environmental Services
ng/kg-day	Units used for estimates of daily acceptable intake of PFAS by humans meaning nanograms of chemical per kilogram body weight per day. 1 ng/kg-day is the same as 0.000001 mg/kg-day.
ng/L	Units for water concentration of PFAS meaning nanograms of chemical per liter of water. Equivalent to parts per trillion (or ppt).
NOAEL	No Observed Adverse Effect Level is the highest administered dose in an experiment with no observed adverse effects.
NJ DWQI	New Jersey Drinking Water Quality Institute, a technical group of scientists and engineers who develop recommendations for drinking water standards for the State of New Jersey Department of Environmental Protection.
PPAR	Peroxisome Proliferator-Activated Receptors are nuclear receptors that regulate a large number of genes. There are several subtypes including alpha (α) and gamma (γ).
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)
RfD	An Oral Reference Dose is an estimate of a daily oral intake not anticipated to cause adverse health effects over a lifetime (including 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.
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 3 to 5 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 unbound or free fT3 or the total of bound and unbound 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	Units of PFAS concentration in human blood serum meaning micrograms of chemical per liter of serum. Serum is the clear liquid that can be separated from clotted blood. Equivalent to parts per billion (or ppb).

Summary

In July 2017, a letter from ten organizations requested that the Washington State Department of Health (department) establish drinking water standards for per- and polyfluoroalkyl substances (PFAS). The department forwarded the letter to the State Board of Health (board) with a recommendation to accept it as a petition to amend chapter 246-290 WAC. The board accepted the petition in October 2017, and initiated the rule-making process. 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 department developed draft recommended state action levels (SALs) for five PFAS detected in Washington drinking water. We recommend these five SALs as indicators to identify and mitigate PFAS contamination in public drinking water supplies.

Our approach to developing SALs involved evaluating primary PFAS scientific literature and reviewing recent assessments by federal and state agencies. We built off recent high-quality science assessments. We found sufficient information to recommend SALs for PFOA, PFOS, PFNA, PFHxS, and PFBS. The first four of these PFAS are highly bioaccumulative in humans and produce developmental toxicity or effects of developmental concern in laboratory animals. To address developmental concerns, our action levels considered exposure pathways specific to early life stages, including placental and lactational transfer using a model the Minnesota Department of Health developed.

We developed the SALs from health protective values for five PFAS. These values were derived from studies in laboratory animals with support from epidemiological data when available. The primary health concerns with these PFAS are liver toxicity, reproductive and developmental toxicity, immune toxicity, alterations in thyroid hormone levels, and altered serum lipids. The International Agency for Research on Cancer considers PFOA "possibly carcinogenic to humans." Other PFAS are less studied.

The SALs for five PFAS proposed in Table 1 define levels in daily drinking water expected to have no appreciable health effects for the general population, including sensitive subgroups. They are comparable to a maximum contaminant level goal (MCLG) in the Safe Drinking Water Act. Taking action at these levels is consistent with the mission of providing safe and reliable drinking water.

Table 1 Proposed State Action Levels (SALs) for per- and polyfluoroalkyl substances (PFAS)

Individual PFAS	Proposed State Action Level for Drinking Water
PFOA	10 ng/L
PFOS	15 ng/L
PFNA	14 ng/L
PFHxS	70 ng/L
PFBS	1,300 ng/L

PFAS frequently appear as mixtures in drinking water. The mixture may contain multiple PFAS, including those with an SAL and those without. We do not have good information on the toxicity of PFAS mixtures. Therefore, when water exceeds any one of the state action levels, we recommend that water systems employ mitigation effective for a wide range of PFAS. For example, activated granular carbon and anion exchange resin filtration both effectively remove many PFAS. New mitigation methods under research may further improve PFAS removal from water. Using action levels as a proxy for other PFAS in a mitigation technology approach provides a reasonable 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 will consider adopting a broader grouped approach to regulating PFAS mixtures as the science and methodology evolve.

Background

We know of five areas in Washington with PFAS contamination in groundwater supplies used for drinking water.

Several Washington communities have detected PFAS in their drinking water supplies. These are the cities of Airway Heights and Issaquah, and communities around Joint Base Lewis-McChord, Naval Air Station Whidbey Island, and Fairchild Airforce base.

During 2013–2015, 132 public water systems in Washington conducted monitoring for six PFAS. The systems included all 113 large Group A systems that serve more than 10,000 people and 19 smaller systems. The systems tested cover 94 percent of Washington residents served by public water systems. Only one water system, the City of Issaquah, had a well that exceeded EPA's lifetime health advisory level (70 ng/L) for PFOA and PFOS.[1] The military identified additional areas of contamination on and around several military bases during voluntary testing.

A type of firefighting foam that contained a PFAS called aqueous film-forming foam, or AFFF, is a key suspect at all these sites. In other states, PFAS sources of drinking water contamination include firefighting foam used at military bases, civilian airports, and oil refineries; industrial manufacturing sites where PFAS were made or used to make coated papers and textiles; lands that accepted biosolids, which contained industrial PFAS waste; and landfills that accepted industrial PFAS waste.[2, 3]

PFAS are highly persistent in the environment.

Perfluoroalkyl acids, such as PFOS, PFOA, PFNA, PFHxS, and PFBS, are essentially nondegradable in the environment and will persist in soils and groundwater. Other PFAS compounds can break

down in the environment to form these PFAS. These other PFAS are sometimes called precursor chemicals.[3]

Some PFAS are highly bioaccumulative in people.

The human body readily absorbs some PFAS from food and water, but only slowly eliminates them (PFOA, PFOS, PFHxS and PFNA). As a result, they accumulate in human blood serum, liver, lung, bone, and other locations in the body.[4, 5] These PFAS can pass through the placenta and accumulate in fetal tissue.[6] The body excretes PFBS more rapidly and it appears to be much less bioaccumulative in people. However, several studies indicate that PFBS in drinking water can increase serum levels of PFBS in consumers.[7-9]

A "half-life" is one way to measure the bioaccumulative nature of a substance. For these PFAS, a half-life is the time it would take for the serum concentration to drop by half after removing a major source of exposure (such as when exposure to contaminated drinking water stops). Estimates from different studies (below) vary depending on the age and gender of the population, the level of PFAS exposure, the level of continuing background exposure, and the length of follow-up.

- PFOA: 0.3 to 3.9 years[10]
- PFOS: 3.3 to 4.6 years[10, 11]
- PFNA: 2.5 to 4.3 years[12]
- PFHxS: 5.3 to 7.1 years[10]
- PFBS: 27 days[13]

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. (The body excretes PFBS more quickly so it is not commonly detected in serum of the general population.) Figure 1 shows the average PFAS levels in U.S. serum over time. More information is available in CDC's PFAS biomonitoring factsheet.

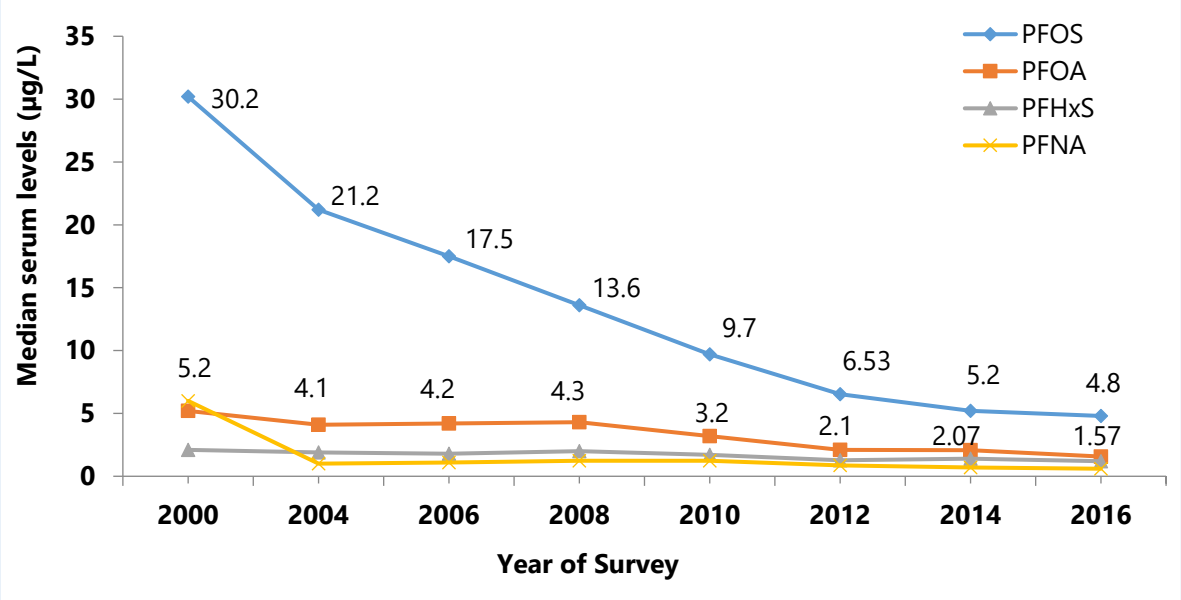


Figure 1: *Time trend of median serum levels of four PFAS in representative samples of U.S. residents >12 years old. Source: CDC National Health and Nutrition Examination Survey (NHANES).*[14]

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

Over the last 20 years, major U.S. industries phased a number of highly bioaccumulative PFAS out of production and most uses. Some uses were allowed to continue. These PFAS continue to be produced in other countries and may be 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 1).

PFAS in drinking water can contribute significantly to consumer exposure.

PFAS in drinking water can contribute significantly to human exposure.[7, 10, 16, 17] For people without PFAS in their drinking water, researchers believe the primary pathways are diet, indoor dust, and air.

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

PFAS risk assessors use predictive models to estimate average serum levels due to a PFAS concentration in daily drinking water. These models predict that in adults, serum levels will increase gradually over a period of years until the serum level reaches a plateau (a condition called steady state). Figure 2 shows an example of predicted serum PFOA level in adults consuming drinking water with PFOA at 10 ng/L.

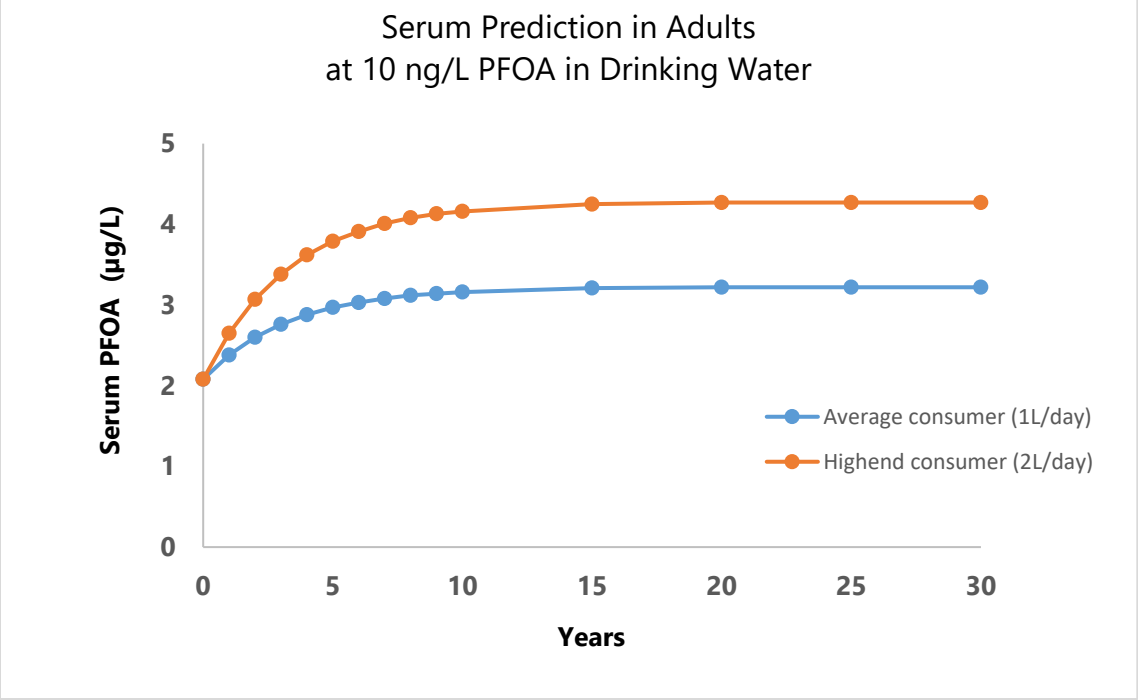


Figure 2 *Predicted average PFOA serum levels in adults with PFOA in drinking water at 10 ng/L using the Bartell model.[18] The graph, based on an average starting PFOA serum level of 2.0 µg/L, represents average background levels of PFOA in serum in the U.S. population. It plots two scenarios of adult drinking water intake: an average drinking water ingestion rate of one liter per day and a high-end consumer drinking two liters per day.*

Exposures to PFAS in drinking water can be higher in breastfed infants than in adults.
Mothers with PFAS in their daily drinking water can pass some of the PFAS they absorb onto their babies during pregnancy and breastfeeding. Another source of exposure for infants is formula mixed with tap water that contains PFAS.

PFAS serum levels in bottle-fed and breastfed infants can increase rapidly because their intake of breastmilk or formula is high compared to their body weight. This is despite the fact that PFOS, PFOA, PFHxS, PFNA are usually present at a much lower concentrations in breast milk (1–12 percent) compared to mother's serum.[19] We strongly recommend that nursing mothers continue to breastfeed because of the many known health benefits of breastfeeding.

The SALs support recommended exclusive breastfeeding for baby's first six months.
We developed our SALs to protect the benefits of breastfeeding and to prevent nursing and formula-fed infants from exceeding the health protective levels for PFAS. It is important to protect infants because research in laboratory animals indicates that early life stages are likely to be sensitive to the toxicity of these five PFAS.

Health effects of PFAS still under study.
We based our public health advice on toxicity of PFOA, PFOS, PFNA, PFHxS, and PFBS observed in laboratory animals with supporting information from health studies of people exposed to PFAS. Toxicity seen in animal testing include liver and kidney damage, certain types of immune suppression, reduced fertility, damage in reproductive organs, reduced survival and growth of offspring, altered development of offspring exposed during pregnancy and early life, and altered

hormone levels and metabolic changes. Long-term exposure to PFOA and PFOS caused certain tumors in animal testing.

Health researchers are still investigating the potential health effects that PFAS in drinking water could have on people. Some studies indicate that drinking water with higher levels of PFAS may increase serum cholesterol levels, lower birth weight in babies, increase liver enzyme levels, suppress immune response to vaccines, alter levels of thyroid and sex hormones, increase the risk of thyroid disease and blood pressure problems in pregnancy, and cause reproductive problems and certain cancers. The types of effects seen and the strength of the evidence vary by PFAS. For details, see the supporting information for each PFAS SAL.

We have limited ability to measure all PFAS contaminants in water or to assess their effect on health.

Only 18 chemicals in the large PFAS class (>4700 members) are measured in the current validated method for drinking water (EPA 537.1). New methods under development at EPA would expand that number to 26.[20] It is unknown how many other PFAS could occur in drinking water.

We still know relatively little about the potential toxicity of most PFAS. EPA issued toxicity assessments and drinking water advice for PFOS and PFOA in 2016. The 2018 ATSDR Toxicological Profile on PFAS included information on 14 PFAS and issued draft health-based guidance for four: PFOS, PFOA, PFNA and PFHxS.[19] The EPA Integrated Risk Information System (IRIS) program released draft toxicity assessments for PFBS and GenX in December 2018 and plans to develop assessments for PFDA, PFNA, PFHxS, PFHxA, and PFBA.[19]

The five recommended SALs include the most commonly detected PFAS in drinking water with sufficient toxicity information. Until we have better tools and toxicity information, we recommend using these five SALs as indicators of PFAS occurrence in drinking water. When a water system exceeds an SAL, we recommend that it use mitigation options effective for many PFAS.

Some 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.[21] The two most commonly installed PFAS removal technologies for public water systems are granular activated carbon (GAC) and anion exchange resins (AER). Both can remove 90 to 99 percent of most PFAS listed in the EPA database. Both types of filtration require ongoing water monitoring for efficacy and periodic replacement of filter media.

GAC is made from organic materials with high carbon contents such as wood, coconut husk, lignite, and coal. When water flows through this substance, the PFAS bind to the carbon. These filters have high efficacy for a number of PFAS. Efficacy depends on the type of carbon used, the depth of the carbon bed, flow rate of the water, temperature, occurrence of organic matter, and other contaminants and constituents in the water. GAC works best on PFAS with longer carbon

chains like PFHxS, PFOS, PFOA, and PFNA. PFAS with shorter carbon chains, like PFBS, "breakthrough" more quickly.

Anion exchange resins (AER) effectively remove many PFAS contaminants. AER removes negatively charged contaminants from water by passing it through a bed of synthetic resins (small beads). AER exchanges negatively charged PFAS ions with negatively charged ions on the resin surface. Water systems must regenerate the resin bed periodically to maintain efficacy. Experience shows AER to have a high capacity for many PFAS and is more effective than some GAC systems at removing short-chain PFAS.

Federal research 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.[20, 22] 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 will consider adopting a broader grouped approach to regulating PFAS mixtures as the science and methodology evolve.

Introduction to Approach and Methods

Health-based values of acceptable daily intake for five PFAS in drinking water.

To support rulemaking, the department 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 EPA and state reference doses (RfDs) and ATSDR minimal risk levels (MRLs). We describe the values specific to each of the five PFAS chemicals in the supporting information for each SAL.

The health-based values we selected are based on non-cancer effects in laboratory animals. Specifically, these were adverse effects on immune function, altered development of offspring, and reduced serum levels of thyroid hormones. Points of departure for other endpoints, such as liver toxicity and reproductive toxicity, were sometimes only slightly less sensitive. There are limited studies of carcinogenicity for these five PFAS. Both EPA and the New Jersey Drinking Water Quality Institute (NJ DWQI) derived values based on cancer data for PFOA. NJ DWQI derived a cancer-based value for PFOS as well. These cancer-based values were not meaningfully different from health protective values based on non-cancer endpoints. Both EPA and NJ DWQI selected non-cancer endpoints as the basis for their health protective values because the underlying data were more robust and suitable for a dose-response assessment. We concurred with EPA, ATSDR, and a number of states that non-cancer endpoints had a better evidence base for supporting health protective values.

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 health outcomes and a specific PFAS in populations with simultaneous exposure to multiple PFAS. When multiple PFAS occur in public drinking water, the individual PFAS will be highly correlated with each other in serum samples from the community. In addition, our analytical methods have only measured about a dozen PFAS in water and serum. Unidentified PFAS in drinking water may be contributing 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.[23] Most of the epidemiological studies focus on PFOA and PFOS with limited study of other PFAS.

The cross-sectional study design of most PFAS epidemiological studies limits their use in determining causality. In fact, researchers believe 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 excretion pathways in women. Conditions like kidney disease that can reduce glomular filtration rate may lead to higher serum PFAS because it impairs a major excretion pathway.

Another concern is using a single serum sample to quantify PFAS exposure. Serum levels reflect exposure across recent months to years, but will not provide information on a historical peak exposure or fluctuations in serum over time. A single serum will not necessarily reflect the level in serum that preceded the onset of a disease or condition. Some studies, like the C8 Health Project, did exhaustive exposure reconstruction to overcome this limitation. 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 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), how a chemical effects specific tissues (PPARα activation[1] in rodent vs. human liver tissue), and the adverse effects produced (serum cholesterol reductions in rodents vs. increased cholesterol in humans). 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). The toxicokinetics differ between species, strains and sex of experimental animals. 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.[25] Although PFBS is less bioaccumulative in humans, estimated serum elimination rate in humans is still on the scale of weeks compared to hours in rodents. For this reason, internal dose (serum level) rather than administered dose was generally used to determine the point of departure in animal studies (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.

[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. 24. 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.

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. We provide the equation (below) used to calculate the concentration of a contaminant in drinking water that will be protective of human health assuming year-round exposure (365 days/year). The Safe Drinking Water Act calls the health-protective drinking water level a "maximum contaminant level goal" (MCLG). We used this same approach to calculate our SALs using internal dose rather than external dose for four of the five PFAS.

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

EPA provides a decision tree for deriving the RSC for water quality standards.[26] EPA recommends a default RSC of 0.20 (20 percent) contribution to the RfD 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 3).

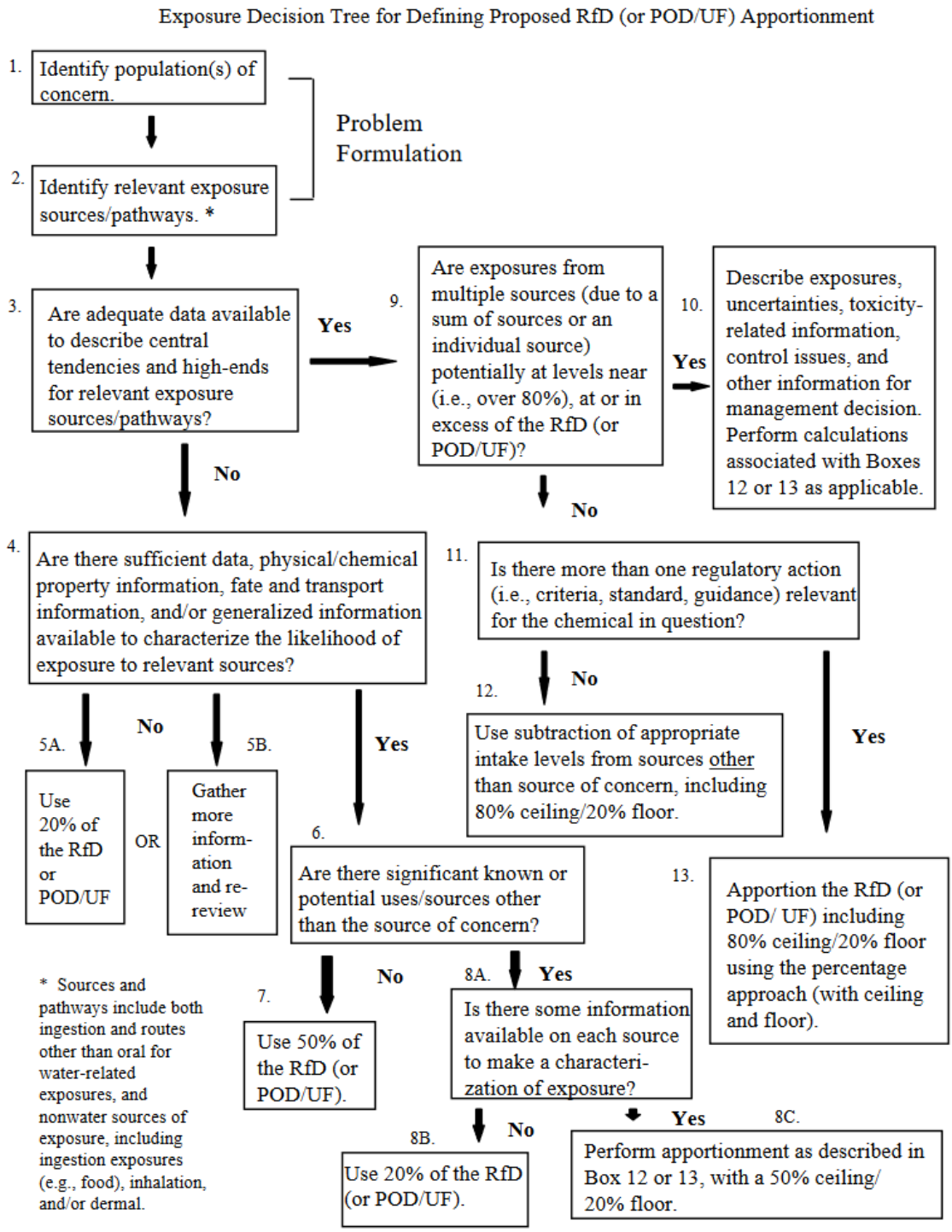


Figure 3. *EPA Exposure Decision Tree for defining apportionment of the RfD between different regulated sources of exposure.*[26]

In Box 1, we identified the developing fetus and infant as the most sensitive life stages of concern for the four PFAS with recommended limits for daily intake (e.g., RfDs, MRLs) based on developmental and thyroid hormone concerns. The MRLs for PFOA and PFNA were based on developmental endpoints in rodents. PFBS and PFHxS RfDs were based on reduced thyroid hormone concentrations in rodents. The critical role that thyroid hormones play during rapid

growth and brain development makes reduced thyroid hormone levels a concern for developmental effects (see individual PFAS summaries for more detailed discussion).

A secondary population of concern for PFOA, PFNA, PFBS, and PFHxS was developing children (birth through puberty). Childhood exposures may be relevant to altered immune responses to childhood vaccines and to altered development at puberty. Since maternal serum drives fetal and lactational exposure, women of reproductive age and children were the subpopulations targeted for protection (Box 1).

For PFOS, the RfD was based on immune suppression in adult male mice, so we derived an RSC for adults. Of secondary concern was PFOS exposure to the fetus and infant as the serum level (internal dose) at the NOAEL for developmental toxicity was not much higher (see PFOS summary for more details).

In Box 2, pathways of exposure to drinking water included direct ingestion by children and adults, and indirect exposures to fetuses and infants 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 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).

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. We lack robust data on the pathways of exposure for this age group, but a Norwegian Institute of Public Health study assessed indoor air levels, dust levels, and breast milk levels for a number of six-month-old infants. They estimated that breast milk contributes on average 83 percent of an infant's total daily intake of PFOA and 94 percent of PFOS.[27] We assumed few other exposure sources for this age group (birth to six months) and answered "no" to Box 6.

Box 7 recommends an RSC of 50 percent, which is higher than the default of 20 percent. Lacking stronger data on infant (birth to six months) exposures to these PFAS, we agreed that a 50 percent RSC was appropriately conservative.

For older children and adults, there are significant potential PFAS sources other than drinking water ("yes" to Box 6).[9, 28-30] PFBS is part of the PFAS chemistry replacing phased-out PFAS in the current U.S. marketplace. We did not have sufficient information on specific applications of PFBS-based chemistry to estimate human exposure. Because PFBS clears more readily from human serum, we may need a different biomarker such as PFBS in urine to estimate a distribution of daily exposure in the U.S. population. Because of these uncertainties, we answered "no" to Box 8A and selected the 20 percent default (Box 8B) for the PFBS RSC.

For the other four bioaccumulative PFAS, we used several lines of evidence to answer "yes" to Box 8A and estimate the total amount of exposure other than drinking water (Box 8C). CDC biomonitoring surveys provide distributions for these four PFAS in the serum of the U.S.

population three years old and older.[14] Biomonitoring data provides an indication of total exposure from all sources. Based on limited data from two surveys of drinking water in the U.S. (described below), we made a conservative assumption that the current 95th percentile of PFAS in serum of the general U.S. population represents exposure to sources other than drinking water. This is a protective assumption because 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).

Two surveys of U.S. drinking water support our assumption that most of the PFAS exposure measured in the CDC NHANES biomonitoring study comes from sources other than drinking water. Those surveys show low percentages of drinking water systems with significant PFAS contamination. The first was a national survey of drinking water conducted in 2013-2015, called the Unregulated Contaminant Monitoring Rule, Round 3 (UCMR3). This survey infrequently detected six PFAS (including PFOS, PFOA, PFHxS, PFNA, and PFBS).[1] In 2016, using UCMR3 data, Hu et al. estimated that 16.5 million people (~5 percent of the U.S. population in 2014 at the time of the survey) had detectable levels of at least one of six PFAS measured in their drinking water.[2] This estimate is limited by the relatively high laboratory reporting limits for UCMR3 samples and the exclusion of most medium and smaller public water systems and all private wells from the survey (reporting limits ranged 20-40 ng/L for PFOA, PFOS, PFHxS, and PFNA).

The State of Michigan recently conducted comprehensive water testing across their state with lower laboratory detection limits and 14 PFAS in the test panel. They tested drinking water at 1,114 public drinking water systems, 461 schools, and 168 childcare providers and Head Start programs. Ninety percent of samples had no detectable PFAS, 7 percent had detections of total PFAS <10 ng/L, 3 percent had total PFAS levels between 10-70 ng/L, and two systems (0.1 percent) had >70 ng/L of PFOA and PFOS combined.[31] The Michigan water-testing project did not calculate the percentage of the population served by water systems with detectable PFAS in their drinking water.

Box 8C recommends the subtraction method to calculate an RSC with a ceiling of 50 percent and a floor of 20 percent. For highly bioaccumulative PFAS, such as PFOA, PFOS, PFHxS, and PFNA, serum concentration is the best measure available for combined exposure from all sources. 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 > 12 years of age. An NHANES survey of children aged 3-11 years in 2013-14 provided estimates for ages 3-11 years. We used these estimates because

we expect that serum levels of PFAS in Washington residents will be similar to national norms. 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.[32] Levels measured in this population were comparable to the distribution in NHANES for the same time period.[33]

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.[34] 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		default	20%
Females ≥ 12 yrs. [d]	< 0.1		default	20%
6-11 year olds	0.13		default	20%
3-5 year olds	< 0.1		default	20%
Infants	-		Box 7	50%

[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 [35]. < 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 [26].

[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 elevated drinking water ingestion rates for adults (90th percentile) from the EPA Exposure Factors Handbook. If a sensitive subpopulation is identified, drinking water ingestion rates are selected specific to their expected consumption.

The Minnesota Department of Health (MDH) developed a toxicokinetic model for infant intake for several bioaccumulative PFAS in drinking water. The model predicts a starting infant serum at birth as a proportion of maternal serum and includes two scenarios of infant nutrition. The first scenario assumes exclusive breastfeeding through 12 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. MDH obtained external peer review of their model from six academic, government, and private industry experts and published the model in 2019 in a peer-reviewed journal.[36]

MDH developed the model to account for the rapid accumulation of PFOA and other bioaccumulative PFAS in infant serum during the first year of life.[37-40] The model assumes that exposure to PFAS contamination in a community water supply is chronic and that maternal serum levels have reached steady state prior to pregnancy due to chronic exposure. The model uses placental transfer ratios that represent the central tendency of empirical data from paired maternal and cord serum PFAS levels. Breastmilk transfer ratios represent the central tendency of observed ratios of PFAS concentrations in breast milk vs. maternal serum levels in studies of paired maternal serum and breast milk samples. MDH applied a life stages approach to infant intake of breastmilk and drinking water to better model infant serum levels through childhood. The formula fed scenario assumes that infants ingest infant formula prepared with contaminated tap water from birth (95th percentile of intake) and transition to 95th percentile drinking water ingestion rates through childhood and into adulthood. The breastfed scenario assumes exclusive breastfeeding at a 95th percentile intake for the first 12 months of age and consumption thereafter of contaminated tap water into adulthood at the 95th percentile intake rate for each life stage. The model assumes a gradual decline in breast milk concentration of PFAS over the course of lactation. Non-drinking water sources of exposure to PFAS are addressed 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.

Chemical-specific volume of distribution in the MDH model is age-adjusted based on differences in the extracellular water content in children as a percentage of their body weight. The volume of distribution of the chemical was multiplied by age adjustment factors ranging from 2.4 for newborns to 1.0 for children over one year old.

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.[41-43] Michigan Department of Health and Human Services (MDHHS)[44] and the New Hampshire Department of

Environmental Services (NHDES)[45] recently adapted the model for PFNA and employed the model to derive their state recommendations on PFOA, PFOS, PFHxS, and PFNA.

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 about the first six months with continued breastfeeding alongside introduction of appropriate complementary foods for one year or longer. This might include complementary foods, such as juices and infant formula mixed with tap water. The department actively supports these recommendations and conducts several 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 2015, 59 percent of mothers reported exclusive breast-feeding through three months and 29 percent reported exclusive breast-feeding through six months. Seventy 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. The MDH developed its model to predict a reasonable maximum exposure resulting from a specific level of PFAS in drinking water. It assumes that infants consume either drinking water at the 95th percentile or breastmilk at an upper percentile during the first year of life. It assumes that mothers and children >one year of age are drinking water at the 95th percentile over many years. These drinking water ingestion rates come from surveys of a representative population asked about their water consumption in the last two days.[46] 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. For this reason, 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 fifteen years prior to pregnancy. We retained the MDH model assumption of 95th percentile water ingestion by mothers over the 12-month lactation period and 95th percentile ingestion of formula or breastmilk by infants.

Table 3, on the following page, 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.

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	2.5
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-d)[c]					
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			
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[d]	Upper (95th)	47			
Women of childbearing age[d]	Upper (90th)	35			
Breastmilk ingestion rates[e] (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] *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).*

[d] *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).*

[e] *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 deriving health protective values, the relative source contribution, and drinking water ingestion rates 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 proportion of the RfD allotted to drinking water sources. We did not use the MDH model for PFBS because we had insufficient information to model infant exposures and a lower concern about the breastmilk pathway of exposure given the more rapid clearance of PFBS from the body. Instead, we used the same approach EPA used in its 2016 health advisory for PFOA and PFOS. We divided the PFBS RfD by the 95th percentile drinking water ingestion rate for lactating women from the 2019 EPA Exposure Factors Handbook, Table 3.3. We multiplied this term by a default of 20 percent RSC to derive a SAL of 1,300 ng/L. 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 (2018)	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	3	ATSDR MRL (2018)	Developmental effects in mice.	50%	MDH Model w/ MDHHS inputs[c]	14 ng/L
PFHxS	9.7	MDH RfD (2019)	Reduced thyroid hormone (T4) in rats (developmental concern).[d]	50%	MDH Model[a]	70 ng/L
PFBS	300	EPA RfD 2018 (w/MDH 2019 DAF)[e]	Reduced thyroid hormone (T4) in mice (developmental concern).[c]	20%	0.047 L/kg-d	1,300 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

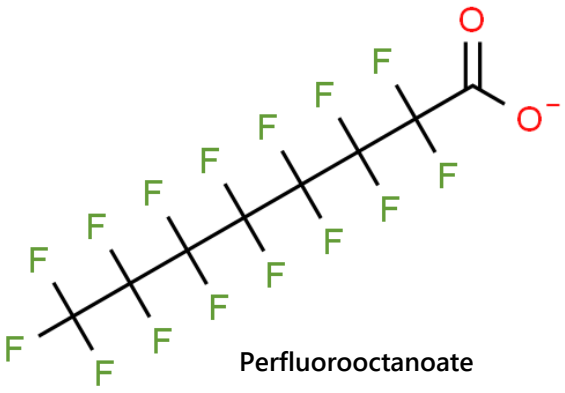
[c]MDHHS is the Michigan Department of Health and Human Services

[d]Thyroxine (T4) is a thyroid hormone

[e]MDH developed a PFBS-specific dosimetric adjustment factor (DAF) from empirical data to account for the difference in half-life between PFBS in rodents and PFBS in humans. We used this DAF rather than EPA's body scaling approach.

Supporting Information—How We Derived Each SAL

Deriving the State Action Level for PFOA



Perfluorooctanoate

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 above. 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.[15, 19, 47] Under a stewardship agreement with the U.S. EPA, major domestic manufacturers of PFOA voluntarily phased-out their production between 2006 and 2015. PFOA and precursor chemicals may still be produced globally. Proposed EPA restrictions on continuing uses in the U.S. have not yet been adopted.[15] PFOA is essentially nondegradable in the environment. Its persistence and water solubility enable it to leach into groundwater from surface soils.

In national surveys, nearly every person tested had detectable levels of PFOA in their blood serum. The average serum levels in the U.S. have declined by 60 percent since the phase-out of PFOA and precursors in the U.S. began in 2006.[35] The latest 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 12 and older) and 4.17 µg/L as the 95th percentile of the population distribution.[14] 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 humans studies ranged from 2.3 to 3.9 years with very little difference reported between sexes.[48] The long half-life of PFOA in humans is attributed to resorption of PFOA following filtration by the kidney.[49] In humans, PFOA appears to accumulate most in liver, kidney, blood serum, lung, and bone.[4, 50]

Food and drinking water contamination are thought to be the major pathways of nonoccupational exposure to PFOA. 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. Exposure to PFOA may also be higher 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,[51-54] immunotoxicity[55-57] reproductive and developmental toxicity,[53, 58-62] and altered thyroid hormones.[63] 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. The strongest and most consistent associations between PFOA exposure and adverse health effects in humans are elevated serum cholesterol,[64, 65] reduced birth weight,[66, 67] reduced antibody response to vaccines[68] and increased serum liver enzymes.[69-73] Studies also report associations between PFOA exposure and altered development of reproductive tissue and delayed puberty,[74, 75] higher serum uric acid,[76-78] altered thyroid hormone levels and thyroid disorders,[79-82] pregnancy-induced hypertension and preeclampsia[83-85] and ulcerative colitis.[86, 87]

PFOA is not considered genotoxic or mutagenic but studies in laboratory animals have reported increased incidence of tumors in liver, testicular, and pancreatic tissues as well as ovarian tubular hyperplasia.[88-90] PFOA exposure was positively associated with increased incidence of kidney and testicular cancers in a large epidemiological study (the C8 Health Project). This study investigated health outcomes in nearly 70,000 people who lived near a West Virginia manufacturing plant and had high levels of PFOA in their public drinking water supply.[91, 92] Studies of the general population have looked for but not found associations between serum PFOA levels and a range of human cancers.[93-96] In 2016, EPA classified PFOA as having "suggestive evidence" of carcinogenic potential in humans based primarily on Leydig cell tumors in rats[54, 89] and increased incidence of renal and testicular cancer in the C8 study.[50] The International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic to humans (Group 2B)[97] EPA modeled cancer risk from dose-response data for Leydig cell tumors in rats in the Butenhoff et al. 2012 study and derived a cancer slope factor of 0.07 per mg/kg-day. EPA concluded that their lifetime health advisory (70 ng/L in drinking water) is protective against *de minimus* cancer risk (one additional cancer in an exposed population of a million people).[50] The NJ Drinking Water Quality Institute calculated a different cancer slope factor (0.021 per mg/kg-day) from the same data set but also concluded that the MCL proposed was protective of *de minimus* cancer risk.[98] Non-cancer endpoints, rather than cancer risk, have commonly provided the foundation of health protective values for PFOA.

Review of Health Protective Values

DOH reviewed the available health protective values (RfD, MRL, target serum levels) for daily ongoing human intake of PFOA. We focused on risk evaluations that were high quality and comprehensive, that considered scientific research, and were conducted by U.S. federal and state agencies. These included a reference dose (RfD) derived by EPA in 2016, a draft minimal risk level (MRL) derived by ATSDR in 2018, and target serum levels derived by NJ DWQI and NH DEP, which are analogous to an RfD except on a serum basis. These values are in Table 5 below.

Table 5. Health Protective Values for PFOA Reviewed by WA

Source	Critical Study	Critical Effect	Human Equivalent Dose	Uncer-tainty Factors (UF)[a]	Oral RfD, MRL[b], or Target Serum Level[c]	Exposure Duration
EPA 2016[88]	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)	300 10-UF_H 3 -UF_A 10-UF_L	20 ng/kg-day (RfD)	Chronic
NJ 2017 [47, 98]	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		300 10-UF_H 3 -UF_A 10-UF_D	14.5 µg/L (target serum level) 2 ng/kg-day[d] (RfD)	Chronic
ATSDR 2018 *Draft* [19]	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)	300 10-UF_H 3 -UF_A 10-UF_L	3 ng/kg-day (MRL)	Inter-mediate (2-52 wks.)
NH 2019 [45, 99]	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		100 10-UF_H 3 -UF_A 3-UF_D	43.5 µg/L (target serum level) Or 6.1 ng/kg-day[e] (RfD)	Chronic

[a]Uncertainty factors: UF_H= intra-individual 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= 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.

[c]NJ DWQI and NH 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]NH DES used a dosimetric adjustment factor of 0.000149 L/kg-day to calculate an RfD from target serum level. 0.0435 mg/L x 0.000149 = 0.000006 mg/kg-day.

EPA and ATSDR selected developmental endpoints as the basis for their health-based values for PFOA. The New Jersey Drinking Water Quality Institute and New Hampshire Department of Environmental Services chose an increase in relative liver weight as the critical effect for PFOA (see Table 5 above).

EPA based its RfD on **Lau et al. 2006**,[58] 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 reduced 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.[58]

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.[51, 55, 100] The candidate RfD based on developmental effects from Lau et al. 2006 was as low or lower than the other RfDs.

EPA's point of departure was the LOAEL of 1.0 mg/kg-day. Altered bone development and timing of puberty observed are not likely to be secondary to reduced growth. With the help of a toxicokinetic model developed by Wambaugh et al. 2013, EPA estimated an average maternal 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. Because of slow excretion of PFOA in humans, the modelled steady state in human serum is not reached until five to ten years of exposure. 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 (10-fold uncertainty factor to account for variability in sensitivity among humans, a three-fold factor for uncertainty for extrapolating from animals to humans, and a 10-fold uncertainty factor for using a LOAEL rather than a NOAEL).[88] This resulted in a chronic oral RfD of 20 ng/kg-day.

ATSDR selected a different developmental study as the basis of their minimal risk level (MRL). **Koskela et al. 2016 and Onishchenko et al. 2011**, a single study published in two papers,[101, 102] 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. 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 4–5x higher than in controls. Subtle changes in bone morphology and mineral density were observed. Their observations of skeletal changes into adulthood at 0.3 mg/kg-day 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 recent 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.[63]

ATSDR selected the LOAEL of 0.3 mg/kg-day 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 an average serum level of 8.29 mg/L in humans after years of exposure. Modified inputs to the model included a PFOA volume of distribution of 0.2 L/kg and a half-life of 3.8 years in human serum based on observations in Olsen et al. 2007.[69] The Olsen study had a longer follow-up time than the Bartell et al. 2010 study[103] 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.[103] The resulting human equivalent dose was 0.00082 mg/kg-day. A ten-fold uncertainty factor was applied to account for variability in sensitivity among humans, a three-fold factor was applied for uncertainty in extrapolating from animals to humans, and a ten-fold uncertainty factor was applied for use of a LOAEL rather than a NOAEL. The resulting MRL was 3 ng/kg-day. The serum level associated with intake at the RfD (27.6 µg/L) was derived by dividing the average serum level of 8.29 mg/L by the combined uncertainty factor of 300.

New Jersey Drinking Water Quality Institute (DWQI) is the scientific body that conducted the risk assessment and recommended drinking water limits to the state of NJ. Both NJ and NH based their health-based value on liver effects observed in **Loveless et al. 2006**. This was a 14-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.[104] Male rats appeared to be less sensitive. The LOEL for increased relative liver weight in male rats was higher (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. The LOELs observed for reduction in serum cholesterol and serum triglycerides

were 0.3–1.0 mg/kg-day depending on the isomer mixture (serum levels at LOELS were 20–51 mg/L.[104]

These results were supported by similar liver observations in a 4-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.[52]

In Loveless 2006, liver weights increased steadily across the range of doses while peroxisomal β-oxidation activity increased sharply at the lower doses and plateaued. Given this misalignment of dose-response curves, Loveless et al. hypothesized that peroxisomal proliferation is not the sole cause of increased relative liver weight in mice and rats.

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.[98] 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. Specific uncertainty factors were for variability in human response (ten-fold), animal-to-human extrapolation (three-fold), and potential for other toxicities at lower doses (ten-fold). 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. New Hampshire 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.

EPA and ATSDR followed the Hall criteria established by an expert group of scientists to determine adversity of liver effects [105]. Both EPA and ATSDR noted that the liver weight changes observed at low doses in mice did not meet criteria for being an adverse effect and were primarily mediated by PPARα activation for which human liver is less sensitive. 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.[51, 52, 100] 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.[19, 50]

An area of uncertainty for PFOA is the functional relevance of delayed and reduced mammary gland development observed in certain strains of mice.[60-62, 106-109] NJ DWQI added a ten-fold database uncertainty factor for this finding. ATSDR and EPA concluded that this endpoint was of unknown functional significance and needed further investigation. 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.[62] This endpoint may need to be reconsidered as more data emerges. 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.[61] This HED is higher than the ATSDR HED (0.000821 mg/kg-day) based on other developmental effects (see Table 5).

Human Relevance

A systematic review of fetal growth by Johnson et al. 2014 found "sufficient evidence" that PFOA reduces fetal growth in humans. Their statistical meta-analysis of nine epidemiological studies on birth weight using birth weight as a continuous variable showed a 18.9 gram reduction in birth weight for every 1 µg/L increase in maternal sera or cord PFOA levels.[66] A downward shift in birthweights across an exposed population might result in more children classified as low birth weight (defined as <2500 g), which is a known risk factor for diseases later in life. However 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.[83, 84] Recent analysis of the Flemish Environmental Health Survey suggested that PFOA might amplify effects of other environmental pollutants on low birth weight.[110] It has also been suggested that low glomerular filtration (GFR) rate may explain some of the association between low birth weight and higher serum PFOA observed in epidemiological studies. Individuals with low GFR have higher serum levels of PFOA as well as lower birth weight.[111] The systematic review by Johnson et al. considered this hypothesis and concluded there was not sufficient supporting evidence for this hypothesis and it did it explain the results observed in experimental animals.[66]

There are limited skeletal observations in human studies. PFOA has been measured in bone in adult human cadavers[4, 5] and associations were reported between PFOA and lower bone density in women[112] and smaller bone size and mass in British girls.[113]

A number of epidemiological studies have investigated neurodevelopmental outcomes associated with elevated PFOA exposure. While a few studies reported positive associations with hyperactivity[114, 115] most reported null or even inverse associations.[116-119]

Few epidemiological studies have evaluated associations between PFOA and altered development of reproductive tissue or time of puberty onset. Associations between higher serum PFOA and reduced testosterone levels in boys (six-nine years old) and delayed puberty in girls (eight-eighteen years old) were observed in two cross-sectional studies of children in the C8 Health Project.[74, 120] In studies in the general population with lower serum PFOA levels, age of menarche in girls was associated with *in utero* exposure (maternal serum PFOA) in one study[121] but not another.[122] Altered male reproductive development was reported in young adult males exposed to PFOA prenatally and throughout childhood in a community with high levels of PFOA in drinking water near a fluoropolymer plant in Veneto, Italy. Compared to a reference population, young adult males had reduced testicular volume and penile length and shorter anogenital distance.[75] A study of young adult Danish males (aged 19-21) born in 1988-89 reported associations between *in utero* exposure (maternal serum PFOA) and lower sperm

concentration and total sperm counts and higher serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH).[123] No association between maternal serum PFOA and anogenital distance in three-month-old male Danish infants was observed in the longitudinal Odense Child Cohort.[124]

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.[125] Similar results were reported in Timmerman et al. 2017 in a Faroe Islands cohort and by Ramono et al, 2016 in a Cincinnati cohort both of which did control for previous breastfeeding duration.[126, 127] 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 enrolled primarily nulliparous women to control for possible confounders of parity and prior breastfeeding duration.[128]

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.[19, 59]

Epidemiological studies support an association between gestational exposure to PFOA and small reductions in fetal growth in humans.[66] Epidemiological evidence is still limited regarding PFOA exposure and skeletal changes, neurodevelopmental outcomes, altered pubertal development, male reproductive toxicity, or mammary gland impairment.

Increases in serum cholesterol in human populations exposed to PFOA is one of the more sensitive and robust findings from epidemiological studies.[64, 129] Rodents have not served as a good model for this effect as they generally show reduced serum cholesterol following PFOA exposure. However, more recent investigations suggest that rodents fed a high fat diet similar to typical U.S. dietary intake, also showed hypercholesterolemia.[130] A recent review by the European Food Safety Authority (EFSA) conducted benchmark dose modelling on several large epidemiological studies for the cholesterol endpoint. $BMDL_5$ for 5 percent increase in mean serum cholesterol across adult populations exposed to PFOA were 9.2-9.6 µg/L.[129]

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.[60, 131] Overall, toxicity studies available for PFOA demonstrate that early life stages are sensitive to PFOA-induced toxicity.[50] Based on the rodent data, we expect fetal and infant periods to have the highest sensitivity to developmental effects. Infant and later childhood developmental periods

could also be sensitive as these are periods of rapid growth and development. Rodent data show that pubertal development may be a sensitive window for PFOA.

Relative Source Contribution (RSC): 50 percent

RSCs were developed for children and adults for all five PFAS evaluated (see Table 1) with the subtraction method and the EPA Exposure Decision Tree described in EPA's methodology.[26] 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).

EPA and New Jersey used the default of 20 percent RSC for PFOA. Minnesota and New Hampshire followed the Exposure Decision Tree approach and the subtraction method to derive RSCs of 50 percent.

Water Intake Rate: MDH model

EPA selected a water-consumption rate representative of the higher maternal drinking water intake needed to support pregnancy and lactation. Specifically, EPA used the recommended value for the 90th percentile ingestion of drinking water for lactating women, 0.054 L/kg-day, from the 2011 EPA Exposure Factors Handbook (Table 3-81: consumers only estimate of combined direct and indirect community water ingestion).

Infants and young children have higher drinking water intake per pound body weight than adults.[46] In addition to drinking water, infants rely on breastmilk that will contain PFOA proportional to maternal serum. Minnesota Department of Health developed a model to predict serum levels in children, via placental and lactational transfer from maternal serum, as a result of PFOA in community drinking water. Minnesota also modeled exposure of infants fed formula mixed with drinking water that contains PFOA.[43]

We used the model inputs we discussed in the Introduction to Approach and Methods. We assumed chronic exposure to PFOA in drinking water and water intake rates at the 90th percentile for adults and for children >one year old. To calculate maternal PFOA level at pregnancy, we assumed 15 years of pre-exposure. The resulting maternal serum (2.5 µg/L) was used to calculate the starting serum at birth for infants (maternal serum x placental transfer ratio, which was 2.2 µg/L.

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 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 (Figure 4). A drinking water level of 10 ng/L PFOA was needed to keep serum levels of infants and children at or below 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 12.7 µg/L. Formula-fed infants reached 3.5 µg/L PFOA in serum (assuming infant formula was prepared with the drinking water). Serum levels in infants did not exceed their RSC of 50 percent of the RfD.

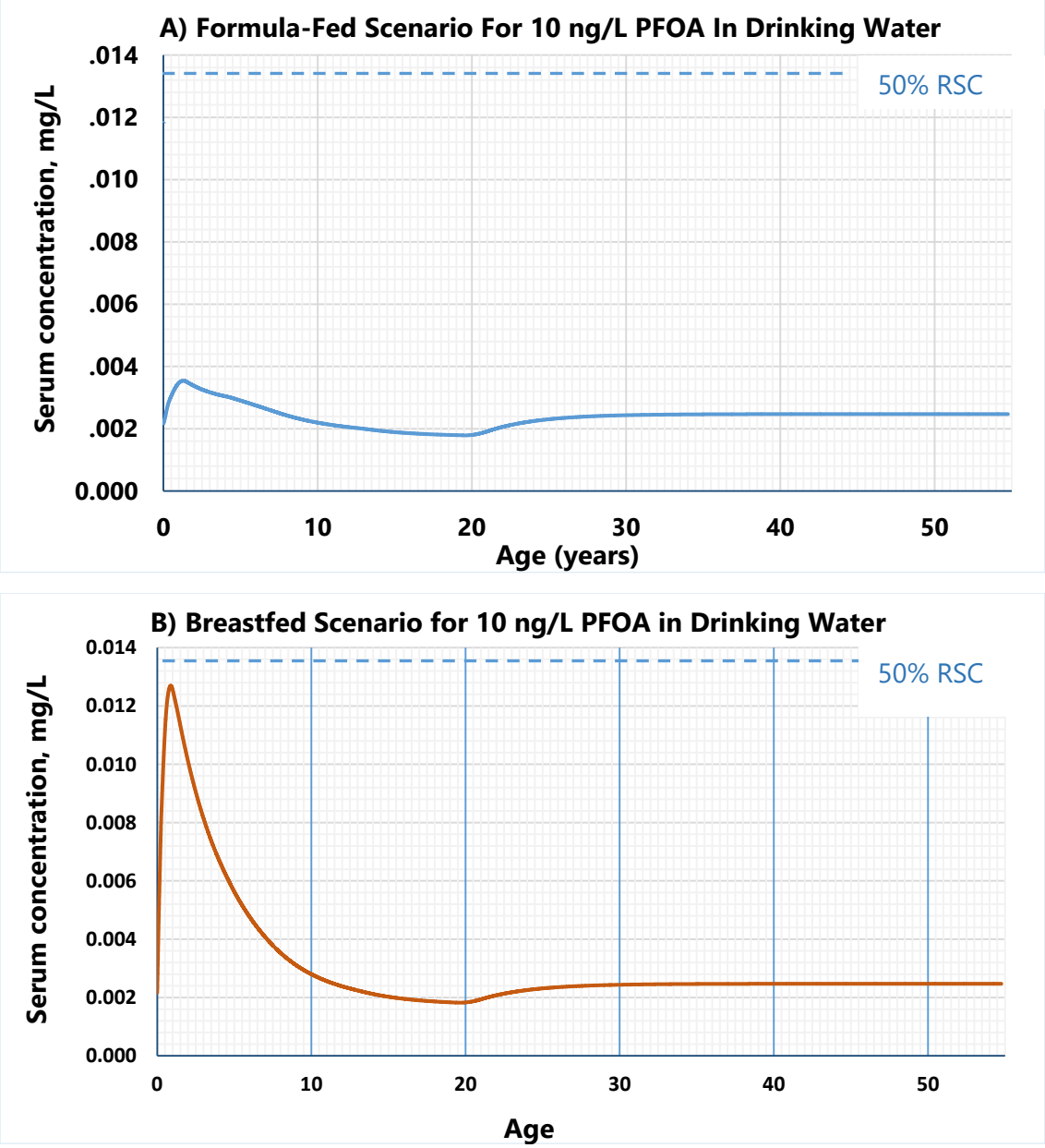
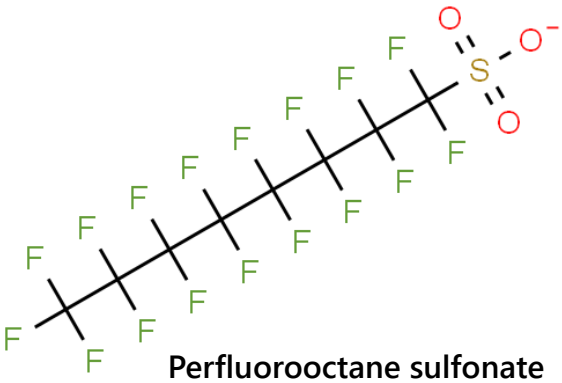


Figure 4. *Model predicted PFOA serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFOA in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the rest 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. 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, 132] Some U.S. commercial uses were allowed to continue (e.g., AFFF, metal plating, aviation fluids, photograph development). PFOS production also continued in other countries. PFOS is essentially nondegradable in the environment. It persistent in the environment and can leach into groundwater from surface soils.[133]

In national surveys, nearly every person tested has detectable levels of PFOS in their blood serum. The phase-out in U.S. production resulted in a decade of steady declines in serum levels in the U.S. (see Figure 1). 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.[14] The latest CDC NHANES survey from 2015-16 reported 4.72 µg/L as the mean serum level of PFOS in the U.S. general population (aged 12 and older) and 18.3 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. PFOS is bioaccumulative in humans because it is readily absorbed and only slowly excreted. 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.[10, 134] Men appear to have slower elimination rates than women.[10, 11]

The primary types of toxicity observed in experimental animals exposed to PFOS are developmental toxicity [135-137], immune suppression [138-142], liver and kidney toxicity [143-145] and disruption of thyroid and other hormones [146-150]. PFOS does not appear to be mutagenic or genotoxic but chronic rodent studies observed liver, thyroid and mammary gland tumors [151].

The most consistent findings from human epidemiological studies are positive associations between serum PFOS and higher serum cholesterol, [79, 152-154] reduced antibody response to vaccines,[155, 156] and reduced birth weight.[157] Other endpoints of concern with less evidence

include elevated uric acid,[76, 77] altered energy metabolism and glucose intolerance,[158-160] altered hormone levels,[161-163] thyroid disease,[81, 164, 165] and chronic kidney disease.[76, 166]

Data relevant to cancer risk of PFOS are limited. The EPA concluded there is "suggestive evidence for carcinogenic potential" in humans based on the liver and thyroid adenomas observed in the chronic rat study by Butenhoff et al. 2012.[151] This study reported a dose-dependent increase in hepatocellular adenomas in female rats and liver tumors in males at the highest dose.[151] Thyroid follicular cell adenomas and carcinomas were also observed in both the male and female rats but according to both NJ DWQI and EPA evaluators, are of unclear biological significance and lacking in a clear dose-response relationships.[132, 167] Mammary gland tumors in female rats were observed but also lacked a dose-response pattern.[132] Some occupational studies suggest an association with bladder, colon, and prostate cancer but these cancers were not associated with PFOS in studies in the general population nor in communities exposed to PFOS in drinking water. [19, 132] Preliminary studies of breast cancer are inconclusive.[93, 168] EPA did not include quantitative cancer risk assessment in their 2016 PFOS evaluation citing insufficient information. NJ DWQI derived a cancer slope factor in their 2018 assessment from the dose-response seen for hepatocellular tumors in female rats in the Butenhoff study. NJ evaluators concluded that their RfD based on non-cancer endpoints was also protective for cancer risk.[133] The International Agency for Research on Cancer (IARC) has not classified PFOS with respect to cancer. As with PFOA, non-cancer endpoints, rather than cancer risk, have provided the foundation of health protective values for PFOS.

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 risk evaluations that were high quality and comprehensive, that considered current scientific research, and were conducted by U.S. federal and state agencies. This included reference doses (RfDs) derived by EPA and the Minnesota Department of Health, a draft Minimal Risk Level (MRL) derived by ATSDR, and target serum levels derived by NJ DWQI and NH DES. Target serum levels are analogous to an RfD except on a serum basis. These values are 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, Target Serum Level[b]	Exposure duration
EPA 2016[132]	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 NOAEL: 7.4 mg/L LOAEL: 29.7 mg/L	0.00051 mg/kg-day	30 10-UF^H 3 -UF^A	20 ng/kg-day (RfD)	Chronic
NJ 2018[167, 169]	Dong et al. 2009	NOAEL (0.0083 mg/kg-day) for reduced immune response in adult mice (decreased plaque-forming cell response). 60-day study. Serum level measured 24 hrs. after last dose at the NOAEL: 0.675 mg/L LOAEL: 7.1 mg/L	22.5 µg/L (target serum level) (0.675÷30)	30 10-UF^H 3 -UF^A	22.5 µg/L (target serum level) 1.8 ng/kg-day (RfD)	Chronic
ATSDR 2018[19] draft	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 NOAEL = 7.4 mg/L LOAEL = 29.7 mg/L	0.000515 mg/kg-day	300 10-UF^H 3 -UF^A 10-UF^D	2 ng/kg-day (MRL)	Intermediate (2-52 wks.)
MN 2019[41]	Dong et al. 2011	NOAEL (0.0167 mg/kg-day) for immune endpoints (increased IL-4, reduced antigen response) in adult male mice. 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	100 10-UF^H 3 -UF^A 3-UF^D	3.1 ng/kg-day (RfD)	Short-term and chronic
NH 2019[45, 99]	Dong et al. 2011	NOAEL (0.0167 mg/kg-day) for immune endpoints (increased IL-4, reduced antigen response) in adult male mice. Serum level measured 24 hrs. after last dose at the NOAEL: 2.36 mg/L LOAEL: 10.75 mg/L	0.000302 mg/kg-day (2.36 mg/L x 0.000128 L/kg-day DAF)	100 10-UF^H 3 -UF^A 3-UF^D	23.6 µg/L (target serum level) 3.0 ng/kg-day (RfD)	Chronic

[a] Uncertainty factors: UF_H= intra-individual 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= 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.

[c] NJ used a clearance factor (8.1×10^{-5} L/kg-day) to calculate an RfD of 1.8 ng/kg-day from the target serum level.

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[136] with support from Luebker et al. 2005b.[137]

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 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.[136]

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.[137] 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 1 and 5 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 LOAEL of 0.4 mg/kg-day (external dose) for Luebker et al. 2005a was associated with a mean maternal serum level (internal dose) of 41 mg/L during gestation, which dropped to 26 mg/L at the end of gestation (GD 21). EPA applied toxicokinetic models to calculate an average maternal serum level (internal dose) over the duration of exposure and a daily intake in people that would result in an average equivalent serum level. 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.[132]

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,[145, 170] developmental neurotoxicity in rats from Butenhoff et al. 2009,[171] and reduced pup weight and neonatal mortality in another rat study by Lau et al. 2003.[135] 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 SRBC 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 immmunotoxicty data in setting the RfD."[132]

Risk assessors at ATSDR selected developmental effects as the most sensitive effect that ATSDR was confident in modelling. 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.[19] They agreed with EPA that immune endpoints were a concern, but cited lack of sufficient data for toxicokinetic modeling on the specific strains of mice used in the immunotoxicity assays. Instead of ignoring the immune data quantitatively, however, ATSDR applied a ten-fold uncertainty factor for database deficiency (UF_D) to account for lower immune LOAELs in mice.

New Jersey, New Hampshire and Minnesota risk assessors based their RfDs on immunotoxicity endpoints in mice in Dong et al. 2009 and 2011, which are descried briefly below. Several mouse studies have shown that PFOS exposure reduces antibody responses to sheep red blood cell antigen, alters immune cell populations, and suppresses immune function in adult mice.[138-142] When mice received PFOS exposure during pregnancy, similar immune effects were observed in their offspring at eight weeks of age.[172] 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.[155]

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. 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 the LOAEL, sheep red blood cell-specific IgM plaque forming cell response was reduced and continued to decline 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).[138]

Minnesota Department of Health (April 2019) and New Hampshire (June 2019) selected a different critical study by **Dong et al. 2011**,[139] which had a similar LOAEL but a higher NOAEL (0.0167 mg/kg-day) than Dong et al. 2009. This study in male mice had the same design as Dong et al. 2009 but 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. At the LOAEL (0.08 mg/kg-day (external dose); serum level 10.75 mg/L (internal dose) IgM antibody response to SRBC antigen challenge was reduced as was secretion of interleukin 4, a cytokine associated with T_H2. Cytokines associated with T_H1 declined but were not statistically significant except at the highest dose tested (0.8 mg/kg-day; serum level 51.7 mg/L).[139] Serum levels of IgG and IgE were also elevated at the highest dose. Overall, there was a significant imbalance observed with excess type 2 responses and deficient type 1 responses. The average serum level in mice at the NOAEL was 2.36 mg/L at the end of the 60-day experiment. MDH multiplied this serum concentration 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). Minnesota applied a ten-fold uncertainty factor (UF_H) for human variability in response and a three-fold uncertainty factor (UF_A) for possible differences between the mouse and humans. They applied an additional three-fold factor (UF_D) for database uncertainty based on the need for a more complete assessment of developmental exposures and immune effects and T4 thyroid hormone reductions. They 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.[150, 173]

Their resulting RfD was 3.1 ng/kg-day and corresponding reference or target serum level was 24 µg/L.[41] New Hampshire used the same inputs and approach as MDH. The NH RfD of 3.0 ng/kg-day differs slightly because MDH rounded their DAF and NH did not.

Human Relevance

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

A large number of epidemiological studies in humans have investigated reproductive and developmental outcomes. These were reviewed by EPA in 2016 and ATSDR in 2018.[19, 132] Large numbers of births (over 80,000) in the Danish National Birth Cohort were analyzed for associations between PFAS and birth outcomes. The odds ratios of preterm birth were about two-fold higher in the top three quartiles for PFOS exposure compared to the lowest quartile. For every doubling of PFOS in serum, birthweight declined 45 grams.[67]} 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.[85] 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). These studies found no association between preconception PFOS maternal serum level and low birth weight babies (< 2,500 g) or pre-term births. However, higher PFOS exposure was associated with lower birth weights and with higher

risk of pregnancy-induced hypertension.[174] There was no association with miscarriage among pregnancies overall but a slight association with PFOS and miscarriage in nulliparous women.[175] 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.[85, 122]

Two meta-analyses of epidemiological studies support the observation of lower birth weights. Koustas et al. 2014 found higher PFOS exposure was consistently associated with lower birth weights in a systematic review.[157] 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.[111] Verner et al. also 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.[111]

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. Doubling of serum PFOS level was inversely associated with serum testosterone in boys and estradiol in girls indicating delayed sexual maturation.[74] 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.[120] Prenatal PFOS exposure was associated with decreased odds of earlier age at menarche in a British birth cohort[122] and no association with markers of puberty in girls or boys in two other studies.[121, 123] 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). Compared to the lowest exposure tertile, 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.[176]

In adults and children, PFOS exposure has been associated with suppressed antibody response to vaccines in a number of studies in different populations.[177-181] For example, an investigation of childhood response to vaccines from birth cohorts in the Faroe Islands showed that PFOS exposure in prenatal and early infancy periods were associated with lower antibody responses to childhood diphtheria and tetanus immunizations. These authors reported a 19 percent to 29 percent decrease in tetanus antibody concentrations at age five for each doubling of the PFAS exposure in early infancy. [177, 181] This study also reported that 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.

There are limited studies of PFOS exposure and the risk of infectious disease. In several longitudinal birth cohort studies, prenatal PFOS exposure (measured as higher maternal or cord

blood PFOS) correlated with indicators of increased infectious disease during childhood including: higher risk of hospitalization for infectious disease for girls but not boys,[182] higher number of days with fever,[183] and more lower respiratory tract infections.[184, 185] In one study, general infections such as ear infections and common cold in girls were decreased with higher PFOS cord blood levels.[184, 186] 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.[187] 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 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.[156] 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."[156]

Washington State Recommendation: 3.0 ng/kg-day

We concurred with Minnesota Department of Health and the New Hampshire Department of Environmental Services on their derivation of the RfD for PFOS. The RfD without rounding of the DAF is 3.0 ng/kg-day. The RfD is based on immune effects in Dong et al. 2011. 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 in rodents. 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[138, 188]).

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. Also supporting this outcome is an assessment by the European Food Safety Authority published in December 2018.[129] This assessment modelled serum levels of PFOS associated with 5 percent changes (BMD_5) in vaccination response, birth weight, and total serum cholesterol in epidemiological studies. The BMD_5 for vaccination response in children was lower than the BMD_5 for reduced birth weight or increased total cholesterol indicating a more sensitive effect.

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 and children, 20 percent adults
RSCs were developed for children and adults for all five PFAS evaluated (see Table 1) with the subtraction method and the EPA Exposure Decision Tree. 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 reflects the higher PFOS serum levels in men in the general population. Because the immune effects in rodents were observed in adult rodents, we used a 20 percent RSC for 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.

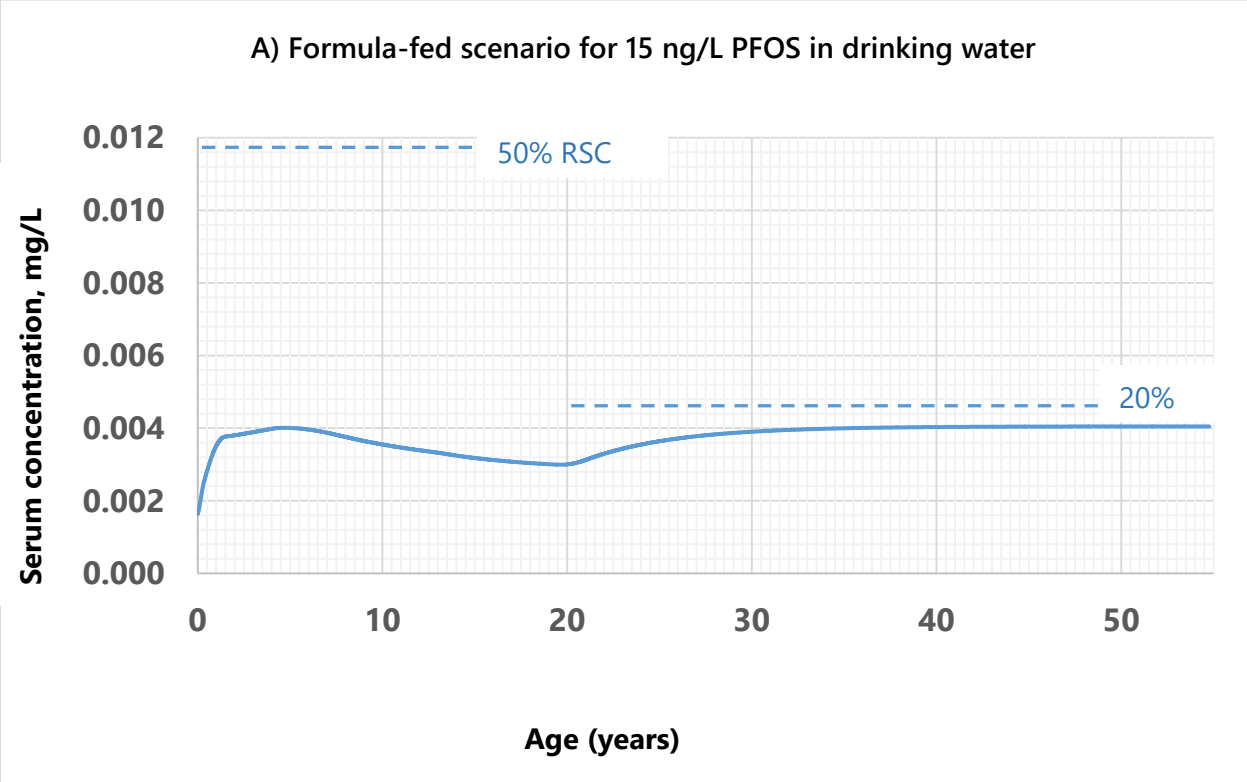
EPA and New Jersey used the standard default of 20 percent RSC for PFOS. Minnesota and New Hampshire followed the Exposure Decision Tree approach described in EPA's methodology (USEPA 2000) and the subtraction or the percentage method to derive RSCs. New Hampshire derived an RSC of 50 percent. Minnesota derived an RSC of 50 percent for infant and young children, 30 percent for older children, and an RSC of 20 percent for chronic exposure in the rest of the population.

Water Intake Rate: MDH model
Infants and young children have higher drinking water intake per pound body weight than adults[46] In addition to drinking water, infants rely on breastmilk, which will contain PFOS proportional to maternal serum. Minnesota Department of Health developed a model to predict serum levels in children, via placental and lactational transfer from maternal serum, resulting from PFOS in community drinking water. Minnesota also modeled exposure of infants fed formula mixed with drinking water that contains PFOS.[41]

We used the model inputs we discussed in the Introduction to Approach and Methods. These were assumptions of chronic exposure to PFOS in drinking water and water intake rates at the 90th percentile for adults and for children >one years old. To calculate maternal PFOS level at pregnancy, we assumed 15 years of pre-exposure. The resulting maternal serum (4.1 µg/L) was used to calculate the starting serum at birth for infants (maternal serum x placental transfer ratio), which was 1.6 µg/L.

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.

Figure 5 provides the model outputs below. A drinking water level of 15 ng/L PFOS was needed to keep serum levels of adults at or below the 20 percent RSC for drinking water sources. The peak serum level predicted for breastfed infants resulting from15 ng/L PFOS in drinking water was 8.2 µg/L. Formula-fed infants reached 4.1 µg/L PFOS in serum (assuming infant formula was prepared with the drinking water). Serum levels in infants did not exceed their RSC of 50 percent of the RfD.



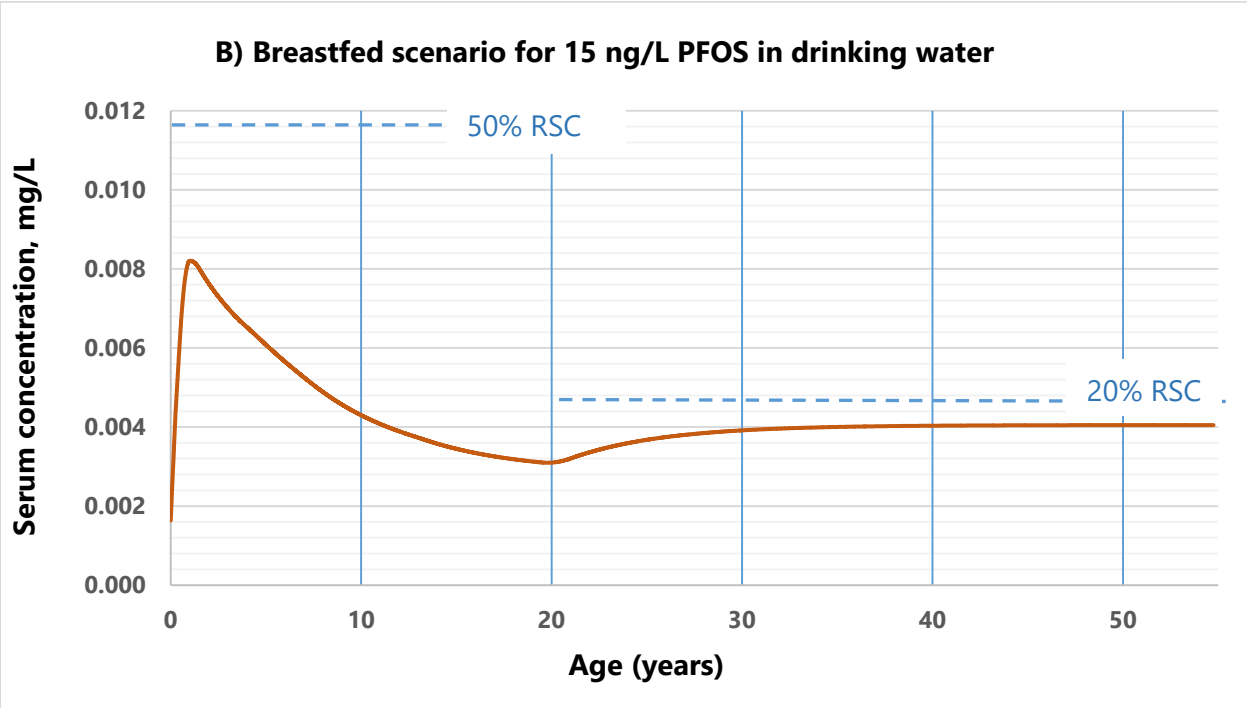
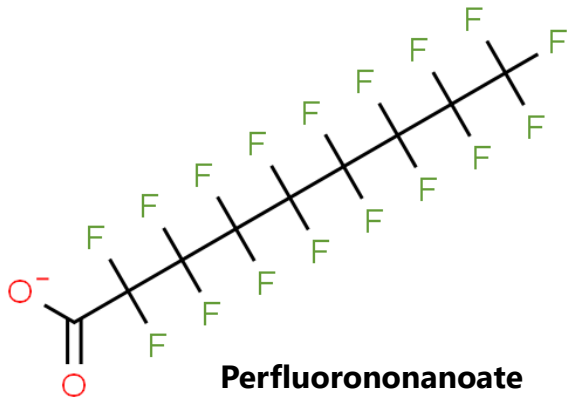


***Figure 5**. Model predicted PFOS serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFOA in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the 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, 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



Perfluorononanoate

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).[189] PFNA may be present in PVDF at low concentrations (100-200 ppm). PVDF was used to line industrial chemical tanks and pipes, to coat internal electronic components, and for biomedical membranes, monofilament fishing line, and architectural coatings. PFNA was phased out of U.S. production by 2015 under an EPA stewardship agreement, but may still enter the U.S. in imported materials.[15] PFNA may also result from breakdown of precursor chemicals such as 8:2 FTOH (fluorotelomer alcohol) and 8:2 diPAP (polyfluoroalkyl phosphoric acid diester) used in carpet and textile coatings and grease proof food contact papers.[189] PFNA has been occasionally detected in public water systems impacted by AFFF firefighting foam.[190]

PFNA and precursors have been released to the environment from manufacturing plants and from industrial, commercial and consumer products. Once released into the environment, volatile precursors such as FTOHs can be transported by air. The nonvolatile PFNA anion can be transported long distances in ground, surface and ocean waters. If PFNA or precursors are released to or deposited on surface soils, PFNA can leach to groundwater. PFNA has been detected in drinking water near a PVDF manufacturing plant in NJ.[189] In WA, PFNA has been detected in Issaquah in drinking water and groundwater that may have been impacted by firefighting foam.[191]

PFNA is widely detected at low levels in blood serum of the general U.S. population. In the most recent national survey by the CDC, mean and 95th percentile serum concentrations were 0.58 µg/L and 1.90 µg/L respectively.[35] Diet is considered the major source of exposure in humans.[192] 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.[17] PFNA is bioaccumulative in people. Its elimination half-life in humans is 2.5-4.3 years by one estimate.[12] Strong correlations have been observed between PFNA concentrations in maternal and cord blood serum, and between concentrations in maternal blood serum and mother's milk.[9]

The toxicity of PFNA is less studied than PFOA or PFOS but the general types of rodent toxicity observed are similar.[19, 63, 193] In mice and rats, the liver is sensitive to PFNA toxicity. Liver effects include increased liver weight, hepatocellular hypertrophy, increased serum liver enzymes, and liver cell damage/necrosis.[63, 194-197] 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.[63, 198-200] In female rodents, it reduced the fertility index, pregnancy rate, and the number of live pups at birth.[197, 201] Developmental toxicity observed with PFNA exposure included reduced growth, delayed development, and reduced survival of pups.[195, 201-203] 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.[63, 204-206] PFNA dramatically 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] [63]

Epidemiological studies relevant to PFNA were reviewed by NJ DWQI and ATSDR.[19, 207] The limited evidence available suggests an association between PFNA exposure and increased serum cholesterol but not with other lipid alterations (HDL, LDL, triglycerides). Neither NJ DWQI nor ATSDR found consistent associations between serum PFNA and increased liver enzymes. More recently published studies from Sweden, China, and the U.S. report small associations between serum PFNA and some liver function biomarkers.[208-210]

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,[178, 180] higher number of reported respiratory infections or common cold in children,[178, 185] and asthma in children.[211] Asthma and allergic diseases were not associated with PFNA in a number of other studies.[178, 184, 185, 212]

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.[67, 213] 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.[214] Other reports include associations in prospective studies between higher serum PFNA and increased risk of miscarriage,[215] lower birth weights,[213] altered timing of puberty onset for boys and girls,[176] and altered bone mineral density in girls at 17 years old.[113] 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.[120]

These findings are preliminary as they 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.

[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.

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.[95]

Review of Health Protective Values

DOH reviewed the available health protective values (RfD, MRL, target serum level) for daily ongoing human intake of PFNA. We focused on risk evaluations that were high quality and comprehensive, that considered scientific research, and were conducted by U.S. federal and state agencies. These included a target serum level derived by NJ DWQI in 2015, a draft minimal risk level (MRL) derived by ATSDR in 2018, and a target serum level and RfD derived by NH DES in 2019. These values are presented in Table 6 below.

Table 6. 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[189]	Das et al. 2015	$BMDL_{10}$ for increased liver weight in mouse pups with prenatal exposure. LOEL: 1 mg/kg-day for liver weight increase Maternal serum level at $BMDL_{10}$= 4.9 mg/L		1000 UF_H=10 UF_A=3 UF_S=10 UF_D=3	4.9 µg/L (target serum level) (4.9 mg/L /1000) or 0.74 ng/kg-day (RfD)	Chronic
ATSDR 2018[19]	Das et al. 2015	NOAEL of 1 mg/kg-day for reduced pup weight and developmental delays in mice. Modelled TWA maternal serum at NOAEL = 6.8 mg/L at LOAEL = 10.9 mg/L	0.001 mg/kg-day (6.8 mg/L x DAF[c])	300 UF_H=10 UF_A=3 UF_D=10	3 ng/kg-day (MRL)	Intermediate (2–52 wks.)
NH 2019 [45, 99]	Das et al. 2015	$BMDL_{10}$ for increased liver weight in mouse pups with prenatal exposure. LOEL: 1 mg/kg-day. Maternal serum level at $BMDL_{10}$= 4.9 mg/L		100 UF_H=10 UF_A=3 UF_D=3	49 µg/L (target serum level) (4.9 mg/L/100) or 4.3 ng/kg-day (RfD) (49 µg/L x DAF[d])	Chronic

[a]Uncertainty factors: UF_H= intra-individual 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]DAF is a dosimetric adjustment factor. ATSDR derived DAF = V_d x (Ln(2)/$T_{1/2}$)/absorbance factor humans = 0.2 L/kg x (Ln(2)/900 days) = 0.001518 L/kg – day. ATSDR paired their developmental POD with the shorter half-life for women of reproductive age from Zhang et al 2013.
[d]NH DES dosimetric adjustment factor = V_d x (Ln(2)/$T_{1/2}$) = 200 ml/kg x (Ln(2)/1570 days) = 0.0883 ml/kg-day. NH used a half-life estimate for older women and men from Zhang et al 2013.

ATSDR, NJ DWQI, and NH DES selected the same critical study, but the three assessments differed in the endpoint selected and/or the uncertainty factors applied.

In the developmental study by **Das et al. 2015 [195],** bred female CD-1 mice received daily oral PFNA dosing (1, 3, 5 and 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.

Serious systemic and reproductive toxicity were observed at the highest dose (10 mg/kg-day). Pregnant mice at this dose lost weight and 100 percent 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. 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. Dose-dependent development delays in eye opening, preputial separation, and vaginal opening were monotonic across all doses and statistically significant at ≥ 3 mg/kg-day.[195]

Maternal liver weight was increased at all dose levels. At delivery, there were no reductions in birthweight, skeletal abnormalities, or visceral abnormalities except for an increase in relative fetal liver weight at all doses. 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,[195] 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] [216].

ATSDR estimated the time-weighted average (TWA) maternal serum levels across pregnancy for each dose group in the Das et al. study. Specifically they estimated TWA values from the areas under the curve calculated using the trapezoid rule. Liver weight increase (in dams and pups) was the most sensitive effect reported in Das et al. but ATSDR was concerned about its relevance for humans given the evidence for significant PPARα activation and the higher susceptibility of the rodent liver compared to the human liver for this endpoint. ATSDR analyzed

[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.

serum levels associated with developmental points of departure in Das et al. and two other developmental toxicity studies.[201, 203] 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).[201] 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.[203] The TWA serum level (6.8 mg/L) at the NOAEL in the Das study was selected as the point of departure.

ATSDR calculated a human equivalent dose expected to result in serum level of 6.8 mg/L at steady state. This was 0.001 mg/kg day. This was divided by a 300-fold uncertainty factor to derive a draft MRL of 0.000003 mg/kg-day or 3 ng/kg-day. The uncertainty factors were ten-fold for human variability, three-fold for differences between mice and humans, and a ten-fold factor for database uncertainty. Database concerns included the limited scope and number of studies that evaluated intermediate-chronic duration exposures and the lack of 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).

NJ DWQI based their health protective value on increased maternal liver weight at GD 17 in Das et al 2015.[189] Liver weight was increased in a dose-dependent manner in maternal, fetal, and postnatal mice and was statistically significant at 1 mg/kg/day. Liver was assessed for gene expression but not for other evidence of pathology. NJ chose to model maternal liver because both serum level and liver effects were measured at the same time point. In contrast, dose-response modelling of maternal serum associated with development effects measured later in offspring was less certain. A $BMDL_{10\%}$ (lower 95th percentile confidence limit on the benchmark dose) for a 10 percent increase in liver weight was 4.9 mg/L in maternal serum. An uncertainty factor of 1,000 was applied to the BMDL to derive a target serum level of 4.9 µg/L. This included uncertainty factors of 10 for human variability (UF_H), 3 for differences between mice and humans (UF_A), 10 to account for extrapolation of a chronic standard from a short-term (17-day) study (UF_S), and 3 for gaps in the toxicological database (UF_D). Data gaps included lack of chronic or cancer studies. Also adding to uncertainty were a 13-week subchronic study in rats and a two-generation study (18-21 weeks) in rats where liver and kidney effects were observed at lower administered daily doses of a PFNA mixture than in Das et al. 2015.[196, 197] These two longer duration studies tested a commercial mixture that contained 74 percent PFNA and 26 percent other PFAS with carbon lengths of C8-C13.

New Jersey derived their MCL in drinking water from a target serum level of 4.9 µg/L by assuming that serum levels within a population consuming contaminated drinking water will reach 200 times the concentration of PFNA in daily drinking water. This ratio represented a central tendency estimate derived from a study in Chinese adults that indicated PFNA clearance from the body was about two times longer than PFOA.[12] 200:1 is twice the ratio NJ used for PFOA.

New Hampshire used the $BMDL_{10}$ derived by NJ as their point of departure but used a dosimetric adjustment factor (DAF) to convert rodent serum level to a human equivalent dose. NH applied an uncertainty factor of 100 to derive an RfD of 4.3 ng/kg-day. The factors included

a ten-fold factor for human variability, a 3-fold factor for differences between mice and humans, and a three-fold factor for database limitations. In addition to the data gaps cited by NJ DWQI, NH DES cited lack of immunotoxicity testing results suitable for establishing a dose-response relationship.

No serum half-life clearance studies in humans were available for PFNA. 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 collected paired blood and urine samples.[12] Younger females (age ≤50 years), had significantly lower levels of PFNA and other PFAS than women >50 years or men. The estimated arithmetic mean elimination half-live 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). ATSDR selected a half-life estimate of 2.5 years for women of childbearing age to pair with their developmental endpoint. NH selected 4.3 years as a half-life estimate to pair with their liver endpoint.

An endpoint with emerging evidence for relevance at low doses is reduction of serum and testicular testosterone and male reproductive injury in mice and rats. 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 and above, estradiol levels were increased and testicular cells contained apoptotic features including crescent chromatin condensation and chromatin margination.[217] 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 (measured serum level at day 29 was 380 mg/L). In the same experiment, testosterone levels were increased in females ≥ 1.56 mg/kg-day (measured serum level at day 29 was 26.4 mg/L).[63] Recently published studies in Parkes mice by Singh and Singh show that PFNA reduced serum testosterone levels, altered sperm viability and sperm production, and produced degenerative changes in the seminiferous tubules.[199, 200, 218] The LOAEL for these outcomes was 5 mg/kg-day in a gestation exposure study (NOAEL = 2 mg/kg-day), 2 mg/kg-day in a 14-day prepubertal exposure study (no NOAEL), and 0.5 mg/kg-day in a 90 day study (NOAEL = 0.2 mg/kg-day). 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 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.[200] 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.

Human Relevance

PFNA is structurally similar to PFOA and some of the observed rodent toxicity is similar. It is reasonable to assume that reproductive and developmental outcomes in rodents are relevant to humans. There has been inadequate investigation of the potential health outcomes of PFNA in human populations. PFNA is widely detected along with PFOA, PFOS, and PFHxS in human serum samples, but usually at lower concentrations. Still, some of the epidemiological

associations between PFNA exposure and health outcomes are stronger than for PFOA or other PFAS.[219]

ATSDR did not consider the 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 to liver effects observed in rodent studies.[105] 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."[19]

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.[201]

Because of much higher expression of PPARα in rodent liver compared to human liver and lack of similar PPARα mediated liver cell proliferation in humans, EPA has cautioned that liver weight increase in rodents exposed to PFAS may not be relevant to humans unless it is accompanied by fatty acid steatosis, necrosis, and other clearly adverse effects in the liver.[24, 105, 132]

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 targets of PFAS. PPARα and γ 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.[220, 221] As such, a PPAR-mediated pathway of developmental effects in rodents should be considered potentially relevant to human reproduction and fetal and child development.

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.[120, 222-225] 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.

Washington State Recommendation: 3 ng/kg-day

We selected the ATSDR MRL of 3 ng/kg-day as the basis for public health advice for PFNA in drinking water. It is based on sensitive developmental effects seen in mice and includes a full ten-fold factor for database uncertainty (UF_D), which we agree is appropriate for PFNA. Lack of chronic toxicity testing and emerging evidence of male reproductive toxicity in rodents supports

use of a ten-fold factor for the UF_D. Although the liver is a sensitive target of PFNA activity in rodents, we agreed with ATSDR and EPA in applying the Hall criteria to the liver effects observed. The RfD derived from developmental effects was lower than one derived from clearly adverse liver effects. Finally, the data set for PFNA is relatively sparse; however, PFNA is similar to PFOA in chemical structure and observed rodent toxicity. PFOA has an equivalent RfD based on a more robust toxicological dataset. The PFOA dataset includes 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 for all five PFAS evaluated (see Table 1) using the subtraction method and the EPA Exposure Decision Tree described in EPA's methodology.[26] The RSCs for PFNA were 50 percent for infants, children, and adults. The target or reference 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: 11.4 µg/L (22.7 µg/L x 0.50).

An RSC of 50 percent is in concordance with RSC determinations in three other states: NJ, NH and Michigan. [44]

Water Ingestion Rate: MDH model

Infants and young children have higher drinking water intake per pound body weight than adults.[46] In addition to drinking water, infants rely on breastmilk, which will contain PFNA proportional to maternal serum. Minnesota Department of Health developed a model to predict PFAS serum levels in children, via placental and lactational transfer from maternal serum, as a result of PFAS in community drinking water. Minnesota also modeled exposure of infants fed formula mixed with drinking water that contains PFAS.[43]

We employed the MDH model for PFNA using the inputs developed by the Michigan Department of Health and Human Services for serum half-life, placental and breastmilk transfer ratios, and volume of distribution.[44] These are presented in Table 3 in the Introduction to Approach and Methods. For drinking water ingestion rates, we assumed chronic exposure to PFNA in drinking water and water intake rates at the 90th percentile for adults and for children >one years old. To calculate maternal PFNA level at pregnancy, we assumed 15 years of pre-exposure. The resulting maternal serum (3.2 µg/L) was used to calculate the starting serum at birth for infants (maternal serum x placental transfer ratio), which was 2.2 µg/L.

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 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 below (Figure 6). A drinking water level of 14 ng/L PFNA was needed to keep serum levels of infants and children at or below the 50 percent RSC for drinking water sources. The peak serum level predicted for breastfed infants as a result of 14 ng/L PFNA in drinking water was 11.3 µg/L. Formula-fed infants were projected to reach 4.1 µg/L PFNA in serum (assuming infant formula was prepared with the drinking water).

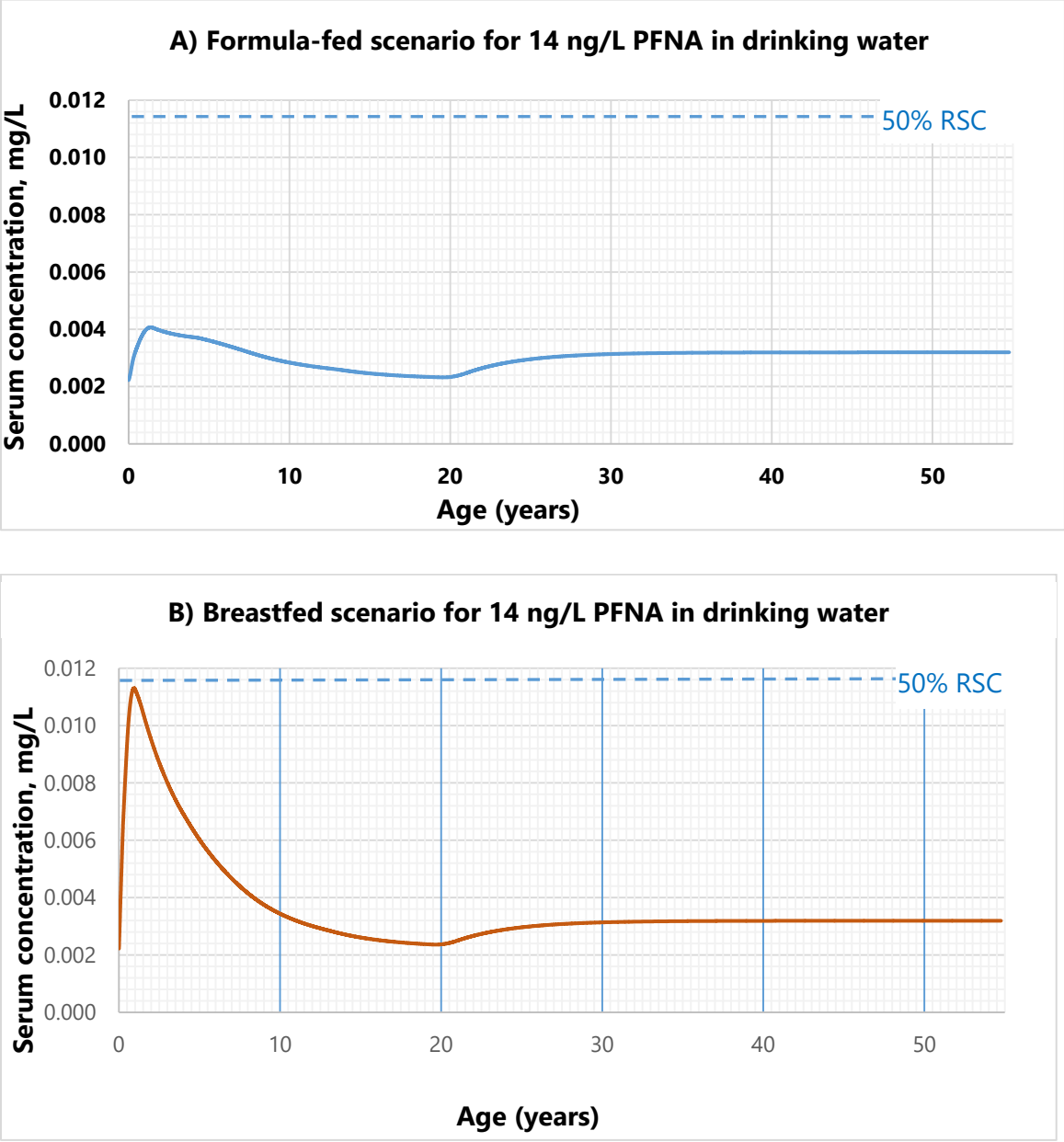
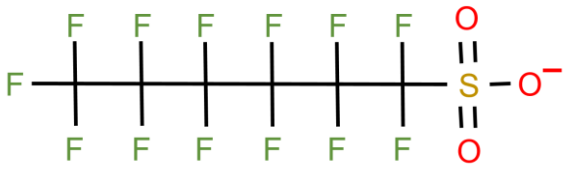


***Figure 6**. Model predicted PFNA serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFNA in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the 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



Perfluorohexane sulfonate

PFHxS is structurally similar to PFOS, but has six rather than eight fully fluorinated carbons. In drinking water it occurs as its anion perfluorohexane sulfonate (shown here). PFHxS along with its salts and precursor compounds have been used in certain class B firefighting foams; in waterproof and stain proof coatings for carpet, leather, upholstery and other textiles; in cleaning and polishing agents; as a mist suppressant in metal plating; and in electronics and semiconductors manufacturing. It was phased out of production by its major U.S. producer (3M) in 2002, but is still produced globally. PFHxS was also an unintentional byproduct in manufacturing of PFOS-related chemicals.[226]

PFHxS and precursors have been released to the environment from manufacturing plants[227] and from commercial products such as aqueous film forming foam used at military bases and airports. It may also be released indoors from products like carpet protection treatments.[228] PFHxS is extremely persistent in the environment. Once released, PFHxS persists in soils and can leach into groundwater from surface soils. In groundwater, it is typically a nonvolatile anion. Volatile precursors can be transported by air. PFHxS frequently co-occurred with PFOS when detected in U.S. public drinking water samples.[190] In Washington state drinking water, PFHxS has been found with PFOS is several areas where firefighting foam is the suspected source of PFAS contamination.[229]

The general population is exposed to PFHxS and precursors chemicals through the diet, by inhalation of indoor air and dust, and by use of certain consumer products. If PFHxS is in daily drinking water, it is likely to be a significant contributor to exposure. PFHxS is poorly excreted from the human body. Median serum half-life was 7.1 years in a group of retired fluorochemical workers followed for 5.0 years and was estimated at 5.5 years in a group of over 100 men and women exposed to PFHxS in their drinking water.[10, 11] PFHxS is one of four PFAS routinely measured in people. 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.[35]

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.[230-232] Several studies have reported thyroid cell damage and reduced T4 and T3 thyroid hormone levels in rodent studies.[230, 233, 234] Reproductive and developmental effects have been reported in some studies such as reduced litter size [232] and reduced birth weight [233] but have not been consistently observed. One study reported altered spontaneous behavior and habituation in adult mice that had received a single dose of PFHxS on postnatal day ten.[235] A key data gap is the lack of immune toxicity testing in animal studies.

According to ATSDR's 2018 draft assessment, the weight-of-evidence for epidemiological studies supports associations between PFHxS exposure and liver damage (as evidenced by increases in serum enzymes and decreases in serum bilirubin levels) and decreased antibody

response to vaccines.[19] There is also limited and somewhat inconsistent evidence of associations between higher PFHxS exposure and increased risk of hyperactivity in children[114, 116] and reduced T4 levels in pregnant women and male infants.[162, 236]

The carcinogenicity of PFHxS has not been investigated.

Review of Health Protective Values

DOH reviewed the available health protective values (i.e., RfDs, MRL, target serum) for daily ongoing human intake of PFHxS. We focused on risk evaluations that were high quality and comprehensive, that considered scientific research, and were conducted by U.S. federal and state agencies. These included a draft minimal risk level (MRL) derived by ATSDR in 2018, a target serum level and reference dose (RfD) derived by the New Hampshire Department of Environmental Services in June 2019, and an RfD derived by the Minnesota Department of Health (MDH) in April 2019. These are presented in Table 6 and discussed below.

Table 6. 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
Draft ATSDR 2018[19]	Butenhoff et al. 2009; Hoberman and York 2003[230]	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]	300 UF_H=10 UF_A=3 UF_D=10	20 ng/kg-day (MRL)	Intermediate (2–52 wks.)
NH 2019 [45, 99]	Chang et al. 2018[232]	BMDL for decreased litter size and reproductive toxicity in mice NOAEL: 0.3 mg/kg-day LOAEL: 1.0 mg/kg-day Maternal serum level at BMDL: 13.9 mg/L		300 UF_H=10 UF_A=3 UF_S=3 UF_D=3	46.3 µg/L (target serum level) Or 4.0 ng/kg-day (RfD)	Chronic
MDH 2019[42]	NTP 2019[193]	$BMDL_{20\%}$ for reduced serum thyroxine (T4) in rats. LOAEL: 0.625 mg/kg-day. Serum level at BMDL: 32.4 mg/L	0.00292[d] mg/kg-day	300 UF_H=10 UF_A=3 UF_D=10	9.7 ng/kg-day (RfD)	Short-term and chronic

[a]Uncertainty factors: UF_H= intra-individual 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 3102 days (8.5 years) and a volume of distribution of 0.287 L/Kg for PFHxS.

[d]The MDH 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 1935 days (5.3 years) and a volume of distribution of 0.25 L/kg.

ATSDR 2018 conducted an extensive review of both the epidemiological and toxicological data available 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. Pups did not have lower birthweights or reduced growth despite dose-dependent increases in liver and serum levels of PFHxS at birth and at PND 22. Observations of pups were limited; they were not evaluated for developmental delays, thyroid gland weight, serum thyroid hormones, or for neurobehavioral outcomes. 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 as assessed by the functional observational battery in the parental rats. In the adult 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[230] 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 females.[230] Thyroid hormones were not measured.

Female rats may be protected somewhat by their more rapid excretion of PFHxS, which produced much lower serum levels in the parental females than the males. The authors noted that thyroid follicular epithelial hypertrophy/hyperplasia observed in this study often accompanies hepatocellular hypertrophy in rats. Thyroid follicular cell adenomas have been observed in long-term oral exposures in rats to the structurally similar compound PFOS.

ATSDR considered the thyroid effects adverse and relevant to humans. The 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 use of 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 immunotoxicity testing and lack of longer duration studies.[19]

Since the ATSDR assessment, three new high quality studies on PFHxS have become available. A reproductive and developmental study in mice by Chang et al. 2018 was selected as the critical study for New Hampshire's assessment. A 28-day subacute toxicity study in male and female adult rats by the National Toxicology Program (NTP) published in 2019 was selected as the critical study for the MDH assessment. A reproductive and developmental study in rats by Romhoj et al. 2018 supported the MDH assessment. We describe these three studies below.

The **Chang et al. 2018 study**[232] 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), liver weight was increased in F0 males and females at 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.[232]

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 the F1 generation, there were no treatment-related effects on postnatal survival or developmental delays noted. Anogenital distance in males at PND 1 was increased in all treated groups but did not show a dose-response relationship. All findings except liver effects were considered equivocal or of unclear significance by the study authors. 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.[232]

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 fT4, TT4, or 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.[232]

Ramhoj 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 at GD 15 compared to controls after only seven days of exposure 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.[233]

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.[193]

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.[193]

New Hampshire risk assessors selected the slight decrease in mean litter size in Chang et al. 2018 as the critical effect for their target serum level and RfD. They noted that a comparable study design conducted in rats by Butenhoff et al, 2009, did not observe statistically significant reductions in implant sites or litter size but that serum levels were lower in the female rats due to more rapid excretion of PFHxS. New Hampshire used a benchmark dose method to derive a BMD of 41.2 mg/L serum level and a BMDL of 13.9 mg/L in serum in female mice from the Chang et al. data. The BMDL is below the serum level at the NOAEL for this effect in Chang et al. (27 mg/L; 0.3 mg/kg-day) They applied a total uncertainty factors of 300 (10x for human variability, 3x for uncertainties between rodents and humans, 3x for extrapolation from a subacute study to a chronic standard, and 3x 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.

The Minnesota Department of Health selected reductions in thyroid hormones as their critical effect preferring a replicated result (in two strains of rats) with support for thyroid cell damage in male rats (Butenhoff et al. 2009) and slight but statistically significant increase in relative thyroid weight in developmentally exposed female mice (Chang et al, 2018).

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 tT4 (33.6 mg/L) and fT4 (32.4 mg/L). MDH applied a dosimetric adjustment to the $BMDL_{20}$ for fT4 to calculate a human equivalent dose. MDH applied a 10x uncertainty factor for human variability and a 3x uncertainty factor for interspecies extrapolation. They applied a 10x factor for database uncertainty to account for lack of immunotoxicity testing and lack of a two-generation developmental study. Their final RfD is 9.7 ng/kg-day (corresponding reference serum level = 108 µg/L).[42]

Human Relevance

T4 is the form of maternal thyroid hormone that passes through the placenta and is deiodinized in fetal tissue to form T3. The fetus relies exclusively on maternal thyroid hormone until the fetal thyroid develops at weeks 18-20 of pregnancy. Thyroid hormone is 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 attention deficit hyperactivity disorder).[237-241] It is still not known whether deficits in neonatal thyroid hormone are associated with neurobehavioral and cognitive deficits.

In the general population, a number of studies have looked for associations between thyroid hormones and serum PFAS. Two large studies in NHANES populations found no association

between PFHxS serum levels and TSH, free or total T4 or T3.[82, 242] 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 [81]. 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.[161] Studies in populations with higher PFHxS exposures are needed.

Because thyroid insufficiency is of special concern during brain development, Ballestero 2017 conducted a systematic review of ten epidemiological studies that examined serum PFAS in relation to thyroid hormones in pregnant women, infants, and older children.[162] It included five studies that analyzed the association between prenatal levels of PFHxS thyroid hormones and one that evaluated postnatal exposure. The authors concluded that there was some evidence of a positive association between maternal TSH and maternal PFHxS serum level. For example, Wang et al. 2014 measured maternal PFHxS in the third trimester and observed a positive association with maternal TSH. On average, there was a 5.2 percent increase in maternal TSH per 1 µg/L increase in maternal PFHxS. In the studies reviewed by Ballesteros et al., associations between maternal PFHxS and maternal free T4 and total T4 were generally inverse but not statistically significant.[162] A more recent large study by Preston et al. 2018 measured serum thyroid hormones and PFHxS early in pregnancy and thyroid hormones in their infants (neonatal blood collected by heel stick at day two).[236] Maternal PFHxS was associated with lower fT4 (as measured by an fT4 index) during early pregnancy. High maternal PFHxS (top quartile) was also associated with lower postpartum total T4 in male neonates.[236]

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.[161] 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.[163, 165]

Evidence for neurobehavioral effects is mixed in human observational studies. Positive associations between serum level of PFHxS in children (aged 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.[114, 116] 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.[116] Several studies have looked for and not found associations between prenatal PFHxS exposure (maternal serum) and ADHD or autism in school-aged children.[243-245] This is somewhat consistent with the finding that parent-reported behavioral problems at children age seven in a Faroe Islands cohort were associated with higher serum levels of other PFAS in children's serum at five and at seven years old but were not associated with the child's prenatal exposure.[246]

Washington State Recommendation: 9.7 ng/kg-day

We selected 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 reduced T4 in pregnant rats and their offspring in Ramhoi et al. 2018. The reduction in litter size observed in mice by Chang et al. was not supported by two studies in rats. Although the absence of reproductive toxicity in Butenhoff et al. and Ramhoj et al. could possibly be explained by lower serum levels in the rat studies, we preferred to base public health advice on a replicated result.

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 rodents exposed during gestation and nursing. Neither Chang et al. 2018 nor Butenhoff et al. 2009 conducted neurobehavioral testing in the developmentally exposed F1 generation. A study in mice by Viberg et al. 2013 administered a single oral dose of PFHxS at postnatal day ten (laboratory mice are typically weaned at PND 21). This experiment reported altered spontaneous behavior and habituation behavior in offspring when tested in adulthood. The LOAEL and NOAEL for this study were 9.2 mg/kg and 6.1 mg/kg.[235]

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. In a Norwegian study of nursing women and their children, Papadopoulou et al., 2016 found that PFHxS (and other PFAS) increased in the serum of breastfeeding infants by 3–5 percent per month.[247]

Relative Source Contribution: 50 percent

RSCs were developed for children and adults for all five PFAS evaluated (see Table 1) 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 and New Hampshire also derived an RSC of 50 percent for PFHxS.

Water Intake Rate: MDH model

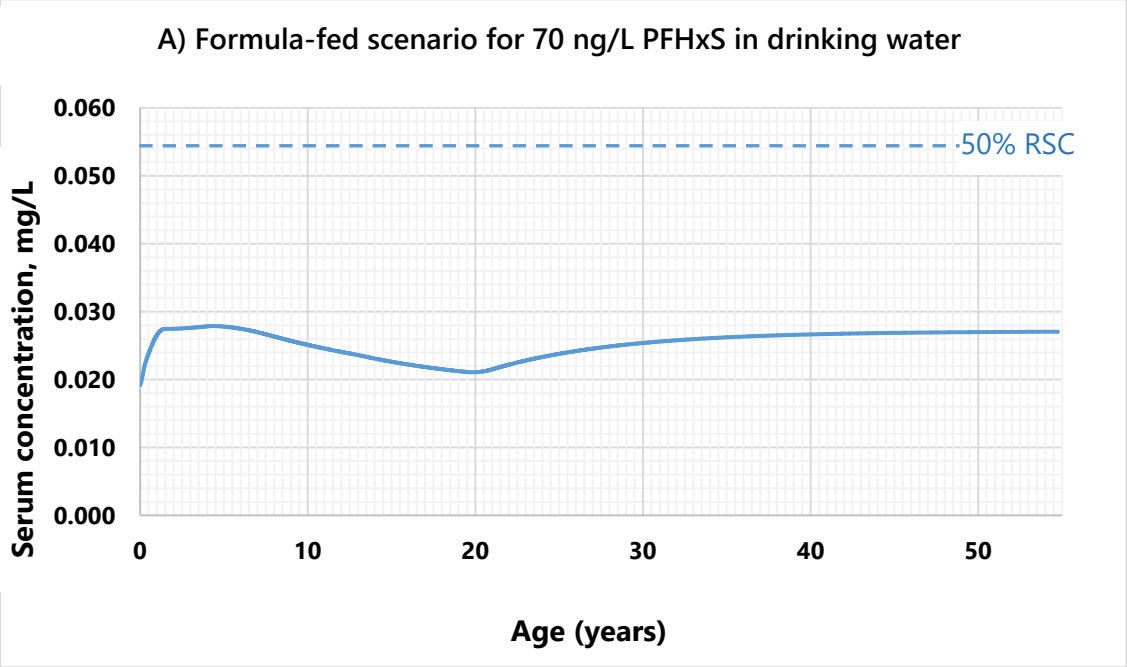
Infants and young children have higher drinking water intake per pound body weight than adults.[46] In addition to drinking water, infants rely on breastmilk, which is expected to contain PFHxS proportional to maternal serum. Minnesota Department of Health developed a model to predict serum levels in children, via placental and lactational transfer from maternal serum, as a result of PFHxS in community drinking water. Minnesota also modeled exposure of infants fed formula mixed with drinking water that contains PFHxS.[41]

We used the model inputs we discussed in the Introduction to Approach and Methods. We assumed chronic exposure to PFHxS in drinking water and water intake rates at the 90th percentile for adults and for children >one years old. To calculate maternal PFHxS level at pregnancy, we assumed 15 years of pre-exposure. The resulting maternal serum (27.4 µg/L) was

used to calculate the starting serum at birth for infants (maternal serum x placental transfer ratio), which was 19.2 µg/L.

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.

We provide the model outputs below (Figure 7). A drinking water level of 70 ng/L PFHxS was needed to keep serum levels of breastfed infants at or below the 50 percent RSC for drinking water sources. The maximum serum level predicted as a result of 70 ng/L was 53.7 µg/L in breastfed children and 27.9 µg/L in formula fed children (assuming infant formula was prepared with the drinking water).



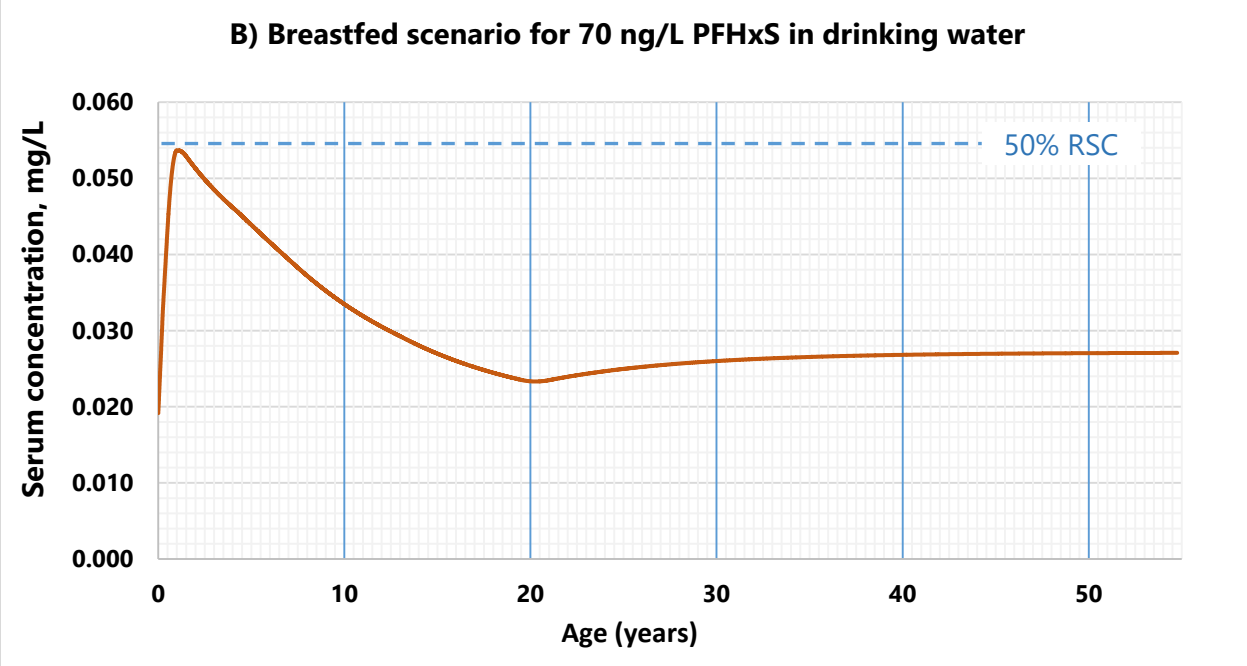
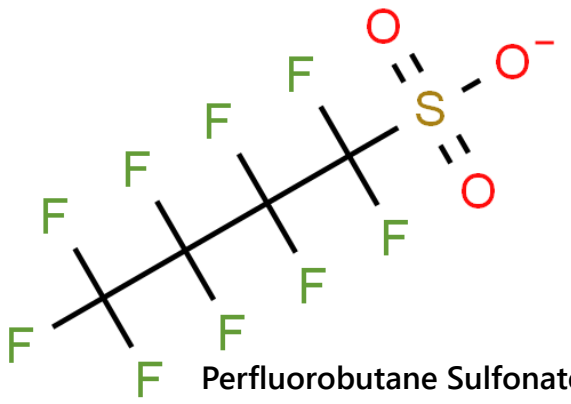


Figure 7. *Model predicted PFHxS serum level (mg/L) in A) formula-fed and B) breastfed infants resulting only from exposure to PFHxS in community drinking water. For formula-fed infants, 95th percentile water intake was assumed for the first year followed by 90th percentile water intake during the rest 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 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 a number of PFBS-based chemicals (e.g., substituted perfluorobutanesulfonamides, perfluorobutanesulfonamidoalkanols and perfluorobutanesulfonamidoalkyl acrylates/methylacrylates). These are replacements for PFOS-based chemistry in consumer products such as stain-proof textiles, waterproof clothing and shoes, and leather protection. They may also be used in paints and repellent coatings for stone, tile, and other porous hard surfaces. PFBS was in older firefighting foams and may be in current mist suppressants used by the chrome plating industry. PFBS has been detected in foods, food contact papers, indoor dust, and drinking water.[248, 249]



Perfluorobutane Sulfonate

PFBS appears to be cleared from human serum much more rapidly than PFOA, PFOS, PFHxS, and PFNA. A small occupational study estimated a half-life in human serum of 27 days.[250] It is infrequently measured in human blood above the laboratory detection limit of 0.1 µg/L in the general U.S. population.[35] A 2015 study of American Red Cross adult blood donors from six U.S. cities reported detection of PFBS in 8 percent of plasma samples. The 95th percentile serum level was 0.02 µg/L and the maximum was 4.2 µg/L [18]. Higher frequencies of detection (over 30 percent) have been reported in the serum of women or children in communities with PFBS in their drinking water.[7, 8] A small sample of fluoropolymer manufacturing workers reported PFBS in serum at 92—921 µg/L following regular work duties.[250] PFBS has been detected in mixtures of PFAS in drinking water in Washington state. The source of these PFAS mixtures appears to be firefighting foam.

The toxicological data for PFBS are not as robust as for PFOS and PFOA, but there are high quality studies in mice, rats, and monkeys with repeated oral doses of PFBS or its potassium salt (K-PFBS). Rodent studies include a two-generation study of reproduction and development in rats,[251] three gestational exposure studies in mice and rats,[252-254] a 90-day oral study in rats[255] and mice,[231] and 28-day oral studies in rats.[193, 256] These studies observed 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 lipids and hematological profiles. Very little information is available on potential immune toxicity or carcinogenicity of PFBS.[249] Using a structured literature review, EPA identified seven epidemiologic studies that report on the association between PFBS and human health effects. Statistically significant positive associations between PFBS and asthma, serum cholesterol, and high-density lipoprotein levels were reported in at least one study.[249]

Review of Health Protective Values

DOH reviewed the available health protective values (i.e., RfDs, MRL) for daily ongoing human intake of PFHxS. We focused on risk evaluations that were high quality and comprehensive, that considered scientific research, and were conducted by U.S. federal and state agencies. These included an RfD developed by the Environmental Protection Agency (EPA) in 2018, an RfD developed by the Minnesota Department of Health (MDH) in 2017 to support drinking water advice, and an RfD developed by the Michigan Department of Health and Human Services (MDHHS) in 2019 to support public health screening values in water. These are presented in Table 7 below. We did not identify sufficient information to evaluate PFBS for cancer endpoints.

Table 7. Health Protective Values for PFBS Reviewed by WA

Source	Critical study	Critical effect	Human Equivalent dose (mg/kg-day)	Uncertainty factors (UF)[A]	Oral RfD[B] (mg/kg-day)	Exposure duration
EPA 2018 draft [249]	Feng et al 2017[252]	$BMDL_{20\%}$ (28.19 mg/kg-day) for reduction of thyroid hormones (total T4) in newborn female offspring of mice dosed during pregnancy (GD 1-20)	4.2[c] (body weight scaling)	100 UF_H=10 UF_A=3 UF_D-3[d]	0.042	Subchronic
EPA 2018 draft [249]	Feng et al 2017[252]	$BMDL_{20\%}$ (28.19 mg/kg-day) for reduction of thyroid hormones (total T4) in newborn female offspring of mice dosed during pregnancy (GD 1-20)	4.2[c] (body weight scaling)	300 UF_H=10 UF_A=3 UF_D=10[e]	0.014	Chronic
MDH 2017[257]	Feng et al. 2017[252]	NOAEL (50 mg/kg-d) for altered maternal thyroid hormones, reduced pup growth and developmental delays in female mice following dosing (GD1-20) LOAEL: 200 mg/kg-day	0.158 (50/317 DAF) [f]	100 UF_H=10 UF_A=3 UF_D-3[g]	0.0016	Short-term (1-30 days)
MDH 2017[257]	Leider et al 2009a; York et al. 2003[251, 254]	$BMDL_{10}$ (45 mg/kg-day) for 10% increase in mild hyperplasia in kidney in both parent and offspring in 2-generation rat study. NOAEL: 30 mg/kg-day LOAEL: 100 mg/kg-day	0.129 (45/350 DAF)[g]	100 UF_H-10 UF_A-3 UF_D-3[g]	0.0013	Subchronic (>30 days – 10% lifetime)
MDH 2017[257]	Leider et al 2009a; York et al. 2003[251, 254]	$BMDL_{10}$ (45 mg/kg-day) for 10% increase in mild hyperplasia in kidney in both parent and offspring in 2-generation rat study. NOAEL: 30 mg/kg-day LOAEL: 100 mg/kg-day	0.129 (45/350 DAF)[g]	300 UF_H-10 UF_A-3 UF_D-3[g] UF_s -3	0.00043	Chronic (>10% lifetime)

MI DHHS 2019[44]	Leider et al 2009b[255]	$BMDL_{10}$ (78.7 mg/kg-day) for 10% increase in incidence of kidney hyperplasia in female rats in a 90 day oral study	0.225 (78.7/350 DAF)[h]	1000 UF_H-10 UF_A-3 UF_D-3[g] UF_s -10	0.00023	Chronic

[a]Uncertainty factors: UF_H= intra-individual 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]RfDis a reference dose
[c]The animal doses in the study, converted to human equivalent doses (HEDs), were used in the BMD modeling. EPA derived HEDs by applying a dosimetric adjustment factor (DAF) to each dose group where HED = dose × DAF. DAFs for each dose are calculated as follows: $DAF=(BWa^{1/4} \div BWh^{1/4})$, where BWa=animal BW and BWh=human BW. DAFs were calculated using dam terminal BWs (BWa) as reported by the study authors.
[d]Database deficiencies cited by EPA were a lack of developmental neurotoxicity studies given the known influence of thyroid hormone on neurodevelopment and a lack of studies evaluating immunotoxicity.
[e]EPA increased the UF_D to 10 for chronic exposures citing additional uncertainty regarding how longer-term exposures might affect hazard identification and dose-response assessment for PFBS via the oral route.
[f]The DAF derived by MDH is the ratio of human serum half -life/female mouse serum half-life = 665 hours/2.1 hours = 316.
[g]A database uncertainty factor of 3 was applied because of an absence of a study evaluating neurodevelopmental effects following developmental PFBS exposure. Thyroid hormone alterations during critical fetal and neonatal life stages can lead to adverse neurodevelopmental effects in offspring.
[h]Instead of body weight scaling, MDH and MDHHS 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.

EPA and MDH evaluators concurred that the most sensitive adverse effects in available animal studies were reductions in thyroid hormones, kidney toxicity, and developmental toxicity such as delayed maturation, reduced pup growth, and persistent hypothyroxinemia in offspring. MDH developed short-term, subchronic, and chronic oral RfDs in 2017 based on altered thyroid hormones and kidney toxicity observed in developmental studies in mice and rats (see Table 7). EPA published draft oral subchronic and chronic toxicity values for PFBS in November 2018 for public comment. These EPA values have been through internal and external peer review, but are not final Agency policy. EPA selected Feng et al. as the critical study, but also evaluated results from a high quality study by the National Toxicology Program that was just recently published. Both teams of risk assessors concluded that the data were insufficient to establish either an inhalation RfC or cancer screening value. In February 2019, the Michigan Department of Health and Human Services published a health screening value for PFBS in drinking water based on EPA's 2014 provisional assessment of PFBS used in the Superfund program. The MDHHS assessment did not consider the more recent assessment by EPA in December 2018 and was not further evaluated by Washington. We describe the three critical studies in the EPA and MDH assessments below.

Feng et al. 2017 administered oral doses (50, 200, and 500 mg/kg-day) of potassium perfluorosulfonate (K-PFBS) daily to pregnant mice on gestation days 1-20. Offspring were allowed to nurse. Female offspring were monitored at birth (PND 1), puberty (PND 30), and adulthood (PND 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 GD 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 PND 30. The NOAEL for altered thyroid hormones was 50 mg/kg-day.

A number of other adverse effects had the same LOAEL and NOAEL in the Feng et al. study. Pups were underweight compared to controls into adulthood at the LOAEL and had delayed eye opening, delayed vaginal opening, and delayed first estrous. At PND 60 (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 decreased and luteinizing hormone was increased at puberty (PND 30) compared to controls. Serum progesterone was decreased in adulthood (at PND 60). The NOAEL was 50 mg/kg-d for all adverse effects noted. Maternal serum PFBS, measured twelve hours after the last dose on GD 20, averaged 74 mg/L at the NOAEL and 332 mg/L at the LOAEL. Because the half-life of PFBS in female mice is estimated to be two hours, measured serum levels likely underestimate the average maternal serum levels during gestation. EPA used the administered dose rather than the serum at that dose as the point of departure for risk assessment.[252]

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 LOAEL for significant reductions in T4 and T3 hormone levels was 62.6 mg/kg-day.[193]

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) was allowed to nurse until PND 22 and dosed from weaning through mating, gestation and a lactation period. The second generation of offspring (F2) was not dosed directly, but allowed to nurse and sacrificed at three

weeks. The study reported dose-dependent increases in microscopic mild to moderate kidney hyperplasia in males and females of the parent generation. The LOAEL was 100 mg/kg-day. Thyroid hormones T3 and T4 were not measured.[251, 254]

EPA evaluators noted that similar patterns of decrease in total T3, total T4, and free T4 levels occurred in pregnant mice, their offspring, adult male rats, and adult non-pregnant female rats exposed to PFBS. Thyroid hormones T3 and T4 are essential for normal fetal and postnatal growth and development and reductions in T3 and T4 may underlie the adverse effects in growth and development observed in the Feng et al. study. Increased TSH in response to lowered T3 and T4 was observed in pregnant mice and their offspring, but were not evident at all time points in mouse offspring. TSH did not differ from controls in adult male or female rats in the NTP study. A pattern of reduced T4 without concomitant rise in TSH is consistent with human diagnosis of hypothyroxinemia. This condition is relevant to humans as it has been associated with impaired neurodevelopment and cognition later in life when the condition occurs during pregnancy.[249]

EPA and Minnesota risk assessors differed in their accounting for the observed difference between rodents and humans in PFBS serum half-life. EPA defaulted to body weight scaling to derive a human equivalent dose from the animal dose. MDH used the limited data available on serum half-lives in mice, rats, and humans to derive a chemical-specific dosimetric adjustment factor (DAF). The resulting difference in the human equivalent dose (HED) is substantial.

EPA calculated the DAF = Body weight $_{animal}^{1/4}$ ÷ $BW_{human}^{1/4}$. Body weight for humans was assumed to be 80 kg, which represents the mean weight for adults ages 21 and older and includes estimates of body weight of pregnant women reported in EPA's Exposure Factors Handbook (U.S. EPA, 2011). The body weights for the animals were taken from measurements in the specific experiments. The resulting dosimetric adjustment based on body weight scaling used by EPA was 0.149 for mice[5] (in the Feng et al. study) and 0.234 for rats (in the Lieder et al. 2009a study).

MDH relied on several published studies of serum half-life of PFBS in mice, rats, and monkeys following single IV or oral doses. The human data came from a small group of occupationally exposed adult volunteers (five men, one woman) who were monitored during a 180-day break in PFBS production.[13] The mean serum elimination half-life for PFBS in these workers was 27.7 days (GM 25.7 days) and ranged 13–46 days for the six individuals. PFBS serum elimination curves in humans best fit a one-compartment model.[13] The corresponding MDH dosimetric adjustment factor (DAF) is a ratio of serum half-life in animals to serum half-life in humans[6]. The

[5]The EPA DAF for Feng et al. = ($BWa^{1/4}$ ÷ $BWh^{1/4}$) = ($0.0399^{1/4}$ ÷ $80^{1/4}$) = 0.149. The HED was calculated by multiplying the dose by the DAF = 200 × 0.149 = 29.9 mg/kg-day.

[6]MDH expresses the equation as HED = POD/DAF where the DAF = $T_{1/2}$ human /$T_{1/2}$ animal. Their adjustment factors for extrapolating to humans from an administered dose in male rats is 203 (665/3.28

DAF_{mice} = 2.1 hrs. $_{female\ mouse}$ /665 hrs. $_{human}$ = 0.0032 (in the Feng et al study). DAF_{rats} = 1.9 hrs. $_{female\ rat}$ /665 hrs. $_{human}$ = 0.0029 (in the Lieder et al study). Use of the MDH adjustment results in a human equivalent dose that is about 50 times lower than the body weight scaling method used by EPA.

EPA cited the low detection frequency of PFBS in human serum in the general population as evidence that PFBS is likely excreted efficiently in humans. EPA determined that the available data in humans and animals was insufficient to estimate an interspecies DAF especially in repeated dosing scenarios. We agree that there is insufficient data for toxicokinetic modelling however, the EPA assumption that excretion rates in rodents and humans are essentially the same is not supported by the rodent and human data available. Human serum clearance data shows substantially longer clearance time in humans than in rodents. Serum elimination rates of PFBS in rodents are similar to other PFAS and are unlikely to be substantially longer following repeated dosing. We preferred the DAF approach of MDH, which used the data available to calculate HEDs from the administered dose or BMDL. This approach was also used by the Michigan Science Advisory Workgroup in their recent recommendations for Health-based drinking water values.[44, 258]

We concurred with the uncertainty factors in EPA's 2018 assessment. EPA applied a 100-fold uncertainty factor for subchronic exposures and a 300-fold uncertainty factor for chronic duration exposure. Uncertainty factors included a 10x for human variability and a 3x for interspecies uncertainty. For subchronic exposures EPA used a 3x UF for database deficiencies, noting the lack of developmental neurotoxicity studies and the lack of immune toxicity studies. For chronic duration exposures, EPA used a 10x UF for database deficiencies citing an additional concern that long-term exposure studies in animals are lacking.

Relevance to Humans

Studies investigating the effects of PFBS exposure on thyroid hormone levels in humans were lacking. The rodent data on this endpoint was consistent for reductions in T4 and T3 across two species of rodents, both sexes, adult and early life stages, and different exposure durations (20–90 days). The altered thyroid hormone levels observed are also consistent with the abnormal and delayed development seen in young rodents. EPA reviewed the evidence and importance of T4 for proper human development in their assessment. Thyroid hormones are critical for normal fetal and early life development in many bodily systems. They are "critically important in early neurodevelopment as they directly influence neurogenesis, synaptogenesis, and myelination."[249] EPA concluded that the available evidence "supports a hazard" and considered the thyroid a potential target for PFBS toxicity in humans.

EPA selected a 20 percent decline in total T4 as their point of departure. Their rationale for this included a finding that "Neurodevelopmental and cognitive deficits have been observed in

hr), in female rats is 350 (665/1.9 hr), in male mice is 202 (665/3.3), and in female mice is 317 (665/2.1). To compare to EPA in the text, we expressed the MDH DAF as 1/DAF.

children who experienced a 25 percent decrease in maternal T4 during the second trimester *in utero* (Haddow et al., 1999). In other studies, mild-to-moderate thyroid insufficiency in pregnant women was defined as having serum T4 levels below the 10th percentile for the study population, which was associated with a 15 percent to 30 percent decrease relative to the corresponding median (Finken et al., 2013; Julvez et al., 2013; Román et al., 2013; Henrichs et al., 2010). Similarly, decreases in mean maternal T4 levels of ~10 percent to17 percent during pregnancy and lactation have been found to elicit neurodevelopmental toxicity in rat offspring (Gilbert et al., 2016 Gilbert, 2011). The lower end of the range of T4 changes associated with untoward developmental health outcomes (e.g., 10 percent) commonly falls within normal experiment-to-experiment variation in control values. A BMD of 20 percent of control mean was determined to be a minimally biologically significant degree of change when performing BMD modeling on thyroid hormone alterations in pregnant females and associated offspring."[249]

EPA evaluation of other endpoints concluded that available evidence from rodents and humans supports a hazard for developmental and kidney toxicity and is equivocal for reproductive toxicity, hepatic effects, and effects on lipid or lipoprotein homeostasis.[249]

Washington State Recommendation: 300 ng/kg-day

We recommend using the EPA 2018 assessment of PFBS toxicity with the dosimetric adjustment factor developed by MDH 2017. The EPA 2018 toxicological assessment was comprehensive and incorporated recent data available for PFBS from the National Toxicology Program. We concurred with EPA on thyroid hormone reduction as the most sensitive critical effect and with selection of Feng et al, 2017 as the critical study. We deferred to EPA on selecting a 20 percent reduction in thyroid hormone in the $BMDL_{20}$ as the best compromise between clearly functional deficits in hormone level and measurement variability in human studies. The permanent reduction in thyroid hormones following *in utero* exposure in Feng et al. was associated with development delays and reproductive abnormalities. This study was supported by the 28-day NTP study showing reduced thyroid hormones in male and female adult rats with a LOAEL of 62.6 mg/kg-day.

We concurred with the EPA uncertainty factors, but preferred the MDH approach to calculating a dosimetric adjustment factor to account for the likely difference in mouse clearance of PFBS and human clearance of PFBS. In experiments reviewed in the EPA assessment, rats and mice had serum clearance half-lives of several hours for both sexes. The only study of humans reported mean serum clearance half-lives of 25.8 days.[13, 249] Much longer serum half-lives in humans vs. rodents are observed for other PFAS. The MDH approach uses the data available to estimate the ratio rather than relying on a default that adjusts mostly for differences in body size and assumes similar excretion rates. Applying the MDH dosimetric adjustment factor (DAF) to the mouse $BMDL_{20}$ results in a Human Equivalent Dose (HED_{POD}) of 0.089 mg/kg-day and an RfD of 0.0003 mg/kg-day (or 300 ng/kg-day).

- $BMDL_{20}$ x DAF_{MDH} = HED_{POD} 28.2 mg/kg-day[7] x 0.00315 = 0.089 mg/kg-day
- HED_{POD} ÷ UFs = RfD 0.089 ÷ 300 = 0.0003 mg/kg-day

Special populations. It is important to protect the developing fetus and children from overexposure to PFBS via drinking water. A number of developmental effects were observed in animal studies with PFBS. Maternal intake of drinking water will affect fetal exposure and lactational transfer. Infants and children also have higher drinking water intake than adults.

Relative Source contribution: 20 percent

RSCs were developed for children and adults for all five PFAS evaluated (see Table 1) with the subtraction method and the EPA Exposure Decision Tree. Daily exposures to PFBS from nondrinking water sources are not well understood. PFBS is a replacement for phased-out PFAS and is likely to be present in newer firefighting foams, stain and waterproofing products and other current use products. 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 serum level trends. While long-chain PFAS showed declining trends, the trend for PFBS showed a doubling of serum level every six years over this time period.[259] Because PFBS is eliminated more rapidly from human serum, PFBS in serum is not a good indicator of cumulative intake from all sources over time.

Using the EPA Exposure Decision Tree, we derived a default RSC of 20 percent for PFBS.

Water Intake Rate: 0.047 L/kg-day

A placental and lactational model for PFBS has not been developed. PFBS has been detected in serum of pregnant women;[259] however, cord blood PFBS did not correlate well with paired maternal serum in a recent study by Wang et al. 2019.[260] PFBS was detected in less than half the studies identified by the MDH that evaluated breastmilk for its presence. At this time, there is insufficient information to model infant exposures and a lower concern about this pathway given the more rapid clearance of PFBS from the human body.

We considered an ingestion rate of 0.047 L/kg-day, which is the 95th percentile community water intake for lactating women from the 2019 update to the EPA Exposure Factors Handbook, (Table 3-2, consumers only). This will also sufficiently protect pregnant women as the 95th percentile intake for pregnant women is 0.038 L/kg-day.

- RfD x RSC ÷ drinking water ingestion rate = SAL
- 0.00030 mg/kg-day x 0.2 / 0.047 L/kg-day = 1.28 µg/L (chronic SAL) rounded to 1.3 µg/L

[7]The EPA converted administered dose to HED dose before conducting benchmark dose modelling. To determine the administered dose at the $BMDL_{20}$, we divided the HED_{POD} by the EPA DAF from body scaling 0.149 at the 200 mg/kg-day dose. $BMDL_{20\ Mouse}$ = 4.2 mg/kg-day ÷ 0.149 = 28.19 mg/kg-day.

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August 2020 addendum to the Draft Recommended State Action Levels for Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water: Approach, Methods and Supporting Information (November 2019)

RE: Change to PFBS SAL

In response to comments on our draft SALs, we reconsidered the calculation for the PFBS SAL. Specifically, we revised it to include infant intake of drinking water.

The reference dose for PFBS was based on developmental toxicity in rodents. In our initial draft SAL, we assumed 95th percentile drinking water intake for lactating women, which is slightly higher than the intake for pregnant women (and therefore protective of pregnant women). Maternal PFBS intake determines the amount of PFBS available to the fetus and breastfed child.

0.00030 mg/kg-day (RfD) x 0.2 (RSC) / 0.047 L/kg-day = 0.00128 mg/L, rounded to 1.3 µg/L

In the revised SAL, we considered all early childhood life stages to better protect infants whose infant-formula is mixed with tap water and young children who have a higher intake of drinking water per kilogram of body weight than adults. These are shown in the table below.

Life stage	Drinking water Intake rate (L/kg-day)[a]	Relative Source contribution or RSC (%)	(RfD[b]/DW intake)*RSC=SAL (mg/L)
Infants (<1 year)	0.174 (95th)	50	0.000862
1 to <2 years old	0.049 (90th)	20	0.001224
2 to <3 years old	0.051 (90th)	20	0.001176
3 to <6 years old	0.039 (90th)	20	0.001538
Pregnant women	0.038 (95th)	20	0.001579
Lactating women	0.047 (95th)	20	0.001276

[a] *Intake rates from 2019 EPA Exposure Factors Handbook Chapter 3*
[b] *RfD = Reference Dose which is 0.0003 mg/kg-day for PFBS*

Consistent with assumptions for other PFAS SALs we used 95th percentile drinking water intake rates for infants, pregnant women and lactating women and 90th percentile drinking water rates for all chronic periods. The RSC of 50 percent for infants is also consistent with our other PFAS SALs (see Table 2 in the WDOH, Draft Recommended State Action Levels for PFAS in Drinking Water: Approach, Methods, and Supporting Information, November 2019.

Our new draft SAL for PFBS is 0.860 µg/L (or 860 ppt).

0.00030 mg-kg-day x 0.5 / 0.174 L/kg-day= 0.000862 mg/L, rounded to 0.860 µg/L