Great Lakes Consortium for Fish Consumption Advisories

Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines

September 2025

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List of Acronyms and Abbreviations

ALT: alanine transaminase

ATSDR: Agency for Toxic Substances and Disease Registry

BMD: benchmark dose

BMDL: Benchmark Dose Limit

CVD: cardiovascular disease

DHA: docosahexaenoic acid

EFSA: European Food Safety Authority

EPA: eicosapentaenoic acid

g: gram

HDL: high-density lipoprotein

HFPO-DA: hexafluoropropylene oxide dimer acid

HPV: health protection value

IRIS: Integrated Risk Information System

kg: kilogram

L-FABP: liver fatty acid binding protein

LDL: low-density lipoprotein

LXR: liver X receptor

MCL: maximum contaminant level

mg/kg-day: milligrams per kilogram per day

MI SAW: Michigan Science Advisory Workgroup

MOE: margin of exposure

ng/g: nanograms per gram

ng/kg-d: nanograms per kilogram per day

ng/mL: nanograms per milliliter

NHANES: National Health and Nutrition Examination Survey

non-HDL: non-high-density lipoprotein cholesterol

NRSA: National Rivers and Streams Assessment

O3PUFA: omega-3 long-chain polyunsaturated fatty acid

PCBs: polychlorinated biphenyls

PFAS: per- and poly-fluoroalkyl substances

PFBS: perfluorobutane sulfonate

PFDA: perfluorodecanoic acid

PFHxS: perfluorohexanesulfonic acid

PFNA: perfluorononanoic acid

PFOA: perfluorooctanoic acid

PFOS: Perfluorooctane Sulfonate

POD: point of departure

POD-HED: POD-human equivalent dose

PUFA: polyunsaturated fatty acid

RfD: reference dose

RSC: relative source contribution

TC: total cholesterol

TK: toxicokinetic

UF: uncertainty factor

UF_A: animal to human extrapolation

UF_D: database deficiencies

UF_H: sensitive human subpopulations

UF_L: use of a lowest-observed-effect-level rather than a no-observed-effect-level

UF_S: use of a sub-chronic study

USEPA: United States Environmental Protection Agency

USFDA: United States Food and Drug Administration

VKM: Norwegian Scientific Committee for Food and Environment

VLDL: very low-density lipoprotein

Executive Summary

In 2019, the Great Lakes Consortium for Fish Consumption Advisories (Consortium) published the *Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines* (Best Practice) (Great Lakes Consortium for Fish Consumption Advisories, 2019), which recommended fish consumption guidelines based upon the 2016 United States Environmental Protection Agency (USEPA) Office of Water reference dose (RfD) for PFOS of 20 nanograms per kilogram per day (ng/kg-d) (USEPA, 2016). The 2019 Best Practice has served as an important resource for fish advisory programs in the Great Lakes Basin. Since its publication, authoritative bodies have developed/updated PFOS toxicity values that are much lower than the 2016 USEPA RfD. New information has also become available about fish consumption health benefits, background PFOS exposure, PFOS cancer risk, fish PFOS bioavailability, toxicity of other per- and poly-fluoroalkyl substances (PFAS), and other important considerations.

To serve the needs of Consortium fish advisory programs, a Best Practice Workgroup (the Workgroup) was established to conduct a review of new toxicity values and other relevant information for PFOS fish consumption guidelines. This review served as a foundation for recommended updates to the Best Practice. Consortium members considered proposed updates from the Workgroup and provided written comments to the Workgroup. The 2025 Best Practice reflects the proposed updates and subsequent input from Consortium members. It does not necessarily imply endorsement of the Best Practice by all Consortium member entities.

Many studies of PFOS exposure to humans and animals have been published since 2019, as have multiple toxicity assessments. Of the assessments considered, the Workgroup agreed that the 2024 USEPA PFOS toxicity review, in support of 2024 maximum contaminant levels (MCLs) in drinking water, included the most up-to-date and comprehensive toxicity information available for PFOS. USEPA ultimately relied on studies that evaluated human developmental and cardiovascular outcomes to derive points of departure (PODs), POD-human equivalent doses (POD-HEDs), and an RfD. The Workgroup agreed that USEPA's reliance on the most sensitive endpoints in humans and identification of critical studies were reasonable and appropriate, and that the PODs and POD-HEDs were appropriate starting points for the development of a PFOS health protection value (HPV) for fish consumption guidance. Upon review of these critical studies, the POD-HEDs, and the RfD, the Workgroup identified conservative assumptions and uncertainties related to the magnitude of effects. Additionally, the Workgroup questioned the need to account for intra-human variability when the POD-HEDs were already based on sensitive populations, especially in consideration of the commensurate loss of numerous health benefits associated with fish consumption.

The Workgroup also considered evidence that PFOS in fish tissue is not as bioavailable as in water. Measured human serum levels of PFOS in fish consumers (and therefore associated toxicity) are much lower than the levels predicted by USEPA toxicokinetic (TK) parameter values and standard fish meal sizes. Based on this information, the Workgroup concluded that adjustment for reduced PFOS absorption (bioavailability factor) was appropriate for the development of fish consumption guidelines.

Therefore, the Great Lakes Consortium for Fish Consumption Advisories *2025 Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines* adopts the 2024 USEPA POD-HED of 1 ng/kg-d as an HPV, specifically for fish consumption, and applies a 0.25 bioavailability factor for fish consumption guidelines. Resulting PFOS concentrations corresponding to meal frequency categories for all populations are shown in **Table 1** below as the 2025 PFOS Best Practice Fish Consumption Guidelines.

Consortium members may choose which meal advice categories to implement. Through this Best Practice, the Consortium promotes consistency in the fish consumption guidelines for PFOS across the Great Lakes basin.

Table 1. 2025 PFOS Best Practice Fish Consumption Guidelines for All Populations

PFOS in Fish Fillet (ng/g)	Meal Frequency
0 – 2.5	4 or More Meals/Week
> 2.5 – 5	2 Meals/Week
> 5 - 10	1 Meal/Week
> 10 - 40	1 Meal/Month
> 40	Do Not Eat

See Derivation of Fish Consumption Guidelines for PFOS for more details about the assumptions, inputs, and calculations used to derive these PFOS guidelines in fish.

While the primary determinants of the recommended PFOS fish consumption guidelines are summarized above, several other potentially influential topics were considered by the Workgroup. Because of the practice of accounting for other sources of exposure when setting contaminant concentration guidelines, action levels, or standards for environmental media, the Workgroup considered application of a relative source contribution adjustment for the HPV or guidelines, concluding that such an adjustment was not appropriate. The Workgroup also decided that cancer risk levels at the guideline levels were within reasonable bounds. The Workgroup considered fillet tissue to be the best tissue from which to evaluate exposure and risk. Because fish cleaning and cooking methods can be effective in reducing exposure to some contaminants [e.g., polychlorinated biphenyls (PCBs)], the Workgroup summarized the literature on this topic and concluded that cooking and cleaning does not consistently change fish tissue PFOS concentrations/amounts. The Workgroup also evaluated PFOS data to characterize “background” concentrations for relatively unimpacted waters, demonstrating that commonly encountered background levels were not far below the recommended Eat up to One Meal per Month guideline. The Workgroup considered recently published USEPA toxicity values for several other PFAS and fish data from New York State and concluded that a small percentage of fish would have unacceptable risk levels for these PFAS, and PFAS exposure via consumption for the majority of these fish would be reduced by following the advisories for PFOS. Finally, because a breastfed infant can experience a spike in PFOS exposure, the protectiveness of the HPV for that population was considered.

This 2025 Best Practice describes the rationale for PFOS fish consumption guidelines as recommended by the Consortium. The rationale considers both the risks of PFOS exposure and the health benefits of fish consumption. As with the 2019 Best Practice, it is expected that Consortium entities could choose to adopt all, none, or select elements of the updated Best Practice recommendations. The Consortium acknowledges that scientific understanding of the adverse health effects and risk assessment of PFAS is continually evolving. To ensure that guidance remains current and evidence-based, the Consortium will actively monitor emerging research and developments. This will inform timely revisions to this edition of the PFOS Best Practice, ensuring that advice on the safe consumption of Great Lakes fish reflects up-to-date and reliable science.

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Background

The Great Lakes Consortium for Fish Consumption Advisories (Consortium) is a collaboration of fish advisory program staff from U.S. state governmental health, water quality, and fisheries agencies bordering the Great Lakes: Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin. Staff from the Ministry of the Environment, Conservation and Parks of the Province of Ontario, Canada, the Great Lakes Indian Fish and Wildlife Commission, and the Saint Regis Mohawk Tribe also participate.

The Consortium originally formed in the early 1980s on an ad hoc basis to share contaminant data and coordinate fish sampling. Later, the group was formally established as the Council of Great Lakes Governors' Fish Consumption Advisory Task Force as a part of the Great Lakes Toxic Substances Control Agreement of 1986.

The Task Force was charged by the Council of Great Lakes Governors with developing common fish advisories for important sportfish species that range widely in open waters of the Great Lakes (i.e., Lakes Superior, Michigan, Huron, Erie, and Ontario). In response, the Task Force developed the *Protocol for a Uniform Great Lakes Sport Fish Consumption Advisory* (Protocol) for assessing risks and issuing fish consumption advice so that the advice issued by each state bordering the Great Lakes would be consistent in protecting the health of people who eat fish from the Great Lakes (Great Lakes Sport Fish Advisory Task Force, 1993). Following development of the Protocol, the Consortium continued to operate less formally through grant funding, and currently functions with United States Environmental Protection Agency (USEPA) Great Lakes Restoration Initiative grant funding to:

- Provide the primary forum for Great Lakes fish advisory programs to collaborate on data and guidance for developing fish consumption advisories.
- Use, share, and advance credible data and science about contaminants in fish and fish consumption advisories.
- Evaluate the risks and benefits of consuming Great Lakes fish to develop common methods for determining consumption advice and harmonize consistent advice for shared waters of the Great Lakes Basin.
- Develop common educational messaging to incorporate into fish consumption advice.
- Establish and utilize best practices for communicating risks and benefits and influencing the behavior of fish consumers.

Members work through these collaborative approaches:

- Share and review data on contaminants in fish.
- Identify and share assessment methods.
- Promote consistency of health risks and benefits assessments utilized by members to develop fish consumption advice.
- Share and coordinate approaches for health education and community engagement.

Previously, the 2019 *Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines* (Great Lakes Consortium for Fish Consumption Advisories, 2019) was based on the 2016 USEPA Drinking Water Health Advisory reference dose (RfD) of 20 nanograms per kilogram per day (ng/kg-d) (USEPA, 2016). Since 2019, the body of scientific literature has increased and become available to reevaluate the guidelines.

This 2025 *Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines* (Best Practice) is intended to reflect the best science for application to fish consumption guidelines and consistent methods for advice determination. Differences in actual advice may exist due to differences in species occurrence, contaminant concentrations, and other implementation factors. This Best Practice will be reviewed every three years, or as new science becomes available to the Consortium.

PFOS Fish Consumption Guidelines and Standards

Many states, including the Great Lakes states and the Canadian province of Ontario, have established fish consumption guidelines at various consumption frequencies and corresponding PFOS concentrations that are meant to be health protective based on noncancer risk assessments. A selection of these guidelines is presented in **Table 2**. Do Not Eat guidelines for the general population vary from 28 to 800 nanograms PFOS per gram of wet weight tissue (ng/g). All U.S. state advisories have recently been summarized by the Environmental Council of the States (ECOS, 2024). For comparison, in 2022, the European Union established maximum levels for PFOS in commercial fish meat for infants and young children of 2 ng/g, and for adults depending upon species of 7 ng/g (e.g., trout, wild salmon, pike, etc.) and 35 ng/g (e.g., perch, smelt, whitefish, etc.) (EU, 2022).

Table 2. PFOS Fish Consumption Guidelines for Select States/Provinces (ECOS, 2024) and 2025 Best Practice

State/Province	1 Meal per Month Guideline (ng/g) All (general/sensitive) Populations	Do Not Eat Guideline (ng/g) All (general/sensitive) Populations
2025 Best Practice*	10	40
Alabama	**	800
Connecticut	8	31
Illinois	51	200
Indiana	50	200
Maine	14	60
Maryland	136	408
Massachusetts	**	(183/81)
Michigan	75	300
Minnesota***	(50/20)	(200/50)
New Jersey	17	(204/17)
New York	50	(200/50)
Ontario, CA	100	(200/50)
Washington	9.5	28.2
Wisconsin	50	200

*Consortium member states/province have various consumption categories for which they provide advice; guidelines may vary accordingly.

**Does not have 1 Meal per Month Guideline category for fish consumption.

***(MDH, 2024) Updated after release of ECOS, 2024

Risk Assessment

The 2019 Best Practice guidelines (Great Lakes Consortium for Fish Consumption Advisories, 2019) were directly based on the 2016 USEPA RfD (USEPA, 2016). Direct application of USEPA's 2024 PFOS RfD of 0.1 nanograms per kilogram per day (ng/kg-d) would result in a Do Not Eat guideline of approximately 1 ng/g PFOS in fish tissue. With common method reporting limits ranging approximately from 0.1 to 2 ng/g PFOS for fish tissue, many PFOS detections could trigger Do Not Eat advisories. Fish PFOS concentrations measured in waters of New York State (n = 1292) indicate that >88%[1] of fish have PFOS concentrations exceeding 1 ng/g. Assessments with other PFOS fish datasets come to similar conclusions (i.e., that the majority of samples would exceed a Do Not Eat guideline directly based on the 2024 USEPA RfD). Thus, the vast majority of waterbodies and freshwater sportfish in Great Lakes states would have concentrations triggering Do Not Eat advice. Fish consumption rates in the U.S. and Canada continue to be below those recommended for a healthy diet by public health agencies (Cave et al., 2020; Hu and Chan, 2020). The high percentage of fish with PFOS exceeding concentrations equivalent to the USEPA RfD suggests that basing fish consumption advice directly on the USEPA RfD would severely restrict or eliminate consumption of fish as a healthy low-cost source of protein for many people, with corresponding potentially adverse effects on public health. The lack of feasibility indicates the need for a closer examination of USEPA's underlying assessment as well as consideration of fish consumption health benefits and other factors in setting fish consumption guidelines for PFOS.

Direct application of the 2024 USEPA RfD to fish consumption advice would vastly limit availability of freshwater fish for consumption.

Cancer

Fish consumption guidelines developed by states and tribes have traditionally not been based on specific cancer risk targets. Reasons for this include the uncertainty in cancer risk assessment, low level of cancer risks associated with exposure compared to the background rate of cancer, and the inherent protection against cancer risk provided by protection against risk of noncancer health end points for sensitive populations (Great Lakes Sport Fish Advisory Task Force, 1993). Other reasons given have been correspondingly much lower fish consumption guidelines and the fear the public has about cancer – both of which could result in reduced fish consumption and accompanying benefits (Stone et al., 2009). Furthermore, cancer risk estimates assume exposure over a lifetime, which may not be consistent with typical anglers' exposure scenarios.

In 2024, USEPA determined that PFOS is *Likely to Be Carcinogenic to Humans*, based on one animal model for multiple sites and both sexes, as well as supporting evidence from human studies (USEPA, 2024a). Epidemiological evidence supports a plausible association between PFOS exposure and liver cancer, which is consistent with evidence of liver cancer in animals. The International Agency for Research on Cancer considers PFOS as "possibly carcinogenic to humans" (Zahm et al, 2023). An assessment of cancer risk at the recommended 10 ng/g PFOS guideline triggering Eat up to One Meal

[1] 88% is an underestimate because most of New York State's data is associated with a detection limit of 2 ng/g. There are a considerable number of samples which would have actual concentrations above 1 ng/g but are not included in the calculation because they are below the 2 ng/g detection limit.

per Month advice is presented later in this Best Practice. As before, the Workgroup decided to use noncancer endpoints for the PFOS Best Practice consumption guidelines.

Noncancer

Noncancer health effects associated with PFOS exposure include chronic immunological, developmental, cardiovascular, and hepatic effects observed in humans and animals. Several toxicity assessments and non-cancer toxicity values for PFOS have been published by authoritative bodies since the 2019 Best Practice, some based on animal studies and others on human studies.

- 2019: The Michigan Science Advisory Workgroup (MI SAW) published a toxicity value of 2.9 ng/kg-d based on immune effects in adult male mice (MI SAW, 2019).
- 2020: The European Food Safety Authority (EFSA) finalized an assessment of per- and poly-fluoroalkyl substances (PFAS) that included a toxicity value of 0.62 ng/kg-d for the sum of PFOS and three other PFAS based on immune effects in children (EFSA, 2020).
- 2021: The Agency for Toxic Substances and Disease Registry (ATSDR) finalized its *Toxicological Profile for Perfluoroalkyls* including a PFOS RfD of 2 ng/kg-d based on developmental effects in rats (ATSDR, 2021).
- 2024: Minnesota published a serum reference concentration of 2.6 nanograms per milliliter (ng/mL) based on decreased birth weight from a study of developmental effects in humans (MDH, 2024).
- 2024: California finalized its assessment and published an RfD for PFOS of 0.64 ng/kg-d based on cardiovascular effects [elevated total cholesterol (TC)] in human adults (CA EPA, 2024).
- 2024: The USEPA Office of Water finalized an assessment that included an RfD for PFOS of 0.1 ng/kg-d based on cardiovascular effects (elevated TC and developmental effects in humans) (USEPA, 2024a).

The underlying studies, the point of departure human equivalent dose (POD-HED), the uncertainty accounted for, and the final toxicity values are summarized in **Table 3** (animal studies) and **Table 4** (epidemiology data). Of these assessments, the 2024 USEPA assessment is the most up to date and comprehensive.

Table 3. Available Noncancer Toxicity Values for PFOS using Animal Studies

Source	Study	Species	Effect	Point of Departure Human Equivalent Dose (POD-HED)[a] (ng/kg-d)	Uncertainty Factors (UF)[b]					Total Uncertainty	Toxicity Value (ng/kg-d)
					UF_A	UF_H	UF_S	UF_L	UF_D		
MI SAW (2019)	Dong et al. (2009)	Mice	Immune effects in adult males (decreased plaque forming cell response)	87	3	10	1	1	1	30	2.9
NHDES (2019)	Dong et al. (2011)	Mice	Immune effects in adult males (increased IL-4 and decreased SRBC specific IgM levels)	302	3	10	1	1	3	100	3.0[c]
ATSDR (2021)	Luebker et al. (2005)	Rats	Developmental effects (delayed eye opening and decreased pup body weight)	515	3	10	1	1	10	300	2[d]
USEPA (2024a)	Zhong et al. (2016)	Mice	Immune effects (decreased plaque forming cell response to SRBC)	288	3	10	1	1	1	30	10[e]
	NTP (2019)	Rats	Immune effects (extramedullary hematopoiesis in the spleen)	291	3	10	10	1	1	300	1[e]
	Luebker et al. (2005)	Rats	Developmental effects (decreased pup body weight)	365	3	10	1	1	1	30	10[e]
	Butenhoff et al. (2012)	Rats	Hepatic effects (individual cell necrosis in the liver)	3,450	3	10	1	1	1	30	100[e]

[a] The HED applies a chemical specific clearance factor to the serum-based point of departure. The clearance factor is based on the chemical's volume of distribution and the half-life. Each authoritative body may have a different clearance factor based on their choice of half-life and volume of distribution.

[b] UF_A = animal to human extrapolation; UF_H = sensitive human subpopulations; UF_S = use of a sub-chronic study; UF_L = use of a lowest-observed-effect-level rather than a no-observed-effect-level; UF_D = database deficiencies.

[c] This RfD was originally derived by the Minnesota Department of Health in 2019, but they have since updated their toxicity evaluation for PFOS (see **Table 4**). This RfD was also used for Pennsylvania (PADEP, 2021) Maximum Contaminant Levels (MCLs) and Washington State Action Levels (Washington State, 2019; Washington State, 2023).

[d] This RfD was also used by Illinois EPA (2021) and Maine (2025).

[e] USEPA (2024a) identified these as candidate RfDs; however, they were not selected as the RfD.

Table 4. Available Noncancer Toxicity Values for PFOS using Epidemiology Data

Source	Study	Population	Effect	POD-HED (ng/kg-d)	Uncertainty Factors (UF)	Toxicity Value (ng/kg-d)
EFSA (2020)[a]	Abraham et al. (2020)	Infants (Germany)	Immune effects (decreased diphtheria antibodies)	0.63[b]	1	0.63 (sum of four PFAS)
MDH (2024)	Wikström et al. (2020)	Mother/infant pairs from the Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy cohort (Sweden)	Developmental effects (decreased birth weight)	Not developed; used serum level 7.7 ng/mL	3 (UF_D)	Not developed, used reference serum concentration 2.6 ng/mL
CA EPA (2024)	Steenland et al. (2009)	Adults from the C8 Health Project Study (USA)	Cardiovascular effects (elevated TC odds ratios)	6.39	10 (UF_H)	0.64
USEPA (2024a)	Budtz-Jørgensen and Grandjean (2018)	Children (Faroe Islands)	Immune effects (anti-tetanus antibody response)	2.71	10 (UF_H)	0.3
	Timmermann et al. (2021)	Children (Greenland)	Immune effect (anti-tetanus antibody response)	1.78	10 (UF_H)	0.2
	Budtz-Jørgensen and Grandjean (2018)	Children (Faroe Islands)	Immune effects (anti-diphtheria antibody response)	1.83	10 (UF_H)	0.2
	Timmermann et al. (2021)	Children (Greenland)	Immune effects (anti-diphtheria antibody response)	1.03	10 (UF_H)	0.1
	Sagiv et al. (2018)	Mother/infant pairs from Project Viva cohort (USA)	Developmental effects (low birth weight)	6.00	10 (UF_H)	0.6
	Wikström et al. (2020)	Mother/infant pairs from the Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy cohort (Sweden)	Developmental effects (low birth weight)	1.13	10 (UF_H)	0.1

Source	Study	Population	Effect	POD-HED (ng/kg-d)	Uncertainty Factors (UF)	Toxicity Value (ng/kg-d)
	Gallo et al. (2012)	Adults from the C8 Study (USA)	Hepatic effects (elevated ALT)	7.27	10 (UF_H)	0.7
	Nian et al. (2019)	Adults from the Isomers of C8 Health Project (China)	Hepatic effects (elevated ALT)	1.94	10 (UF_H)	0.2
	Dong et al. (2019)	NHANES Population (USA)	Cardiovascular effects (increased TC)	1.20	10 (UF_H)	0.1
	Steenland et al. (2009)	Adults from the C8 Health Project Study (USA)	Cardiovascular effects (increased TC)	1.22	10 (UF_H)	0.1

Toxicity values selected by each authoritative body are highlighted in yellow.

[a] EFSA (2020) chose to evaluate all four PFAS that were identified in serum in the study population. The point of departure is a mixture of PFOA, PFOS, PFNA, and PFHxS; however, the primary PFAS were PFOA (mean serum concentration was 16.8 ± 6.6 ng/mL) and PFOS (mean serum concentration was 15.2 ± 6.9 ng/mL) when compared to serum concentrations of PFHxS and PFNA (mean serum concentrations were 2.1 ± 1.3 and 0.6 ± 0.2 ng/mL, respectively).

[b] EFSA (2020) modeled a maternal intake that would reach the serum level in a breastfed infant.

ALT = alanine transaminase

PFOA = perfluorooctanoic acid

PFNA = perfluorononanoic acid

PFHxS = perfluorohexanesulfonic acid

NHANES = National Health and Nutrition Examination Survey

Conservative Assumptions Inherent in USEPA's Toxicity Assessment

In support of their 2024 drinking water PFOS maximum contaminant levels (MCLs), USEPA identified developmental and cardiovascular effects as the two critical effects with nearly identical POD-HEDs and RfDs. The reasoning and uncertainty behind these chosen studies indicate that the resulting POD-HEDs and RfD are conservative for fish consumption advisories.

Closer examination of USEPA's toxicity assessment demonstrates uncertainties and inconsistencies, and more conservative assumptions than USEPA used when estimating the economic benefits of PFAS drinking water regulations.

Developmental Effects

The basis for USEPA's developmental health outcome RfD for PFOS is the association between PFOS exposure during pregnancy and decreased birth weight as found in a range of epidemiological studies from Sweden, China, and the United States (USEPA, 2024a). These studies provide candidate POD-HEDs ranging from 0.87 to 6.0 ng/kg-d equivalent to exposures resulting in a 5% increase in the number of infants with birth weights below 2,500 g (Darrow et al. 2013; Starling et al. 2017, Sagiv et al., 2018; Chu et al. 2020; Wikstrom et al., 2020; Yao et al. 2021). USEPA chose the Wikstrom et al. (2020) evidence of decreased birth weight in 1533 mother/infant pairs from Sweden for the POD-HED of 1.13 ng/kg-d (USEPA, 2024a). This study was in part selected because PFAS levels were measured early in pregnancy before other confounding factors associated with gestation might influence PFAS measurement. However, this study and the other studies selected for developmental RfD consideration have limitations in several respects that may affect the way a toxicity value could be applied to fish consumption advisories. For example, the Wikstrom et al. (2020) study found statistically significant associations between low birth weight and five PFAS, including PFOS. The associations were all along similar dose response curves, with the second and third quartiles of exposure not achieving a significant difference from the (lowest) reference quartile, and a statistically significant difference occurring only for the highest quartile. USEPA used the beta coefficient for PFOS to represent the slope between PFOS serum concentration and low birth weight to calculate the benchmark dose (BMD) associated with a 5% effect level (consistent with USEPA's general approach when performing dose-response modeling for an endpoint resulting from developmental exposure) and associated 95% lower limit on the BMD or benchmark dose limit (BMDL). This BMDL (7.7 ng/mL) became the basis for POD-HED derivation via reverse dosimetry toxicokinetic (TK) modeling (1.13 ng/kg-d) and ultimately the RfD itself (0.1 ng/kg-d) after application of a 10-fold uncertainty factor (UF) to account for variability in sensitivity among humans. Thus, the beta coefficient from Wikstrom et al. (2020) is the key parameter upon which the RfD is based.

Based on the Wikstrom et al. (2020) beta coefficient as expressed by USEPA (-8.4 g birth weight per ng PFOS/mL serum) (USEPA, 2024a), exposure at the RfD of 0.1 ng/kg-d would result in a reduced birth weight of approximately 7 g (not accounting for life stage modeling). For its economic analysis of the health benefits of PFOS drinking water regulation, USEPA estimated from a metanalysis of 29 medium or high confidence studies a beta coefficient of -3.0 g birth weight per ng PFOS/mL serum, less than half that used for its derivation of its developmental RfD (USEPA, 2024b). In calculating the beta coefficient used by USEPA for the RfD determination, Wikstrom et al. (2020) corrected for various potential confounders. However, the contribution of other PFAS to the beta coefficient calculated for PFOS was

not reported. Thus, the beta coefficient attributed to PFOS may reflect the cumulative effect of multiple PFAS, given the statistically significant slopes found for four other PFAS and the likelihood that their serum levels were correlated with PFOS (not evaluated in Wikstrom et al., 2020). The other developmental studies that USEPA reviewed also did not consider potential confounding for multiple PFAS, with the exception of Starling et al. (2017), which found that the negative association between PFOS and birthweight was completely attenuated after correcting for the other PFAS. However, in response to comments on its 2023 draft toxicity assessment, USEPA did consider the potential for confounding by other PFAS and concluded that while confounding was an important source of uncertainty, there was no evidence "that the observed associations between PFOS and birth weight deficits are fully attributable to confounding by co-occurring PFAS," leaving open the possibility that some of the observed association could be due to confounding by other PFAS. Other potential confounders of the PFOS-low-birth-weight slope not considered by USEPA are mercury and polychlorinated biphenyls (PCBs). Both have been found to be correlated with PFOS exposure in studied populations due to fish consumption being a common primary exposure route for these contaminants (Weihe et al., 2008). Further, they are known to impair in utero development (Dack et al., 2021; Patandin et al., 1998), but neither one was assessed in maternal serum in Wikstrom et al., 2020, nor in the other studies that USEPA considered for its developmental outcome RfD.

There are other uncertainties with respect to the relationship between serum PFOS and low birth weight in humans. Among these are the use of a linear regression model in studies like Wikstrom et al. (2020) rather than a non-linear model for a non-cancer endpoint, given the limited understanding of the appropriate dose response curve shape. Further, there is a great degree of variability in the reported relationship across epidemiology studies, with some studies reporting no association. An additional uncertainty is the comparison of low birth weights across species, given that this PFOS effect is found in controlled animal studies but at a much-reduced potency compared to human epidemiology studies. As documented in USEPA (2024) and shown above in **Table 3** and **Table 4**, PFOS exposure in rats produced decreased pup birth weight at a POD-HED that is 365-fold greater than the human-based POD (Luebker et al., 2005; USEPA 2024a; Wikstrom et al., 2020). Even with additional UFs applied to the animal-based POD for birth weight, the candidate animal-based toxicity value is 100-fold greater than the human-based toxicity value (**Table 3** and **Table 4**; Luebker et al., 2005; Wikstrom et al., 2020). There is no known mechanistic or model-sensitivity-based reason for this large difference in POD-HEDs for developmental effects across species. Thus, while the animal evidence of a PFOS-induced decrease in birth weight supports a causal effect in humans, it also raises a question about the human-based potency. Dose-response relationships can be better understood in animal studies in which the timing, duration and quantity of exposure are well known and in which most confounders (e.g., maternal smoking, co-exposure to other PFAS, mercury and PCBs) are not present. Finally, EFSA evaluated some of the same studies and concluded that "the decrease in birth weight after adjusting for confounders is not large and the potential longer-term consequences of this decrease are unclear" (EFSA, 2020).

However, it is prudent and precautious risk assessment practice to base an RfD upon human evidence when that evidence suggests a greater potency in humans than in animals. Thus, the human-based RfD is appropriate for many applications, although its appropriateness for fish consumption advice, for which health benefits should be considered, may be more limited. The magnitude of uncertainty in the RfD based on Wikstrom et al. (2020) can be characterized as 10-fold due to the use by USEPA (2024a) of a 10-fold intraspecies UF for deriving the RfD. A 10-fold intraspecies UF may be overly conservative in

this case because the critical effect (low birth weight) already includes a sensitive human subpopulation (i.e., newborns).[2] The magnitude of uncertainty also happens to agree with the difference between the human-based draft RfD of 0.1 ng/kg-d and the lowest of the variety of animal-based RfDs for different endpoints. These RfDs are 1 ng/kg-d for splenic immune effects in rats (NTP, 2019; USEPA, 2024a) and higher (for other immune, developmental, or liver effects in rats and mice) (USEPA, 2024a). This level of uncertainty and precaution built into the USEPA draft RfD of 0.1 ng/kg-d based on the birth weight outcome suggests that an allowance for PFOS exposure of up to 10-fold higher than the RfD would still be below modeled effect levels in human and measured effect levels in animal studies.

Cardiovascular Effects

USEPA, in their supporting documentation for drinking water MCLs, concluded that effects on cholesterol and lipid homeostasis are the predominant cardiovascular effects associated with PFOS exposure, with supporting evidence of associations with blood pressure (USEPA, 2024a). USEPA identified a health-outcome-specific POD of 1 ng/kg-d for cardiovascular effects based on a BMDL of 5% extra risk (due to the severity of cardiovascular-related health effects associated with increased cholesterol) for TC exceeding the adverse level of 240 mg/dL as reported by Dong et al., 2019 (USEPA, 2024a). TC is the sum of the cholesterol content of low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL). Non-high-density lipoprotein cholesterol (non-HDL), which consists of LDL and VLDL cholesterol, is the form of cholesterol that is the best predictor of risk of coronary heart disease (Brunner et al., 2019). USEPA also cited previous studies that reported associations between LDL, triglycerides, TC and PFOS. (The forms of cholesterol are important because fish consumption and omega-3 long-chain polyunsaturated fatty acid (O3PUFA) intake are associated with decreased non-HDL but not TC. See Cardiovascular Benefits below.) In the Dong et al. (2019) study, TC, HDLC and LDLC were directly extracted from National Health and Nutrition Examination Survey (NHANES) 2003-2014 data. Participants who reported taking lipid medication were excluded. Dong et al. (2019) recommended an RfD of 2 ng/kg-d for PFOS; USEPA reevaluated the data in Dong et al. (2019) and determined an RfD of 0.1 ng/kg-d. The difference between the two RfDs was largely due to a 10x UF applied by USEPA for intra-human variability to be protective of vulnerable individuals. However, the study population for Dong et al. (2019) were adults 20-80 years of age, an age range that contains those most vulnerable to elevated cholesterol and chronic cardiovascular disease (CVD). Less influential factors contributing to the RfD differences were variations in POD, volume of distribution and half-life. Dong et al. (2019) determined an increase in TC of 0.4 mg/dL per ng/mL increase of serum PFOS based on all NHANES years analyzed (USEPA, 2024a), implying that exposure at the RfD would result in an increase of TC of 0.27 mg/dL. This value was five-fold higher than a pooled slope estimate of 0.08 mg/dL TC per ng/mL serum PFOS based on a metanalysis used by USEPA for its assessment of the economic benefits of its drinking water regulations (USEPA, 2024b). This association was not corrected for the effect of other PFAS or other chemicals which could increase cholesterol. This difference reflects the underlying contrast between the more precautionary approach of USEPA's risk assessment and the requirements for its cost-benefit analysis to be accurate and reliable.

Steenland et al. (2009) did report that considering both perfluorooctanoic acid (PFOA) and PFOS in the same model attenuated effects of individual PFAS by 20-30%. In the Dong et al. (2019) study, no

[2] Some authoritative bodies have chosen to assume no intra-human UF in the derivation of an RfD based on an epidemiology study that examined a vulnerable population (e.g., EFSA, 2020).

association was found for PFOS and LDL or HDL. Non-HDL cholesterol was not considered. Some concerns have been raised as to whether associations found between PFOS exposures and cholesterol are causal, or due to shared effects of variability in enterohepatic cycling (EFSA, 2020; Anderson et al., 2021). USEPA (2024a) noted that the mechanisms underlying the positive associations between PFOS and serum TC in humans have yet to be determined. Finally, it should be noted that elevated TC is not actually a cardiovascular disease, but an indicator of cardiovascular risk.

Effects Summary

The USEPA carried out a thorough assessment of not only developmental and cardiovascular effects but also hepatic and immune effects, drawing upon multiple lines of evidence from human, animal, and in vitro studies (USEPA, 2024a). In general, the human studies for these four health effects upon which USEPA relied for the development of candidate RfDs avoid uncertainty inherent in the extrapolation from animals to humans and thus can serve as a basis for dose response assessment. However, the human studies USEPA relied upon to develop candidate RfDs all share similar uncertainties. Human studies cannot control for the multiple potentially confounding variables that can contribute to an observed association. Like the developmental endpoint study USEPA used, the critical immune effects study was conducted on a vulnerable population (children), and yet USEPA applied a 10-fold UF to account for vulnerable populations (human variability), as it did with the other outcomes. The critical immune effects study POD-HED was identical to the POD-HEDs for the developmental and cardiovascular endpoints, but the hepatic POD-HED was seven times higher than those for the other three outcomes. The dose-response curves for all four critical endpoints were assumed to be linear, even though the true curves were more likely to be non-linear, which would have resulted in higher POD-HEDs. POD-HEDs from animal studies for all effects reviewed by USEPA, absent all the confounders of human studies, were orders of magnitude higher than the human POD-HEDs, with no clear explanation as to why humans might be more sensitive than animals. Overall, considering these and additional uncertainties, USEPA's RfDs and their underlying POD-HEDs are conservative for fish consumption advisories.

Risk-Benefit Considerations

The potential health benefits of fishing and fish consumption are many and diverse. There are benefits that are difficult to define, such as the psychological benefits of time spent in nature, cultural benefits where local fish harvesting is a key aspect of a community's "way of life", social benefits including opportunities for bonding with family and friends, and the pleasure and satisfaction of harvesting one's own food. But the health benefits with the most support in the literature are nutritional benefits.

Fish consumption provides substantial health benefits, including developmental and cardiovascular benefits. Nutritional and immeasurable psychological (quality of life) benefits are also important for consumers.

Nutritional Benefits

The health effects of exposure to contaminants from eating fish may be modified by fish nutrients, including omega-3 fatty acids (VKM, 2022; Ginsberg and Toal, 2008; Gochfeld and Burger, 2005; Stern and Korn, 2011; Groth, 2016; Mahaffey et al., 2011). These nutrients co-occur with contaminants and

have benefits on a wide range of human health endpoints including neurodevelopment, blood lipids and cardiovascular health, and immune system function (Mozaffarian and Rimm, 2006; Strain et al., 2015; Innis, 2007; Ginsberg et al., 2015). Fish consumption guidelines should take into consideration the benefits of fish consumption and the degree to which this may offset the adverse effects of fish contaminants.

The precedent for considering nutritional benefits of fish consumption, along with risk, in setting consumption thresholds dates back at least as far as the 1984 U.S. Food and Drug Administration (USFDA) reduced tolerance limit for PCBs in fish for commerce, which concluded that the tolerance limit would "strike a proper balance between protecting consumers from the risks associated with exposure to PCBs, and the loss of food due to the lowered tolerance" (49 Fed. Reg. 21519, May 22, 1984). While various publications have evaluated risks and benefits of consuming fish with mercury and other contaminants, thus far only two recent reviews have considered the risks and benefits of consuming fish with PFAS (Hamade, 2024; VKM, 2022). The assessment of the Norwegian Scientific Committee for Food and Environment (VKM) only considered PFAS immune effects but was not able to consider immune system benefits (VKM, 2022). Nonetheless, VKM concluded that fish intake is beneficial and protective against several health outcomes that present important public health challenges, with the evidence for beneficial effects of total fish intake stronger than for fatty fish intake, along with strong evidence for beneficial effects of O3PUFAs.

Some freshwater fish species, including trout and salmon, are a rich source of O3PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wang et al., 2016; Dellinger et al., 2018). While O3PUFA concentrations in Great Lakes fish are not as high as the most O3PUFA-rich commercially available marine species (e.g., Atlantic mackerel and herring with over 1,500 mg EPA+DHA/100 g tissue), they are comparable to high O3PUFA commercial freshwater species with over 1,000 mg/100 g EPA+DHA (Neff et al., 2014). All 15 Lake Erie species examined had nutritionally desirable ratios of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (> 0.4) and n-3 fatty acids to n-6 fatty acids (>1). Some studies like Dellinger et al. (2018) reported even higher levels of EPA+DHA, e.g., over 1,500 mg/100g in lake trout. O3PUFAs are antioxidant and anti-inflammatory and thus beneficial to several systems in the body (Mahaffey et al., 2008; Williams et al., 2017, Mendivil, 2021; Zaloga, 2021). Like O3PUFAs, proteins, peptides, and amino acids in fish also have health benefits (Mohanty et al., 2019). In addition, fish is a significant source of vitamin B12, and essential micronutrients like copper, manganese, zinc, iodine and selenium which can be important for enzyme function (Torfadottir and Ulven, 2024; Khalili Tilami and Sampels, 2018). Fish are also an important source of vitamin D (Khalili Tilami and Sampels, 2018), with some studies indicating higher concentrations in freshwater fish than marine fish (Tirakomonpong et al., 2019). Some fish species have high levels of vitamin A (Torfadottir and Ulven, 2024). The nutritional content of fish is responsible for many health benefits for fish consumers, including beneficial effects on the same systems that PFOS may adversely affect.

While the nutritional health benefits of fish consumption have been well studied and documented over the years, the shared molecular interactions of O3PUFAs and PFOS have not been the focus of much research and can provide new insight into balancing risks and benefits for fish consumers. O3PUFAs and PFOS are molecules with long hydrophobic carbon chains and terminal acidic groups. Due to these structural similarities, O3PUFAs and PFOS both engage in similar interactions with cellular proteins and phospholipids in cell membranes. Many human health effects attributed to PFOS may be directly or

indirectly related to its binding to proteins in lieu of their natural fatty acid ligands (Zhao et al., 2023a). For example, there is evidence that PFOS and O3PUFAs are agonists and antagonists, respectively, for the nuclear receptor LXR (liver X receptor) which is involved with lipid metabolism (USEPA, 2024a; Jalil et al., 2019). O3PUFAs in the cell membrane decrease membrane thickness, packing density, and rigidity, while PFOS increases membrane thickness, density, and rigidity (Harayama and Shimizu, 2020; Vásquez et al., 2014; Ayee et al., 2020; Matyszewska et al., 2008; Liu et al., 2016). Thus, the intake of both O3PUFAs and PFOS through fish consumption may have mutually opposing biological effects, although more research is needed in this area. PFOS adverse effects have been demonstrated primarily in animal exposure studies and in human epidemiology studies involving contaminated drinking water. There are few epidemiology studies describing the effect of PFOS on human health from fish ingestion. Thus, the extent to which PFOS adverse effects will be observed in fish eating populations is uncertain.

Cardiovascular Benefits

The most sensitive toxic effects of PFOS are on fetal development, immune function, lipid metabolism and liver toxicity. Omega-3 fatty acids have beneficial effects in each of these areas and, thus to some degree, may offset the adverse effects of PFOS in fish.

The potential risk for higher cholesterol and related CVD effects from the ingestion of PFOS in fish should be considered in light of the well documented benefits of fish consumption on similar endpoints. Beneficial effects on the cardiovascular system are probably the best studied health benefit of fish consumption. Fish consumption studies generally have shown reduced CVD incidence and associated mortality (VKM, 2022; He et al., 2004; Alhassan et al., 2017; Zheng et al., 2011; Del Gobbo et al., 2016; Jayedi et al., 2018; Jayedi et al., 2019; Zhao et al., 2019; Jayedi and Shab-Bidar, 2020; Jayedi et al., 2021; Mohan et al., 2021; Torfadottir and Ulven, 2024; Ricci et al., 2023). Fish consumption and O3PUFA studies do not show reduction in TC (the USEPA 2024 PFOS critical endpoint for cardiovascular effects), but do show reduction in non-HDL cholesterol and serum triglycerides and an increase in healthy HDL (e.g., Wang et al., 2023; McMullan et al., 2023). Non-HDL cholesterol is considered a better indicator of cardiovascular risk than TC (Brunner et al., 2019). Elevated serum triglycerides increase CVD risk, while higher HDL reduces cardiovascular risk. An analysis of 20 different prospective cohort or dietary intervention studies found that omega-3 fatty acids consistently had an effect on decreasing acute myocardial events (Mozaffarian and Rimm, 2006). A benefit slope from this evidence (14.6% decrease in CVD mortality per 100 mg/day chronic ingestion of omega-3 fatty acids) was incorporated in a fish risk-benefit model, which showed substantial CVD benefit from ingestion of most species of commonly available commercial fish, in spite of the mercury content of these fish (Ginsberg and Toal, 2009). A review of the metabolic and heart health benefits of fish also highlights the evidence that lean fish, even though they may not contribute as much O3PUFAs as some marine species, contain nutrients that can be beneficial to cardiovascular health (Tørris et al., 2018).

In addition, several authoritative bodies recommend fish consumption for cardiovascular benefits. The American Heart Association continues to recommend 1 to 2 fish meals per week to reduce the risk of congestive heart failure, coronary heart disease, ischemic stroke, and sudden cardiac death (Rimm et al., 2018). The Dietary Guidelines for Americans advise eating 8 ounces (equivalent to 227 grams or ½ pound) of fish per week based on heart health and other benefits (USDA and HHS, 2020). The 2023 Nordic Nutrition Recommendations recommend eating 300-450 g fish per week, citing cardiovascular health benefits (Torfadottir and Ulven, 2024). Thus, a substantial blood lipid and cardiovascular benefit

would likely be lost if highly restrictive fish consumption advice were put into place. This lost benefit should be at least qualitatively considered for PFOS-based consumption advisories aimed at preventing cholesterol elevation and cardiovascular risk. This is especially the case given that the fish oil benefit appears to impact disease while (as summarized above) the evidence for PFOS effect is only at the level of blood lipid perturbation but not actual worsening of CVD.

Developmental Benefits

The benefits of fish consumption in reducing adverse birth outcomes have also been well studied. Numerous epidemiological studies and reviews report reduced risk of developmental outcomes like low birthweight with intake of fish consumption or O3PUFAs. For example, one review of 26 studies reported that maternal supplementation with O3PUFAs during pregnancy was associated with a weighted mean of 42.55 g higher birth weight, compared to the control (Li et al., 2018a). Another review of 19 European birth cohort studies found that women with fish consumption greater than three times per week gave birth to neonates with 15.2 g higher birth weight (Leventakou et al., 2014). One study found that consumption of freshwater fish was significantly associated with decreased risk of low birth weight (Wei et al., 2023). A recent National Academies of Sciences, Engineering, and Medicine review concluded that fish consumption by women during pregnancy is likely associated with several health benefits, including improved birth outcomes (NASEM, 2024). The benefits of fish consumption that support healthy fetal development and reduce the incidence of low birth weight would be lost if fish consumption was decreased to reduce the risk of low birth weight due to PFOS exposure through highly restrictive fish consumption advisories. Thus, the developmental benefits of fish consumption should be considered in setting PFOS fish consumption guidelines. Overall, **the Consortium recognizes the potential risks/benefits of consuming fish when recommending the PFOS fish consumption guidelines in this document.**

Derivation of Fish Consumption Guidelines for PFOS

Consortium fish consumption guidelines are generally based on a combination of a health protection value (HPV), exposure factors, and other considerations. Fish consumption guidelines for PFOS should: 1) be based on an HPV that prevents doses above toxic effect levels and balances loss of health benefits of fish consumption against reduced risk (e.g., to account for intra-human uncertainty), and 2) consider the human bioavailability of PFOS in fish. The PFOS HPV is used to calculate fish consumption guidelines for each meal frequency category assuming body weight and consumption rates with an additional modifier for reduced human absorption of PFOS from fish. The following sections discuss each of these parameters in more detail.

Derivation of a Health Protection Value for PFOS in Fish

The USEPA POD-HEDs were based on conservative interpretations of human toxicity data with significant uncertainty. The developmental POD-HED was based on a study of mother/neonate pairs, a subpopulation that is clearly a vulnerable subpopulation. The cardiovascular POD-HED was based on the NHANES population survey of U.S. adults aged 20 - 80 years, a large study population inclusive of vulnerable individuals (e.g., elderly, genetically predisposed, etc.). Adults are not a traditional vulnerable subgroup according to USEPA's definition, although arguably they are the vulnerable subgroup for

cardiovascular effects and increased TC. Young adults generally have higher cardiovascular risks than children, and risk for adults increases with age (Candelino et al., 2022; Rodgers et al., 2019).

Some authoritative bodies that have developed toxicity values have not accounted for uncertainty associated for intra-human variability (i.e., setting a UF_H =1) when the study population was a vulnerable subpopulation. One example is EFSA in their 2020 toxicity assessment of PFAS, which concluded that an immune effects study POD-HED could be used with no intra-human or other uncertainty adjustment because the study population was a vulnerable subgroup (children). Nonetheless, the USEPA RfD was developed with ten-fold UFs to the POD-HEDs to account for sensitive subgroups for both developmental and cardiovascular effects of PFOS (USEPA, 2024a). In contrast, when USEPA conducted its economic benefits analysis for PFOS reduction, the Agency applied 3- to 5-fold lower slope factors (substantially less potency) for both the developmental and cardiovascular endpoints, respectively, with no adjustment for uncertainty (USEPA, 2024b). This illustrates USEPA's view of the uncertainty in its developmental and cardiovascular POD-HEDs when applied to risk/benefit analysis. When considering all the critical study POD-HEDs and toxicity values summarized in **Table 3** and **Table 4** of this 2025 Best Practice, a PFOS HPV of 1 ng/kg-d is based on POD-HEDs for the most sensitive effects and is associated with ranges of margins of exposure (MOEs) for human and animal study POD-HEDs of 1-7 and 55-3,450, respectively. These ranges of MOEs are not unlike the MOE ranges reported in the Consortium PCB Protocol (Great Lakes Sport Fish Advisory Task Force, 1993).

Eliminating local fish consumption due to implementation of guidelines directly based on the 2024 USEPA RfD would remove an important low-cost source of protein, vitamin D, omega-3 fatty acids, and other nutrients for many people, which could result in adverse impacts on public health, including cardiovascular and developmental endpoints. The quantitative offset of PFOS toxicity provided by fish consumption benefits has not been determined, but the magnitude of the offset is likely to be substantial (Hamade, 2024). An HPV should prevent doses above toxic effect levels and balance loss of health benefits of fish consumption against reduced risk (e.g., to account for intra-human uncertainty).

In developing a toxicity value such as an RfD, it would be in keeping with traditional risk assessment practice to apply an UF to the POD-HED that is greater than one to account for intra-human variability, particularly for the cardiovascular endpoint, which is not based on a study of a vulnerable group traditionally identified in human health risk assessment. Traditionally, an RfD does not consider the potential loss of health benefits. However, consistent with past Consortium Protocols, the HPV is a protective value specifically for the fish consumption exposure pathway and is not intended to be an RfD. The HPV is based on a weight-of-evidence assessment and professional judgment that considers both risk and benefits. As such, it could be construed as incorporating risk management considerations. As noted in the Consortium PCB Protocol (Great Lakes Sport Fish Advisory Task Force, 1993), while an HPV is not an RfD, it is not meant to undermine the value of RfDs and traditional risk assessment practice for other exposure pathways. In identifying an HPV, the Workgroup felt that it was reasonable to consider the intra-human uncertainty in assessing possible risk of toxicity together with the likely risk of lost benefits of fish consumption impacting the same health endpoints, possibly at a similar magnitude.

The effect levels (POD-HEDs based on BMDLs) identified by USEPA for cardiovascular and developmental effects were 1.20 ng/kg-d and 1.13 ng/kg-d, respectively, both rounding to 1 ng/kg-d. A fish consumption HPV of 1 ng/kg-d PFOS would prevent doses above effect thresholds, pose acceptable risks

for cardiovascular and developmental endpoints, and have reasonable MOEs when considering other toxicity effect levels. The Workgroup believes that the choice of this HPV would provide reasonable protection against toxic effects while avoiding unnecessary loss of fish consumption health benefits. **For all populations (general and sensitive), the Consortium is using 1.0 ng/kg-d POD-HED (USEPA, 2024) as the PFOS HPV for fish consumption guidelines in this Best Practice.**

Exposure Parameters

Regulatory requirements of some state and federal programs may dictate that specific exposure factors (e.g., body weight) be used in the risk assessment process. However, fish advisory programs, due to their non-regulatory nature and in consideration of the benefits of fish consumption, have traditionally maintained more autonomy in choices of exposure factors. Some state fish advisory programs may rely upon different values for exposure parameters, and these choices may or may not have significant effects on calculated guidelines. PFOS concentration ranges corresponding to meal frequency categories in this Best Practice accommodate the different body weight assumptions, rounding conventions, and approaches to calculating consumption used by Consortium members. **Ultimately, after rounding, the various approaches arrive at similar guidelines.**

Body Weight

Due to changes in the U.S. population, body weight assumptions used in risk assessment have changed over time and are being adopted at different rates by the state agencies. Historically, the 70 kg adult was widely used in calculations as the average weight for adults in the U.S. Most Consortium states currently use 70 kg and the 2019 Best Practice did as well (Great Lakes Consortium for Fish Consumption Advisories, 2019). This value has been re-evaluated by ATSDR and USEPA, and they now recommend using 80 kg for an adult body weight (ATSDR, 2023; USEPA, 2011). Some Consortium states have adopted the updated 80 kg body weight (New York and Michigan), and other states are considering it. To follow the latest federal guidance and population trend, the Workgroup decided to use 80 kg in this Best Practice for an adult body weight.

Consumption Rate

Few studies have surveyed typical fish meal sizes. The average meal size in two studies reported in the USEPA Exposure Factors Handbook was around 117 and 114 g (4 ounces) (USEPA, 2011). Recommendations for fish consumption for a healthy diet from the Dietary Guidelines for Americans 2020-2025 are at least 8 ounces of fish per week for adults and 8 to 12 ounces for those who might become or are pregnant or breastfeeding (USDA and HHS, 2020). Meal size should be considered when interpreting fish consumption meal frequencies and transparent when communicating fish consumption recommendations.

Meal size for fish consumption advice has typically been 227 g (equivalent to ½ pound or 8 ounces) of an uncooked fish fillet. In its PCB Protocol, the Consortium recognized that the 227 g meal size appeared to be the most widely used for exposure assessment, often with the caveat that any overestimate provides an additional "margin of safety" (Great Lakes Sport Fish Advisory Task Force, 1993). In general, contaminant exposures on a per-kg-body-weight basis tend to be higher for infants and young children than for adults. However, the assumption in previous Consortium Protocols with meal size is that it changes proportionally with body weight [See *A Protocol for Mercury-based Fish Consumption Advice* (Great Lakes Consortium for Fish Consumption Advisories, 2007) (Mercury Protocol)]. Thus, while this assumption may underestimate exposure in young children, the overestimate of typical meal size for

adults balances out this potential excess risk. All agencies within the Consortium utilize a 227 g meal size for fish consumption purposes, and this Best Practice will continue to rely on this assumption.

Mathematically, the conversion of the number of meals into daily intake rate presents options for rounding and assumption for the duration of a month. For example, a half-pound meal per week could be rounded to a consumption rate of 32.4 g/day or 32 g/day, and a half-pound meal per month could be considered a quarter of that since there are 4 weeks/month (8.1 g/day or 8 g/day) versus the 2019 Best Practice value of 7.4 g/day (assuming 30.7 days/month) (Great Lakes Consortium for Fish Consumption Advisories, 2019). This Best Practice is using rounded consumption rates (8 g/day, 32 g/day, etc.).

Bioavailability

The toxicity of PFOS has been assessed in human studies based on effects corresponding to PFOS concentrations in serum, as an indicator of PFOS in systemic circulation. Bioavailability is the fraction of the external dose of a chemical that is absorbed and reaches systemic circulation. The Workgroup is not aware of any published studies of bioavailability of PFOS in any exposure medium for humans, nor any studies estimating bioavailability of PFOS in food in animals. However, serum concentrations resulting from dietary exposure to PFOS can be compared to that in water in animal studies. While USEPA reported ~100% bioavailability of PFOS in solution administered to rats by oral gavage (USEPA, 2024c), comparing studies in rats suggests that dietary exposure results in significantly lower PFOS serum concentrations compared to oral gavage exposure (NTP 2019; Lefebvre et al., 2008). Correspondingly, toxicity appears to be less when route of exposure is dietary compared to oral gavage. For example, immunotoxic effects were observed at much higher doses of PFOS in mice following dietary exposure (Qazi et al., 2010) compared to gavage exposure (Peden-Adams et al., 2008).

Inferences about PFOS bioavailability can also be drawn from PFOA due to their structural similarity (ITRC, 2023; USEPA, 2017). Bioavailability of PFOA in animal-based food for mice ranged from 15% (shrimp) to 37% (mutton) of that of PFOA in water, with less bioavailability in high lipid foods, possibly due to competitive sorption of free fatty acids with PFOA on to protein transporters (Li et al., 2015). A later study reported that the protein content of food co-ingested with PFOA-spiked soil reduced bioavailability of PFOA (30-43% for high protein foods) in mice, with mechanistic evidence suggesting that dietary protein decreased liver fatty acid binding protein (L-FABP) expression and associated PFOA binding and bioaccumulation (Cui et al., 2022).

PFOS binding to transport proteins such as serum albumin, L-FABP and membrane phospholipids is associated with bioaccumulation and half-life in humans (Cao et al., 2019; Peng, 2024; Lu, 2021; Zhao et al., 2023a; Chen and Guo, 2009). Fatty acids in fish compete for the same binding sites on these transport proteins. Thus, reduction of PFOS binding to albumin, L-FABP and other proteins through competition with fatty acids could reduce PFOS serum concentrations and bioaccumulation in target organs. Onteeru et al. (2022) reported an inverse association between fish oil supplement use and serum PFOS, but not total PFAS, in older adults, females, and in early calendar years for NHANES survey of adults 25 and older between 2007 and 2014. Retention (bioaccumulation) of PFOS in humanized mice was reduced by a high fat diet that included 23% O3PUFAs (Hamilton et al., 2021). Lin et al. (2020) reported that among prediabetic adults in the U.S., intake of high O3PUFA fish was associated with lower plasma concentrations of PFOS. Kishi et al. (2015) reported negative associations of relatively low PFOS levels with maternal fatty acid levels including saturated (palmitic acid), monounsaturated

(palmitoleic and oleic acids), omega 6 (linoleic acid and arachidonic acid), and omega 3 (alpha-linolenic acid) fatty acids during pregnancy in a Japanese birth cohort.

Bioavailability of a chemical through the oral exposure route can be no higher than bioaccessibility, which is the amount of a substance that is released from its matrix in the gastrointestinal tract and becomes available for absorption. Based on an assessment of oral bioaccessibility of PFAS using an in vitro gastrointestinal model with oral, gastric, and intestinal stages in succession for spiked homogenized fish samples, Zhao et al. (2023b) reported approximately 40% bioaccessibility of PFOS in steamed salmon and 60-65% in steamed carp and snakehead. Bioaccessibility in oil-fried fish was a few percentage points higher, and raw fish higher still. PFOS in "native" fish tissue samples, resulting from environmental exposure rather than spiking, might be expected to have even lower bioaccessibility due to having had more time to form strong bonds with the matrix. Alves et al. (2017) reported 62% bioaccessibility for PFOS in unspiked raw market flounder resulting from four stage in vitro digestion (salivary, gastric, duodenal and bile). Based on these limited studies, a central tendency of approximately 50% of PFOS in fish across species and preparation methods may be available for absorption from the human gastrointestinal tract. Once PFOS is initially absorbed, competition with fatty acids for transport proteins may accelerate its elimination and reduce its equilibrium concentration in serum.

Absorption of PFOS from fish is likely to be much less than that from drinking water. Exposure predictions for fish consumers based on 100% bioavailability, standard meal size, and USEPA 2024 TK parameter values overestimate serum levels by about 4-fold.

An unpublished study by Ginsberg et al., used TK parameter values (USEPA, 2024a) and detailed information on PFOS fish tissue concentrations and fish consumption rates from three other studies to estimate human serum levels, and compared those estimated levels to human biomonitoring results from the same studies (**Figure 1**). In one study, anglers consumed fish from waters in northern Norway in which brown trout and flounder were shown to have elevated PFOS levels due to aqueous film-forming foam releases (Hansen et al., 2016). The study reported the PFOS levels of 59 individuals consuming these fish, which were broken into different categories of fish consumption and further characterized by the amounts and types of fish eaten per year (over a 5-year period) from the affected waterbody. This information allows for an estimate of PFOS exposure (ng/kg-d) through chronic fish consumption. Biomonitoring results show a continuous trend of increasing serum PFOS across groupings of consumers with increasing fish consumption from the affected waterbodies. The geometric mean serum concentration in the highest group of fish consumers (n = 16) was 28 µg/L but can be adjusted down to 22 µg/L to account for the lower end of the distribution (20th percentile) of background levels of PFOS in serum of local residents who did not consume fish from the affected waters. The PFOS exposure from fish consumption in this high consumer group (based upon geometric mean fish PFOS concentrations) can be estimated as 11.1 ng/kg-d. Applying the one compartment TK model for PFOS uptake, distribution and elimination used by USEPA, which assumes an elimination half-life of 3.4 years and volume of distribution of 230 mL/kg (USEPA 2024a), yields a predicted serum concentration of 87 µg/L PFOS. This prediction is a 3.95-fold overestimate of the measured concentration. The Hansen et al. (2016) data are summarized in **Figure 1** along with predicted versus actual serum concentrations reported in two other fish ingestion datasets in which PFOS biomonitoring is also available. Collectively, these three studies suggest the amount of PFOS in human serum from consumption of fish is approximately 25% of that which would be predicted based

on the tissue concentrations and fish consumption rates. Considered together, evidence of competitive binding with transport proteins, studies of bioaccessibility, and comparisons of external dose to serum levels suggest that bioavailability of PFOS from fish may be on the order of 25%.

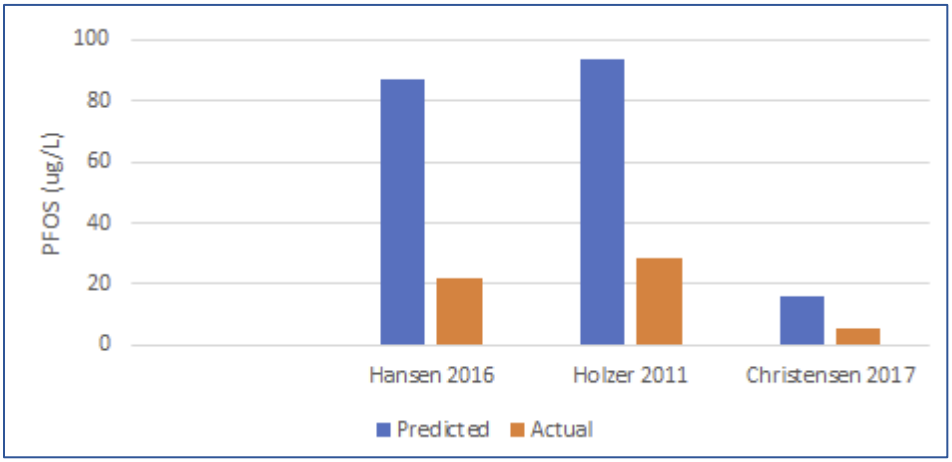

Figure 1. Predicted vs. Actual PFOS Serum Concentrations Among Fish Consumers

Similar overestimates (3-fold and 3.3-fold respectively) are apparent in the Christensen et al. (2017) and Holzer et al. (2011) datasets. Christensen et al. (2017) reported PFOS biomonitoring results for 154 Wisconsin anglers whose fish meal frequency information (19 Great Lakes fish/year) suggested a PFOS daily dose of 2.1 ng/kg-d assuming that the median level of PFOS in Great Lakes fish is 12.4 ng/g (USEPA 2015 data as summarized in Barbo et al. 2023). Holzer et al. (2011) assessed serum PFOS in 105 anglers who frequently consumed fish from Lake Mohne, Germany, which had PFAS impacts from local agricultural practices. A portion of the modeling overestimation is likely from using the default 227 g meal size as Hansen et al. (2016) provided a meal size estimate of 150 g for their subjects and Holzer provided PFOS ng/kg-d intake estimates more compatible with 100 g of fish ingestion per meal. As discussed previously, the factors that may affect the relationship between PFOS fish concentration and resulting serum concentration in consumers are limited bioaccessibility and bioavailability, and potential effects of fatty acids in fish on PFOS TKs.

Recommended PFOS Fish Consumption Guidelines

Assuming a half-pound meal size per week (52 meals/year) (and therefore a consumption rate of 32 g/day) and a body weight of 80 kg, the 1 ng/kg-d HPV, after adjusting for apparent 25% bioavailability, would be equivalent to weekly consumption of fish with a PFOS concentration of 10 ng/g. Thus, eating fish weekly with concentrations up to 10 ng/g would be expected to maintain total exposure below effect levels. A fish concentration of PFOS higher than 10 ng/g would trigger the next level of restrictive advice (e.g., Eat up to One Meal per Month). A similar calculation for monthly consumption results in a PFOS concentration of 40 ng/g. A fish concentration higher than 40 ng/g would trigger a state's next level of restrictive advice (e.g., Do Not Eat). **The Consortium recommends using the 1.0 ng/kg-d HPV, with an adjustment for apparent bioavailability of 25%, for fish consumption guidelines for the general and the sensitive populations for PFOS** (**Table 5**)**.** These guidelines rely upon a number of risk and benefit considerations, mathematical approximations, professional judgments, and risk management decisions.

Table 5. 2025 Best Practice PFOS Fish Consumption Guidelines and Parameters

Target Population and Effect	PFOS Health Protection Value (HPV) (ng/kg-d)	Body Weight (kg)	Modifying Factors	Meal Frequency Category	Fish Guideline: PFOS Concentration (ng/g)
All Populations, Developmental and Cardiovascular Effects	1.0	80	25% bioavailability in fish tissue	4 or More Meals/Week	0 – 2.5
				2 Meals/Week	>2.5 – 5
				1 Meal/Week	>5 – 10
				1 Meal/Month	>10 – 40
				Do Not Eat	>40

$$\text{Fish Guideline}_{\text{meal frequency category}}\left(\frac{\text{ng}}{\text{g}}\right) = \frac{\text{HPV}\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times \text{Body Weight (kg)}}{\text{Consumption rate (g/day) x Bioavailability factor}}$$

Exposure Parameters

1. HPV = 1.0 ng/kg-d (developmental and cardiovascular effects)
2. Body weight of consumer = 80 kg adult
3. Fish fillet meal size = 227 g (raw)
4. Consumption rate by meal frequency category: 225 meals/year or 128 g/day (4 or more meals/week), 64 g/day (2 meals/week), 32 g/day (1 meal/week), 8 g/day (1 meal/month), and Do Not Eat
5. PFOS bioavailability through fish consumption = 25%; i.e., bioavailability factor = 0.25

Meal Frequency Advisory Categories Calculations

Table 6 shows the calculations for meal frequency advisory categories. The goal is to restrict PFOS exposure to 80 ng PFOS/day (1.0 ng/kg-d x 80 kg) by using the HPV. To account for the reduced bioavailability of PFOS through fish ingestion (25%), PFOS exposure is divided by 0.25. Calculations are rounded to one significant figure.

Table 6. Meal Frequency Calculations

Meal Frequency	Calculation $\frac{\text{HPV}\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times \text{Body Weight (kg)}}{\text{Consumption rate (g/day) x Bioavailability factor}}$	PFOS Guideline (raw fillet)	Fish Guideline Range: PFOS Concentration (ng/g)
4 or more meals/week	$\frac{1\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times 80\text{ (kg)}}{128\text{ (g/day) x }0.25}$	2.5 ng/g	0 – 2.5
2 meals/week	$\frac{1\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times 80\text{ (kg)}}{64\text{ (g/day) x }0.25}$	5 ng/g	>2.5 – 5
1 meal/week	$\frac{1\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times 80\text{ (kg)}}{32\text{ (g/day) x }0.25}$	10 ng/g	>5 – 10
1 meal/month	$\frac{1\left(\frac{\text{ng}}{\text{kg}\cdot\text{d}}\right) \times 80\text{ (kg)}}{8\text{ (g/day) x }0.25}$	40 ng/g	>10 – 40
Do not eat	Not applicable	>40 ng/g	>40

Additional Considerations for PFOS and Fish Consumption

While the primary determinants of the recommended PFOS fish consumption guidelines are summarized above, several other potentially influential topics were considered by the Workgroup. Because of the tradition of accounting for other sources of exposure when setting contaminant concentration guidelines, action levels, or standards for environmental media, the Workgroup considered application of a relative source contribution (RSC) adjustment for the HPV or guidelines, concluding that such an adjustment was not appropriate. The Workgroup also decided that cancer risk levels at the guideline levels were within reasonable bounds. The Workgroup considered fillet tissue to be the best tissue from which to evaluate exposure and risk. Because fish cleaning and cooking methods can be effective in reducing exposure to some contaminants (e.g., PCBs), the Workgroup summarized the literature on this topic and concluded that cooking and cleaning does not consistently change fish tissue PFOS concentrations. The Workgroup also evaluated PFOS data to characterize “background” concentrations for relatively unimpacted waters, demonstrating that commonly encountered background levels were not far below the recommended Eat up to One Meal per Month guideline. The Workgroup considered recently published USEPA toxicity values for several other PFAS and fish data from New York State and concluded that a small percentage of fish would have unacceptable risk levels for these PFAS, and PFAS exposure via consumption for the majority of these fish would be reduced by following the advisories for PFOS. Finally, because a breastfed infant can experience a spike in PFOS exposure, the protectiveness of the HPV for that population was considered. These topics are discussed below in more detail.

Relative Source Contribution

In non-cancer risk assessment, there may be an exposure threshold above which there are observable adverse effects. Populations that eat fish from a specific waterbody may have various "background" sources of exposure to PFOS (including fish consumption from other waterbodies), in addition to consumption of fish from a specific waterbody, that contribute to total exposure. Therefore, total exposure inclusive of background exposures may be considered when determining exposure guidelines based on non-cancer endpoints, such as fish consumption guidelines for PFOS. This consideration could result in limiting the RSC of fish consumption to reduce the likelihood that total exposure exceeds a risk target. Although USEPA guidance recommends a default RSC of 20% when inadequate information is available to quantify exposure to a contaminant from all sources, available information suggests that an RSC for PFOS would be much higher. In **Table 7**, we calculated exposure for an 80 kg adult eating one meal per month of fish containing 25 ng/g PFOS (the middle of the range of potential exposures in the One Meal per Month concentration range of 10-40 ng/g). We then calculated an RSC of 82% equal to the ratio of the fish consumption exposure to the total of that exposure and "background" exposure based on NHANES data on population PFOS levels in blood serum. For PFOS fish consumption guidelines, an RSC of 82% could be applied to be protective of background sources of exposure to PFOS (including fish consumption from other waterbodies). However, the Consortium has not considered an RSC in the Protocols for mercury or PCBs. Further, PFOS was phased out of production in the U.S. and exposure to PFOS has been declining. Drinking water regulations for PFOS and other PFAS have become increasingly stringent and are expected to reduce drinking water exposure for the general population. Considering that PFOS in human serum has been declining by 21.3 % per two-year NHANES cycle from 1999-2000 through 2017-2018 (Sonnenberg et al., 2023), and that a decline is likely continuing after 2018, more current serum PFOS levels in the U.S. would likely suggest a higher RSC than 82%.

This analysis indicates the dominant PFOS exposure pathway would be fish consumption for someone eating one meal per month from a waterbody with a One Meal per Month advisory, and thus further consideration of background exposure and an RSC limit is not needed. However, considering potentially lower bioavailability of PFOS from other exposure sources, or using a higher percentile of the NHANES distribution to represent background exposure, would result in a lower RSC. These uncertainties suggest the need for future research to refine these estimates, including an assessment of PFOS exposures from various sources (e.g., drinking water, other foods, etc.). The Consortium concluded not to use an RSC when deriving the 2025 Best Practice but recommends revisiting this topic in future work based on more research.

Table 7. Fish Consumption Relative Source Contribution (RSC) to Total Exposure of PFOS

Background Blood Serum Geometric Mean Concentration for PFOS (ng/mL)	Background Exposure (ng/kg-d)*	Exposure from Eating One Fish Meal/Month Containing 25 ng/g PFOS (ng/kg-d)	RSC of Fish Consumption to Total Exposure (%)
4.25	0.544	2.5	82%
Source/Derivation: NHANES, 2017-2018, age 20 and older	Source/Derivation: One compartment PFOS TK model, USEPA 2024 parameter values	Source/Derivation: Midpoint of 2025 Best Practice range	Source/Derivation: $\frac{\text{Fish Exposure} * 100}{\text{Fish Expos.} + \text{Background Expos.}}$

* $\text{Background Exposure}\left(\frac{\text{ng}}{\text{kg*day}}\right) = \text{Background serum conc.}\left(\frac{\text{ng}}{\text{ml}}\right) * \text{Vol. of Distribution}\left(\frac{\text{ml}}{\text{kg}}\right) * \frac{\ln(2)}{t_{1/2}\ (\text{days})}$

TK parameter values used by USEPA 2024: 230 mL/kg (Thompson et al., 2010) and 1241 days (3.4 years) (Li et al., 2018b) were used for the volume of distribution and half-life ($t_{1/2}$), respectively. The calculated clearance for PFOS is 0.000128 L/kg-d.

Cancer Risk

The guidelines are supportive of the 10^{-6} to 10^{-4} lifetime cancer risk range, which is commonly considered for cancer risk screening in risk assessment practice across multiple media (USEPA, 2020). Using the cancer slope factor of 39.5 $(\text{mg/kg-d})^{-1}$ (USEPA, 2024a) and considering 25% bioavailability of PFOS in fish, the excess cancer risk for weekly consumption of a ½ pound of fish fillet with 10 ng/g PFOS over a lifetime is $4.0\text{x}10^{-5}$. If the bioavailability is not accounted for, the excess cancer risk is $1.6\text{x}10^{-4}$. For comparison, the Consortium PCB Protocol HPV equated to an excess cancer risk over a lifetime of approximately $1.3\text{x}10^{-4}$ at one meal per week considering 50% cooking loss (Great Lakes Sport Fish Advisory Task Force, 1993). Considering the estimated cancer risks at the guidelines, the benefits of fish consumption, and the Consortium's historical approach taken for other contaminants, the PFOS Best Practice is based on noncancer endpoints.

Fillet Concentration as an Indicator of Exposure

PFOS is distributed differently among fish tissues compared to other contaminants. While it is found in muscle tissue like mercury, it has also been found in fish skin and can have high concentrations in blood and particularly the liver (Honda et al., 2018). Nonetheless, the standard Consortium fish flesh sample collection protocol of using a raw, skin-on or skin-off fillet, with an exception for certain species as described in the PCB Protocol, is likely to adequately characterize the PFOS levels in the most commonly eaten portions of fish (Great Lakes Sport Fish Advisory Task Force, 1993). Given that PFOS levels in fish liver are generally greater than PFOS levels in muscle tissue (Nilsen, et al., 2024), communication efforts may want to convey that whole fish or products made from whole fish (e.g., fish paste) should not be consumed from waters which have moderately elevated levels of fish PFOS (i.e., those not requiring Do Not Eat advice).

Exposure Reduction for Cleaning and Cooking

Unlike PCBs and other organochlorine contaminants, PFOS does not preferentially accumulate in fatty tissue in fish. Therefore, methods of removing fat through skinning, trimming, and cooking that would

result in a substantial reduction in exposure for PCBs (assumed exposure reduction factors of 50% and 30% depending upon the species per the Consortium's PCB Protocol) are not expected to result in any reduction of exposure for PFOS (0% exposure reduction) (Great Lakes Sport Fish Advisory Task Force, 1993). Steaming reduced the concentration of PFOS by 53% in tuna but slightly increased the concentration in plaice (Barbosa et al., 2018). Bhavsar et al. (2014) reported that higher PFOS concentrations in freshwater sportfish increased (due to moisture loss) or had no change after baking, broiling, or frying. Hu et al. (2020) reported PFOS concentration in grass carp was increased by 32.2%, 44.6% and 23.1% after steaming, frying, and grilling and remained nearly the same after boiling, likely due to at least in part changes in moisture content. Kim et al. (2020) reported that cooking reduced total PFAS concentrations in mackerel but did not detect PFOS in either raw or cooked mackerel. A recent meta-analysis of ten studies concluded that cooking reduced PFAS concentrations in seafood by 29% on average but did not report changes specifically for PFOS (Vendl et al., 2022). Vassiliadou et al. (2015) suggested that PFOS in fish increased after broiling, baking, and frying. Another study found that boiling, frying, and baking of crab, flathead, and prawn slightly increased PFOS (consistent with water loss) and concluded that cooking does not consistently reduce PFAS concentrations (Taylor et al., 2019). Overall, reported effects of cooking on PFOS concentrations and PFOS bioaccessibility have been variable and as such challenging to practically incorporate in the fish consumption guidelines.

Background or Commonly Encountered PFOS Levels in Fish

Levels of PFOS in fish can vary by orders of magnitude. Knowledge of this distribution can help formulate fish consumption advisory benchmarks that would be health protective while still allowing relatively safer consumption of appropriate types of fish from waterbodies where levels are much lower. Considering the history of PFAS production and usage, fish tend to exhibit lower levels of PFOS in relatively pristine and remote waterbodies, which are primarily influenced by atmospheric deposition. Waterbodies in populated areas potentially impacted by surface water runoff, wastewater treatment plant effluent, and other potential sources may have higher levels (Hu et al., 2016).

Fish PFAS monitoring data (from all the Great Lakes and other in-state waterbodies) collected by Great Lakes state agencies were examined. To understand the distribution of the datapoints at lower PFOS levels, data from the Great Lakes, suspected PFAS affected in-state locations, and PFOS measurements over 40 ng/g (i.e., the Do Not Eat guideline presented in this document) were excluded from the analysis. About 3,800 PFOS monitoring datapoints for about 67 fish species from over 290 locations were considered. The bottom 25% of the measurements were below 4 ng/g, and the median concentration was 9 ng/g. Approximately 10% of the measurements fell between non-detect and 2.5 ng/g while 9.5% ranged from 2.5 to 5 ng/g. See **Figure A1** in the Appendix for the graphs depicting these data. It should be noted, though, that some PFOS data may have been compromised due to interference from naturally occurring substances (EGLE, 2024).

Fish PFAS levels monitored in U.S. rivers and streams by the USEPA were also examined to understand concentrations of PFOS at over 500 "non-urban" locations. The USEPA conducted national monitoring efforts for PFAS in freshwater fish using a probability design during the years 2008-2009, 2013-2014, and 2018-2019 with lower analytical detection limits applied in the latter two surveys. The 2013-2014 and 2018-2019 National Rivers and Streams Assessments (NRSA) involved the random selection of river sites across the lower 48 states for fish tissue sampling. The 2013-2014 assessment sampled 353 sites, while the 2018-2019 assessment included 290 sites, both assessing rivers with established fish populations (Stahl et al., 2023). Out of the 643 sites, data for 519 sites classified in the dataset as "non-urban" were

included in this review. The most frequently caught species in both studies were channel catfish, smallmouth bass, and largemouth bass. Collected fish were sent to EPA-designated laboratories for fillet preparation and homogenization before analysis for chemical contaminants such as mercury, PCBs, and PFAS. Results indicated that all samples contained detectable levels of mercury and PCBs, with PFAS found in 99.7% of the samples in 2013-2014 and 95% in 2018-2019 (Stahl et al., 2023), highlighting ubiquitous contamination across the sampled rivers. In terms of specific PFOS measurements, the lowest 25% of 519 measurements were below 2.1 ng/g, while the median concentration was at 4.5 ng/g. Approximately 30% of the measurements fell between non-detect and 2.5 ng/g, while 23% ranged from 2.5 to 5 ng/g. These data are graphed in **Figure A2** of the Appendix. Notably, the median level of total targeted PFAS in freshwater fish could be higher than levels in store bought fish (Barbo et al., 2023; Ruffle et al., 2020).

PFAS Mixtures

Reduction in exposure to PFOS to meet PFOS consumption guidance would likely result in reduction in exposure to other PFAS.

Adverse noncancer effects observed following exposures to PFOS are similar to those of some other PFAS (e.g., perfluorobutane sulfonate (PFBS), PFHxS, PFNA) and include outcomes in several shared biological systems (e.g., hepatic, thyroid, lipid synthesis and metabolism, developmental and immune) (USEPA, 2024d). However, data on modes of action are limited or entirely lacking for many PFAS. USEPA recommends that in the absence of data indicating shared mode of action, PFAS that elicit similar adverse effects should be assumed to act in a dose-additive manner unless data demonstrate otherwise (USEPA, 2024d; USEPA, 2024e). The Consortium Best Practice for Risk Assessment of Chemical Mixtures concluded that additive effects of chemicals should only be considered if a target organ *and* mode of action were shared (Great Lakes Consortium for Fish Consumption Advisories, 2018). As a general observation, PFOS seems to contribute 75% or more of the total commonly measured PFAS concentration in fish (e.g., median of 81% and 73% of total 13 measured PFAS in the 2013-14 and 2018-19 NRSAs, respectively (Stahl et al., 2023), especially at higher PFOS concentrations. The relative contribution can be lower at low PFOS concentrations (e.g., <20 ng/g), particularly if another PFAS is the primary local source contaminant.

In April 2024, USEPA established RfDs and legally enforceable MCLs in drinking water for six PFAS, including chemical-specific MCLs for PFBS, PFOA, PFOS, PFHxS, PFNA, and GenX [also known as hexafluoropropylene oxide dimer acid (HFPO-DA)] (40 CFR Parts 141 and 142, 2024). These RfDs were converted to RfD-equivalent fish concentrations that would trigger a One Meal per Month fish consumption advisory and then compared to New York State PFAS fish concentrations (**Table 8**). The data illustrate that a small minority of fish would exceed the RfDs for PFOA (>7%) and PFHxS (2%) with PFBS and PFNA having no exceedances. Therefore, where there are exceedances, the resulting advisory would be more restrictive than One Meal per Week e.g., Eat up to One Meal per Month or Do Not Eat. Also shown is that PFOS is the most frequently detected PFAS and that the majority of results would exceed a One Meal per Week fish consumption advisory. Similarly, USEPA (2024f) reported at least 92% of fish exceeded 0.25 ng/g PFOS in the 2018-19 NRSA dataset. Considering that most PFAS in various environmental media (including fish) tend to be correlated with one another, reduction in exposure to PFOS to meet PFOS consumption guidance would likely result in reduction in exposure to other PFAS. This tends to be true even when correlations are poor, although there may be instances where the reduction in exposure to other PFAS may not be adequately protected by the PFOS guidelines. Note that

fish exceeding RfD-equivalent concentrations for PFOS and PFOA are underestimated because the RfD-equivalent concentrations are below the reporting limits for these compounds.

Table 8. Individual fish from New York State waters with PFAS levels exceeding RfD-equivalent fish concentrations (would trigger at least a 1 Meal per Month advisory)

PFAS	RfD (ng/kg-d)	RfD-Equivalent Fish Concentration (ng/g)	% New York fish Exceeding RfD-Equivalent Concentration	# New York fish Exceeding RfD-Equivalent Concentration	# Total Fish
PFBS	300	750	0	0	1292
PFNA	3	7.5	0	1	1292
GenX	3	7.5	NA	NA	NA
PFHxS	2	5.0	2	30	1292
PFOS	0.1	0.25	>88*	>1140*	1292
PFOA	0.03	0.08	>7*	>88*	1292

NA = Not applicable, New York State does not monitor GenX.

*Underestimate due to method reporting limit being greater than the RfD-equivalent concentration.

In July 2024, USEPA finalized the Integrated Risk Information System (IRIS) toxicological review for perfluorodecanoic acid (PFDA) and related salts along with an RfD of 0.002 ng/kg-d (USEPA, 2024g). The RfD was based on developmental and immune effects, specifically decreased birth weight in male and female infants and decreased serum antibody concentrations for tetanus and diphtheria in children (Wikstrom et al., 2020; Grandjean et al. 2012; Budtz-Jørgensen and Grandjean, 2018). In addition to the UF of 10 for intra-human variation, USEPA applied an UF of 3 for evidence-based deficiencies to the POD-HED of 0.06 ng/kg-d. Similar to USEPA's use of Wikstrom et al. (2020) for the PFOS MCL RfD (USEPA, 2024a), no correction was made for potential confounding by other PFAS or other contaminants for the PFDA IRIS RfD based on birth weight. For the immune endpoint RfD, consideration was given to potential confounding by PCBs, but no adjustment was made for mercury or other contaminants. A PFDA model adjusted for PFOS and PFOA did result in considerably lower beta coefficients (37% lower for tetanus and 67% lower for diphtheria) and higher immune endpoint BMDs (Appendix C.1.1 in USEPA, 2024g), but USEPA did not use these adjusted results in the determination of their POD-HED.

PFDA levels tend to be lower in fish than PFOS levels, but PFDA is frequently detected. According to data from USEPA's 2013-14 and 2018-19 NRSAs, PFDA was detected in 84% of fish fillet samples with 50th and 75th percentiles of PFDA levels of 0.580 ng/g and 1.16 ng/g, respectively, or about twenty-fold lower than PFOS levels (Stahl et al., 2023). As with other PFAS, exposure to PFDA in fish would likely be reduced by consumption advisories based on PFOS. For example, if the guideline development approach for PFOS based on POD-HED and reduced absorption was applied to PFDA, guidelines for PFDA would be 17-fold lower than those for PFOS. Considering these hypothetical PFDA guidelines and 1292 fish samples analyzed for PFOS in New York through 2023, PFOS guidelines would be protective of PFDA concentrations in 83% of fish samples.

A conservative approach for applying a PFOS fish consumption guideline would be to apply it to the sum of all measured PFAS. However, laboratories may vary in the specific PFAS (and the number thereof) included in their analysis, and this approach for application of the guideline could result in the identity

and number of the specific compounds analyzed affecting the total concentration. Further, laboratories seem to be offering analytical results for an increasing large number of PFAS. Additionally, because of the dominance of PFOS in fish, the added complexity of summing PFAS before comparing the guideline may result in little added conservatism. Finally, data suggest that some PFAS (e.g., PFBS) have much lower toxicity than PFOS (USEPA, 2024a). The Province of Ontario is the only Consortium member organization that currently applies its PFOS guidelines to the sum of PFAS; no Great Lakes state routinely applies their PFOS guidelines to the sum of PFAS.

However, given the timing of the finalization of the IRIS PFDA RfD with respect to the Workgroup's development of the 2025 PFOS Best Practice, PFDA may require further consideration beyond the scope of this assessment. Taking the time to conduct that additional assessment would delay lowering the 2019 Best Practice PFOS guidelines with potential impacts on public health.

For these reasons, and due to the paucity of data indicating a shared mode of action across multiple PFAS consistent with the Best Practice for Risk Assessment of Chemical Mixtures, **the Consortium recommends that fish consumption advice only be based on PFOS levels at this time. However, the Consortium also recommends that the literature be reviewed every 3 years (or sooner if critical new information becomes available) to evaluate if other PFAS should be included in a mixtures calculation.** Members will submit new evidence that emerges for Consortium review.

PFOS Exposure for a Breastfed Infant

Breastfed infants can have increased PFOS exposure compared to formula-fed infants (e.g., Abraham et al., 2020). USEPA took this into account when calculating the POD-HED for immune effects in children. The internal serum POD was converted to an external dose (POD-HED) with assumed exposure parameter values for drinking water using the updated Verner model, which "explicitly models the mother from her birth through the end of breastfeeding, which allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy."

The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. For the one-year breastfeeding period, the exposure to the child was only through lactation and serum levels in the child were greater than serum levels in the mother. After one year, the exposure to the child (relative to body weight) was set to the same value as the mother. The model provided predictions up to a child age of five years when the serum concentrations used to determine the POD were collected, and reverse dosimetry was used to determine the POD-HED that results in the POD serum concentration.

USEPA did not take breastfeeding exposure of infants into account for the cardiovascular and hepatic endpoints because they were both based on studies of adults. The USEPA POD-HEDs used to develop candidate RfDs for cardiovascular effects based on Dong et al. (2019) and the hepatic effects based on Nian et al. (2019) were based on simple steady-state TK calculations. For the USEPA POD-HEDs for developmental effects (Wikstrom et al., 2020), the study population was infants at birth, so the breastfeeding of infants was not taken into account, although lifetime exposure of the mother was modeled from birth through gestation.

The POD-HED selected for immune effects of 1.03 ng/kg-d accounted for the breastfed infant and was similar to the POD-HEDs for cardiovascular (1.20 ng/kg-d) and developmental effects (1.13 ng/kg-d), and lower than the POD-HED for hepatic effects (1.94 ng/kg-d) (USEPA, 2024a, Table 4-8). Thus, USEPA

considers the overall POD-HED of 1 ng/kg-d to be protective of these four critical effects, including exposure to the breastfed infant for the only outcome for which that exposure pathway is directly relevant (exposure leading up to five-year-old serum levels associated with immune effects).

PFOS exposure in breastfed infants incorporates maternal placental transfer of maternal PFOS during gestation and from consumption of breastmilk, which can lead to PFOS exposure peaks during infancy. Currently available TK models for drinking water exposure have demonstrated that exposure peaks during infancy and declines as breastfed children age into adulthood across a lifetime of exposure, thus making early childhood a sensitive window of exposure to PFAS. Current validated TK models in the scientific literature assume the primary source of exposure to PFAS is from drinking water consumption with negligible non-drinking water exposures (including dietary exposures). The Minnesota Department of Health has modeled PFOS exposure to the breastfed infant and concluded that the cumulative dose over time (equated to a health-based drinking water concentration) would have to be lower by 47% to protect breastfed infants at peak serum levels compared to formula fed infants (MDH, 2024). USEPA (2024a) modeled a chronic maternal POD-HED from the five-year-old serum concentration (POD) using reverse dosimetry, but there is no indication whether any exceedances of the POD were observed in the model output prior to five years of age. The USEPA defines an RfD as an estimate of the daily oral exposure to a toxic substance that is unlikely to cause adverse health effects over a lifetime and considers the 2024 PFOS RfD to be "protective of effects that may occur in sensitive populations (i.e., embryo and fetus, infants, and young children)." It is generally not applicable to short term exposures. However, applying it to a sensitive window of exposure is not necessarily inconsistent with current risk assessment practices. As one of the co-critical effects identified for PFOS is a developmental endpoint and can potentially result from a short-term exposure during critical periods of development, USEPA concluded that the overall RfD for PFOS is applicable to both short-term and chronic risk assessment scenarios (USEPA, 2024a).

While the breastfed infant has been primarily considered for drinking water exposure scenarios, breastfed infants may also be exposed to PFOS through maternal fish consumption. In a birth cohort study of a Faroese fishing community, where elevated exposures to marine contaminants occur via ingestion of traditional foods such as whale meat, whale blubber and fish, serum concentrations of PFAS were reported in children (including breastfed infants) at birth and up to 60 months of age (Mogensen et al., 2015). Children in this cohort were breastfed exclusively for a cohort median of 4.5 months, followed by partial breastfeeding with supplementary baby food for a cohort median of 4 months. In addition, more than two-thirds of the children consumed whale by age 5. The peak of PFOS exposure in children occurred at 11 and 18 months of age (median PFOS serum concentration of 23.2 and 24 ng/mL, respectively), and declined at 60 months of age (median PFOS concentration of 13.3 ng/mL). The median maternal serum concentration of PFOS for this cohort was 19 ng/mL (Needham et al., 2011). Thus, the median maternal exposures and median infant exposures for this cohort are within a similar range. However, when using linear mixed models to assess the trajectory of serum-PFAS concentrations during months with and without breastfeeding, adjusting for confounders such as body size, extending the duration of exclusive breastfeeding from the cohort median (4.5 months) to an assumed 6-month duration was associated with increases in PFOS serum concentrations. Study findings are limited by the small sample size of total children (n = 81), variations in same sizes for each sampling age, and complete observations from all examinations were available for an even smaller subset of data (n = 12). However, the mixed model results show that children in this subset of data would have PFOS trajectory exposure peaks ranging approximately between 8 and 60 ng/mL, with 9 of those children with peaks greater than approximately 18 ng/mL. The study does not provide much detail on the breastfeeding patterns in these 12 children but notes that the child with the lowest serum concentration was not breastfed at all,

whereas the child with the highest serum concentration was breastfed exclusively for 6 months and then partially breastfed for the following 5 months. Thus, while the median infant PFOS concentrations are within similar range of mean maternal serum concentrations for this cohort, the mixed model results show that breastfed infants can have higher PFOS concentrations than the median maternal serum concentration. However, additional studies may be needed on the relationship between serum PFOS concentrations in mothers exposed to PFOS primarily through diet (fish consumption) and corresponding serum concentrations in breastfed infants.

Lifetime models such as those used by Minnesota and USEPA are used for, and have been validated for, drinking water exposures. While it could be possible to modify existing two-generation first-order models to address fish consumption as the primary source of exposure, the Consortium is not aware of publications of validated TK models using this approach. Validation measures are based on comparisons of predicted and measured serum to ensure that the model and model assumptions are adequately predictive. Given that certain TK parameters may differ between drinking water and fish ingestion, such as relative absorption, ingestion rates, and differences in patterns of consumption (exposure frequency), validation would be an important step in considering use of this approach. While it may be reasonable to assume that an exposure peak during infancy may occur in breastfed infants of mothers whose primary exposure to PFOS occurred via fish consumption, these differences in TK assumptions used for fish versus drinking water consumption could potentially impact the extent of the exposure peak during infancy (i.e., the height and width) and the declines in those levels across lifetime exposure as children age into adulthood. Thus, additional studies in this area would be helpful in evaluating breastfed infant exposures corresponding to maternal fish consumption.

In addition to concerns about peak exposure to a breastfed infant from a mother's exposure, the Consortium has discussed concerns related to an infant's concurrent breastfeeding and consumption of fish. The Workgroup has not considered concurrent breastfeeding and consumption of local freshwater fish by infants. Infants typically begin eating solid food at 6 months of age, and most mothers discontinue breastfeeding when infants are under 1 year of age, although some continue beyond this age. At this point, the Workgroup is not aware of data indicating fish consumption rates for infants. In the absence of that data, the Best Practice assumes that the guidelines are protective of infants and all vulnerable subgroups (and therefore also the general population). However, for some infants, concurrent breastfeeding and consumption of local fish may occur. This could lead to exposures in excess of the HPV. For this small subpopulation, targeted risk communication could be considered. For example, breastfeeding mothers who eat local fish could be advised not to also incorporate local fish into food for their infants. These considerations should be further explored in future Consortium work.

Potential Considerations for the Next PFOS Guidelines Review

As more information on PFAS toxicity, fish consumption benefits, and other relevant topics becomes available, the Consortium will review this information and update its approach for this fish consumption Best Practice as appropriate. Some potential considerations for future review were identified through comments from Consortium members on proposed updates for the Best Practice and are presented below. Others may include the need to establish guidelines for other PFAS like PFDA, and how to handle fish consumption exposures to mixtures of PFAS, especially if the relative proportion of PFOS declines over the coming years. Future research could be conducted on maternal PFOS exposure through fish consumption and subsequent exposure to the breastfed infant, and whether the breastfed infant is adequately protected. Due to concerns about potential short-term exceedances of an HPV for a

breastfed infant who also consumes local fish, thought could be given to future development of messaging discouraging feeding of food containing local fish to infants. More research should be conducted to quantify the offset of toxic effects of PFOS with the benefits of fish consumption. The appropriateness and applicability of the Best Practice PFOS guidelines and underlying assumptions for tribes and cultural/traditional practices could benefit from additional consideration in the future. Outreach and education Consortium members should help develop approaches to transparently communicate scientific information and uncertainty related to PFAS in fish to maintain public trust. Outreach messaging could also be developed to communicate to the public that there are PFOS exposures other than fish consumption (e.g., consumer products) that could be reduced through healthy choices.

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Appendix

Figure A1. Distribution of fish PFOS measurements collected by the Great Lakes states for sites that are not in the Great Lakes and not identified in the dataset as suspected of being contaminated with PFAS, as well as measurements that were not over 40 ng/g. a) boxplot by location, b) histogram of measurements in bin sizes of 2.5 ng/g, and c) boxplot of all "filtered" measurements.

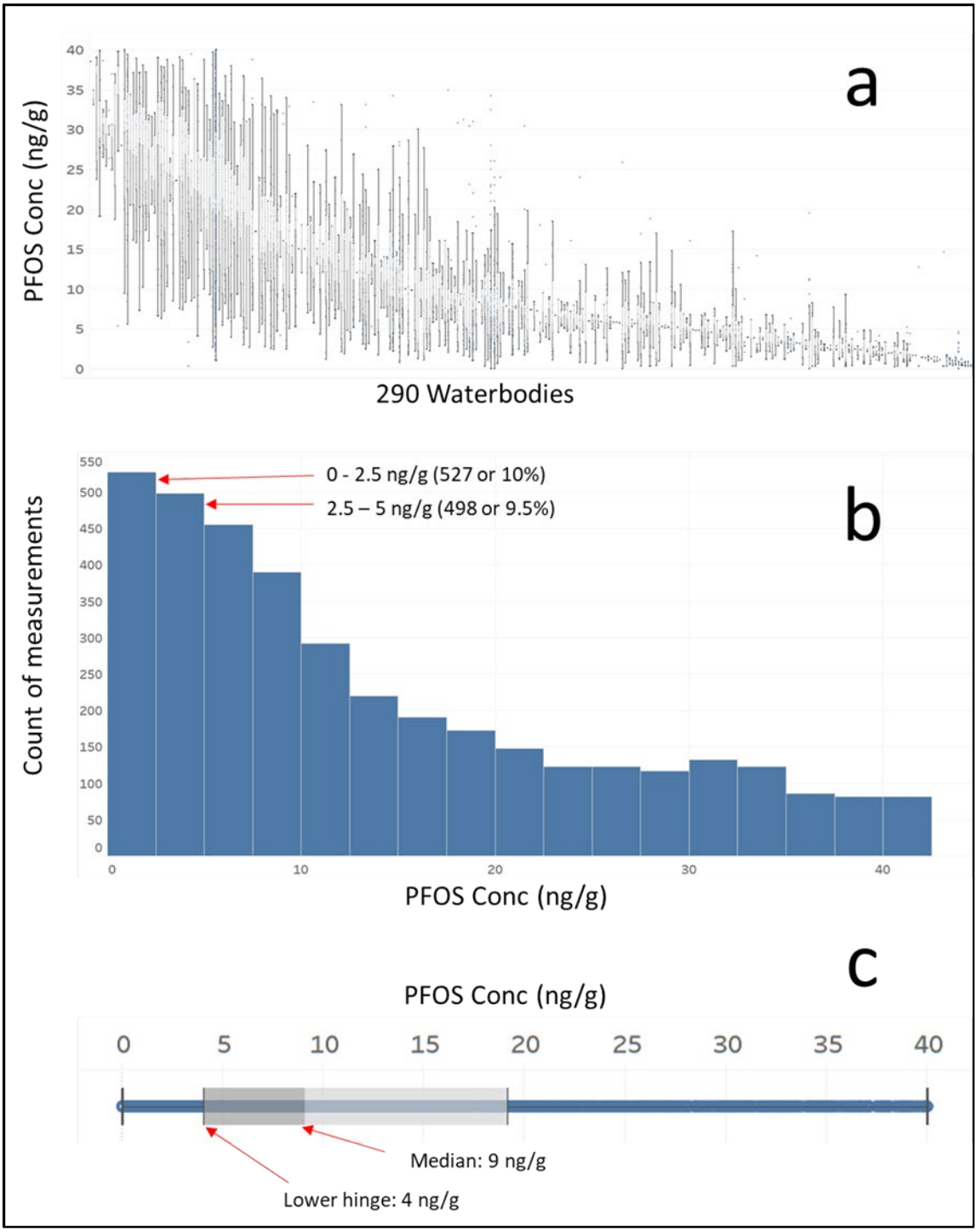

Figure A2. Distribution of fish PFOS measurements collected by the USEPA at "non-urban" locations during the 2013-2014 and 2018-2019 National Rivers and Streams Assessments. a) boxplot by location, b) histogram of measurements in bin sizes of 2.5 ng/g, and c) boxplot of all measurements.